SUCCESSFUL TREATMENT OF LIVER-REFRACTORY ANEMIA WITH SYNTHETIC LACTOBACILLUS CASEI FACTOR

By Jan Waldenström, M.D.

The treatment of pernicious anemia that is refractory to liver has been one of the most difficult problems in hematology. In many of these cases the result of treatment with liver extracts is excellent for some time and everything seems well. Later the patient responds less and less to antianemic treatment and the final outcome is usually fatal. Before discussing the favorable results of treatment with folic acid in such conditions, it may seem appropriate to treat some questions of nomenclature briefly. For example, it is evident that the name "folic acid" is not a synonym for the synthetic lactobacillus casei factor. For the sake of brevity, however, it is used here in this meaning.

In 1935 and 1936, Isräels and Wilkinson introduced the expression achrestic anemia to characterize cases showing a pernicious anemia-like blood picture but having a normal gastric acidity, no disturbance of the gastrointestinal tract, no involvement of the central nervous system, no pyrexia or evidence of hemolysis, and a lack of or a poor response to antianemic treatment. The bone marrow was described as megaloblastic.

Since the first delimitation of this picture, a few cases have been published by various authors but not by as many as might be expected from the comparatively large series of cases described by the original authors. On the other hand, their experience with uncomplicated pernicious anemia is unusually large. The incidence of achrestic anemia in Manchester, England, was stated to be 1 per cent of 600 cases of typical pernicious anemia. It is, however, hard to say anything definite about the real frequency of this disease in other populations. According to our opinion it would seem to be too early to decide whether these cases constitute a homogenous group or are instances of different pathological mechanisms (cf., e.g., Davis and Davidson). The term achrestic anemia has not been universally adopted. In the German literature Schulten (1939), in his textbook on diseases of the blood, does not mention the term and Heilmeyer (1941) regards the entity as not yet well founded. He seems to believe that these cases should be regarded as instances of aplastic anemia. In Switzerland, Rohr (1940) does not treat the question of achrestic anemia in his monograph on the bone marrow. In American literature there is some discussion about the real connection of these cases with pernicious anemia. Wintrobe (1942), for instance, takes up this problem in his Clinical Hematology and thinks that the cases show more resemblance to the cases of progressive hypochromia described by Thompson. The latter are discussed under the title aplastic anemia. It is obvious that this is true of a large number of cases that are regarded as liver-refractory pernicious anemia.

In their second paper, published in 1940, Isräels and Wilkinson described further cases of achrestic anemia. The incidence was still about 1 per cent in their total
material of 1100 patients with pernicious anemia. The 6 new patients were divided into two groups, 3 being regarded as typically achreptic. The others resembled somewhat pernicious anemia in pregnancy. All the patients had a typically megaloblastic marrow, as shown convincingly in a number of photomicrographs. It was found that the young patients reacted somewhat better on liver treatment.

Case reports of achreptic anemia have also been published by Abrahamson and Thompson (1937), Wauchope and Leslie-Smith (1938), Hynes (1939), and, probably, by Dameshek and Valentine (1937).

A very interesting report on 6 cases of liver refractory anemias has recently been made by Davis and Davidson (1944). Three cases in this paper are regarded as achreptic anemia by the authors. The cases were treated with proteolyzed liver after it had been shown that they were refractory to potent liver extracts. The reactions were favorable. The authors are of the opinion that proteolyzed liver perorally supplies some hematopoietic factor lacking in fractionated liver extracts. All the patients had previously received large doses of potent liver extracts, and the authors think it likely that they needed an additional factor for normal blood formation. The question of several factors will be discussed later in this paper.

Since 1937, when we had the opportunity of following the development of a typical instance of achreptic anemia in Upsala, we have been convinced that this represents a clear-cut syndrome. I shall give the case history briefly, as it shows some interesting features.

Case 1. A woman, born in 1891. Father died of gastric carcinoma. Mother probably also had carcinoma. Since 1925 dyspeptic troubles with vomiting, anorexia, and loss of weight. Admitted to the Surgical Clinic in 1926. No achlorhydria, no melena. Later, increasing number of stools, rather loose. Admitted to the Medical Clinic in 1928. Very thin. Tongue normal. Hemoglobin 57 per cent; R.B.C., 4.2 M.; W.B.C. 5,000; platelets, 350,000. Some normoblasts in the blood smear. No achlorhydria. Treated with arsenic and iron. In November 1929, suddenly blind in right eye; pregnant. Admitted to the Obstetric Clinic. Anemia. R.B.C., 2.26 M.; thrombosis central artery of retina; later thrombosis in both legs. Postpartum increasingly anemic with exessively low blood values. R.B.C., 0.68 M.; 123 normoblasts/100 W.B.C., and large numbers of megalocytes. Treated with blood transfusions, iron, liver extracts, liver diet and arsenic. The blood values slowly improved. In 1930, R.B.C. 4.0 M. Readmitted to the Medical Clinic in February 1934 for diarrhea. Three to four loose, foul smelling movements daily. Quite emaciated. Very pale. Blood pressure 95/50. Hemoglobin, 30 per cent; R.B.C., 1.4 M.; W.B.C., 4,400. Normoblasts 31/100 W.B.C. Treatment with campolon gave rather good results, with a hemoglobin of 80 per cent. R.B.C., however, only increased to 3.5 M. and later declined in spite of continued treatment. In July 1935, back pain, loss of weight, glossitis. Later, fever with some coughing. Admitted in January 1936. Tongue smooth; neurologically negative; blood pressure 130/95; hemoglobin, 80 per cent; R.B.C., 2.43 M.; W.B.C., 4,300; neutrophiles, 37 per cent; lymphocytes 58 per cent; monocytes 5 per cent. Normoblasts 35/100 W.B.C., i.e., a very considerable leucopenia. Large numbers of Howell-Jolly bodies (54/500 R.B.C.). Platelets, 182,000; reticulocytes 4 per cent. Some hydrochloric acid after caffeine. The spleen could not be seen on roentgenograms. The patient was treated with campolon. Readmitted in March 1937. She had had periods of diarrhea with fever. Still severe hyperchromic anemia that did not react to campolon. X-rays of the spine showed considerable osteoporosis with biconcave vertebrae. The patient was treated with large periods of campolon injections and heparin and heparin perorally but these gave no lasting effect. She was also given transfusions but her general condition deteriorated. In spite of the intense antianemic treatment the bone marrow contained typical megaloblasts. She ran a slight fever and had an increased sedimentation rate. Later a lump was palpated in the abdomen to the left but the roentgenogram showed no signs of gastric carcinoma. The later development was dominated by an acute right-sided otitis that never subsided. The patient was transferred to the Otolaryngic
Department, where she later died. It was found that the palpable masses in the abdomen were caused by enlarged retroperitoneal glands which were diagnosed by the pathologists as Hodgkin's disease. There was found a marked atrophy of the spleen, confirming the clinical diagnosis of hypersplenism.

COMMENTS

The syndrome represented by this patient showed some remarkable features. She had a hyperchromic, megaloblastic anemia that responded rather well to treatment with liver extracts for several years. The peculiar thing was that her gastric juice contained free hydrochloric acid. A large number of nucleated red cells in the blood and erythrocytes containing Howell-Jolly bodies was regarded as evidence of splenic atrophy. This diagnosis was confirmed at autopsy. The clinical diagnosis in this case was nontropical sprue of the type associated with atrophy of the spleen, but it could just as well have been achreptic anemia. It must be regretted that analyses of fecal fat were not performed, nor was a glucose tolerance test made. The combination of chronic diarrhea with osteoporosis, liver refractory megaloblastic anemia, and atrophy of the spleen in the absence of achlorhydria places the patient in the group of nontropical sprue without any doubt (see also case 5).

The combination of idiopathic steatorrhea and atrophy of the spleen has been known for only a comparatively short time. Dünner, Hirschfeld, and Geraldy and Hotz and Rohr discussed the possibility of atrophy of the spleen in these conditions but were unable to prove it at autopsy. The first anatomical confirmation was given by Engel, who published a case of nontropical sprue from Sweden in 1931. The patient died in 1934 and an atrophic spleen weighing only 12 Gm. was found. The histologic picture showed severe sclerosis with only small remnants of the pulp in the form of follicles. No signs of any specific disorder were found. Re-examination of blood smears by Engel in 1939, when the case was again published, showed that 8 per cent of the erythrocytes contained Jolly bodies. At the discussion following Engel's paper, Strandell gave a short account of a woman suffering from liver-refractory hyperchromic anemia with leucopenia and thrombocytopenia but without achlorhydria. Seven per cent of the erythrocytes showed Jolly bodies. The spleen weighed only 60 Gm. at autopsy. Another case history was briefly related by G. A. Johansson. Hyperchromic anemia, some leucocytosis, very marked thrombocytosis, and large numbers of Jolly bodies were regarded as possibly indicating atrophy of the spleen. A glucose tolerance test showed a very slight increase after a dose of 1 Gm./Kg. body weight. It is thus possible that this condition is not extremely unusual. Engel has shown that a number of previously published cases with atrophy of the spleen that had signs of chronic enteritis may well be interpreted as nontropical sprue. The biological importance of these interesting findings will not be discussed here. From a diagnostic point of view it is important to know that atrophy of the spleen may be one of the symptoms that should be looked for in cases of liver-refractory macrocytic anemia.

I have previously suggested that nontropical sprue may be the diagnosis in some instances of achreptic anemia. Since this case was seen, we have not had another instance of achreptic anemia in ten years. During this time about 200 new cases of uncomplicated pernicious anemia from the province of Upsala have been diagnosed.
in the Medical Clinic. The incidence of liver-refractory pernicious anemia has therefore been 2/120, a number that fits in quite well with Wilkinson’s 1 per cent in Manchester. In our country this may therefore be the approximate frequency of the condition. The other cases published in this paper come from other parts of Sweden and should therefore not be counted in this connection. Our case of liver-refractory anemia had the following history.

**Case 2.** W. B. Woman born in 1890. Hereditarily nothing of importance. No maladies since 1908, when she had a chronic nontuberculous mastitis. In 1939, chronic eczema. In 1942, hypochromic anemia treated with iron. Regarding the later development of blood values, see fig. 1. Admitted to the Medical Clinic in 1933 with signs of pernicious anemia. Tongue completely smooth; spleen enlarged. W.B.C., 3,100; platelets 112,000. After two months of treatment with campolon and iron, the patient was dismissed with normal blood values. Readmitted in November 1939 for symptoms relating to the mucous membranes with glossitis, dysphagia and perlèche. Otherwise in good condition. Unusually large fissures in the corners of the mouth. Tongue absolutely smooth, as though polished. Also signs of general stomatitis. Skin somewhat diffusely pigmented with numerous dark patches. Heart: systolic murmur over the whole precordium. Liver and spleen not palpable. No urobilinuria. Blood pressure, 110/75. Icterus index, 3. It was regarded as probable that the patient suffered from iron deficiency and she was treated with large doses of iron without any definite improvement of the oral symptoms. In 1940 she was again treated with large doses of iron without any definite effect. Later dry yeast for one period, riboflavin 6 mg. daily for a considerable time, and pyridoxine in injections during different periods—all this without lasting effect on the fissures or the tongue papillae. She was therefore readmitted to the clinic in June 1943. Tongue still completely smooth. She was now treated with "becozym" but without any real improvement. In March 1945 she was rather ill with anorexia, vomiting, and diarrhea. Severe stomatitis. Gait unsteady with paresthesias in hands and feet. Blood values steadily decreasing (fig. 1) in spite of continued treatment. Sternal puncture: megaloblastic marrow. No Jolly bodies in smears of the periphery. blood. Admitted to the Medical Clinic on October 26, 1945. Tongue completely smooth with aphthoul ulcers. Spleen somewhat enlarged, easily palpable. Blood pressure rather low (95/65). For blood values see figure 2. Bone marrow as before. The patient was treated with injections of liver extracts and later with transfusions (fig. 2). The result of the liver treatment as regards hemoglobin and R.B.C. was negative but there was an increase in platelets from 38,000 to 143,000. Roentgenogram of the esophagus showed no varices or other pathological changes. Sternal puncture: scattered megaloblasts. Takata reaction negative. Quantitative blood bilirubin, 0.7 mg. per cent. Icterus index, 2. Red cell fragility, 0.44-0.26 per cent NaCl. Roentgenogram of the thoracic spine showed no osteoporosis. Serum calcium, 8.6 mg. per cent. No occult blood in the stools. An analysis of fecal fat in the Department of Physiological Chemistry (Professor G. Blix), showed a content of 18.5 per cent with 49 per cent free fatty acids, 41.5 per cent neutral fat and 9.5 soaps. Fractional test meal: no hydrochloric acid after caffeine. After 0.5 mg. histamine: free acid 14, total acid 28. The patient later showed a rise in temperature (maximum 39.7° C) with marked decrease of W.B.C. (minimum of 1.100). She was treated with penicillin, 12,000 units X 8 for 6 days. The high temperature slowly subsided but remained subfebrile and the sedimentation rate was high, 70-80 mm./hr. The patient was given several transfusions but her status remained very unsatisfactory.

When she was discharged on December 17, 1945, it was noted in the case history that the possibility of a splenic anemia must be taken into account. Readmitted on January 23, 1946. Very tired. No glossitis. Insomnia, coughing, tachycardia, swelling of the legs, slight diarrhea, no paresthesias, quite emaciated. Weight, 42 Kg; tongue absolutely smooth and glistening; fissures now healed. Blood pressure,
Liver palpable 6 cm. below the costal margin; spleen a finger-breadths below the costal margin. Neurologically nothing objective. For blood values, see figure 3. N.P.N., 24 mg. per cent. Icterus index 4. Takata negative. Blood proteins, 5.1 per cent; albumin, 2.5; globulin, 2.6; fibrinogen, 0.3 per cent.

At this time, Dr. Gunnar Ågren of the Department of Physiological Chemistry obtained some samples of folic acid through the courtesy of A. B. Ferrosan. Because of the very marked leucopenia, it was decided to try the preparation on this patient. As regards the effect, see figure 3.

The patient was dismissed in a rather satisfactory condition after a total dose of 2,300 mg. of folic acid. No further treatment was given and the patient was followed in the Out-Patient’s Department. Her general condition of health has been good and she has been able to do her usual household work.

Readmitted in August 1946 as the status of her tongue was not satisfactory. There were found a few very low papillae on the back of the tongue and several small ulcers. Big fissures in the corners of the mouth. Liver and spleen still increased in size. As the serum iron value was persistently low, the patient was given iron parenterally and as intravenous injections in a dosage of 10 mg. of iron once daily. This treatment did not materially change the condition of her mucous membranes. She was discharged on iron therapy but later studies of the tongue status showed no improvement and the serum iron values remained low. Her last blood status was taken 20 months after her folic acid treatment, except a period of 7 days in September when she was given a daily dose of 15 mg. against her glossitis. It was Hb., 91 per cent; R.B.C., 4.4 M.; W.B.C., 3,500; and serum iron 447 per cent.

COMMENTS

The anemic condition began as hypochromic anemia in 1932. She is thus an instance of the transition from hypochromic to hyperchromic "pernicious" anemia. This condition is very common in Upsala, having been seen in 28 cases of a total of 137 female cases with pernicious anemia from the province (Waldenstrom, 1944). When she came back in 1933 she was treated with both liver extract and iron and it seems certain that it was the liver that brought about the improvement, as the anemia was definitely macrocytic. It must be regretted that reticulocyte counts were not made nor were sternal punctures performed at this early stage of the disease. In the meantime her blood values were kept very satisfactorily on continuous treatment with liver extracts with intervals of about six to 8 weeks. It should be noted, however, that both in 1939 and in 1941 an occasional leucocyte count showed strikingly low values. In the year 1944 the values for the red cells were normal as before, but in 1945 there was a marked decline and in the autumn of 1945 the patient became desperately ill in spite of large doses of potent liver extracts. This is shown in figures 1 and 2. From these charts it may be noted that heptomin in large doses gave a slight drop in serum iron and a very feeble reticulocyte peak but no improvement of either the red or the white cell counts. The patient had to be given several transfusions to keep her alive. (It should perhaps be noted that another patient with pernicious anemia in the same ward reacted normally on heptomin at the same time.)

The result of folic acid treatment was prompt, as is clearly seen from figure 3. The reticulocyte response was rather good. The platelets rose in the usual manner and the serum iron values decreased very markedly. The increase in the red cell count was rapid and considerable. The temperature came down to normal values and the sedimentation rate also became normal in a short time. The white cells increased but the values were rather variable and there has been a persistent tendency to leucopenia. This is especially worth noting as the leukopenia was regarded as a special indication for treatment with folic acid.
During twenty-three days the patient received 100 mg. folic acid daily perorally. After that time all treatment was stopped but the erythrocyte count continued to increase to high values (about 5.0 M.) and has remained normal for ten months without any further treatment than iron and 5 mg. folic acid thrice daily for seven days.

It is thus obvious that a patient who has become completely refractory against otherwise
potent liver extracts may react well on large oral doses of folic acid. The long-lasting effect of this therapy speaks in favor of the assumption that folic acid itself may be stored for long times in the body or is the initiator of some secondary mechanism that keeps on promoting blood cell production in the presence of antipernicious anemia factor from liver. It is of interest to note that Davis and Davidson found good therapeutic effect with proteolyzed liver after ineffective administration of liver extracts.

A chapter of special interest is the condition of the tongue in this case. Because of the fact that the lingual mucosa was refractory to all sorts of specific treatment it was assumed that the patient might suffer from some hitherto unknown deficiency. The folic acid however was obviously not what was needed by the tongue nor was iron effective after massive folic acid administration.

Several other features should be especially noticed. At present it is impossible to tell if this patient really had a histamine-fast achlorhydria on her first examination. The amount of duodenal regurgitation was probably considerable as the samples of gastric juice were of a yellow color. When re-examined in 1945 with a special technic worked out by Hallèn in order to avoid duodenal contamination, the gastric juice contained hydrochloric acid after caffeine. It is thus obvious that in this respect the patient resembled achrestic anemia. On the other hand there are no symptoms indicating an idiopathic steatorrhea and there is definitely no atrophy of the spleen.*

* Dr. H. Grundsell of Kristinehamn has kindly allowed me to use his data on this patient and referred her to the clinic.
Another instance of liver-refractory anemia is the following:

Case 3. C. B. Woman born in 1905. *Mother died of gastric carcinoma. Otherwise there was nothing important as regards heredity. Since 1920, first period of diarrhea; treated with pancreatic preparations. In November 1944, ulcers on both legs. About 1940, first period of diarrhea; treated with pancreatic preparations. In November 1944, ulcers on both legs with eczema. Tongue: low papillae. Hb., 63 per cent; R.B.C., 1.3 M.; sedimentation rate, 32 mm./hr. Test meal showed free hydrochloric acid. Later discontinued treatment. Re-examined in January 1946. Increasingly tired. Very pale; glossitis, dyspepsia, palpitations, slightly icteric. Liver and spleen not palpable. Hb., 38 per cent; R.B.C., 1.9 M.; color index, 1.0; W.B.C., 3,300. Differential count: neutrophiles 44 per cent, eosinophiles 1 per cent, lymphocytes 54 per cent, monocytes 1 per cent, reticulocytes 10 per cent. Sternal puncture: megaloblastic marrow. After histamine, free hydrochloric acid, 60 units. As the diagnosis of pernicious anemia seemed certain, the patient was given 4 + 4 ml. of a potent liver extract on 2 consecutive days. Her reticulocytosis never exceeded 5 per cent. She was later given a new injection of 4 ml. heptomin.; Hb., 37 per cent; R.B.C., 1.5 M. The result of liver treatment was regarded as unsatisfactory and the patient was sent to a hospital on January 28, where she was treated as a probable instance of aplastic anemia. On admittance her Hb. was 22 per cent; R.B.C., 1.2 M.; W.B.C., 1,700. In spite of intensive therapy with liver extracts and blood transfusions, no real improvement occurred. Severe leucopenia; at one stage not more than 420 cells. The patient was treated with blood transfusions, repeated injections of liver extracts, and nucleotide. Blood values on dismissal: hemoglobin, 40 per cent; R.B.C., 2.1 M.; W.B.C., 3,200, with improved general condition. The patient was regarded as an instance of aplastic anemia and a relapse was regarded as probable. In the end of April: hemoglobin, 50 per cent; R.B.C., 1.9 M.; W.B.C., 3,000, with some nucleated red cells. Reticulocytes, 10-15 per cent. The patient was given 2 + 1 ml. soluble liver extract (Lederle) on 3 consecutive days. No reticulocytosis. Two weeks later: Hb., 66 per cent; R.B.C., 2.7 M. On May 12: Hb., 64 per cent; R.B.C., 2.4 M. After that time R.B.C. was constantly about 2.5 M. The patient was admitted to the Medical Clinic in Upsala on June 8, 1946, as a possible instance of achreatic anemia. She felt much better than she had before. Her glossitis had disappeared and her menstruation, which had ceased during the period from December to March, had reappeared. The color of the skin was brownish, with stronger pigmentation on the face, on the hands, knees, and feet. The medial aspects of both malleoli were strongly pigmented and showed ulcerations. No pigmentation of the abdomen or in the folds of the hands. No perlèche. Tongue papillae low. Heart normal. Muscular sensibility in toes and fingers normal. Tactile and vibratory sensation normal. Serum calcium, 9.8 mg. per cent; potassium, 18.9 mg. per cent; sodium, 128 mg. per cent. Total protein, 8.8 per cent, albumin, 4.5 per cent, globulin 4.3 per cent, fibrinogen 0.35 per cent. Takata reaction positive. Formol-gel reaction, negative. Fasting blood sugar normal. Glucose tolerance test, very flat with initial value 113 mg. per cent, maximum value 140 mg. per cent, 2.5 hours after the ingestion of 55 Gm. of glucose. For blood values, see figure 4. No Jolly bodies in the smears of the peripheral blood. Sedimentation rate, 92 mm./hr. As the diagnosis of nontropical sprue seemed probable, analyses of the fat in the feces were performed in the Department of Physiological Chemistry. On June 11, 1946, the total fat was found to be 37.5 per cent, with free fatty acid, 42.7 per cent; neutral fat, 43.7 per cent; and soaps, 13.6 per cent. The analyses was repeated later on July 9. Total fat, 25.6 per cent, with free fatty acid 19.1 per cent, neutral fat 21.7 per cent, and soaps 43.1 per cent. Roentgenograms of the spinal column showed no osteoporosis. Fractional test meal: free hydrochloric acid = 30 after caffeine. The patient was given folic acid 25 mg. daily for 13 days, then 50 mg. for a week, and finally 200 mg. daily for 12 days. The total dose was thus nearly 1 gm. (The folic acid for the treatment of this patient was generously supplied by the Lederle Laboratories, New York, through Dr. Guy Clark, for which the author expresses his sincere thanks.)

The patient was discharged on July 12, 1946. The results of treatment are seen in figure 4. After a month's time without further therapy the patient returned to the hospital in very good general condition and with practically normal blood values. The blood showed: Hb., 77 per cent, R.B.C., 4.1 M.; W.B.C., 4,000. Sedimentation rate, 36 mm./hr. (Fig. 4).

* [In fact, the association of splenomegaly with a more or less refractory anemia and persistent leucopenia makes one suspect the possibility of a hypersplenic condition.—Editor]
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COMMENTS

Several interesting points should be noted. It is evident that the reticulocyte response was rather slight, but that the decrease in serum iron was marked. There was also a certain increase in the platelets. These three changes together indicated that the therapy may have been successful and it was expected that the red cells would improve. This was not a quick result, however, and the effect of folic acid therapy was at first regarded as a failure. The exact diagnosis in this case is not easy to make. Naturally she may be regarded as an instance of achrestic anemia according to Wilkinson. It has earlier been pointed out that there is probably a certain connection between achrestic anemia and nontropical sprue. This patient showed a few symptoms that might indicate some disturbance of this type. She had had diarrhea for several years. There was found a hyperchromic anemia with megaloblastic marrow, in spite of abundant hydrochloric acid in the gastric juice. Her serum calcium was normal and there were no signs of osteoporosis. The percentage of fat in the feces was rather high. This fact cannot in itself be used to prove the presence of idiopathic steatorrhea. On the other hand, the glucose tolerance test showed a very flat curve with an increase of only 2.6 mg. per cent after 55 Gm. glucose. Also, the curious pigmentation on the arms may be an indication of nontropical sprue. The excellent effect of large doses of folic acid perorally has lasted for several months, as in case 2.

Case 4 differed from the others in that she had a histamine-fast achlorhydria and showed no signs of leucopenia.

Case 4. Woman born in 1886.* Hereditarily nothing of importance; previous maladies of no interest. In 1931 the patient became very tired. Various medications were without benefit. She was admitted to

* Dr. R. Berlin referred the patient to the Clinic and kindly gave the author access to his notes on the history. Dr. Berlin recently treated a patient with macrocytic anemia, 1.6 M., and megaloblastic bone
a hospital in 1934. Clinically, typical pernicious anemia with complete achlorhydria was found and a normal remission ensued following the administration of campolon, a brand of liver extract. Injections of liver extracts were discontinued in 1937, and she was treated with hepaforte perorally instead, which the patient stated she had been taking regularly. In November 1944, relapse with severe anemia (Hb., 45 per cent; R.B.C., 1.4 M.). Sternal puncture showed typical megaloblastosis. With liver extract parenterally, reticulocytosis was normal, but very slow and incomplete increase of the blood values took place (after 3 weeks Hb. was 64 per cent, R.B.C., 3.0 M.). The patient was treated with large doses of different liver extracts, 350 ml. in all during a period of 8 months with only a temporary rise of R.B.C. above 3.0 M. In May 1946, the highest red cell value was 3.4 M. No signs of complications such as carcinoma, chronic renal or liver disease or myxedema. From November 1945 to September 1946, 130 ml. of liver extracts in all were given intramuscularly.

Subjectively, the patient felt rather poorly; occasional lingual disturbance was present; no paresthesias of the legs. She felt rather nervous—"no pep." Admitted to the Medical Clinic in Upsala on October 8, 1946. On examination she appeared tired, thin, and pale. Weight, 49 Kg. Tongue reddish with atrophic papillae. Internal organs otherwise normal except considerable decrease of vibratory sensation in the legs. No albuminuria or urobilinuria. No occult blood in the stools. For blood values, see figure 5. No Howell-Jolly bodies in the smears of the peripheral blood. Sternal puncture: scattered megaloblasts. Takata reaction normal. N.P.N., 30 mg. per cent. Histamine-fast achlorhydria. X-rays of the stomach normal.

The patient was treated with 25 mg. folic acid daily and there was rapid amelioration in the status of the tongue. The patient felt much stronger (fig. 5) and was discharged on October 30, 1946. Later examinations by Dr. Berlin showed the values: November 8, Hb. 84 per cent, R.B.C. 4.1.; December 3, Hb. 90 per cent, R.B.C. 4.1 M. The patient had then taken a further course of folic acid (15 mg. daily for 3 weeks).

![Blood counts](image)

**Fig. 5—Case 4. Blood counts.**

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marrow (heptomin 8 ml. for 4 consecutive days). This had no effect. Injections of liver extracts were repeated without any results. The patient was therefore treated with a transfusion and hepaforte perorally. No reaction. After a week's treatment, folic acid (20 mg. daily) was given. Maximum reticulocytosis 26.7 per cent on the fifth day. After a month, R.B.C. 4.5 M. This effect has lasted. The patient also suffers from chronic arthritis.
COMMENTS

The diagnosis in this instance seemed to be uncomplicated pernicious anemia. In spite of this she never showed normal erythrocyte or hemoglobin values, even after massive doses of liver extracts. It was therefore regarded as desirable to try folic acid. In this instance the dosage was much smaller and lasted only for two weeks. In spite of this the response was rapid. The difference in the effect of folic acid as compared to liver extracts cannot therefore be explained solely on a quantitative basis. The result has been lasting and the patient is at present on continuous treatment with folic acid in moderate doses. The immediate effect of the therapy showed itself both as a slight increase in reticulocytes and as a very marked drop in serum iron values.

The following case belongs in the same group as case 1.

Case 3. Woman born in 1906.* Nothing of importance hereditarily. As a child she was very small and delicate. She was said to have suffered from rickets. At the age of 22 she had an optic neuritis but this has never relapsed nor had she had any other symptoms of organic neurological disease. About 1943 she felt especially tired and her doctor told her that she was anemic. She noticed that her skin became darker. Examined by Dr. Grundsell in June 1945. She was quite emaciated, strongly pigmented but showed no other signs of Addison’s disease. Blood pressure, 140/90. Plasma sodium, 315 mg. per cent. Sedimentation rate, 39 mm./hr.; Hb., 65 per cent; R.B.C., 3.2 M. In the beginning of May 1946, she began to have dyspeptic troubles with four to five bulky stools daily. Large numbers of fatty acid crystals were found in the feces. She was later treated for steatorrhea at the Carolina Hospital in Stockholm. In October 1946, she felt pains in her back. These pains often lasted for about an hour. Her back was quite stiff after such an attack. No menstrual disturbances of special importance.

Admitted to the Medical Clinic in Upsala on December 1946. Very thin (44 Kg.), with a wrinkled face. Skin darkly pigmented, hair and eyes also strongly pigmented. A number of brown spots were present on the arms and legs which, according to the patient, had developed after insect bites and punctures. Tongue normal. Some small aphthous ulcers in the mouth. Abdomen distended with gas, no palpable masses. Neurological examination negative. No albuminuria or urobilinuria. Prothrombin index, 76. Serum calcium, 8.8 and 9.2 mg. per cent. Total protein, 5.4 per cent. Serum iron, 71 gammas; Hb., 70 per cent; R.B.C., 3.6 M.; W.B.C., 3,800–3,200. Differential count normal. About 1.5 per cent of the red cells contained Howell-Jolly bodies. Scattered normoblasts. Reticulocytes, 6 per cent, platelets, 281,000. Sedimentation rate 42 mm./hr. Afebrile. X-rays of the spleen failed to show a definite shadow—*"The spleen is probably small."* Spinal column and pelvis showed no definite osteoporosis.

The feces were bulky, usually grayish in color, mostly semisolid, sometimes formed. An analysis of fecal fat was performed in the Department of Physiological Chemistry† on samples from December 7 and December 10. The total fat was 66.4, viz. 60.6 per cent; of this 38.4 viz. 37.7 per cent were free fatty acid; 36.6 viz. 30.6 per cent neutral fat and 25.0 viz. 41.8 per cent soaps. A glucose tolerance test with 44 grams of glucose showed a fasting value of 76 mg. per cent. The highest value reached was 106 mg. per cent after 45 minutes. A similar experience was made with butter (50 Gm.). Before the test the total lipids of the blood were 720 mg. per cent. There was present 155 mg. per cent total cholesterol, 34 mg. per cent of this was free. Choline was 30 mg. per cent and lipid phosphorus 6.9 mg. per cent. Four hours after the tolerance test, the total lipids were 730 mg. per cent, the total cholesterol 142 mg. per cent, free 39/choline 32.5 mg. per cent, lipid phosphorus 7.5 mg. per cent.

The electrocardiogram showed a rather long Q-T of 0.42 at a heart rate of 65 per minute. Because of the slightly decreased calcium values in the blood and the possible latent tetany, the E.K.G. was repeated.

* Dr. H. Grundsell kindly referred this patient to the Clinic and gave the author access to his notes about the history.
† The analyses were kindly performed by Dr. G. Brante.
At a rate of 75 per minute, Q-T was 0.40. The patient was given 10 ml. of 20 per cent calcium (Sandoz). The heart rate was then 65/min., Q-T 0.36. Five minutes later, it was 60/min., Q-T 0.37.

The patient was treated with folic acid perorally without any obvious influence on the stools. She felt decided subjective improvement however. Her hemoglobin and red cell values after this treatment were completely unchanged. After six days of treatment the serum iron was still 90/7 per cent. Injections of folic acid (15 mg. daily) were therefore started. The serum iron decreased to 32/7 per cent, but there was no decided reticulocytosis nor any increase in red cell count. The macroscopic appearance of the feces remained unchanged and their weight was the same as before. Analyses of the fecal fat still showed 5.7 per cent total fat, consisting of 17.6 per cent free fatty acids, 45.9 per cent neutral fats and 36.5 per cent soaps.

It was thought that liver extracts might possibly have some therapeutic value after treatment with folic acid. Heptomin (4 ml.) was therefore given on 4 consecutive days. There was no reticulo- or thrombocytic response; no improvement of the red cell value and no gain in weight. The serum iron came down to very low values of 15/7 per cent. After two weeks of observation the patient was given large doses of iron because of the low serum iron value. This gave no further reticulocyte response nor did it increase the hemoglobin or the red cells. After one week, rather large doses of casein digested with enzymes from the spleen, according to a method described by Ägren, were given. This week there was a marked increase in weight of 1.6 Kg. to 47 Kg. The iron medication was stopped. As the weight decreased during this period, the patient was given no more digested casein. She was discharged with large doses of iron.

COMMENTS

The diagnosis is not difficult to make. The patient probably suffers from the peculiar type of idiopathic steatorrhea that is combined with atrophy of the spleen (cf. case 1). The leading symptom in this case was not so much the anemia as the steatorrhea and extreme emaciation. Atrophy of the spleen is indicated by the presence of large numbers of Howell-Jolly bodies. The connection between these findings and the other symptoms of idiopathic steatorrhea has already been discussed. The influence on the Q-T interval in the electrocardiogram by intravenous calcium therapy might indicate the presence of a latent calcium deficiency.

This patient showed a slightly hyperchromic or normochromic anemia of moderate severity. Treatment with folic acid perorally had no effect on the anemia nor did it give any reticulocytosis or decrease in serum iron. It thus seems probable that the anemia in this case was also achrestic against folic acid. Whether the suspected atrophy of the spleen plays some part in this mechanism is difficult to judge.

DISCUSSION

The most interesting problem in this connection is the question of why the patients 2, 3, and 4, who had lost the ability to react to treatment with liver extract, responded to folic acid. Many explanations are possible. The simplest would be that the dosage of folic acid was greater than the previous amounts of liver extract given. The difference would thus be purely quantitative. This seems improbable for two reasons. First, the dosage of liver extracts had been very considerable. Second, case 4 showed good response on doses of folic acid that are regarded as quite small. This is also in accordance with our own experience of folic acid treatment in 15 cases with uncomplicated pernicious anemia. Some qualitative difference should therefore be looked for.

Another explanation would be to assume a difference of absorption. It seems
LIVER-REFRACTORY ANEMIA AND L. CASEI FACTOR

hard to understand however that an injected substance should be less well utilized than one that is administered perorally. At least one of the cases (4), had also been treated with liver preparations per os without any effect. Therefore this explanation does not seem very convincing. The only way to look upon the matter is to regard folic acid and liver extract as different in their action. Other hypotheses could be formulated but would probably be found lacking in factual foundation. I shall therefore abstain from the further treatment of this subject until more information has been collected.

It seems obvious that case 5 suffers from a normo- or hyperchromic anemia in connection with nontropical sprue that is refractory to injected liver extract and to folic acid both in the form of tablets and injections. This would thus indicate that there may be cases of achrestic anemia both as regards the liver factor and folic acid. Whether they will respond to some other factor is not yet known but will be investigated. The question whether there are cases refractory to folic acid which respond to liver extracts cannot be answered as yet. We have been in a position to treat some 15 cases with uncomplicated pernicious anemia in relapse with folic acid. All responded well to this treatment but our experiences must be much greater before anything definite may be said. In the presence of such complications as chronic arthritis and iron deficiency, the effect of folic acid was diminished.

SUMMARY

Four cases of liver-refractory macrocytic anemia are described. Three were treated with folic acid perorally and reacted well.

The first was an instance of nontropical sprue with atrophy of the spleen that was diagnosed clinically and confirmed at postmortem examination. The megaloblastic anemia also became completely refractory to crude liver extracts and liver digested with gastric juice.

The second case was successfully treated with liver extracts for twelve years. After that time she suddenly became refractory with severe leucopenia. Folic acid in large doses had an excellent effect and the patient has kept relatively normal blood values for about a year without further treatment of importance. It should be noted that the morbid condition of the tongue could not be influenced by folic acid.

The third case should probably be classified as idiopathic steatorrhea. Folic acid gave a full remission of the macrocytic anemia.

The fourth case resembled typical pernicious anemia more closely, as a histamine-fast achlorhydria was present. This patient had been refractory to liver treatment but reacted well to folic acid in ordinary doses.

The fifth case was a typical instance of idiopathic steatorrhea with probable atrophy of the spleen. She had a moderate anemia that was refractory to liver extracts. Folic acid perorally and intramuscularly neither influenced the anemia nor the fecal fat.

In a series of cases of uncomplicated pernicious anemia, the immediate response to folic acid in doses of 20 to 30 mg. daily was good. Full remissions as regards
hemoglobin and erythrocytes could be expected with a total dosage of 400 to 600 mg.

Certain cases of pernicious anemia refractory to liver extract may respond to folic acid. This indicates that the two substances probably have different mechanisms of action.

REFERENCES


SUCCESSFUL TREATMENT OF LIVER-REFRACTORY ANEMIA WITH SYNTHETIC LACTOBACILLUS CASEI FACTOR

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