CLINICAL AND LABORATORY STUDIES
OF
22 PATIENTS WITH MEGALOBLASTIC ANEMIA

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ABSTRACT

Clinical and laboratory studies of 22 patients with megaloblastic anemia were reported herein.

The patients were grouped on the basis of pathogenesis of megaloblastic anemia.

Group A: 12 patients with idiopathic megaloblastic anemia.
Group B: 10 patients with secondary megaloblastic anemia.
   In 5 patients megaloblastic anemia was associated with gastrectomy, in 3 patients with operation of small intestine and in 2 patients with drugs.

The common presenting symptoms were palpitation and general fatigue.
The erythrocyte count was ranged between 860,000 and 4,980,000 per mm$^3$.
Patients with severe anemia mostly showed leucopenia and thrombocytopenia. When anemia was severe, promegaloblasts and basophilic megaloblasts were recognized in bone marrow. The serum iron was usually normal or raised, and high levels of LDH were recognized. The Schilling test for 14 cases revealed vitamin B$_{12}$ absorption ratio to be so low as 0.34-6.1 per cent.

Hematological findings were rapidly improved by administration of vitamin B$_{12}$ in all cases except 2 cases with severe heart failure.

There are not so many megaloblastic anemia in Japan. So-called pernicious anemia reported first by Addison and Biermer has seldom been reported and only few cases are reported annually. This might be attributable not only to the racial difference$^1$ but also to the fact that most Japanese people use habitually multivitamin tablets containing vitamin B$_{12}$ and folic acid. Since the number of patients with secondary megaloblastic anemia caused by gastrectomy, intestinal operation and drugs, has been gradually increasing in
recent years, the secondary megaloblastic anemia has been called attention.

During the period from 1963 to 1971, we had treated 22 cases of megaloblastic anemia. Not a few cases of them were treated as disorders of cardiovascular system, gastrointestinal tract or nervous system.

This report is dealt with laboratory and clinical investigations on these 22 cases.

Patients:

The subjects were 22 patients with megaloblastic anemia who were admitted to the 2nd Tokyo National Hospital from 1963 to 1971. The greater part of the patients had hyperchromic anemia and megaloblastic haematopoiesis in bone marrow, and they were mostly given vitamin B₁₂. The patients who had so-called megaloblastoid cells in bone marrow in the progress of leukemia and erythroleukemia or during administration of anti-leukemic drugs were excluded. The subjects consisted of 1 patient under 20 years old, 3 of 21~30 years, 1 of 31~40 years, 6 of 41~50 years, 5 of 51~60, 2 of 61~70 and 4 over 71 years old (10 males and 12 females). These patients were classified into two groups of the idiopathic and the secondary megaloblastic anemia. Ten patients with secondary megaloblastic anemia all ranged from 30 to 60 years in age. Idiopathic patients consisted of 6 males and 6 females.

Group A......12 patients with idiopathic megaloblastic anemia, who were divided into 3 groups by ages.

A-I: (Case 1-4)
4 patients over 70 years old.
Case 1 and 2 died during this investigation.

A-II: (Case 5-8)
4 patients ranged from 58 to 67 years in age.

A-III: (Case 9-12)
4 patients ranged from 18 to 41 years in age.
Case 9 and 11 were treated occasionally by vitamin B₁₂ and folic acid for past 10 years and 3 years respectively.

Group B......10 patients with secondary megaloblastic anemia who can be divided into 3 groups by causes.

B-I: (Case 13-17)
5 patients who developed this disease several years after gastrectomy of gastric ulcer.

B-II: (Case 18-20)
3 patients who developed this disease after operation of
small intestine or abdominal radiotherapy.

**B-III: (Case 21–22)**

In this group megaloblastic anemia was probably induced by the long-term administration of drugs.

In idiopathic group A-1, megaloblastic anemia was induced by impaired secretion of intrinsic factor due to the atrophy of gastric mucosa because of their advanced ages. For group A-III, megaloblastic anemia was caused by functional disorder of intrinsic factor owing to the presence of intrinsic factor antibody and parietal-cell antibody rather than secretory disorder of intrinsic factor. For group A-II both the secretory and the functional disorders may participate in the cause of megaloblastic anemia.\textsuperscript{2,3,4}

Group B-I caused megaloblastic anemia by secretory inability of intrinsic factor owing to gastrectomy. Group B-II developed this disease from the absorption disturbance of intrinsic factor combined with vitamin B\textsubscript{12}, in spite of their normal abilities of intrinsic factor secretion.

For group B-III, the causes of megaloblastic anemia seem to be disorders of folic acid absorption, its activation disturbance and biosynthetic disturbance of nucleic acid, although those are different by the nature of drugs administered.

Familial megaloblastic anemia which occurs in childhood is caused by the congenital intrinsic factor deficiency. Most of the megaloblastic anemia which occurs in pregnancy is ascribable to the lack of folic acid, and the association of megaloblastic anemia with sprue and steatorrhea is said to be caused by absorption disorder of intrinsic factor-vitamin B\textsubscript{12} complex.\textsuperscript{4,5,6} We could not find these cases in our series.

**CLINICAL FINDINGS**

Main symptoms of 22 patients are shown in Table 1. The most common symptom was palpitation, which was seen in 14 patients and was not always in parallel with the severity of anemia. General fatigue was seen in 11 patients. Most of them complained edema of lower extremities, anasarca, anorexia and headache. These complaints of palpitation and general fatigue in patients with megaloblastic anemia were more frequent and severe than those in patients with other anemias including iron deficiency anemia, aplastic anemia and hemolytic anemia. Paraesthesia was seen in 5, and ataxia in 2 patients. Four cases out of 9 patients who complained sore tongue could scarcely eat.
Table 1

Chief complaints and past history of 22 cases

<table>
<thead>
<tr>
<th>Group</th>
<th>Cases</th>
<th>Chief complaints</th>
<th>Past history</th>
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<td>A1</td>
<td>1</td>
<td>83(M)</td>
<td>dyspnea, edema</td>
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<td>2</td>
<td>73(M)</td>
<td>cyanosis, dyspnea</td>
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<td>4</td>
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<td>anorexia, edema</td>
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<td>67(F)</td>
<td>hemorrhagic tendency, general fatigue</td>
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<td>disturbance of gait</td>
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<td></td>
<td>8</td>
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<td>palpitation, hemorrhagic tendency</td>
</tr>
<tr>
<td>AII</td>
<td>9</td>
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<td>palpitation</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>22(M)</td>
<td>general fatigue, palpitation</td>
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<tr>
<td></td>
<td>11</td>
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<td></td>
<td>16</td>
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<td>17</td>
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<tr>
<td>B1</td>
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<td>headache</td>
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<tr>
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<tr>
<td>BII</td>
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<tr>
<td></td>
<td>22</td>
<td>44(F)</td>
<td>palpitation</td>
</tr>
</tbody>
</table>

Group A-I:

Group A-I consisted of 4 patients over 70 years old.

Case 1 and 2 who had been treated for heart failure for more than 1 year, were admitted to our hospital because of cyanosis and dyspnea. Both cases had significant anemia, tongue atrophy, low voltage and depression of ST segments of ECG, and atrophic gastritis on X-ray were revealed. Duodenal diverticulum was found in case 1, and a slight jaundice in case 2. Each case also showed megaloblastic hyperhaematopoiesis in bone marrow. Despite of blood transfusion and administrations of heart drugs and vitamin B12, case 1 died in 23 days and case 2 died in 3 days after hospitalization.
Before the liver therapy was established by Minot and Murphy\(^3\) in 1926, megaloblastic anemia drove all patients to death, as its another name, “progressive pernicious anemia” showed. The direct cause of death was said to be heart failure and hemorrhage, according to Huser\(^4\). However, since vitamin B\(_{12}\) and folic acid were discovered, cases of death became so rare that the name of pernicious anemia is not suitable. However, on rare occasions as case 1 and 2, patients died from this disease when they were treated only for cardiovascular disorder. These cases suggested that the older patients should be treated carefully.

Case 3 who had been treated for heart failure for about a year before admission showed moderate anemia, atrophy of the tongue, absence of tendon reflex and high megaloblastic crisis of bone marrow. This patient showed a clinical improvement by vitamin B\(_{12}\), but one year later, he had difficulty in swallowing and was found to have esophagus cancer. After operation, he has been subjected to radiotherapy.

Case 4 was admitted to our hospital from anorexia, anasarca, anemia, and first-sized tumor in the epigastrium showed 16.2% megaloblasts in bone marrow. X-ray examination of gastrointestinal tract showed wide-spread cancer all over corpus ventriculi. A large amount of vitamin B\(_{12}\) and blood transfusion were given and total gastrectomy was carried out, and thereafter the administration of vitamin B\(_{12}\) was followed.

It may be difficult to find a direct relationship between esophagus cancer and megaloblastic anemia in case 3. Megaloblastic anemia in patient with gastric cancer as in case 4 can be understood to be caused by impaired secretion of intrinsic factor as in case 1 and 2.

It is characteristic that only secretion of intrinsic factor but also secretion of gastric acid and pepsin are impaired in adult, particularly in aged persons in this disease. On the other hand, many younger patients have not impaired secretion of gastric acid and pepsin but of intrinsic factor.

It can be said that the absorption disturbance of vitamin B\(_{12}\) due to lack of intrinsic factor interferes enzym activities in heart muscle and that anemia induced by the DNA synthetic disorder due to lack of vitamin B\(_{12}\) also worsen the heart function.

Group A-II:

Group A-II consisted of 1 male and 3 female patients ranged in age from 50 to 70. They were aged in the middle of A-I and A-III. Therefore, coexistence of the inducing factors of A-I and A-III was suspected.
All cases in this group had general fatigue due to anemia. Case 5 and 8 also showed hemorrhagic tendency and case 7 had a remarkable funicular myelosis. Mild fever and lower abdominal pain were recognized in case 5. Diarrhea, redness and pain of tongue and intercostal neuralgia were noted in case 6 and epigastralgia was found in case 8.

In the past history, case 5 had a cholecystectomy from cholelithiasis 5 years ago and then she had anemia and was treated with iron. Case 7 had infiltration of the lungs at the age of 34, and had an operation for ectopic pregnancy at 42. Diagnosed as megaloblastic anemia with funicular myelosis, she had been treated with vitamin B₁₂ injection (15γ, 8 times) since 2 years ago, when she had paresthesia on legs and was difficult to walk. Case 8 had often been treated for gastroptosis and chronic gastritis since 10 years ago.

As for the question whether these past histories have any correlation with the occurrence of megaloblastic anemia, the possibility that cholecystectomy or hysterectomy brings some disorders of digestive tract can not always be denied. And gastritis continued for a long time is closely related with the occurrence of this disease.

Case 6 had intercostal neuralgia and paresthesia of legs. Case 7 showed paresthesia in the lower extremities, gastrocnemious tenderness and ankle edema.

Funicular myelosis is one of the common symptoms in megaloblastic anemia. It greatly varies the manifestations with the position and severity of degeneration of spinal cord. When anterior column as well as posterior column are damaged, pyramidal tract lesion, spastic paralysis, hyper-reflexia and/or pathologic reflex can be observed.

When the degenerative changes extend to axis cylinder, even peripheral nervous disorders are not always relieved by treatments, although anemia itself can be improved.

Hemorrhagic tendency was observed in case 5 and 8 due to thrombocytopenia. Case 5 had gingival bleeding and case 8 had mainly subcutaneous bleeding of pectoral region and extremities. Besides these hemorrhages, we often found epistaxis, retinal hemorrhage, genital bleeding, and cerebral hemorrhage which once played the leading part of cause of death with this diseases. It is notable that 2 cases whose administration of vitamin B₁₂ was discontinued showed a remarkable decrease of platelet counts at the earliest time of recurrence.
Group A-III:

This group patients suffered from typical Addison-Biermer type pernicious anemia. No acid was recognized in their gastric juice even after administration of histamine and intrinsic factor was inactive. Intrinsic-factor antibody and parietal-cell antibody were also detected in serum. In case 10 and case 12 intrinsic factor was inactive, and intrinsic-factor antibody and parietal-cell antibody were positive in serum. This type is frequently recognized among relatively younger persons, and it is characteristic that they have no past histories likely to cause this disease.

In our clinical trial, 4 patients (2 males and 2 females) belonged to this group. Case 9 was a doctor who had suffered from pernicious anemia for about 10 years and had been occasionally treated with vitamin B12. Her complaints were general fatigue, intercostal neuralgia, paresthesia of legs and anxiety, and splenomegaly was recognized on physical examination. Case 10 had palpitation and gingival bleeding from 20 years of age, and at 22 he had anemia which was improved by iron therapy, blood transfusion and vitamin B12 (15γ × 6) administration. He recurred severe anemia at 24 and was diagnosed as pernicious anemia and treated with vitamin B12 (30γ × 18, 15γ × 10). At the age of 29, he had anemia again and was sent to our hospital because of general fatigue, sore tongue and weightloss. Physical examination revealed whiteness of hair, splenomegaly (2 finger-breadths) and hepatomegaly (2 finger-breadths) besides severe anemia.

Case 11 had been treated for chronic gastritis and hepatic disorder for several years. But since 6 months ago the patient had a mild jaundice which was not improved by any therapy, and therefore he was sent to our hospital. Anemia was not so severe, and neuralgia in legs and slight jaundice were found. Laboratory examinations proved that these symptoms were caused by megaloblastic anemia and administration of vitamin B12 completely improved the symptoms.

Case 12 (Fig. 1) was hospitalized for epistaxis, anasarca, dyspnea and mental disorder. The patient had been diagnosed as anemia and treated with oral iron for 3 months, but anemia was not improved. This patient was a case of typical pernicious anemia of juvenile type.

Case 9 and 10 had anemia repeatedly for 10 years and were treated with insufficient doses of vitamin B12 on all occasions. Minimum dose of Vitamin B12 for this disease is said to be 2–3γ (5–6γ at highest) daily. However, oral drugs of vitamin B12, even in a large amount, can be absorbed only 0.1–0.5%. And moreover, it must be considered that vitamin B12 is excreted into bile.
through entero-hepatic circulation even in the parenteral administration. Therefore, the patient should be given a large amount of vitamin B₁₂ enough to be stored in body.

In these cases having recurrence of megaloblastic anemia, it is notable that splenomegaly was recognized. It is well known that this disease is accompanied by a mild jaundice due to increase of indirect bilirubin. When hemolytic function is repeatedly stimulated, spleen gradually enlarges to make anemia worse. However, adequate administration of vitamin B₁₂ makes hematological findings and splenomegaly improved obviously.

Group B-I:

This group consisted of 3 male and 2 female patients all who occurred megaloblastic anemia after gastrectomy. They developed this disease 15, 29, 10, 9 and 6 years after gastrectomy respectively, and their ages ranged from 49 to 59 years. It is interesting that almost all patients in this group developed this disease in their fifties.

Case 13 had gastrectomy from gastric ulcer at his age of 43 and had an operation again due to post-operative esophagostenosis at age of 47. He had severe anemia, slight jaundice and hepatomegaly (3 finger-breadths below the costal margin).

Case 13, 15 and 16 developed this disease in 10–15 years after gastrectomy, and they had been treated with iron, blood transfusion and the other hema-
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The patients of this gastrectomy group scarcely showed any severe symptoms of circulatory disorder, neurologic disturbances and/or hemorrhagic tendency. Because they were treated with blood transfusion, iron, vitamin B₁₂ and/or folic acid when they had anemia once or twice after gastrectomy. The larger the area of excision of the mucosa containing parietal cells, the more frequently megaloblastic anemia occurs. In other words, total gastrectomy induces megaloblastic anemia more frequently than partial gastrectomy. There has been lack of agreement in the incidence of megaloblastic anemia after partial gastrectomy. According to Mac Lean⁷ the incidence of megaloblastic anemia after partial gastrectomy is less than 1 per cent whereas Hines⁸ et al described the incidence of 18 per cent.

Therefore, it should be considered that the incidence of megaloblastic anemia depends upon the observation and treatment after operation, though it varies with the size of excision.

Group B-II:

Vitamin B₁₂ in food is absorbed in ileum, when it combines with intrinsic factor in stomach. Megaloblastic anemia is induced by lesions or resection of the sites wherein vitamin B₁₂ is absorbed.

In case 18, tumor in the size of an egg was found in the ileocecal region, and it was removed together with a part of small and large intestines 14 years ago. Thereafter, the patient had the radiotherapy on the operated site. However, 3 months after operation she developed ileus and had enterectomy again. She was admitted to our hospital from fullness of lower abdomen, dullness, sleeplessness and anorexia. At that time she showed severe anemia, slight jaundice.

Case 19 developed intestinal obstruction after appendectomy at the age of 11 and underwent enterectomy and ileotransversostomy. He sometimes
complained diarrhea, vomiting and paresthesia of lower extremities since the age of 16. At age of 17 he had visual disturbance and was diagnosed as optic atrophy. Thereafter the patient had disturbance in the gait along with the increased paresthesia of extremities. Furthermore, he began to have hoarseness and dysphagia, and was treated at a hospital under diagnosis of polyneuritis and paralysis of the left recurrent laryngeal nerve, but the symptoms progressed. The patient was admitted to our hospital having edema of lower extremity, weight loss and anemia for last 6 months.

Case 20 underwent an operation for caecum mobile 15 years ago. The patient got enterectomy because of occurrence of ileus 4 years after the operation.

Thereafter she was healthy. About one year prior to admission she had shown gingival bleeding occasionally. Because gingival bleeding gradually increased and epistaxis and palpitation followed, she was admitted to our hospital.

These 3 cases had enterectomy for ileocecal disorder and remained symptoms of megaloblastic anemia 11–15 years after the operation.

Ileocecal disorders such as tropical sprue, ileitis, stenosis or diverticulum can also be regarded as a cause of megaloblastic anemia. Moreover, pre- and post-operative radiotherapy of tumor of intestine, uterus, or ovarium, can be a cause of megaloblastic anemia.

Group B-III:

This group consisted of 2 females who presumably developed megaloblastic anemia because of long-term administration of drugs.

Case 21, a pharmacist, had been emotionally unstable and had taken meprobamate in 6–9 tablets a day for about 5 years by herself. The patient had hallucination, illusion and hypnobatia-like symptoms for several days after hospitalization.

Case 22 had been administered para-aminosalicylic acid (PAS) for pulmonary tuberculosis for about 18 years since the age of 26, and had received cycloserine in combination with PAS for last 2 years. The patient had pyothorax and developed anemia. Hematological examinations showed megaloblasts in bone marrow and leukocytosis.

Anticonvulsants, especially primidone, diphenylhydantoin and phenobarbital, are well known as the drugs to induce megaloblastic anemia. It is said that these drugs cause disturbance of folic acid absorption and they have antagonizing action against folic acid. They are also said to cause disturbance
of absorption of vitamin B₁₂. Megaloblastic change is also induced by the administration of methotrexate, a folate antagonist. Furthermore, 6MP, cytosine arabinoside, 5FU can also cause megaloblastic change by inhibiting enzymic activity to activate folic acid and by inhibiting pyrimidine synthesis. Among anti-tuberculous agents, PAS and cycloserine are said to induce megaloblastic anemia by impairment of absorption of vitamin B₁₂ as seen in case 22.¹⁰ Meprobamate (benzodiazepine compounds), a hypnotic sedative, is reported to induce aplastic anemia, purura and porphyrinemia. However no megaloblastic anemia induced by meprobamate has ever been reported. Since both minor and major tranquilizers inhibit DNA synthesis, it can be fully considered for meprobamate to have some factors to cause megaloblastic anemia. In addition, chloramphenicol, benzol, etc. are reported as causative of this disease.

Administration of vitamin B₁₂ improved anemia moderately in case 22, resulting disappearance of megaloblasts in bone-marrow. The hypnobatic symptoms in case 21 were also improved immediately after administration of vitamin B₁₂. She gained weight and was relieved from psychiatric symptoms in parallel with the improvement of anemia.

In the above lines, we have reported the clinical cases classified in some groups. Either idiopathic or secondary the megaloblastic anemia may be, every case principally showed remarkable anemia, gastric, psychoneurotic and circulatory disorders.

LABORATORY FINDINGS (Table 2)

1) Hematological Findings

The erythrocyte count of the patients was ranged between 860,000/mm³ and 4,980,000/mm³ (2,130,000/mm³ on an average). Most part of the patients (12 cases) had 1~2 million per mm³ erythrocytes. Fifteen patients (72.8%) had more than 1.0 color index and the mean value of mean corpuscular volume was as slightly large as 109.7 µ³. It was 4 cases that definitely showed hypochromic anemia, and almost all of them had hemorrhagic tendency including genital bleeding, gingival bleeding, etc.

Patients had low reticulocyte count when untreated, but patients having dyspnea generally showed the increased reticulocytes, as seen in group A-1.

The leukocyte count was under 4,000/mm³ in about half of the patients and over 9,000/mm³ in only 4 cases. The average numbers of lobes in the neutrophil polymorphy were 2.5~3.9 (3.1 on an average) which was rather
more than that of normal persons (2.7). In relation to erythrocyte count and leukocyte count, almost all having less than 2 million per mm$^3$ erythrocyte count showed the decrease of leukocytes. The severer the anemia was, the more frequently erythroblasts appeared in blood picture. However, appearance of megaloblasts in peripheral blood was hardly recognized.

As to platelet count, 4 cases had less than 50,000/mm$^3$, 8 cases 50,000~10,000/mm$^3$, that was about half numbers of patients clearly showed the decrease of platelet count. Patients with low erythrocyte count mostly showed the decrease of platelet count. Indeed, case 17 shown in Fig. 2 had platelet count decreased prior to reduction of erythrocytes and leukocytes when he had recurrence of this disease. In these cases with decreased platelets, it was recognized that bleeding time was prolonged proportionately with the decrease of platelets.

The most characteristic findings of myelogram were the remarkable increase of erythroid cells and appearance of megaloblasts. Megaloblasts were noted in 0.8~46.2% (18.0% on an average) in marrows. These megaloblasts were all negative in PAS staining. Promegaloblasts and basophilic megaloblasts appeared when anemia was severe. Every individual case showed remarkable morphological changes, such as irregular density of nuclear chromatin, abnormal division of nuclei and deformity of cytoplasm. Such morphological variety of megaloblasts may be related to the duration of vitamin B$_{12}$ and folic acid deficiency, degree of those deficiencies and other hemato-
### Table 2

Hematological and biochemical findings of 22 cases

<table>
<thead>
<tr>
<th>Group</th>
<th>Case</th>
<th>RBC (10^6)</th>
<th>Hb (g/dl)</th>
<th>C.I.</th>
<th>MCV (fl)</th>
<th>Ret (%)</th>
<th>Plat (10^9)</th>
<th>WBC</th>
<th>Bleed. T. (min.)</th>
<th>Coag. T. (min.)</th>
<th>Megaloblast % (Bone marrow)</th>
<th>SFe (γ/d)</th>
<th>LDH</th>
<th>Schilling test (%)</th>
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poietic factors.

Also large rod cells were found in myeloid cells in about half of the patients, and those cells were in various shapes similar to those of megaloblasts.

Chromosomal morphology was studied in several cases by means of marrow culture. As the result, any modal irregularities were not recognized except a few cases which showed abnormalities in chromosome structure.

2) Biochemical Studies

Serum Iron:—Most part of the patients had normal or high values. The mean serum iron was 156.7 µ/dl. Case 14, 20 and 22 who had all hemorrhagic tendency did not show any abnormal low value, as compared with those who had posthemorrhagic anemia. From this fact, it can be guessed that there are disorders of iron utilization in megaloblastic anemia. Following the administration of vitamin B₁₂ and other specific therapies, reticulocyte crisis occurs, and erythrocyte count and hemoglobin value increase. Then, serum iron rapidly decreases owing to the acceleration of iron utilization. In that condition the patients with megaloblastic anemia show evidence of iron deficiency.

Serum Copper:—This value was rather high in general, but it decreased to normal by treatment.

Serum LDH:—In 11 cases out of 22 cases serum LDH was measured before treatment. A slight increase was recognized in 4 cases and remarkable increase in 7 cases. The mean value of serum LDH was 2,145 unit; LDH₁ and LDH₂ were especially increased in isozyme pattern.

Gordin¹¹ and McCarthy¹² demonstrated that serum LDH level was related to the severity of anemia and that the origin of high LDH level was the megaloblastic marrow. According to them patients sometimes had serum LDH as much as 10,000 unit, but the value was not greatly increase when anemia was not severe.

Uric Acid:—It is said that the impaired synthesis of nucleic acid in hematopoietic organs results in high level of serum uric acid. The greater part of our patients whose uric acid level measured showed high level. Particularly, administration of vitamin B₁₂ increased reticulocyte count and induced extraordinary high uric acid level due to destruction of unnecessary megaloblasts, and these phenomena make it necessary to administer allopurinol.

Serum Bilirubin:—It is well known that megaloblastic anemia is accompanied with slight jaundice likely caused by increase of indirect bilirubin due
to hemolysis. However, the degree of jaundice differs with cases, and incidentally case 11 showed moderate jaundice.

Other biochemical observations:—GOT and GPT were almost within normal range, although some higher values were recognized in a few cases. Serum cholesterol and cholinesterase were both clearly lower.

3) **Examination of Gastric Juice**

Nine cases subjected to this test showed so severe hypoacidity or anacidity that they did not respond to histamine test. Six cases (group A) examined by X-ray and gastrocamera had all obviously atropic gastritis. Deficiency of intrinsic factor was proved in all of 4 cases studied in the group A-II and A-III, and 2 cases of them were recognized to have intrinsic factor anti-body in serum.

4) **Schilling test**

This test in 14 cases studied revealed vitamin B₁₂ absorption ratio to be so low as 0.34~6.1% (3.8% on an average). The patients with megaloblastic anemia had the absorption ratio of 0~7.3% according to Schilling et al,¹³ and of 0~7.5% according to Callender et al,¹⁴ while that of normal persons showed 16~40%.

5) **Serum Vitamin B₁₂**

Since megaloblastic anemia is mostly induced by deficiency of vitamin B₁₂, decrease of serum vitamin B₁₂ is an essential factor of this disease. Serum vitamin B₁₂ was 100~1,000 pg/ml for normal persons. Serum vitamin B₁₂ in 7 patients with megaloblastic anemia were all under 80 pg/ml. In addition, folic acid level in serum is clearly lower for patients with megaloblastic anemia due to deficiency of folic acid. High serum folate level is sometimes recognized, when vitamin B₁₂ is deficient. Urinary excretion of methylmalonic acid increases after administration of l-valine (precursor of methylmalonic acid), when vitamin B₁₂ is deficient. Increase of urinary excretion of formiminoglutamic acid is an evidence for folic acid deficiency. Latent deficiency of vitamin B₁₂ and folic acid can be proved by increased excretion of these substances in urine. We, indeed, recognized increased excretion of urinary methylmalonic acid both in case 10 and 12.

6) **Findings of ECG and Chest X-Ray Examination**

For major part of the cases, electrocardiogram showed sagging of ST
segments and T waves, low voltage, ventricular hypertrophy and bundle branch block. Particularly, case 1 and 2 showed severe myocardial damage in ECG and died without response to any treatments. X-ray examination of chest frequently revealed cardiomegaly, accentuation of hilar markings and/or edematous shadow in entire lung field.

**TREATMENT AND PROGNOSIS**

Hematological findings were rapidly improved by administration of vitamin B\textsubscript{12} in 20 cases out of 22 cases. Only 2 cases with heart failure died without proving no effectiveness of vitamin B\textsubscript{12}.

Cyanocobalamin, hydroxocobalamin, coenzyme B\textsubscript{12} or methylcobalamin, was administered by parenteral or partly oral routes. The same effectiveness was obtained in any form of vitamin B\textsubscript{12}.

Vitamin B\textsubscript{12} is well known to be effective for improving hematological findings of megaloblastic anemia even in very small doses, even 1\gamma per day. However, even though rapid improvements of hematological findings are obtained by parenteral or oral administration of high dose of vitamin B\textsubscript{12} as seen in Fig. 2, the disease sometimes recurs in 4~5 months after discontinuation of administration. It became clear that both in oral and parenteral routes the absorbed vitamin B\textsubscript{12} was excreted into urine in considerable amount and that it could not all be transported to hematopoietic tissue to be utilized. Therefore, it is necessary to use middle to high doses continuously. We use 500~1,000\gamma per day of vitamin B\textsubscript{12} by injection until anemia is improved and then 1,500~2,000\gamma per day by mouth.

When vitamin B\textsubscript{12} is effectively utilized, reticulocyte crisis, increase of erythrocyte count and hemoglobin, and decrease of serum iron and LDH are obtained.\textsuperscript{15} When serum iron is decreased, it is desirable to give iron together with vitamin B\textsubscript{12}.

Two cases out of 22 cases died from heart failure. The autopsy findings were degeneration of myocardium and remarkable central necrosis of liver, severe atrophy of gastric mucosa and erythroid hyperplasia in bone marrow. The autopsy proved the cause of death to be necrosis of myocardium. Thus, it was found that even high doses of vitamin B\textsubscript{12} could not prevent the patients from death, when patients had degenerations of myocardium and liver. These two patients had been treated as heart disease for a long time before admission. Early diagnosis of megaloblastic anemia might prevent from death. It is especially noteworthy that some patients with megaloblastic anemia are
Megaloblastic Anemia

treated as heart disease.

REFERENCES