Case Reports

Hemoglobin M Disease

A Case Report

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CONGENITAL and chronic cyanotic diseases which has been regarded as morbus caeruleus in the past became enlightened in recent years from the pathoanatomical and hematological points of view.

In 1884, methemoglobinemia was distinguished from cyanotic congenital heart diseases by Francois. Since that time, more than 100 cases have been reported by many authors.¹⁾ What had been reported as "chronische familiäre Methämoglobinämie" by Hörlein and Weber in 1948²⁾ was ascertained to be hemoglobin M disease due to abnormal hemoglobin.

In this paper a case with congenital cyanosis is described, which is considered, after an extensive examination, to be a hemoglobin M disease, the report of which is very rarely found in the literature up to this time.

CASE REPORT

M. S., 6-year-old boy, was admitted to the Kurume University Hospital on May 30, 1959, with a chief complaint of cyanosis.

Birth was normal at full term. He has been found to be pale or cyanotic since his birth.

At the age of 3, he was diagnosed as having no cardiovascular abnormality. During his infancy, he has never had dyspnea, palpitation nor increasing of the grade of cyanosis on exercise.

He has no siblings. Father is 35-year-old bamboo worker and well. Mother, 31-year-old, has been pulmonary tuberculosis, having a partial lobectomy in 1958. Both parents have no cyanosis or cardiovascular diseases. All relatives were observed with on cyanosis (Fig. 1). He has had a measles at the age of 4. He has never experienced gastrointestinal disorders and there was no history of exposure to toxic agents.

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Fig. 1. Family tree of M.S.

Clinical Pictures:

Height 105.1 cm., weight 16.6 kg. He is well developed and no mental disturbance was found. Colour of the skin was bluish. Moderate cyanosis on the nailbeds and mucous membranes was present. No clubbing of the fingers was present. Purpura and exanthema were absent. Axillary temperature was 36.8°C.

Pulse was regular and the rate was about 90 per minute, with a moderate tension. Blood pressure was 100/60 mm.Hg, without significant difference between the right and left arm. Pupills was round and regular. Light reflex was normal. Movement of the eyeball was normal. Palpebral conjunctiva and mucous of mouth was slightly anemic in colour, but there was no hemorrhage. Tonsills were slightly hypertrophied. Chest was not remarkable. The percussion over the lung fields revealed no abnormality and the breath sound was normal. Right lower border of the lung resonance was on the sixth rib and its respiratory movement was normal. Cardiac dullness extended slightly to the right from the sternum. Upper dullness was at the level of the third rib. Left border extended slightly to the left midclavicular line. Heart sound at the apex was clear. Soft systolic murmur was present over the pulmonic area. Pulmonic second sound was accentuated. Abdomen was flat and soft. No organs, masses or rigidity were palpable. Patellar tendon reflex was intact and no pathological reflexes were noted. There was no edema of the lower extremities.

Clinical Findings and Diagnosis:

As indicated in Table I, there was no polycythemia but slight anemia in hematological study. Examinations of urine and feces were normal. Liver function,

Table I. Laboratory Findings

Urinalysis			
Reaction	acid	Sugar	(—)
Colour	normal	Bilirubin	(—)
Albumin	(—)	Porphylline	(—)
Urobilinogen	normal		
Feces			
Occult blood	(—)		
Parasite ova	(—)		
Liver function			
Kunkel		2.2 u.	
Thymol turbidity test		2.3 u.	
Takata reaction		(—)	
Icteric index		6.0	
Non protein nitrogen			
Serum Wassermann reaction		39 mg./100 ml.	
		negative	
Hematology			
R.B.C.		405×10^4	
Hemoglobin		75% Sahli	
		12.2 Gm./100 ml.	
Hematocrit		31%	
Reticulocytes		0.46%	
Platelets		186,300/mm. ³	
Bleeding time		1'30"	
Coagulation time		2'30"	
		9'30"	
W.B.C.		6,400	
Differential count			
Neutrophils		26%	
Eosinophils		3%	
Basophils		0%	
Lymphocytes		65%	
Monocytes		6%	
Plasma cells		0%	
Peroxydase reaction		normal	
Blood Chemistry			
Total protein	7.0 Gm./100 ml.	Total lipids	468 mg./100 ml.
Fraction		Total cholesterol	193 mg./100 ml.
Albumin	50.5%		(Kieth)
α_1 -Globulin	5.7%	Lipoprotein β/α	1.79
α_2 -Globulin	12.4%	Lipids in lipoprotein	169 mg./100 ml.
β -Globulin	12.4%	Na	140.0 mEq./L.
γ -Globulin	19.0%	K	6.4 mEq./L.
A/G ratio	1.02%		

total protein, protein fraction, albumin-globulin ratio, total lipids and total cholesterol were all within normal limits. Electrocardiogram (Fig. 2) was normal.

Fig. 2. Electrocardiogram of M.S. (June 11, 1959)

Fig. 3. Right anterior oblique, posteroanterior and left anterior oblique chest films of patient M.S.

Arterial blood O₂ capacity 12.3 Vol. %
Arterial blood O₂ saturation 77.2 %

Fig. 4. Circulatory dynamics.

Fig. 5. Angiocardiogram taken one film per second using 20 ml. of 76 per cent Urographin,

Chest X-ray films (Fig. 3) showed no pathological findings. Intracardiac catheterization was performed under suspicion of congenital heart disease because of existing cyanosis (Fig. 4). As indicated in Fig. 4, oxygen capacity was 12.3 Vol. per cent and oxygen saturation was 77.2 per cent. No abnormality was present in angiogram (Fig. 5).

The venous blood was chocolate brown in colour, and the unusual colour was not altered by shaking it in the presence of air. Serum was clear and icteric index was 6.0.

Methemoglobinemia was suspected from the results of spectrophotometric and chemical studies by the method of Evelyn and Malloy,³⁾ but no increased methemoglobin or sulfhemoglobin was found in the blood. Two mg. of methylene blue (1 per cent solution) per kilogram of body weight given intravenously at slow rate over a period of 5 minutes^{4),5)} and ascorbic acid given orally from 200 to 300 mg. a day during about one month were ineffective in decreasing the cyanosis.

"Abnorme Methämoglobinämie" reported by Hörlein and Weber in 1948^{2),6)} having been suspected, spectrophotometrical examination of hemoglobin M was made in accordance with the method of P. Gerald.⁷⁾

Heparinized blood obtained by venipuncture was centrifuged. After washing several times with physiological saline solution, red cell was hemolyzed with ad-

dition of distilled water and toluen, and the stroma was removed by high speed centrifugation. Thus, about 10 per cent hemoglobin solution (untreated hemolysate) was made.

Spectrophotometric studies were performed of this solution after treating by following methods with EPS-2 type Hitachi Recording Spectrophotometre.

Fig. 6. Spectral curves of untreated hemolysates in M/15, Ph 6.5 sodium phosphate buffer.

Spectroscopy of the untreated hemolysate is shown in Fig. 6. Immediately after clarification by high speed centrifugation, the spectral absorption curve of the hemolysate in M/15 phosphate buffer (pH 6.5) was obtained. Only the normal maxima for oxyhemoglobin (at 542 and 577 $m\mu$) were present in the visible portion of the spectrum, these maxima were indistinguishable in their location from those of normal oxyhemoglobin. There was no peak at 630 $m\mu$. But in the hemolysate of the patient there were 2 slight inflections at about 500 and 600 $m\mu$, and these spectral characteristics suggested the presence of abnormal pigment and were in agreement with the visual impression of the blood.⁸⁾

Spectral curves of acid methemoglobin form is shown in Fig. 7. This form was prepared by oxidation of untreated hemolysate with potassium ferricyanide. These spectral curves showed a remarkable difference from that of the patient and of normal individual. In the hemolysates of normal subject and both parents of the patient, oxyhemoglobin peaks at 542 and 577 $m\mu$ were absent and the peaks due

Fig. 7. Spectral curves of methemoglobin prepared by oxidation with potassium ferricyanide in M/15, Ph 6.5 sodium phosphate buffer.

to the presence of normal methemoglobin at 500 and 630 $m\mu$ became apparent after the treatment. The failure of the peak to appear at 630 $m\mu$ after oxidation of the oxyhemoglobin of the patient indicated that his methemoglobin differed from this derivative of normal hemoglobin in its spectral characteristics. This spectral curve closely resembles the curve reported by Hörlein and Weber^{2),6)} or Kiese *et al.*⁹⁾ Hemoglobin M content was determined by the method of P. Gerald⁷⁾ as 77 per cent.

The spectral curves produced by the addition of neutralized sodium cyanide to the solution of Fig. 7 are shown in Fig. 8. There was no significant difference between the patient and the normal subjects.

The alkali-resistant fraction was determined by the technic of Singer *et al.*¹⁰⁾ and its values were within normal limits.

COMMENT

"Hemoglobin M disease" is a syndrome characterized by hereditarily transmitted cyanosis in absence of increased amounts of reduced hemoglobin of "normal" methemoglobin.⁷⁾ Usually, cyanosis is attributed to the increase in the reduced hemoglobin produced from cardiac or pulmonary diseases. The colour of cyanosis is dependent upon the absolute amount of reduced hemoglobin and not upon the relative amounts of oxyhemoglobin

Fig. 8. Spectral curves of cyanmethemoglobin prepared by addition of neutralized sodium cyanide in M/15, Ph 6.5 sodium phosphate buffer.

and reduced hemoglobin.¹¹⁾ About 5 Gm. of reduced hemoglobin per 100 ml. of blood is required to produce recognizable cyanosis.¹²⁾

In our case, the only positive finding was the decrease in arterial blood oxygen capacity and oxygen saturation. There was no other finding in physical examination, ECG, fluoroscopic studies and intracardiac catheterization. So there was no evidence of any organic disorders.

In cyanosis due to abnormal pigments within the red cell, 1.5 Gm. of methemoglobin per 100 ml. of blood,⁴⁾ 15 per cent of total pigment of methemoglobin,⁵⁾ or 0.5 Gm. of sulfhemoglobin per 100 ml. of blood cause cyanotic hue.⁴⁾ A considerable amount of research has been reported on the methemoglobinemia—idopathic and secondary^{4), 5), 13)-17)}—and about 100 cases have been observed in Europe and America.¹⁾

In this report of ours, as the plasma was clear and its protein fraction was normal, hemolysis and abnormal serum pigments were excluded as causes of the cyanosis. The whole blood was grossly chocolate brown and when shaken in air for 15 minutes, it remained dark. Spectrophotometric studies about this blood were made by the method of Evelyn and Malloy³⁾ under suspicion of methemoglobinemia, but neither methemoglobin nor sulfhemoglobin were present. Methylene blue injected intravenously and ascorbic acid given orally^{2), 4), 5), 18)} did not result in a decrease of the cyanosis. These findings

excluded a possibility of methemoglobinemia.

It was Hörlein and Weber (1948) who first found methemoglobin with unusual spectral characteristics^{2), 6)} in a German family exhibiting a dominant transmission of a cyanotic condition for 4 generations. In their investigation they emphasized the facts that "neue Methämoglobin" had a new maximum of optical density at 600 $m\mu$ of wave length and that they had failed to detect methemoglobin by the method of Evelyn and Malloy which was dependent on the disappearance of a maximum at 630 $m\mu$ after chemical treatment. By interchanging the heme and the globin with the corresponding components of normal methemoglobin, they found the abnormality exist in the globin fraction. This hemoglobin was designated as hemoglobin M by Karl Singer in 1955.¹⁹⁾ In 1956, another case of this hemoglobinopathy was reported by Kiese *et al.* It was observed that oxygen saturation of the hemoglobin was normal and the speed of reduction of hemoglobin was same as that of normal hemoglobin, but the oxygenation of hemoglobin by oxygen was 2 times faster than normal.⁹⁾

In our case it was a matter of much interest that in spite of the absence of cardio-respiratory disorders, oxygen capacity and oxygen saturation of arterial blood were decreased.

In 1957 Gerald *et al.*⁸⁾ discovered a family of German extraction in New England presenting a cyanotic condition in at least 4 generations, and separated the hemoglobin of patient to hemoglobin A and hemoglobin M by starch electrophoretic technic of Kunkel.²⁰⁾ Piscotta *et al.* in the same year found a Swiss family with hemoglobin M defect.²¹⁾ A 33-year-old woman of the family with cyanosis since birth had splenomegaly and mild hemolytic anemia. Paper electrophoresis of the hemoglobin showed 2 clear cut components—fast and slow electrophoretic migrations. He stated also that the properties of hemoglobin M included slow electrophoretic migration and inability to transport oxygen.

About the foregoing case Gerald gave additional reports of investigation in 1958.⁷⁾ He reported that relative concentration of hemoglobin M was 30 per cent of the total hemoglobin. He classified hemoglobin M according to difference of reaction of hemoglobin M on cyanide.

The alkali-resistant fraction was found within the normal limits by the technique of Singer *et al.*¹⁰⁾ in our case, but Piscotta *et al.* found a hemoglobin F content of 11 per cent of total hemoglobin in his case,²¹⁾ and Gelard found a hemoglobin F content of 1.4 per cent in his case.⁷⁾

Hemoglobin M disease is a kind of molecular disease,^{19), 22), 23)} since spectroscopically and electrophoretically anomalous behaviour is attributable to an abnormality of globin. So a hereditary observation is necessary with a person about the study. Hörlein and Weber, Piscotta *et al.*, Gerald *et al.* found clear dominant hereditary pattern in each case, but such could not be found in our case. Further studies in this point are necessary.

In Japan "Nigramia hereditaria" first described by Tamura and Taka-

hashi^{24), 25)} has been given attention in relationship of hemoglobin M diseases recently.

SUMMARY

We examined a cyanotic patient considered as congenital heart disease and found that it was due to hemoglobin M disease spectrophotometrically. Hemoglobin M was 77 per cent of the total hemoglobin. We could not find the hereditary pattern in our case.

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