SELECTIVE MALABSORPTION OF VITAMIN B₁₂:
REPORT OF A CASE AND STUDIES ON
VITAMIN B₁₂ ABSORPTION

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INTRODUCTION

Although it is quite rare to find megaloblastic anemia in the field of pediatrics, we recently encountered an infant with this disease. The chief symptoms were a pale face, anorexia and malaise. The case was investigated and the result led to the following conclusion: the appearance of the megaloblasts was due to Vitamin B₁₂ deficiency (Vitamin B₁₂ will be abbreviated to V.B₁₂) caused by V.B₁₂ malabsorption in the small intestine, and further, this malabsorption was specific to V.B₁₂. That is, this case was diagnosed as selective V.B₁₂ malabsorption.

There has been no previous report of selective V.B₁₂ malabsorption in Japan and only a few in Europe and America.^{1,2,3,4,5} The clinical elucidation of the mechanism of V.B₁₂ absorption is very difficult because of the many factors involved. We therefore, made several types of tests described in past reports.

REPORT OF THE CASE

Patient: ■■■■■, a 2 year and 10 month old male. (Father's Occupation: farming)
Chief Symptoms: Pale face, anorexia, and malaise.

Previous Health: The child was delivered normally with a birth weight of 3,200 g. Neonatal jaundice showed no abnormality.

Development: He first raised head at 3 months and walked alone at 20 months. No mental retardation was noted.

Nutrition: Formula feeding. Weaning was begun 10 months after birth. Little vomiting or diarrhea.

Immunization: He has already been vaccinated and also received the inoculations of DPT and polio vaccine. Tuberculin reaction was (+).

Family History:

His parents were consanguineous as cousins.

The patient was their second child, his sibling is an elder brother and there were no dead siblings.

There is no record of anemia, jaundice or hemorrhagic tendency in the family.

Present Illness: Abnormality appeared around the age of 2 years and 3 months (March, 1970) when his face became pale and he was easily tired and lethargic. The patient was admitted to a university hospital for close examination from April 19, 1970 to June 9, 1970; anemia and albuminuria were detected. Blood transfusion was effective for the anemia. However, the renal biopsy which was performed to investigate the albuminuria revealed no abnormality, and benign albuminuria was diagnosed. The etiology of the anemia was not elucidated. Anemia and anorexia further developed thereafter, and the patient was admitted to the [REDACTED]

Presenting Condition: The patient's stature was rather small but his weight and height were within normal limits. His face was pale, but there were no signs of jaundice, purpura or edema. Dark brown pigmentation was noted around the nail. The lymph nodes, liver, and the spleen were not palpable. No congenital deformity was noted. His expression was normal. There was no atrophy of the lingual papillae and no neurological sign. Blood pressure 86/40.

RESULTS OF PHYSICAL EXAMINATIONS

Blood examination of the peripheral blood of the patient at the time of his admission gave the following results: Ht, 22%; hemoglobin, 7.7 g/dl; red blood cell count, 233×10^4 ; reticulocyte count, 18%; MCV, $94 \mu^3$; MCH, $33 \gamma\gamma$; MCH, 35%. These figures are typical of slightly macrocytic and of normochromic anemia. Platelet count was 7.8×10^4 ; white blood cell count was rather low at 4,300. Fig. 3 shows Price-Jones' curve: 35% of the red cells of the peripheral

blood, had diameters of 8.1–9.0 μ . Low neutropenia (19%) was associated with a decrease in the activity of alkaline phosphatase (score 127 and positive rate 58%). His parents and elder brother had normal blood.

In bone marrow smear (Fig. 1), megaloblasts represented as much as 33%. They were large, as shown in Fig. 2 (a), and the mature blasts with lumpy chromatin and polychromatic cytoplasm [Fig. 2 (b)] predominated over the immature cells with fine chromatin and blue and abundant cytoplasm; both of them were negative periodic acid-Schiff staining. Sideroblasts were seen in 34.5%, but no ringed form was noted. In the granulocyte series [Fig. 2 (c)], cells which appeared to be giant metamyelocytes were prominent. (See Fig. 2 on page 57)

These morphological findings of erythroblasts and the cells of the granulocyte series corroborated the diagnosis of typical megaloblastic anemia. The abnormal morphology found at the time of his admission (Oct. 15, 1970) was restored to normal at the same time as his recovery from anemia (Jan. 19, 1971).

Examination of serological biochemistry; showed only abnormalities (Fig. 3): Coomb's test was (–); the serum bilirubin normal; serum iron, 154 r/dl; LDH, 1840 u/ml (high); cholestrol, 129 mg/dl (low). No abnormality was noted in the serum protein fraction except IgG which was estimated as 500 mg/dl (low).

These abnormal values also returned to their normal levels following the recovery from anemia.

Fig. 1. Bone marrow smears.

Fig. 3. Laboratory findings.

INVESTIGATION OF THE CAUSE OF THE MEGALOBLASTIC ANEMIA

Quantitative determination of serum V.B₁₂ by the method of L. leichmanii gave the value of 0.11 mr/ml, which was remarkably low compared to the 0.29 mr/ml of his father and 0.48 mr/ml of his mother. Moreover, this was less than half the value of two infants with leukemia who were being treated with anti-metabolite.

Ukyo⁷ has recently reported that V.B₁₂ acts as a coenzyme of isomerase in the pathway from methyl malonyl-CoA to succinyl-CoA, and Cox *et al*⁶ have reported abnormal excretion of methyl malonic acid (MMA) in the urine³⁰ in cases of V.B₁₂ deficiency. This was found to be true in the present case: the patient excreted the abnormally high amount of 123 mg/day. Loading with L-valine, a precursor of methyl malonyl-CoA, has been found to markedly increase urinary MMA¹⁴; in the present case the amount increased to 268 mg/day.

The conclusion from these results, that the megaloblastic anemia was due to V.B₁₂ deficiency, was supported by the striking response to treatment with V.B₁₂. The increased urinary MMA level returned to normal (4 mg/day), and after one intramuscular injection of 1.000 μ g of V.B₁₂ there was remarkable reticulocytosis, apparent the next day, and, within four days, a marked increase in hemoglobin content (Fig. 4).

Fig. 4. Hb and reticulocyte response to V.B₁₂.

V.B₁₂ ABSORPTION IN THE STOMACH AND SMALL INTESTINE

On the stimulus of an intramuscular injection of 1.5 mg/kg of histalog, 54.5 ml of gastric juice at pH 1.8 (HCl 0.93 mEq) was obtained. This gastric juice contained 1830 units of intrinsic factor, which is within normal limits.⁸ (One unit is the amount able to combine with 1 mμg of V.B₁₂). No antibodies for intrinsic factor or parietal cells were found in the circulating blood of either the patient or his parents.

The Schilling test was carried out as follows Co⁵⁷-B₁₂ was given to the patient on Oct. 29, 1970 when he was still anemic, and Co⁵⁷-B₁₂ + intrinsic factor was given on Nov. 5, 1970. The urinary excretion of them was 0.3% and 0.9% respectively. This test was repeated in the same way on Jan. 14 and 20, 1971 in the convalescent stage; the urinary excretion rates were still as low as 2% and 1.5%, which suggested an absorption abnormality. A test described by Hippe³ was made. Pancreatic juice which is considered to be an important factor in absorption in the small intestine was obtained on a stimulus, attending the pancreozyminsecretin test, and determined normal. Pancreatic juice was inoculated with Co⁵⁷-B₁₂ and swine intrinsic factor using a stomach tube. However, the urinary excretion was still 1.0% and showed no significant increase (Table I).

The biopsy of the gastric mucosa, jejunum, and ileum showed a completely normal picture from the view point of morphology and histochemistry, and no

Table 1
Comparative Examination Vitamin B₁₂ Absorption Test (Schilling test)

Test dose		% in Urine		
		A Case (Hippe: 1966) in the Literature Age 16m.	Our Case Age 34m.	Control Age 38m.
Anemic stage	Co ⁵⁷ B ₁₂ alone Co ⁵⁷ B ₁₂ +I·F	0.4 0.5	0.3 0.9	14.8
Convalescent	Co ⁵⁷ B ₁₂ alone Co ⁵⁷ B ₁₂ +I·F		2.0 1.5	
Co ⁵⁷ B ₁₂ +duodenal juice		0.5	1.0 (normal adult duodenal juice, pH 8.2)	
Co ⁵⁷ B ₁₂ + ^{post operative} jejunal juice		0.3		
Co ⁵⁷ B ₁₂ +noma ¹ jejunal juice		5.3		

abnormality of the intestinal canal was noted by macroscopic observation on laparotomy.

Other tests can be summarized as follows: The radiographic appearance of the gastrointestinal tract was normal; I.P. or Fishberg tests of renal function gave normal results; the results of the glucose and fat tolerance tests and the aminoacid absorption test were within normal limits; endocrinic examination with the triosorb, PBI, tetrasorb and ACTH tests revealed no abnormality. I.Q. was greater than 100 and equivalent to that at the age of 3 years and one month.

The examination of chromosomes in cells from the blood and the buccal mebrane revealed that the patient's chromosome is 46xy (the normal male pattern) and his mother's is 46xx (the normal female pattern). Radiographic examination revealed only slight retardation of bone age.

However, the protein content of the urine was abnormally high, the qualitative determination indicated (+)-(#) and the quantitative determination indicated 13 mg/dl-85 mg/dl. The proteinuria of the anemic stage persists throughout convalescence and therefore, we thought that it may be diagnosed as benign proteinuria¹ (unexplained and persistent proteinuria.⁹) The urinary protein fractions showed a pattern similar to the serum fractions, albumin being predominant.

During the period when these examinations were carried out, no hematic administration or transfusion was performed except the intramuscular administration of V.B₁₂ for the Schilling test. The patient however gradually gained

color and his movement became active.

The Schilling test was carried out 5 times, and so in all the patient received the intramuscular administration of 1000 μ g of V.B₁₂ 5 times during the period from Oct. 29, 1970 to March 12, 1971. Subsequent to the first administration anemia disappeared (Jan. 19, 1971), and both the platelet-and leukocyte count returned to normal. The blood was normal in all the tests we made. Since then, the patient has continuously received the oral administration of 250 γ /day of V.B₁₂ for these 20 months, and now maintains a completely normal state hematologically.

DISCUSSION

Since this is the first case of selective malabsorption of V.B₁₂ reported in Japan, particularly extensive tests were made for the diagnosis and also to clarify the etiology of this rare disease. The condition for the appearance of megaloblasts is the existence of abnormality in DNA synthesis by erythroblasts. This can be caused by deficiency in either V.B₁₂ or folic acid.

However, it is quite improbable that both of these should be reduced or deficient, and it is known that if one of them is deficient, the other increases in compensation for it.^{10,11} Therefore, if V.B₁₂ deficiency is detected and a remarkable response to the administration of V.B₁₂ is noted; V.B₁₂ deficient, megaloblastic anemia may be diagnosed.

In our case, there was no marked disturbance of growth or of the central nervous system or vomiting or diarrhea (steatorrhoea) all which are characteristic of folic acid deficiency, and there was no associated protein deficiency. Therefore, alimentary folic acid deficiency⁹ or congenital abnormality in folic acid metabolism¹² could not be suspected. Two other possible causes may be ruled out: Congenital orotic aciduria¹³ in which disturbance of pyrimidine synthesis leads to megaloblastosis was not noted in the urinary findings, and the patient had never been given antimetabolite or anticonvulsants.

Since there was no abnormality in the growth or nutrition of this patient, the case was diagnosed as V.B₁₂ malabsorption.

The cause of V.B₁₂ malabsorption: pernicious anemia, obvious abnormality in the intestinal tract, disturbance of V.B₁₂ utilization by intestinal flora, chronic pancreatitis, and selective V.B₁₂ malabsorption will be considered.

In our case, the tissues of the gastric mucous membrane and the gastric juice were normal, the gastric intrinsic factor was (+), and intrinsic factor antibody and parietal cell antibody were both (-). This would not have been so in a case

of juvenile¹⁵ or congenital^{16,17} pernicious anemia. In other words, there was no obvious defect in the stomach where V.B₁₂ malabsorption occurred; and so this is not the pernicious anemia as seen in childhood.

If the cause of the malabsorption is looked for in parts other than the stomach, abnormality in the intestinal tract may be considered. In our case however, the biopsy of the jejunum and the ileum showed the mucous membranes normal to be in appearance, the macroscopic appearance of the intestinal tract was normal on laparotomy, and the radioactive findings were also normal. Moreover, the collected small intestinal juice was pH 6.8, which did not suggest the V.B₁₂ malabsorption caused by a reduction in bicarbonate compounds secretion due to pancreatitis.¹⁸ When the juice of the small intestine was cultured, 4×10^5 /ml of *Neisseria sicca* was detected. This however, hardly took up V.B₁₂ *in vitro*, and so it is unlikely that intestinal flora were utilizing significant amounts of V.B₁₂.

The patient, though having some retardation of bone age, showed normal growth and revealed no such symptoms as diarrhea and vomiting in the digestive tract. No neurological sign was noted. Inborn error screening test using urine was negative; the fat and glucose tolerance tests were both normal; the amino acid analysis of urine and serum gave normal results. There was no abnormality in renal and endocrinic functions.

Taking these results into consideration, we concluded that there was a functional absorption abnormality in the mucosa of the small intestine which caused selective V.B₁₂ malabsorption, and that this is a congenital disease was made more likely because this patient has the hereditary defect¹⁹ that his parents are consanguineous. Gräsbeck¹ once suggested that proteinuria may be caused by protein-reabsorption insufficiency in the nephro-urinary duct which is a congenital functional defect, and he dealt with both anemia and proteinuria as congenital syndromes. However, it has recently been reported²⁰ that mild glomerulonephritis associating with thickening of the basement membrane in the glomeruli was noted in electron microscopical findings, and it has been concluded that these changes are caused by serious anemia during infancy. Imerslund⁴ observed V.B₁₂ deficiency and proteinuria in the two patients who underwent the resection of the small intestine in infancy, and so he questions of the theory of congenital abnormality.

Since the first report of Herbert²⁰ (1959), selective V.B₁₂ malabsorption has been reported by Gräsbeck,¹ Mohamed,²¹ and Ben-Bassat.⁵ There is no case-report in Japan but there is a report on absorption experiment by Okuda.²² He concluded that V.B₁₂ is combined with intrinsic factor to form a compound; and this is absorbed on to the surface of the intestinal mucosa, where V.B₁₂ in a simple

form or in a form of compound; is absorbed. Thus the mechanism of V.B₁₂ absorption involves three stages and in the study of V.B₁₂ malabsorption, the many factors which are involved in each of the stages should be taken into account.

First, the chemical structure of the compound²³ it self has not yet been elucidated. However, it is well known²⁴ that V.B₁₂ is hardly absorbed unless in the form of the compound. In our case, we took into account the combining ability of intrinsic factor with V.B₁₂, but it was not been confirmed whether the compound had really been formed.

Secondly, the problem of absorption is raised. Absorption is considered to take place in the ileum, where the existence of a moulding mechanism²⁵ of absorption is supposed between the compound and the receptor on the ileum mucosa, and if there is a defect at one side, the absorption is not performed. It is also known²⁶ that dibalent ions like Ca⁺⁺ are necessary for this V.B₁₂ uptake and that the optimal pH is 5.9–8.9²³. The intestinal juice used for our study was pH 6.8, and the pancreatic juice which was collected from normal adults and used for the V.B₁₂ absorption test was pH 8.2. Thus either of them was an adequate material as far as pH is concerned.

Thirdly, there is the problem of how the absorbed V.B₁₂ passes through the mucosal membrane. In the process of transport of V.B₁₂ from the intestinal epithelial cells into the blood vessels, the existence of a "releasing factor"^{23,25} has been proposed. It is also supposed⁸ that intrinsic factor facilitates V.B₁₂ absorption, but this factor itself is not absorbed.

As described above, studies on V.B₁₂ absorption both *in vitro* and *in vivo* have been reported. We, therefore, attempted the V.B₁₂ absorption test (Table 1) according to Hippe.³ Malabsorption was indicated throughout the anemic stage and the convalescent stage, and such increase in Co⁵⁷B₁₂ in the urinary excretion by the addition of intrinsic factor as observed in the our previous case¹⁵ of juvenile pernicious anemia was not found. This proved that the fault was not in the intrinsic factor. Hippe³ collected juice from the ileum of a healthy person and introduced it into the stomach of his patient. This caused the excretion of Co⁵⁷B₁₂ to increase 5.3%. We tried to repeat thus experiment, but were unable to obtain juice from the ileum. Hippe had shown that juice collected from a patient undergoing an operation was ineffective, so we used pancreatic juice from healthy adults. However, no significant result was given.

Gräsbeck²⁴ also observed only a slight increase in Co⁵⁷B₁₂ excretion following the addition of the normal ileum juice which was collected by stimulating with carbamylcholine, and he seems to consider that selective V.B₁₂ malabsorption is a congenital metabolic abnormality.

Although the mechanism of selective V.B₁₂ malabsorption could not be demonstrated by the experiments on absorption in the present study, it is supposed that the factors involved in the V.B₁₂ absorption are complicated as has been mentioned. Regarding the question whether the receptor is a physical entity, the analysis of the mucosal membranes seems impossible. However, the experiment for the "releasing factor" (proteolytic enzyme)¹ which is supposed to act when the V.B₁₂ intrinsic factor compounds are converted to V.B₁₂ peptides is feasible in future; because Okuda²⁷ caused an increase in V.B₁₂ absorption by protease in rats under the condition of intrinsic factor deficiency. If intestinal juice containing protease is given at the optimal pH (e.g., using sodium bicarbonate)¹⁸ significant result may be expected.²⁸

CONCLUSION

We discussed megaloblastic anemia seen in a 2 year and 10 month old male infant. It was concluded that this case was caused by V.B₁₂ malabsorption due to functional abnormality in the small intestine. The results of various examinations led to the diagnosis of selective V.B₁₂ malabsorption.

In order to demonstrate facilitated V.B₁₂ absorption, swine intrinsic factor or pancreatic juice of healthy adults was added; but no significant results were obtained. On the basis that his parents are consanguineous and he revealed benign proteinuria, we considered that this is selective V.B₁₂ malabsorption seen in the literatures in Europe and America and reported as the first case in Japan.

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Fig. 2(a). Megaloblasts (immature form).

Fig. 2(b). Megaloblasts (mature form).

Fig. 2(c). Giant metamyelocyte.