REFRACTORY MEGALOBLASTIC ANEMIA


The term "refractory anemia" was introduced by Bomford and Rhoads (1941) for anemias of a wide variety of types that were refractory either temporarily or permanently to hematinic therapy. In 1943 Davidson, Davis and Innes published a series of papers entitled "Studies in Refractory Anaemia" which dealt with the problem of classification on the basis of examination of the bone marrow by sternal puncture and divided the anemias refractory to liver extracts into two main groups, namely (1) refractory anemias with hypocellular normoblastic marrows, and (2) refractory anemias with hypercellular megaloblastic marrows. Of particular significance was their finding that the prognosis was vastly different in the two groups. Thus, of 16 patients in Group 1, 11 died of progressive anemia within a few months, while of 16 cases in Group 2 all eventually made a complete recovery. Intensive treatment with large amounts of liver extract supplemented with iron and vitamins and repeated blood transfusions was required for long periods if such satisfactory results were to be obtained. The long period of illness during which life was continuously in danger indicated the need for some therapeutic agent which would cause a prompt remission comparable to that obtained with parenteral liver therapy in the relapse stage of Addisonian pernicious anemia.

In this paper the term refractory megaloblastic anemia is confined to cases of megaloblastic anemia which failed to respond hematologically and clinically to the parenteral administration of an amount of liver extract which has been proved to produce an optimal response in cases of Addisonian pernicious anemia. The test preparation employed was Anahaemin, marketed by British Drug Houses Ltd., which has been found by the writer to be potent in a dose of 2 cc. when administered parenterally in a large number of cases studied during the past ten years. Every patient with refractory megaloblastic anemia received at least twice this dose after admission to hospital. In addition many cases had received large amounts of potent liver extracts prior to being referred to us for investigation of their failure to respond. Since infections, intoxications and advanced arteriosclerosis are known to inhibit or delay the response to parenteral liver therapy, patients exhibiting any of these complications were not included in the group of refractory megaloblastic anemia discussed below.

For many years the writer has suggested that chemical purification of liver extracts for parenteral use removes some essential factor which is necessary for the restoration of normal blood formation in certain cases of megaloblastic anemia which have failed to respond to potent liver extract given parenterally. The following case history of a patient seen by the writer nearly fifteen years ago illustrates this problem very clearly.

The patient was a middle-aged business man who had worked in India for many years and had always been in good health until one year before the present illness. His case notes from Calcutta indicated that

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a year previously he had had an attack of dysentery from which he apparently recovered completely. A few months later he began to feel tired and breathless on exertion. His tongue became sore and his bowels loose. The report from a medical specialist in Calcutta indicated that he had a moderate degree of macrocytic anemia and free hydrochloric acid was present in the gastric juice. Despite all treatment, he continued to lose weight and strength rapidly and was sent home to Scotland for investigation of the cause of his illness. When I first saw the patient he was extremely emaciated having lost 6 stone in weight during the previous twelve months. He was passing pale, greasy, bulky stools and the blood picture was typical of Addisonian pernicious anemia. His red cells numbered 1 M. and his Hb. 2.5 per cent. A histamine-fast achlorhydria was present. He was diagnosed as suffering from tropical sprue and given a low fat diet supplemented by vitamins and iron. Parenteral treatment with the liver extract Campolon was started. This resulted in a rise of reticulocytes to 1 per cent but no subsequent increase in red cells or Hb. occurred. The patient was desperately ill and life had to be maintained by blood transfusions. Parenteral liver therapy was continued but was totally ineffective. The patient’s diet was then changed to a high protein diet containing 150 Gm. of protein daily in the form of meat and liver by mouth. Within a week a remarkable improvement in his general condition and hematologic state occurred. Within three months the patient gained nearly 4 stone in weight and his blood count and blood picture were restored to normal. Of particular interest was the finding that free hydrochloric acid was again present in his gastric juice. Contact was kept with this patient for many years and it was found that the complete clinical and hematologic remission was maintained.

This case represents a perfect example of a refractory megaloblastic anemia associated with the sprue syndrome which failed to respond to large quantities of crude potent liver extract given parenterally and showed a dramatic improvement when given liver by mouth.

During the next ten years I occasionally encountered patients with the classic pernicious anemia blood picture who were refractory to parenteral liver therapy but who responded to liver given orally. The problem of refractoriness was brought into prominence during an investigation which was conducted in Edinburgh into cases of pernicious anemia of pregnancy. In this group of megaloblastic anemias we found that refractoriness to potent liver extracts given parenterally is not uncommon. The results of the investigation were published in 1942. (Davidson, Davis and Innes). Of 16 cases with a classic megaloblastic marrow 10 were refractory to liver extracts given parenterally. Shortly after this investigation our attention was attracted to the megaloblastic anemias associated with the sprue syndrome (tropical sprue and idiopathic steatorrhea), and here again we found patients who were either completely or partially refractory to potent liver extracts given parenterally. In addition to cases of refractory megaloblastic anemia associated with pregnancy, and the puerperium and the sprue syndrome we also encountered cases of refractory anemia whose etiology was completely obscure, and to this group we gave the name ‘idiopathic refractory megaloblastic anemia’ and it is with this group that this paper is particularly concerned.

This short introductory note regarding our clinical investigations into refractory megaloblastic anemias over many years is given with the object of indicating why we desired to find a therapeutic agent which would be effective and why we believed that this product could be produced from liver which had not been submitted to a process of chemical purification for parenteral therapy.

The first step in this investigation consisted of predigesting liver with the enzyme papain at a pH of 5.6, thus avoiding the danger of destruction of active principles.
by exposure to acid or alkaline conditions. The product obtained was a light brown powder completely soluble in water. The name ‘proteolysed liver’ was selected for descriptive purposes. Since the walls of the liver cells had been completely disrupted it appeared likely that a high proportion of water soluble constituents would be liberated and hence retained in the final product, and that other active principles present as a protein complex would be set free and so be rendered available for immediate absorption. Clinical tests made with a 70 per cent alcohol soluble fraction of liver before and after digestion with papain supported this conclusion.

It was estimated that 1 oz. of ‘proteolysed liver’ was derived from 6 oz. of raw ‘wet’ liver. The material which has since been marketed under the trade name ‘Hepamino’ was first tested on 5 cases of classic Addisonian pernicious anemia and produced a dramatic response in all instances in a daily dose of ¼ to ½ oz. A report on the method of preparation and its clinical trial was published in 1943 (Davis, Davidson, Riding and Shaw). During the next two years work was extended to testing the preparation in cases of refractory megaloblastic anemia. Thus, in 1944, we described the remarkable results produced in 4 cases of idiopathic megaloblastic anemia and in 1 case of refractory megaloblastic anemia of pregnancy (Davis and Davidson). We also noted its therapeutic failure in cases of macrocytic anemia with a normoblastic marrow.

We suggested as a provisional hypothesis that ‘While failure of maturation of the megaloblasts in the great majority of megaloblastic anemias is due to deficiency of the liver principle of Castle present in fractionated liver extracts, in refractory megaloblastic anemias it results from an additional deficiency consequent on a failure in production or absorption of some unknown factor which is present in adequate amount and assimilable form in proteolysed liver and presumably also in whole liver.’ In the same paper we discussed the possible nature of this factor and came to the conclusion that it was unlikely to be a mineral, an amino acid, or any of the vitamins available at that time for clinical use. We suspected that it might be folic acid or biotin, both of which were known to be present in considerable quantities in liver. From our assessment of the position we felt that folic acid was most likely to be the missing factor and accordingly, in 1944, we wrote to Dr. Riding of Evans Medical Supplies Ltd., asking him to make a preparation of folic acid for clinical trial in refractory megaloblastic anemias. The folic acid fraction sent to us for this purpose consisted of material precipitated by 70 per cent alcohol from a watery extract of liver. This fraction is discarded in the manufacture of parenteral liver extracts as it has been repeatedly shown to be impotent therapeutically. Nevertheless, it is in this fraction that most of the folic acid in liver is stated to occur, as determined by biologic assay. As was to be expected from previous clinical experience, this fraction was found to be impotent when fed in daily doses of 1 oz. to patients with pernicious anemia. Unfortunately at that time no suitable case of refractory megaloblastic anemia was available on which to try the extract. These results suggested that if folic acid was present in the nonproteolysed 70 per cent alcoholic precipitate of liver, it existed in some conjugated form which could not be utilized by a patient with pernicious anemia or, alternatively, that
I 10 REFRACTORY MEGALOBLASTIC ANEMIA

the content of folic acid in the test dose of extract was insufficient. The next step taken was to submit the 70 per cent alcoholic precipitate of liver to papain digestion and see whether this would lead to the liberation of some hematinic factor which was not available in the nonproteolysed 70 per cent alcoholic precipitate. Three cases of Addisonian pernicious anemia were treated with this proteolysed fraction. Of these, 1 case responded moderately well and 2 failed to show any response. The single success achieved suggested that as a result of enzymic digestion some potent material had been liberated but that the amount so liberated was insufficient to produce satisfactory results. From an assay of folic acid in proteolysed liver carried out at a later date, this supposition was almost certainly correct. Accordingly we decided to obtain a more potent source of folic acid and were in the process of investigating measures to achieve this object when the synthesis of folic acid was announced by Angier et al. (1945) and its clinical and hematologic effects in megaloblastic anemias were published by Spies et al. (1945). Through the courtesy of Messrs. Lederle and Dr. Spies, we were fortunate in obtaining adequate supplies of folic acid and have thus been able to confirm the observations made by Spies and other workers in America in regard to its effectiveness in all forms of megaloblastic anemia (Davidson and Girdwood, 1946, 1947). Of particular interest to us was the determination of the smallest dose of synthetic folic acid which would produce a hematologic response in pernicious anemia as this was obviously a matter closely related to the problem of what constituent in proteolysed liver was responsible for its therapeutic activity in megaloblastic anemias refractory to potent parenteral liver extracts. In this connection we should mention that of 5 cases of pernicious anemia treated with 2.5 mg. of folic acid daily all responded satisfactorily. Of 5 cases given 1 mg. daily 1 gave an excellent response, 2 a moderate response and 2 gave no response. The last 2 cases subsequently responded well to a daily dose of 2.5 and 5.0 mg. respectively. The problem of the minimal effective dose of folic acid is referred to again in the discussion.

IDIOPATHIC REFRACTORY MEGALOBLASTIC ANEMIA

During the past six years more than 450 cases of macrocytic anemia have been submitted to a full clinical and hematologic examination including sternal biopsy in the wards and blood clinics under my charge. Approximately 75 cases out of this group were found to be cases of macrocytic anemia with a normoblastic marrow and need not be considered in this paper. They included many examples of the sprue syndrome and cases of chronic hepatitis, aplastic and hypoplastic anemia, macrocytic hemolytic anemia and aleukemic leukemia. Three hundred and fourteen cases were diagnosed as classical pernicious anemia and all responded satisfactorily to parenteral liver therapy, proteolysed liver or folic acid. In addition, parenteral liver therapy was effective in 12 cases of megaloblastic anemia associated with pregnancy and the sprue syndrome. Lastly there were 59 cases of megaloblastic anemia refractory to potent parenteral liver extracts. Since a description has been given in our previous publications of cases associated with pregnancy and the puerperium and with the sprue syndrome it has been decided merely to illustrate representative cases of these groups with graphs showing the effect of treatment
Fig. 1. A woman, aged 37, with refractory megaloblastic anemia associated with idiopathic steatorrhea. Response to folic acid. (See Case History 2.)

Fig. 2. Male, aged 32, with refractory megaloblastic anemia associated with tropical sprue. Response to folic acid.
with proteolysed liver and folic acid (figs. 1, 2 and 3), and confine our observations essentially to the group which we have called "idiopathic refractory megaloblastic anemia."

A patient is placed in this group only if the cause of the megaloblastic anemia cannot be ascribed to direct dietary deficiency, pregnancy or the puerperium, malabsorption from the gastrointestinal tract or hepatic disease. It is obvious that the more thorough is the investigation and the more prolonged the period of observation the fewer will be the cases which will be classified as idiopathic. This point is well illustrated by the following 2 case histories.

**CASE HISTORY I**

First admission. A man, age 61. He gave a two years' history of weakness, breathlessness and anemia but there was no history of diarrhea, of paresthesia, of unsteadiness in walking, or of pain in the tongue. His diet had been satisfactory. A diagnosis of pernicious anemia was made in a neighboring hospital and the patient received 14 cc. of a potent purified liver extract (Anahaemin) during a period of four weeks. The patient's condition continued to deteriorate so he was transferred to our clinic for further investigation and treatment.

When admitted the patient was very weak. His blood figures were as follows: Hb. 2.8 per cent, R.B.C. 950,000 per cu. mm., W.B.C. 5,200 per cu. mm., P.C.V. 13.0 per cent, M.C.V. 136.8 cu. microns, M.C.H. C. 30.0 per cent, reticulocytes 2.2 per cent, C.I. 1.5. The marrow was megaloblastic and a test meal showed that free hydrochloric acid was present. No other abnormality was found and at no time during the period of the first admission to our wards did the patient suffer from looseness of the bowels. He was treated with 10 mg. of folic acid daily and this resulted in a reticulocyte peak of 18.2 per cent, a rapid gain in red cells and transformation of the marrow to the normoblastic state. Eventually the red cell count reached a level of 5 million. A diagnosis of idiopathic refractory megaloblastic anemia was made.

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Second admission. The patient returned to hospital a year later because his ankles were painful and swollen. He had been having repeated courses of folic acid and the red cell count was moderately satisfactory, being 4,110,000 per cu. mm. Examination of the stools revealed that he was now passing two large motions daily and these were pale, greasy and bulky. A fat balance test was carried out and this showed that the patient was absorbing only 59 per cent of ingested fat (normal 90 to 95 per cent). This clearly indicated that the patient was suffering from a malabsorption syndrome and accordingly the diagnosis was revised to that of idiopathic steatorrhea.

CASE HISTORY 2.

First admission. A woman, age 37. Admitted to hospital in March, 1944, when she was five months pregnant. Her hemoglobin was then 56 per cent and red cells 1,370,000 per cu. mm. The bone marrow was megaloblastic. A test meal showed the presence of free hydrochloric acid. A diagnosis of pernicious anemia of pregnancy was made. She failed entirely to respond to 4 cc. of Anahaemin given intramuscularly, but responded to proteolyed liver, an increase of red cells of one million per cu. mm. occurring in twenty days. The patient was then discharged from hospital, but owing to difficulty in obtaining proteolyed liver she did not continue treatment.

Second admission. She was readmitted in April, 1946, with a history of weakness and of intermittent diarrhea of a fatty type; a fat-balance test showed the percentage absorption to be 75 per cent. A test meal again showed the presence of free hydrochloric acid. She had never been abroad, and the dietetic history was normal. No antianemic treatment had been given for eighteen months before the commencement of folic acid therapy. At the start of folic acid therapy her blood findings were as follows: Hb. 40 per cent, red cells 1,370,000 per cu. mm., white cells 7,800 per cu. mm., P.C.V. 19.0 per cent, M.C.V. 138.7 cu. microns, M.C.H.C. 28.9 per cent, reticulocytes 3.5 per cent, C.I. 1.5.

The reticulocyte response and the rise in red cells over a therapeutic period of 14, 21 and 28 days reached the standards demanded by the U.S.P. Board (see fig. 1.).

A diagnosis of idiopathic steatorrhea was made.

These case histories, together with others which we do not think it is necessary to elaborate, suggest that a failure in absorption from the alimentary tract may be a primary fault in some cases of idiopathic megaloblastic anemia. In a few of these patients the poor bodily build and lack of development of the skeleton suggested previous malabsorption from the gastrointestinal tract though no history of diarrhea could be obtained at any time from infancy up to the presenting illness. The absence of such a history may have little significance since we have clearly demonstrated in the sprue syndrome that fat absorption may remain grossly defective although diarrhea is absent and the patient’s general health is good either as a result of folic acid therapy or from spontaneous remission (Davidson, Girdwood, and Innes, 1947). Other possible causes of alimentary dysfunction which are worthy of consideration are an abnormal intestinal flora or chemical or enzymic secretory defects which destroy the antianemic factor or fail to liberate it from its bound form in natural foods.

Some of the clinical and hematologic features of the group of cases labelled idiopathic refractory megaloblastic anemia are given in table 1. The patients were of both sexes and their ages ranged from 12 to 76 years. The chief complaint in all cases was weakness and breathlessness on effort, and physical examination usually revealed nothing of importance other than the signs and effects of severe anemia. Acute glossitis and objective signs of involvement of the central nervous system were absent. Chronic glossitis was frequently noted. The liver, spleen and lymphatic glands were not enlarged. The blood picture and the bone marrow were
REFRACTORY MEGALOBLASTIC ANEMIA

identical with that seen in Addisonian pernicious anemia at corresponding levels of anemia, except in Cases 16, 17, 18 (see table 1). In those cases with histamine-fast achlorhydria the differential diagnosis from pernicious anemia was impossible

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Sex</th>
<th>Age</th>
<th>Initial hematologic response to treatment</th>
<th>Treatment given</th>
<th>Free HCl</th>
<th>Hb</th>
<th>Red cells</th>
<th>Treatment given</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>46</td>
<td>Slow and delayed</td>
<td>Intensive liver, iron, ascorbic acid, transfusion</td>
<td>Present</td>
<td>23</td>
<td>0.88</td>
<td>Slow and delayed</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>20</td>
<td>Slow and delayed</td>
<td>Proteolysed liver</td>
<td>Present</td>
<td>32</td>
<td>1.80</td>
<td>Prompt</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>55</td>
<td>Slow and delayed</td>
<td>Proteolysed liver</td>
<td>Absent</td>
<td>32</td>
<td>1.33</td>
<td>Prompt</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>51</td>
<td>Slow and delayed</td>
<td>Proteolysed liver</td>
<td>Present</td>
<td>32</td>
<td>0.86</td>
<td>Slow and delayed</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>41</td>
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<td>Protelysed liver</td>
<td>Absent</td>
<td>32</td>
<td>0.75</td>
<td>Slow and delayed</td>
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<tr>
<td>6</td>
<td>F</td>
<td>34</td>
<td>Slow and delayed</td>
<td>Protelysed liver</td>
<td>Absent</td>
<td>32</td>
<td>1.91</td>
<td>Slow and delayed</td>
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<tr>
<td>7</td>
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<td>45</td>
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<td>Absent</td>
<td>32</td>
<td>0.89</td>
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<tr>
<td>8</td>
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<td>34</td>
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<td>Protelysed liver</td>
<td>Absent</td>
<td>32</td>
<td>1.34</td>
<td>Slow and delayed</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>51</td>
<td>Slow and delayed</td>
<td>Protelysed liver</td>
<td>Absent</td>
<td>32</td>
<td>1.34</td>
<td>Slow and delayed</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>14</td>
<td>Slow and delayed</td>
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<td>32</td>
<td>1.31</td>
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</tr>
<tr>
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</tr>
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<td>32</td>
<td>1.74</td>
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<td>36</td>
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<td>Protelysed liver</td>
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<td>1.16</td>
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</tr>
<tr>
<td>14</td>
<td>M</td>
<td>46</td>
<td>Slow and unsustained</td>
<td>Anahaemin</td>
<td>Present</td>
<td>32</td>
<td>1.13</td>
<td>Slow and unsustained</td>
</tr>
<tr>
<td>15</td>
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<td>21</td>
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</tr>
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<td>76</td>
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<td>1.13</td>
<td>Slow and delayed</td>
</tr>
<tr>
<td>17</td>
<td>F</td>
<td>65</td>
<td>Slow and delayed</td>
<td>Protelysed liver</td>
<td>Present</td>
<td>32</td>
<td>1.31</td>
<td>Slow and delayed</td>
</tr>
<tr>
<td>18</td>
<td>M</td>
<td>58</td>
<td>Slow and delayed</td>
<td>Protelysed liver</td>
<td>Present</td>
<td>32</td>
<td>1.31</td>
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</tr>
<tr>
<td>19</td>
<td>F</td>
<td>72</td>
<td>Slow and delayed</td>
<td>Protelysed liver</td>
<td>Present</td>
<td>32</td>
<td>1.31</td>
<td>Slow and delayed</td>
</tr>
<tr>
<td>20</td>
<td>M</td>
<td>66</td>
<td>Slow and delayed</td>
<td>Protelysed liver</td>
<td>Present</td>
<td>32</td>
<td>1.31</td>
<td>Slow and delayed</td>
</tr>
<tr>
<td>21</td>
<td>M</td>
<td>46</td>
<td>Slow and delayed</td>
<td>Protelysed liver</td>
<td>Present</td>
<td>32</td>
<td>1.31</td>
<td>Slow and delayed</td>
</tr>
</tbody>
</table>

Cases 16, 17, 18. These 3 cases had a "dimorphic" bone marrow. Although some typical megaloblasts were present the majority of the erythroblasts were normoblasts or cells intermediate in appearance between megaloblasts and normoblasts.

Case 19. Owing to vomiting this patient was initially unable to take adequate quantities of proteolysed liver.

until their failure to respond to parenteral injections of potent liver extract was discovered. In other cases the presence of free hydrochloric acid in the gastric juice was a point of exceptional diagnostic importance. Such cases would conform to the
Fig. 4. Idiopathic refractory megaloblastic anemia in a 12 year old girl. Response to proteolyzed liver.

Fig. 5. Idiopathic refractory megaloblastic anemia in a man aged 53. Response to folic acid.
diagnosis of achrestic anemia as defined by Israels and Wilkinson (1936, 1940). When the disease occurs in patients below the age of 30 and is not associated with pregnancy, suspicion should be aroused that the case is not one of classic pernicious anemia. Likewise, if the state of nutrition is unsatisfactory and the skeleton poorly developed a malabsorption syndrome should be suspected. As has already been emphasized the patients were only placed in the idiopathic group when search for a cause for the anemia had failed to establish any satisfactory etiological explanation. With regard to treatment it should be noted in table 1 that cases 1 to 9 were under our care prior to the advent of proteolysed liver and folic acid. They had a prolonged and dangerous illness lasting weeks or months and many would have succumbed if life had not been supported by repeated blood transfusions while intensive treatment with parenteral liver extract, iron and vitamins was continued. Cases 10 to 21 were treated with proteolysed liver, and cases 21 to 25 with folic acid. Case 21 was treated successfully in his first relapse with proteolysed liver. A second relapse, resulting from cessation of treatment, responded excellently to folic acid. In contrast to cases 1 to 9 whose response to treatment was slow and prolonged, cases 10 to 25 responded to proteolysed liver or folic acid in a manner comparable to that obtained in pernicious anemia with parenteral liver extract therapy. Representative examples of these cases are shown in figures 4 and 5.

Discussion

The parenteral injection of chemically purified liver extracts and the oral administration of liver, liver extracts, proteolysed liver and folic acid produce identical effects in transforming the megaloblastic bone marrow of Addisonian pernicious anemia into the normoblastic state as has been clearly demonstrated by studies of the bone marrow by ourselves and other workers. It may therefore be assumed that some common factor is responsible for this effect. Since daily doses of 1 mg. of synthetic folic acid will accomplish this result it appears likely that folic acid itself is the maturation factor, or plays some essential role in the final stage of the process of maturation. Since purified liver extracts for parenteral therapy are practically devoid of folic acid it seems not unreasonable to suppose that they produce a transformation of the bone marrow through their ability to liberate free folic acid from its conjugated state. Hence it may be postulated that in pernicious anemia the inability of the stomach to produce Castle’s intrinsic factor leads to a failure in the production of an interaction product whose function is to liberate free folic acid. There appears to be no failure in the absorption of conjugated folic acid since an immediate response is obtained to the injection of purified liver extract even when the patient has been partaking of an unsatisfactory diet for long periods.

In contrast the refractory megaloblastic anemias may be considered to be due to a failure in the supply of conjugated folic acid since no response occurs to the injection of large doses of potent purified liver extract. This failure of supply may result from direct nutritional deficiency as occurs particularly in tropical countries such as India (Lucy Wills, 1911) and the anemia may be partially or completely refractory to large doses of purified liver extract given parenterally. Other cases can be explained on the basis of a malabsorption syndrome as is typically seen in tropical
sprue and idiopathic steatorrhea (see figs. 1 and 2). As has already been noted some of these cases may not be recognized because a failure in absorption can occur in the absence of diarrhea. In other cases the possibility exists that abnormalities in the intestinal flora, or chemical and secretory changes in the alimentary tract may destroy folic acid or make it unavailable to the body in some way as yet unknown.

Lastly the liver plays an important role in the storage and possibly the final synthesis of the liberating factor formed from the interaction of Castle's intrinsic and extrinsic factors. It may also be of importance in the storage and liberation of free folic acid. Accordingly it is not surprising that in severe chronic disease of the liver a megaloblastic anemia is occasionally found. In our experience, however, the macrocytic anemia of hepatic disease is accompanied much more frequently by a normoblastic marrow reaction. Accordingly it may be concluded that a deficiency of conjugated folic acid can result from a variety of causes, some of which are known while others can merely be suspected. The resulting megaloblastic anemia will be partially or completely refractory to parenteral liver extracts depending on the relative degree of deficiency of conjugated folic acid. All types of megaloblastic anemia respond to the administration of free folic acid. This is not surprising since by giving free folic acid the need for an interaction to take place between the liberating factor contained in purified liver extracts and conjugated folic acid contained in food is circumvented. What is surprising is the dramatic therapeutic effect produced by the oral administration of folic acid in the malabsorption syndromes such as sprue. We can only assume that the capacity to absorb different substances in this syndrome varies greatly. Thus absorption of fat appears to be particularly poor while the absorption of free folic acid must be nearly perfect since a daily dose of 5 mg. or less will produce the most dramatic clinical and hematologic improvement in sprue cases with a megaloblastic anemia.

Lastly it is necessary to consider why proteolysed liver is usually as effective in the treatment of refractory megaloblastic anemia as is free folic acid.

Proteolysed liver contains the liberating factor present in chemically purified liver extracts for parenteral use. Experience has shown, however, that oral treatment with whole liver is very much less effective than parenteral treatment with liver extract derived from an equivalent amount of liver.

The therapeutic effects of proteolysed liver in refractory megaloblastic anemia cannot therefore be ascribed to its content of this factor. Proteolysed liver is a rich source of amino acids readily available for absorption because of the predigestion of liver protein with papain. We have treated cases of pernicious anemia with a papain digest of beef protein without any response, and hence do not think it likely that the therapeutic activity of proteolysed liver depends on its content of amino acids. It is possible, however, that liver contains some amino acid in high concentration which is necessary for the maintenance of normoblastic blood formation. No evidence of this hypothesis is, however, available. Supplementing the diet with individual amino acids such as methionine and choline has not been found effective in the treatment of the megaloblastic anemias. Proteolysed liver is a rich source of many vitamins, especially of the vitamin B complex including folic acid. The question therefore arises whether the therapeutic effects of proteolysed liver can be
ascribed to its content of folic acid. Before this question can be answered it would be necessary to undertake an assay of its folic acid content by the biologic methods at present in use. Opinion appears to differ widely among experts whom we have consulted in regard to the accuracy of such methods when used for the estimation of folic acid in foods and tissues. The only figures which we have available have been supplied to us by Dr. Riding of Evans Medical Supplies Ltd., who found an average figure of 0.8 mg. of folic acid per oz. of proteolysed liver. This quantity approaches the lowest amount of synthetic folic acid which we have found to be effective in the treatment of Addisonian pernicious anemia. As previously noted, of 5 cases given 1 mg. a day only 1 gave an optimal response, 2 showed no response and 2 showed a moderate response. In contrast the first 5 cases of pernicious anemia treated with proteolysed liver in doses of less than $\frac{1}{2}$ oz. daily (folic acid content 0.4 mg.) all showed an optimal response. It may be safely concluded that the therapeutic effect of free folic acid must have been augmented by other hematinic principles contained in the preparation, e.g. the liberating factor and possibly other members of the vitamin B complex. The lowest dose of folic acid which we have used for the treatment of refractory megaloblastic anemia is 2.5 mg. daily and this dose was effective in the only case in which it was tried; hence we are unable to state what is the minimal effective therapeutic dose in this group of anemias. Even if it be assumed that it is in the region of 1 mg. daily the results achieved by 1 oz. of proteolysed liver daily in refractory megaloblastic anemia were superior to that produced by 1 mg. daily of synthetic folic acid in pernicious anemia. Accordingly we feel that the beneficial effects of proteolysed liver in refractory megaloblastic anemias cannot be explained solely on its content of folic acid, nor on its content of the liberating factor since large amounts of purified liver extract given parenterally are ineffective.

The superior efficiency of orally administered liver or proteolysed liver to liver extract given parenterally in refractory megaloblastic anemia can be explained on one or other of the following hypotheses: (1) That some interaction takes place in the gastrointestinal tract between the ingested liver preparation and gastrointestinal enzymes which leads to a potentiation of hemopoietic factors already present; or (2) that liver and proteolysed liver contain some essential hemopoietic principles, including folic acid and possibly other members of the B<sub>2</sub> complex, which are removed or destroyed in the chemical processes used in the manufacture of liver extracts for parenteral injection.

Additional support for this latter view is suggested by the following observations. We have many cases of tropical sprue and idiopathic steatorrhea who persistently have a moderate degree of macrocytic anemia which is entirely resistant to parenteral liver therapy. The bone marrow presents a picture which is mainly normoblastic but many erythroblasts have an appearance intermediate between a megaloblast and a normoblast. The failure of parenteral liver therapy and iron to restore the marrow picture to normal and the persistence of a macrocytic anemia clearly indicate a lack of some additional hematinic factor. The hope that this factor would be folic acid was not realized as can be clearly seen in the
protocols of our cases published in 1946 (Davidson, Girdwood, and Innes). Proteolysed liver also was found to be ineffective in some of these cases of refractory macrocytic anemia associated with the sprue syndrome. In 2 or 3 cases, however, it caused a considerable increase in the blood level after parenteral liver therapy and folic acid by mouth had failed. In such cases we concluded that proteolysed liver contained some additional hematinic principle whose composition was still unknown. An investigation into the nature of this principle is proceeding. Our therapeutic program in operation at the present time is based on the investigations detailed above. Our practice is to treat all cases of megaloblastic anemia in the first instance with parenteral liver extracts. Should the result be unsatisfactory we prescribe folic acid. If this fails to restore the blood picture to normal we give 1 oz. of proteolysed liver daily. By this means we are able to restore the blood picture qualitatively and quantitatively to normal in the great majority of cases of megaloblastic anemia of all types.

**SUMMARY**

1. Fifty-nine cases of megaloblastic anemia refractory partially or completely to potent liver extracts given parenterally have been investigated in Edinburgh during the past six years. Thirty-four of these cases were associated with pregnancy, the puerperium or the sprue syndrome. No explanation of the cause of the megaloblastic anemia was discovered in the remaining 25 cases.

2. The etiology, clinical features and treatment of 25 cases of idiopathic refractory megaloblastic anemia are described. Attention is directed to the excellent therapeutic effects produced by proteolysed liver or folic acid.

3. The mechanisms involved in refractoriness to potent parenteral liver extracts are discussed.

4. In certain cases of refractory megaloblastic anemia it is suggested that an unknown hematinic principle, in addition to the liberating factor in purified parenteral liver extract and folic acid, is required for the complete restoration of normoblastic blood formation.

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REFRACTORY MEGALOBLASTIC ANEMIA

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