Hartnup Disease

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Two cases of typical Hartnup disease and six presumable carrier members through three generations were studied as the first cases in Japan. Cases 1 and 2 showed the typical clinical and laboratory findings of the disease. We carried out paperchromatographic studies on urine specimens from parents, siblings, maternal first cousins, and maternal and paternal grandmothers and grandfathers.

Two siblings, two maternal first cousins, and maternal and paternal grandfathers who were the first cousins had indicanuria and generalized aminoaciduria. But they had no symptoms suggestive of Hartnup disease. We think they may be carrier members of Hartnup disease.

Hartnup disease which was first described by Baron *et al.*¹ in 1956 is a recessive hereditary disorder of tryptophan metabolism. The disease is characterized by pellagra-like skin rash, mental deterioration and temporary cerebellar ataxia. Laboratory studies revealed the renal aminoaciduria without evidence of any other tubular dysfunction and an excessive urinary excretion of indican and indoleacetic acid. Up to the present time a total of 19 cases²⁻¹⁰ of this disease have been reported. However, no case report has appeared in Japan. The purpose of the present paper is to describe the first cases of Hartnup disease in Japan and to give further information with regard to the clinical manifestations.

CASE REPORTS

Case 1

History: A.M., a girl, was born on September 3, 1948, weighing 3,000 g, after a full-term pregnancy and spontaneous delivery. At the age of 4 years she suffered from frequent diarrhea and vomiting, and was admitted to a local hospital. She suffered from acute nephritis and was treated for 4 months at the age of 6 years. In the summer of 1954, when she was exposed to sunlight she exhibited ataxic gait and tended to fall especially to the right side. But she recovered spontaneously within 2 weeks, and during the rest of the year she was free from such attacks. In May, 1955, an itching rash developed on the areas exposed to

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Fig. 1. Hyperpigmentation on the face of the patient (Case 1).

sunlight, mainly hands, forearms, and face. In the spring of 1956, the rash developed again on the same areas but ataxic gait did not appear. She was not mentally retarded at that time. Twenty days before admission (at the age of 13 years), she suddenly suffered from ataxic gait and was unable to walk, but soon recovered after a little rest. Ten days before admission the rash appeared on the same areas as before. Because the rash and nervous symptoms tended to increase in severity, she was admitted to our Hospital in May 1962.

Physical findings: She was 137 cm tall and weighed 28 kg. The skin showed bilateral dermatitis characteristic of pellagra on the forearms, hands, legs and face (Fig. 1). There was no abnormality in the cardiovascular system. Neither the liver nor the spleen was palpable. The patellar reflexes were hyperactive but no pathological reflexes were elicited. The gait was normal and on Romberg's test she had no tendency to fall. There was no sign suggestive of mental retardation. Nystagmus, diplopia and hand tremor were not found.

Laboratory findings (May, 1962): The hemoglobin concentration was 15.2 g/100 ml, erythrocyte count $462 \times 10^4/\text{mm}^3$, white cell count $10,500/\text{mm}^3$ with a differential count of 0% eosinophils, 20.5% lymphocytes, 2.5% monocytes and

72.5% neutrophils without nuclear shift to the left. The erythrocyte sedimentation rate was 65 mm/hour. Tuberculin reaction was positive, but Wassermann's reaction was negative. Urinalyses for sugar, protein, acetone and urobilinogen were normal. No porphyrin in urine was detected on repeated examinations. Stools revealed normal findings. Serum chloride level was 97.0 mEq/l, sodium 135.0 mEq/l, potassium 4.3 mEq/l, phosphorus 5.4 mg/100 ml and total cholesterol 299.0 mg/100 ml. The intravenous glucose tolerance test showed normal finding. The liver function tests showed a slight hepatic damage: Thymol flocculation test was 6.0 units, zinc sulfate test 26.0 units, Takata's reaction one-positive, bromsulphalein test 7.0% after 30 minutes, alkaline phosphatase activity 9.6 Shinowara-Jones-Reinhart units, glutamic oxalacetic transaminase 86.6 Reitman-Frankel units and glutamic pyruvic transaminase 58.5 Reitman-Frankel units. Total protein in serum was 7.0 g/100 ml and electrophoretic pattern showed no abnormality, consisting of 55.1% albumin, 7.5% α_1 -globulin, 6.5% α_2 -globulin, 12.0% β -globulin and 18.9% γ -globulin. Histological examination of biopsied liver showed the finding of fatty degeneration (Fig. 2), but at this time liver function tests were within the normal range. Thorn's test showed a decrease of 51% in eosinophils. Urinary excretion of 17 ketosteroid was 3.68 mg/day. X-ray examination of the chest showed abnormal shadows in the hilar regions which were considered to be of tuberculous nature.

Fig. 2. Histological finding of the biopsied liver (Case 1). Note fatty degeneration.

Electroencephalographic finding (May, 1963): There was a generalized cerebral dysrhythmia with an increase in both the theta-delta and beta activities.

Chromosomal analysis: No abnormality was disclosed in both the number and configuration.

Clinical course before administration of nicotinamide: Streptomycin, para-aminosalicylic acid and isonicotinic acid hydrazide were given in the treatment of pulmonary tuberculosis. Several days after admission she took a turn for the worse and she talked incomprehensible words. In August, 3 months after admission, she complained of stiffness on the legs and was unable to walk but soon recovered after a little rest. During the summer of 1962, she developed cerebellar ataxia and pellagra-like rash several times, but these signs were improved during the winter. During the summer of 1963, the same attacks were seen, and in August she seemed to be psychologically abnormal. She became anxious, timid, and gradually depressive. In November, 1963, hallucination and delusion became apparent and at last severe and persistent feeling of depersonalization developed. At this time she complained of dizziness, hand tremor and diplopia. On Romberg's test she had tendency to fall, and the deep tendon reflexes were exaggerated. The ataxic gait became severer and she was unable to walk. Liver function tests were normal and no abnormal porphyrin metabolites were found in the urine. In December, 1963, she suffered from severe diarrhea of 3 months' duration and at the same time an atrophy of the hand muscles was seen (Fig. 3). In February, 1963, psychiatric reaction changed from depression to euphoria.

The present case was thought to be clinically most similar to one of Hartnup disease, and then the following investigations were carried out.

Fig. 3. Atrophy of the hand muscles (Case 1).

Fig. 4. Urinary amino acid chromatogram. Typical pattern of aminoaciduria in Hartnup disease (Case 1).

TABLE 1. Plasma amino acid levels (Case 1)

Amino acids	μ moles/l	Amino acids	μ moles/l
Aspartic acid	22	Methionine	14
Threonine	48	Isoleucine	34
Serine	382	Leucine	103
Glutamine		Tyrosine	22
Glutamic acid	155	Phenylalanine	44
Proline	126	Histidine	22
Glycine	266	Lysine	161
Alanine	371	Arginine	25
Valine	136		

Paperchromatographic studies: The paperchromatography of urinary amino acids was carried out by Ghadimi's method.¹¹ Two-dimensional paperchromatogram, using 80% phenol as the first solvent and butanol/acetic acid/water (4:1:5) as the second, revealed the typical pattern of Hartnup disease with urinary excretion of free amino acid nitrogen 1,000 mg/day (Fig. 4). On the other hand, the plasma levels of amino acids were found to be within the normal range (Table 1). The pattern of urinary amino acids was the same before and after commencement of treatment with nicotinamide. In September, 1964, 100 mg of L-proline per kg body weight were orally administered, 24-hour-urine specimens were collected before and following the test dose. Paperchromatogram of urinary amino acids revealed the same pattern and no excess of proline. Paperchromatography of urinary indoles was carried out by Jepson's method.¹² Two-dimensional paperchromatogram with isopropanol/ammonia/water (20:1:2) as the first and butanol/acetic acid/water (12:1:5) as the second revealed marked

Fig. 5. The indole chromatogram of Case 1 before L-tryptophan loading (40 mg/kg).
1, Indican. 2, Tryptophan. 3, Indoleacetic acid. 4, Indole acrylglycine.
5, Urea.

Fig. 6. The indole chromatogram of Case 1 after L-tryptophan loading (40 mg/kg).
1, Indican. 2, Tryptophan. 3, Indoleacetic acid. 4, Indole acrylglycine.
5, Urea. 6, Indole acetylglutamine.

TABLE 2. Urinary excretion of tryptophan derivatives before and after
L-tryptophan loading (40 mg/kg)

Urinary output of tryptophan derivatives	Case 1		Case 2	
	Before loading	After loading	Before loading	After loading
Tryptophan (mg/day)	70.3	93.0	192.0	360.0
Indoleacetic acid (mg/day)	64.6	133.9	12.6	222.5
N-methylnicotinamide (mg/day)	1.4	4.1	2.8	5.0
Indican (Obermeyer's test)	+	++	++	+++

spots of indican, tryptophan, indoleacetic acid (IAA), indoleacetylglutamine (IAcGluNH₂) and indoleacrylglycine (IAcylGly) (Fig. 5). The spots of indican, tryptophan, IAA and IAcGluNH₂ were increased respectively in the specimens of urine after an oral load with tryptophan in a dose of 40 mg/kg (Fig. 6).

Urinary tryptophan derivatives and plasma level of tryptophan before and after loading test, which were presented in Tables 2 and 3, were determined in both

TABLE 3. Plasma tryptophan levels before and after L-tryptophan loading (40 mg/kg)

	Before loading	After loading			
		30 min	60	120	240
Case 1	0.88 mg/100 ml	1.60	2.70	2.50	1.35
Case 2	1.10 mg/100 ml	1.35	2.55		1.05

Cases 1 and 2. The fasting levels of indican, IAA and tryptophan were higher in both Cases 1 and 2 as compared with those of controls. Each level of these after the loading was significantly higher as compared with the fasting levels, especially in Case 2. However, the fasting levels of urinary N-methyl-nicotinamide and plasma tryptophan were lower as compared with normal levels and not so increased after the loading.

Clinical course after administration of nicotinamide: The diagnosis of Hartnup disease was made basing upon the above-mentioned clinical and laboratory findings, and the patient was given orally 30 mg of nicotinamide with vitamin B complex preparation daily. When nicotinamide treatment was started, the skin lesion was slightly pigmented; six months after the treatment the pigmentation was clearly decreased, also the ataxic gait, and other neurological symptoms and psychiatric abnormalities were improved markedly. Six months after the treatment she appeared to be almost normal, showing that the atrophy of the hand muscles was markedly improved, and the electroencephalogram revealed normal patterns.

Case 2

History: K.M., a boy, was born on April 7, 1946, weighing 3,370 g, after a full-term pregnancy and spontaneous delivery. He had an itching rash on the areas exposed to the sunlight, the rash flared up and became moist and wet. Twenty days after the attack, the rash became dry, hyperkeratotic and pigmented. But during the rest of the year he showed no skin lesions. No neurological symptom was complained of. In the spring of 1959, he had rash on the same areas and at the same time his gait became unsteady and he was unable to walk. Because of the rash and nervous signs with increasing severity, he was admitted to a local hospital in June, 1959. His mother said that he gradually took a turn to the worse and became depressive during the hospitalization. Two months after admission, he was able to walk and almost recovered from depressive state. His chest x-ray finding showed abnormal shadows on the hilar regions which were considered to be tuberculous lesions, so he was admitted to our Hospital, in November, 1959.

Physical findings: He was a well developed and well nourished boy. The

Fig. 7. Hyperpigmentation on the face of the patient (Case 2).

skin of the hand and legs was dry and thickened, and the face was hyperpigmented (Fig. 7). Deep tendon reflexes were hyperactive, otherwise the physical and neurological findings were normal. The psychological tests revealed no abnormality.

Laboratory findings: The hemoglobin concentration was 15.0 g/100ml. The erythrocyte and leukocyte counts were within normal range. Tuberculin reaction was positive and Wassermann's reaction was negative. Urinalysis was normal on routine tests. No urinary porphyrin was present on repeated examinations. The liver function tests showed a slight hepatic damage: Thymol flocculation test 3.5 units, zinc sulfate test 14.0 units, Takata's reaction one positive and bromsulphalein test 11.3% after 30 minutes. Total protein in serum was 7.9 g/100 ml and electrophoretic pattern of serum protein showed a hypergammaglobulinemia, consisting of 54.4% albumin, 5.4% α_1 -globulin, 10.9% α_2 -globulin, 7.0% β -globulin and 21.7% γ -globulin. Electroencephalographic finding was normal.

Clinical course after admission: Para-aminosalicylic acid and isonicotinic acid hydrazide were given for pulmonary tuberculosis. During the admission of

four months he had suffered from neither the rash nor the ataxic gait. Psychological tests were normal. The clinical course had been similar to that of Case 1. Paperchromatographic studies were carried out in May, 1964. Paperchromatogram of urinary amino acids revealed the typical pattern of Hartnup disease with urinary excretion of free amino acid nitrogen of 1,764 mg/day. Paperchromatogram of urinary indoles revealed marked spots of indican, tryptophan, IAcrylGly, and those spots were increased after a loading with 40 mg of L-tryptophan per kg body weight.

Family investigation: The family pedigree is presented in Fig. 8. The parents were second cousins and had five children. Both the mother and father were healthy. Paperchromatographic studies of urine specimens for amino acids revealed no abnormality.

Fig. 8. Family pedigree.

T.M., a girl, died of severe diarrhea and vomiting at the age of 2 years.

Ay.M., a well developed 12-year-old girl, had never skin lesions or other symptoms suggestive of Hartnup disease. Physical findings were normal without exaggerated patellar reflex. She was not mentally retarded. Paperchromatographic studies of urinary amino acids and indoles revealed the same pattern as in Cases 1 and 2.

Ka.M., a well developed and well nourished 10-year-old boy, had not suffered from rash and other symptoms. Physical and laboratory findings were normal. Paperchromatographic studies revealed the same pattern as in Cases 1 and 2.

We carried out the loading test with L-tryptophan in 80 mg per kg body weight in these younger siblings. Urinary tryptophan derivatives — tryptophan, indoleacetic acid and indican were apparently increased after the loading, as compared with controls, especially in the younger brother (Ka. M.) (Table 4). Further survey of the pedigree revealed that maternal first cousins, 12- and 13-year-old boys, maternal and paternal grandfathers who were first cousins, also had characteristic amino acid pattern of the urine and elevated urinary indole excretion. All these 4 members were apparently healthy.

TABLE 4. *Urinary excretion of tryptophan derivatives before and after L-tryptophan loading (80 mg/kg)*

Urinary output of tryptophan derivatives	Ka. M.		Ay. M.		Control	
	Before loading	After loading	Before loading	After loading	Before loading	After loading
Tryptophan (mg/day)	89.0	146.0	29.0	85.0	12-38	44-70
Indoleacetic acid (mg/day)	5.1	42.8	1.3	16.6	1.2-3.4	2.0-6.0
N-methylnicotinamide (mg/day)	6.5	14.0	5.5	18.0	4.0-7.0	14.0-26.0
Kynurenine (mg/day)	8.0	14.0	7.4	32.6	2.7-5.7	16.0-28.0
Indican (Obermeyer's test)	+	++	+	+	-	-

DISCUSSION

The clinical manifestations of Hartnup disease vary from case to case but there are two main clinical signs, photosensitive rash typical of pellagra and attacks of cerebellar ataxia. One of the most constant and characteristic biochemical findings in Hartnup disease is a generalized aminoaciduria of renal type. In addition there is an increase of urinary indole derivatives, especially indican, IAA and IAcGluNH₂.

The skin rash which is essential for the diagnosis is localized to the areas exposed to sunlight. It became dry, hyperkeratotic, pigmented and worse during the spring and early summer months. Pellagra is also most commonly seen at this time of the year.¹³ Exposed to sunlight, the rash flares up and becomes moist and wet. The rash markedly responds to nicotinamide or nicotinamide with vitamin B complex therapy.⁷ But in the recent studies¹⁴ the rash improved spontaneously without nicotinamide therapy with increasing age. In our Case 1 when nicotinamide therapy was started, the skin lesions were only slightly pigmented and six months after treatment the pigmentation was decreased.

The neurological disturbances, especially ataxia which is also essential for the diagnosis, are transient and reversible. It usually develops during acute stadium of the disease. Some patients have never shown severe ataxia.¹⁴ Etiology of the ataxia is still obscure. In our Case 2, the patient had recovered from ataxia without nicotinamide therapy. In our Case 1, ataxic gait was temporary and reversible at first, but it gradually became progressive with increasing

age, and she was finally unable to walk. Six months after the nicotinamide treatment, ataxic gait almost disappeared.

Mental disturbances: The psychiatric features of Hartnup disease have ranged from emotional lability in the milder cases to apathy, irritability, depression, and delirium in the severer cases. In their original report Baron *et al.*¹ described that the biochemical defect in Hartnup disease might cause progressive mental deterioration. In our Case 1, emotional lability developed at first, as a psychiatric symptom followed by timid and depressive conditions, then hallucination and confusion, at last depersonalization. With nicotinamide therapy of six months' duration psychiatric symptoms disappeared rapidly. Recent observations¹⁴ suggested that in a certain cases with Hartnup disease spontaneous remission of clinical features ensued with the lapse of time even without treatment. But nicotinamide therapy for preventing the mental disturbances was emphasized by many investigators.^{1,3,9} It has been known that permanent nicotinamide deficiency leads to mental deterioration.¹³

Electroencephalographic investigations are reported in several cases. In Hersov's⁵ and Halvorsens' cases,⁷ pathological electroencephalographic changes were said to be improved following the nicotinamide therapy of several months. In our Case 1, electroencephalogram revealed a generalized dysrhythmia which disappeared five months after the nicotinamide therapy.

In biochemical investigations, the most constant and characteristic biochemical alteration in Hartnup disease is the renal aminoaciduria without any evidence of other defects in renal tubular function. This suggests that there is a specific defect in the transport of amino acids from glomerular filtrate across the proximal renal tubular cells into the tubular capillary blood.¹⁵ In addition, there is markedly increased excretion of alanine, serine, tryptophan, tyrosine, glutamine, histidine, phenylalanine and valine, but no excess of proline. We confirmed it by loading test with 100 mg per kg body weight of L-proline. In their original report Baron *et al.*¹ described an abnormal excretion of indole, especially indican IAA, and IAcrylGly, as a constant finding of Hartnup disease. Recent studies suggested that urinary indole excretion was found to be less constant and that urinary indole excretion may be influenced by urinary pH,^{17,18} age¹⁴ or diet. The indoles and indolic acids are degradation products of tryptophan. Fig. 9 is the schema of the metabolic pathway of tryptophan. In Hartnup disease, intestinal absorption of tryptophan is markedly reduced as compared with normal individuals, so that tryptophan is decomposed by intestinal flora to indole substances, consequently leading to an increased urinary excretion of indican, IAA, IAcrylGly, *etc.* Shaw *et al.*¹⁶ postulated that the action of intestinal microorganisms on tryptophan before absorption was the probable source of the urinary indolic acids in Hartnup disease. In normal individuals, absorbed tryptophan is oxidized by tryptophan pyrrolase in the liver to formylkynurenine

Fig. 9. Metabolic pathway of tryptophan.

and decomposed to nicotinic acid. Baron *et al.*¹ postulated that there might be a diversion in tryptophan metabolism from the kynurenine acid — nicotinic acid — indican pathway. Milne *et al.*¹⁵ confirmed that patient with Hartnup disease excreted about one-tenth of the kynurenine excreted by normal individuals after loading test with tryptophan. In both of our cases, urinary excretions of indican, IAA and IAcrlyGly were increased respectively after loading test with tryptophan; on the contrary urinary excretion of N-methylnicotinamide was not so increased as compared with normal individuals after loading test. Recently, Tada *et al.*¹⁹ reported a case of congenital tryptophanuria with dwarfism which was clinically similar to Hartnup disease but definitely discriminated from this disease in laboratory findings. They said in their report that congenital tryptophanuria with dwarfism was due to a possible defect of tryptophan pyrrolase in the liver. In both of our cases the liver functions were slightly damaged and biopsied liver of Case 1 showed a finding of fatty degeneration. It is difficult to say whether the fatty degeneration of the liver of our case is due to Hartnup disease or tuberculosis.

The inheritance pattern in Hartnup disease cannot be stated with certainty. It is thought to be autosomal recessive transmission.¹⁴ We carried out paperchromatographic investigations of urinary amino acids from grandmothers, grandfathers and first cousins of our patients. Both grandfathers who were the first cousins and two first cousins of our patients had indicanuria and generalized aminoaciduria. We think those cases mentioned above may be carrier members of Hartnup disease. Tryptophan loading tests in these presum-

able carrier members, which are now being carried out, will clarify the mode of inheritance in this family.

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