Abnormal Hemoglobins Discovered in Japan*

Susumu SHIBATA, Iwao IUCHI
and Takaoki MIYAJI
Department of Clinical Pathology, Yamaguchi Medical College, Ube

One day in early September, 1956, we left the Somerset Hotel in Boston, Massachusetts, and walked towards a nearby cafeteria. At this hotel was already held the 6th Congress of the International Society of Hematology for several days and hemoglobinopathy drew the attention of the participants as a noticeable new disease. It was the 7th year since the epoch-making discovery of Hb S by Pauling and his associates,[1] being the period when new abnormal hemoglobins were being reported continuously from various places of the world. We, Japanese, who attended this congress said to each other as we walked over to the familiar cafeteria, "At any rate, such a disease does not exist in Japan. The Japanese have not been in touch with negroes who have sickle cell anemia"

However, among the Japanese hematologists, there was one person who believed in the presence of hemoglobinopathy in this country. This was Prof. Shigeyasu Amano of the Kyoto University. By the request of Prof. Amano, systematic investigations of abnormal hemoglobin were started in 1957 and they are now in the 9th year.

To our surprise, our presumption has been upset completely, for no less than 26 variants of abonrmal hemoglobins have been discovered in Japan during this period, and from the process of the investigation came out also the episode of the elucidation of the etiology of hereditary nigremia which had been a disease of unknown cause in Iwate Prefecture for a long time.

I. HISTORICAL REVIEW

Fukutake[2] is credited with the first trial of the detection of abnormal hemoglobin in this country. He introduced the techniques for the study of abnormal hemoglobins, examining the blood of infants for Hb F in 1953. However, it was not until 1957 that the systematic survey of abnormal hemoglobin was started. The reason is that hemoglobinopathy was then considered a negro's disease and as the Japanese had not had direct contact with negroes historically, it was thought certain that any hemoglobin survey would end in futile efforts with fruitless results.

* This investigation was supported in part by a PHS reseach grant GM-09469-04 from the Division of General Medical Sciences, U. S. Public Health Service.

We[3] commenced our study in Ube in that year, using the conventional paper electrophoresis, alkali denaturation tests, and ferrohemoglobin solubility test and examined about 1000 samples of blood for the period of succeeding three years, but did not encounter even a single case of abnormal hemoglobin, only providing confirmative evidence for the then prevailing view that "hemoglobinopathy does not exist in Japan". Therefore, we thought of closing our survey, but we did not stop, because it was considered imprudent to arrive at any conclusion about the absence of hemoglobinopathy before we examined all the diseases suspicious of hemoglobinopathy which had been recorded in Japan. Thus our attention was attracted to acatalasemia (Takahara's disease)[4] and hereditary nigremia (Tamura-Takahashi's disease[5]).

In Feburuary, 1960, we received a blood sample of a patient with nigremia by special express mail from Prof, Tamura who was in Morioka, Iwate Prefecture. Nigremia is a congenital cyanosis which has been seen among the members of particular families living in a restricted area of Iwate Prefecture since about 200 years ago, and it has been established as a disease entity by Prof. Tamura's extensive study. According to him[6], the etiology of this disease was said to be related to the production of a hemoglobin possessing a special porphyrin nucleus destined to yield hematin-like substance. We immediately prepared a hemolysate and examined it by the conventional technique, but the result was negative for abnormal hemoglobin.

However, just at the time, we[7] had developed a new method of agar gel electrophoresis which proved to be superior to paper electrophoresis in the efficiency of detection of abnormal hemoglobin. So we tried to apply the method to the hemolysate of nigremia. The observation we obtained was a spectacular and unexpected one. That is, in a time as short as twenty to thirty minutes, a beautiful dark greenish brown stripe (to the anode) and a scarlet stripe (to the cathode) appeared on the agar slide[8]. It was easily determined from comparison of the electrophoretic mobility with the hemolysate of a normal person that the scarlet stripe was concerned with Hb A, but it took us a long time to elucidate the nature of the dark greenish brown stripe. We had never seen a hemoglobin with such color, and could never imagine that such might exist. We made further study with chromatography and spectroscopy, looked for the reference books and journals in the library, and finally, a week later, came to the inference that this pigment might belong to Hb M.

The hemoglobin was reported at the 8th Congress of the International Society of Hematology which was held in Tokyo in 1960[9] and was approved by experts as a new type of Hb A. Since then it has been called Hb M_{Iwate}. Frankly speaking, we had not been completely confident of our identification of the abnormal hemoglobin as Hb M before we attended the congress. We had been afraid of a

great adventure in our presumption which might lead us to an erroneous conclusion.

The year 1960 when Hb M_{Iwate} was discovered was an especially memorable year in the history of abnormal hemoglobin study in this country. Not only was nigremia established to be a Hb M disease but also another type of Hb M disease was reported from Kurume.[10] Furthermore, Hb Shimonoseki[11] was discovered and Hb Tokyo[12] was detected in that year.

Nevertheless, needless to say, this was the dawn of hemoglobin survey in this country, and we were then by 10 years or more behind the advanced countries (United States of America and England) in the realm of hemoglobin study. When we just found the first abnormal hemoglobin in Japan, hemoglobin was already being discussed with respect to its molecular structure in those countries. In 1960, at the Congress of the International Society of Hematology (Tokyo), this situation became apparent. At the congress, we were acquainted with the following new knowledges[13], that is,

(1) Globin of hemoglobin is composed of α chain and β chain.

(2) There are several types of different abnormal hemoglobins sharing the same electrophoretic migration. Therefore, identification of hemoglobin by electrophoresis only is not always trustworthy.

(3) Accordingly, when an abnormal hemoglobin is discovered, it must also be examined by the test for the detection of its chain anomaly, and

(4) Finally, the globin must be digested with trypsin into tryptic peptides which are in turn subjected to Ingram's fingerprint technique[14] in order to know the abnormal peptide. The peptide is then analyzed for amino acid composition to elucidate the substituted amino acid in the abnormal chain. Therefore, establishment of amino acid substitution is mandatory for the identification of new abnormal hemoglobin.

It seemed to us that identication of hemoglobins was impossible, because these tests mentioned above were beyond our capability at that time. We returned from the congress, having been discouraged by the recognition that there was a remarkable difference in the level of study between foreign countries and ours.

Fortunately, later, we found that our difficulty could be overcome. In this country, there were several chemists who had been studying animal hemoglobins. Those were Prof. Satake and his associates (Take and Sasakawa) of the Tokyo Metropolis University. We learned some of the techniques from Prof. Satake and applied them with successful results to human abnormal hemoglobins which were discovered in Ube.

II. NEW TECHNIQUES WHICH CONTRIBUTED TO THE PROMOTION OF THE STUDY OF ABNORMAL HEMOGLOBINS IN JAPAN

As will be stated later, hemoglobinopathy is a rarity in this country. So, unique techniques suitable for screening had to be invented in order to improve the efficiency of our survey. The following are the notable methods developed or modified by the investigators in Japan for the purpose of hemoglobin study.

1) Agar gel electrophoresis[7] : — Paper electrophoresis was not satisfactorily efficient in the screening of many samples of hemolysates on account of its limited separability of abnormal hemoglobins from Hb A. So, we modified Wieme's agar gel electrophoresis[15] using tris-EDTA-borate buffer solution (pH 8.6 and 7.0). This was the method which was particularly useful for the demonstration of Hb M_{Iwate} in the hemolysate. Examination of 100 hemolysates can be finished by agar gel electrophoresis (pH 8.6 and 7.0) within a period of time as short as 3 hours.

2) Determination of abnomal chain (Take's urea dissociation paper electrophoresis)[16] : — Globin was perpared from bovine hemoglobin. The globin was dissolved in a barbital buffer solution (pH 8.6) containing urea in a high concentration (7 M) to be dissociated into its α subunit and β subunit. The solution is applied to a filter paper to be subjected to electrophoresis. The α subunit and the β subunit migrated towards the anode with different velocity depending on their individual electric charges.

The method was, for the first time, used with success for the demonstration of α chain anomaly in Hb M_{Iwate} with its purified globin as material[17], and proved to be helpful for the determination of chain anomaly in various abnormal hemoglobins discovered in Ube.

3) Hybridization test with canine hemoglobin : — Dissociation and recombination of the α and β chains of two different hemoglobins give rise to hybrid hemoglobins in addition to the original ones. This is called hybridization[18]. In a country where hemoglobinopathy is frequently encountered it is easy to obtain standard sample of human hemoglobin whose chain anomaly is already known. So, when a new abnormal hemoglobin is to be examined for its chain anomaly, the hemoglobin is subjected to hybridization with standard human abnormal hemoglobin. Unfortunately, this is not feasible in a country like Japan where incidence of hemoglobinopathy is remarkably low.

Itano[19] discovered that human hemoglobin produced hybrid hemoglobins when it was mixed, dissociated and recombined with canine hemoglobin. Shibata and his associates[20] used this human-canine hybridization in combination with agar gel electrophoresis for the detection of abnormal chain of many abnormal hemoglobins with good results. This was more accurate than the urea-dissociation ele-

ctrophoresis. Urea dissociation paper electrophoresis failed to elucidate the chain anomay of Hb M_{Kurume}, but hybridization with canine hemoglobin definitively established its β chain anomaly.[21]

4) Isolation and purification of α and β chains: Fingerprinting of Ingram[14] is an ingenious and powerful tool for the analysis of the chemistry of abnormal hemoglobin. Each abnormal hemoglobin has its own fingerprint. However, there are occasional abnormal hemoglobins whose fingerprints are apparently identical with those of Hb A when their whole globins are analyzed. These hemoglobins must be examined by fingerprinting after their abnormal chains have been isolated and purified.

It is a rather complicated task to isolate the α and β chins. In general, Amberlite chromatography (elution with urea)[22] and counter-current distribution[23] are used for this purpose.

Hayashi[24] devised a very simple procedure which enabled us to obtain α and β chains of considerable purity.

In this method globin is dissolved in a highly concentrated (8 M) urea solution, equal volume of 1.8 M tichloroacetic acid is added, mixed and allowed to stand for two or three hours in order to precipitate β chain. Thus α chain alone is left in the supernatant after cenrifugation. The precipitate (β chain) and the supernatant (α chain) are collected separately in a visking tube to be dialyzed against water so that they may be purified.

Miyaji and his associates[25] successfully determined the amino acid substitution of Hb Shimonoseki by examining the fingerprint of its α chain which was perpared in this way.

III. ABNORMAL HEMOGLOBINS IN JAPAN

Up to the present time as many as 26 variants of abnormal hemoglobins have been recorded in Japan. Almost all are from the families of pure Japanese ancestry, although two variants are found in families of Koreans. These can be classified into two groups, namely, those which show clinical symptoms and those which do not.

The variants showing symptoms are Hb M_{Iwate}, Hb M_{Kurume} and Hb M_{Osaka} which cause cyanosis, and Hb Ube-1 which give rise to hemolytic anemia. Besides, there is Hb Yukuhashi which causes appearance of target cells in the peripheral blood. If there should happen a homozygote for this hemoglobin gene (although its occurrence is extremely unlikely), hemolytic anemia would be seen in such a case.

Other abnormal hemoglobins do not show any abnormal manifestations either clinically or hematologically. However, there is no evidence for the security that they do not cause hemolytic anemia in an unusual situation. Drugs may induce hemolytic anemia in a person with these hemoglobins as seen in Hb Zürich carriers

under incidental therapy with sulfonamide[26,27].

IV. ABNORMAL HEMOGLOBINS ASSOCIATED WITH CLINICAL MANIFESTATION

1) Hb M_{Iwate} : — This is a chocolate brown hemoglobin that was discoverd from the hemolysate of a nigremia patient in Iwate Prefecture by Shibata and his associates[8] in 1960. At first. it was called Hb M_I taking the initital letter of Iwate Prefecture, and later, named Hb M_{Iwate}. The properties of this hemoglobin were described in detail in 1965[28].

Nigremia is a disease of long history which has been existing in the North-Western area of Iwate Prefecture, and recently it has been known to be distributed also in Hokkaido.[29] Tamura[5] was the first investigator who noticed the perculiar absorption curve of the acid methemoglobin type hemolysate of this disease. There was neither peak at 630 mμ nor valley at 600 mμ, both of which are characteristic of the methemoglobin type hemolysate of normal person.

It was in 1948 that Tamura and his associates recognized hereditary nigremia to be a disease of hemoglobin. At about the same time the first family of Hb M disease in the world was described by Hörlein and Weber[30] in Germany. Of course they could not isolate Hb M from the blood of the patient. They prepared globins from the hemolysate—which was obviously a mixture of the globins of Hb A and Hb M—and mixed them with normal hemin to resynthesize the methemoglobin type pigments. The absorption curve of the solution of resynthesized methemoglobin was entirely the same as that of the original methemoglobin type hemolysate. On the ground of this recombination experiment, they presumed that their patient had a modified methemoglobin composed of normal heme and abnormal globin. This hemoglobin was similar to, but not identical with methemoglobin. So it was named Hb M taking M from methemoglobin, seven years after its discovery, by Singer[31] who was interested in this hemoglobin.

Tamura read Hörlein-Weber's report and recognized that there was a close resemblance between hereditary nigremia and the disease found in Germany, but he was not convinced of the identity of the two diseases[32].

Hb M_{Iwate} has a negative charge slightly larger than that of Hb A. As the difference in charge between Hb A and Hb M_{Iwate} is small, this abnormal hemoglobin is not demonstrable by ordinary techniques of Tiselius electrophoresis and paper electrophoresis. Agar-gel electrophoresis of O_2Hb type hemolysate at pH 7.0 gives the best separation : Hb M_{Iwate} migrates to the anode side of Hb A forming a sharply delineated stripe of chocolate brown color. When hemolysate is treated with ferricyanide (to convert hemoglobin into methemoglobin) and then subjected to starch block electrophoresis (pH 7.0), met Hb M_{Iwate} is again separated to the anode side

of met Hb A (brown in color) as a dark gray band. Starch gel electrophoresis is also useful for the demonstration of this hemoglobin in the hemolysates (of O_2Hb as well as metHb type)[33].

Hb M_{Iwate} is separated as a black layer at the top of the red layer of Hb A on Amberlite IRC 50 column chromatography[28]. On carboxymethyl cellulose column chromatography it flows down as a dark colored fluid between eluates of Hb A_0 and Hb A_2[28].

Highly purified O_2Hb M_{Iwate} solution is considerably different in absorption curve from Hb A solution[34]: The α and β absorption bands are less salient because of the appreciably large absorption of light in the non-specific range in addition to a slight inflection around 610 mμ

MetHb M_{Iwate} shows a characteristic absorption curve, in which the protrusion at 630 mμ and the depression at 600 mμ pertaining to metHb A are not seen. Hb M_{Iwate} occupies 30 per cent of the hemoglobin in the blood of nigremia patient. The remainder, 70 per cent, is Hb A. The peculiar absorption curve of acid metHb type hemolysate is interpreted as representing the summation of the interaction of light absorptions by metHb A and metHb M_{Iwate}.

MetHb M_{Iwate} reacts slowly to KCN and rapidly to NaF and NaN_3. Cyan-metHb M_{Iwate} and reduced Hb M_{Iwate} have absorption curves different from those of cyan-metHb A and reduced Hb A, respectively[35].

In Hb M_{Iwate}, His which is the 87th amino acid of the α chain has been substituted for by Tyr[37,37]. Accordingly, this is an abnormal hemoglobin expressed by $\alpha_2^{87Tyr}\beta_2$. The same hemoglobin was later recorded in the United States of America under the name of Hb $M_{Kankakee}$[38,39]. It is distributed also in Israel[40].

The molecule of Hb M_{Iwate} is composed of the abnormal α subunit which is incapable of O_2 transportation and the normal β subunit which is able to carry oxygen. The O_2-combining capacity of Hb M_{Iwate} is less than 50 per cent of that of Hb A, and its oxygen dissociation curve is less sigmoid in shape[41].

2) Hb M_{Kurume}:— This was discovered by Kimura and his associates[10] from a cyanotic boy suspected of congenital heart disease. The hemoglobin was for the first time separated from Hb A on starch block electrophoresis of the metHb type hemolysate by Yamaoka and his associates[11] in 1962. The amino acid substitution of this hemoglobin was studied by Shibata and his associates[21], who disclosed that it was identical with Hb $M_{Saskatoon}$ ($\alpha_2\beta_2^{63Tyr}$).

Hb M_{Kurume} resembles Hb M_{Iwate}, but they are discriminated by the following characters. (1) Hb M_{Kurume} is not separable from Hb A by simple application of O_2Hb type hemolysate to agar gel electrophoresis. Preliminary treatment with ferricyanide to convert hemoglobin into methemoglobin is necessary before the electrophoresis[17,28]. MetHb M_{Iwate} migrates to the anode side of metHb A. (2) MetHb M_{Kurume} and metHb M_{Iwate} have different absorption curves. MetHb

M_{Kurume} has a peak at 600 mμ and a depression at 560 mμ, whereas in met-Hb M_{Iwate} the first peak is seen at 585 mμ without being associated with significant depression around 560 mμ[35]. (3) In contrast to metHb M_{Iwate}[42] metHb M_{Kurume} reacts rapidly to KCN and other ligands[35].

3) Hb M_{Osaka} : — This is a hemoglobin obtained from a cyanotic patient in Osaka by Hayashi and his coworkers[43] in 1964. Detailed chemical study demonstrated that this was identical with Hb M_{Boston} ($\alpha_2^{58Tyr}\beta_2$) or Hb $M_{Gothenburg}$.[44]

The absorption curve of met-Hb M_{Osaka} closely resembles that of met Hb M_{Iwate}, but unlike metHb M_{Iwate}, met Hb M_{Osaka} reacts to NaF and NaN_3 slowly. The most remarkable difference will be seen in fingerprint. In Hb M_{Osaka} or Hb M_{Bostn} the No. 20α an No. 21α spots will be absent in their proper position. Instead, two abnormal spots will be seen, and they are positive for Tyr reaction and negative for His reaction (Figure 1)[45]. By contrast, in Hb M_{Iwate}, the No. 3 spot in the neutral region is shifted nearer the anode side, which is positive for both His and Tyr.

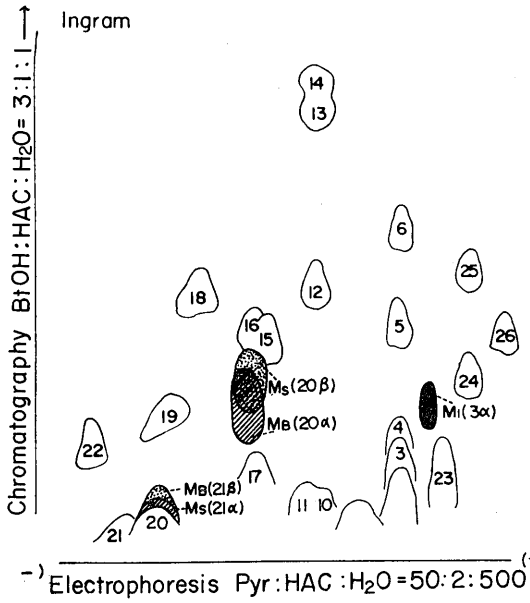

Fig. 1. Comparision of fingerprints (Ingram) of Hb M_{Boston} (Hb M_{Osaka}) Hb M_{Iwate} (Hb $M_{Kankakee}$) and Hb $M_{Saskatoon}$ (Hb M_{Kurume}), as inferred from the studies made by Shibata and his associates[28] and Pik and Raine[65].

Abnormal spots are represented by shaded or black areas. Figures refer to the numbers (Ingram) of the peptide spots.

As in Hb M_{Iwate} O_2-transporting capacity is remarkably diminished in Hb M_{Osaka}[46].

4) Hb Tokyo : This hemoglobin has an electrophoretic migration (pH 8.6) which is presumably almost equal to that of Hb Q and it is not always easily separated from Hb A by electrophoresis. According to Fukutake[12] who discovered this hemoglobin, the proband had hemolytic anemia which was unrelated to the use of drugs. The alkali resistance and solubility of ferrohemoglobin are normal. The hemoglobin is said to have abnormal β chain, and fingerprinting revealed abnormal spots, but their amino acid substitution has not yet been established.

5) Hb Ube-1 : In 1960, Shibata and his associates[47] detected this abnormal hemoglobin from a girl (aged 11) who was ill with hemolytic anemia. She had re-

ceived splenectomy for hypersplenism. Hb Ube-1 is almost equal in electrophoretic migration (pH 8.6) to Hb S. However, it migrates to the anode side of Hb A on agar gel electrophoresis at pH 7.0. The hemoglobin is not so stable as Hb A. The fingerprint is normal notwithstanding that its β chain anomaly is easily demonstrable by hybridization test. Titration of the purified hemoglobin with p-chloromercuribenzoic acid revealed the absence of active SH radical on the β chain. This was interpreted that the 93rd amino acid residue of the β chain, Cys-SH, was blocked.

Hb Ube-1 is susceptible to degeneration even while it is in the red cells forming insoluble aggregates. These are seen as intraerythrocytic Heinz bodies when the blood is stained supravitally with brilliantcresyl blue. The Heinz bodies appear singly in an individual red blood cell. They are seen in about 30 to 40 per cent of erythrocytes. Similar hematological abnormality and abnormal hemoglobin could not be demonstated in other members of the family including her parents, brothers and sisters.

6) Abnormal hemoglobins discovered from the foreigners living in Japan and from the mixed-blood Japanese : — In Tokyo, Hb S was detected from a mixed blood child born to a Japanese mother with a negro father (Morita-Shirai)[48]. Of course, the child was Hb S-trait. Hb S was discovered also from a Ghanan living in Japan[49]. Besides these, Hb D was discovered from a Caucasian residing in Japan[49].

V. ABNORMAL HEMOGLOBINS WITHOUT RELATION TO CLINICAL MANIFESTATION

Most of the abnormal hemoglobins discovered in Japan were encountered in apparently healthy persons or in patients without any symptoms reminiscent of hemoglobinopathy. These can be classified into two groups, the fast-moving hemoglobins which move to the anode more rapidly than Hb A electrophoretically (pH 8.6) and the slow-moving hemglobins which migrate more slowly than Hb A to the anode (Figures 2 and 3).

Table I presents the list of the abnormal hemoglobins found in Japanese people. It includes 9 variants of fast-moving hemoglobins, 14 variants of slow-moving ones and 3 Hb Ms. Some of them belong to the group of α chain anomaly and others to the group of β chain anomaly. Both groups are almost equal in size.

The notable features of these abnormal hemoglobins are as follows.

1) All the cases of hemoglobinopathies hitherto recorded in Japan are invariably heterozygotes. Hemoglobinopathies with symptoms mentioned above constitute no exceptions. Hb A was always demonstrable together with abnormal hemoglobins in the hemolysate of the patients or carriers.

2) Usually the content of abnormal hemoglobins of α chain anomaly in the

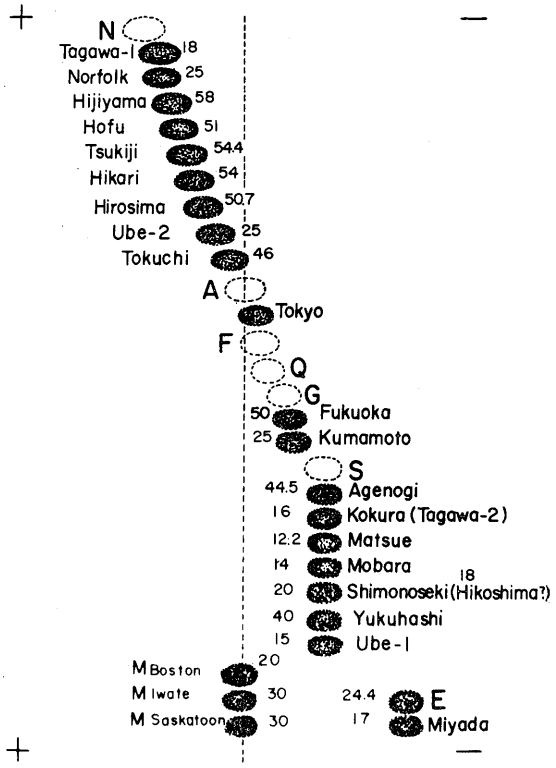

Fig. 2. Electrophoretic sequences (pH 8.6) of abnormal hemoglobins found in Japan, as inferred from the studies made by the Japanese students of hemoglobin.[8, 11, 12, 17, 21, 28, 43, 47, 49, 50, 52, 53, 55, 56, 61, 62, 64]

Figures (18, 25, ...) written close to the spots indicate the content of abnormal hemoglobins in the hemolysates (abnormal hemoglobin contents were 18, 25,. ... per cent, respectively).

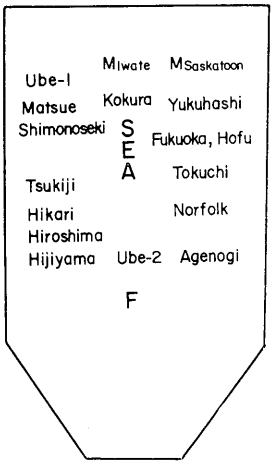

Fig. 3. Sequences on Amberlite IRC 50 chromatogram of principal variants of abnormal hemoglobins found in Japan, as inferred from the studies made by Shibata and his associates[8, 17, 47, 49, 50, 51, 55, 56, 61] and Hanada and his associates.[53, 62]

hemolysate was low, being about 30 per cent or less. In contrast, the amount of those of β chain anomaly in the hemolysate was large, frequently surpassing 50 per cent, particularly in fast moving variants. Hb Hikari[50], Hb Hiroshima[51], Hb Hijiyama[51] and Hb Hofu[51] are such examples. Hb Ube-1 was the exception. Its content in the hemolysate is only 10 to 15 per cent.

3) Amino acid substitution has been studied and established for Hb Hikari ($\alpha_2\beta_2^{61\text{Asp}NH_2}$),[50] Hb Shimonoseki ($\alpha_2^{54\text{Arg}}\beta_2$)[25,52] and Hb Kagoshima (Nishiki, $\alpha_2^{57\text{Asp}}\beta_2$).[53] Hb Shimonoseki and Hb Hikari are internationally well known. Hb Kagoshima (or Hb Nishiki) is identical with Hb Norfolk[54] which was discovered in

Table I. Abnormal hemoglobins discovered in Japan

Hemoglobin	Chain anomaly	Substitution	Abnormal spot on the fingerprint
1) Fast-moving			
Hijiyama[51] (Hiroshima-2)	β	β Tp-13 ?	# 5 absent
Hikari[50]	β	61Lys→AspNH_2[50]	# 20, 21 (19 absent)
Hiroshima[51] (Hiroshima-1)	β	β Tp-14～15	# 17β (# 15 β absent, Baglioni)
Hofu[51]	β		
Norfolk (Kagoshima)[53] (Nishiki [53])*	α	57Gly→Asp[53]	# 20, 21
Tagawa-1[53]	α	α Tp-10	# 18
Tokuchi[60, 61]	β	β Tp-13	# 5
Tsukiji[60, 61]	β	β Tp-13 ?	# 5 ?
Ube-2[60, 61]	α	68AspNH_2→Asp[68]	# 3
2) Hb M			
Iwate[8, 28] (Kankakee)	α	87 His→Tyr[36, 37, 66, 67]	# 3
Saskatoon (Kurume[10, 28])	β	63 His→Tyr[21]	# 20, 21
Boston (Osaka[43, 44])	α	58 His→Tyr[33]	# 20, 21
3) Slow-moving			
Agenogi[51]	β	90 Glu→Lys[69]	Below # 12 (core)
E (Nagasaki)[55]	β	26 Glu→Lys[55]	# 26
Fukuoka*[62]	?		
Hikoshima[53] (Shimonoseki ?)	α	α Tp-6	# 10
Kokura[62] (Umi, D_α Michigan-1)	α	47 Asp→Gly[53]	# 10
Kumamoto[63]	α		
Matsue (Matsue-1)[60, 61]	α	α Tp-9	# 3
Miyada[53, 62]	β	21 Asp→Lys ?	# 26
Mobara[56] (Shimonoseki ?)	α		
Shimonoseki[11, 62]	α	54 GluNH_2→Arg[51, 52]	# 10
Tokyo[12]	β	β Tp-13 ?	# 5 absent
Tagawa-2[53] (Kokura ?)			
Ube-1[47]	β	93 Cys blocked[47]	No (in the core)
Yukuhashi[62]	β	β Tp-8 ?	double # 22 ?

* Detected also in the blood of Koreans.

England.

4) Hb E-hemoglobinopathy is frequently seen among the people of the countries of the South Pacific Area. Japan is surrounded by these countries and historically there has been mutual communication of peoples since the ancient time. Accordingly, it is naturally expected that Hb E will be found also in Japanese. However, hemoglobin survey had been fruitless with respect to this expectation until 1964, when a slow moving hemoglobin reminiscent of Hb E was discovered in the blood samples collected from a Japanese family living in Nagasaki by Shibata and his associates.[55] The abnormal hemoglobin was studied by hybridization test, fingerprinting and amino acid analysis of the abnormal peptide. The studies have established its amino acid substitution which is the same as that seen in Hb E ($\alpha_2 \beta_2^{26Lys}$). This is the first and the only one instance of Hb E-hemoglobinopathy ever found in Japan.

VI. GEOGRAPHICAL DISTRIBUTION AND FREQUENCY

About 130,000 Japanese have received systematic examination of abnormal hemoglobins in several districts of Japan for the past 9 years and 26 variants of abnormal hemoglobins have been discovered. Therefore, it is estimated that a new variant of abnormal hemoglobin is encountered at every 5,400 examinations. Hemoglobinopathy is therefore thought to be a rare disease in Japan. The individual variants of abnormal hemoglobins were usually seen exclusively in the family pertaining to the relevant hemoglobin. Hb Hikari,[50] Hb Kokura[53] and Hb Kagoshima[53] constitute the exceptions. Hb Hikari was detected in two families living in Yamaguchi Prefecture which have no marital relation. Hb Kokura was also found in two families living in Kokura and Umi, Fukuoka Prefecture. Hb Kagoshima which is the same as Hb Norfolk was encountered in 3 families residing in Kagoshima (Kagoshima Prefecture) and Nishiki (Yamaguchi Prefecture). One of the families is said to be of Korean ancestry.

Hb Fukuoka is concerned with a Korean family living in Fukuoka.

When the incidence of hemolgobinopathy is calculated on the ground that the number of all the carriers of abnormal hemoglobins, including the probands and their family members harboring the abnormal hemoglobins, are compared with the total number of persons examined, the frequency of this disease is estimated to be between one per 2,000 and one per 3,000.

In figure 4 is illustrated the geographical distribution of abnormal hemoglobins in Japan. It is apparent that hemoglobinopathy is more frequent in Kyushu and Chugoku than in other districts. Only five variants are distributed outside of Chugoku-Kyushu : namely, Hb M_{Osaka} (Osaka), Hb Tsukiji (Tokyo), Hb Mobara[56] (Chiba Prefecture) and Hb M_{Iwate} (Iwate Prefecture and Hokkaido).

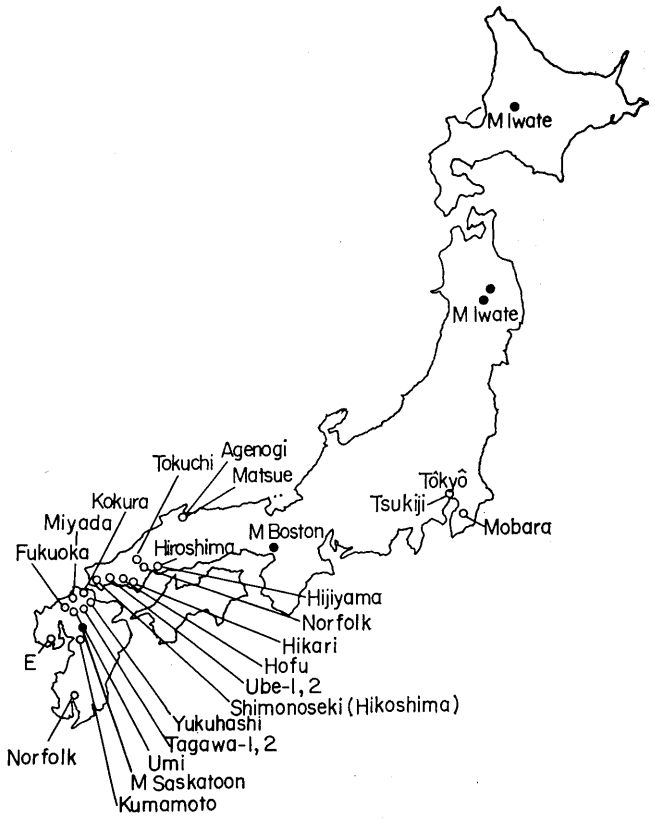

Fig. 4. Georaphical distribution of abnormal hemoglobins in Japan.

In our opinion, this does not mirror the true distribution of hemoglobinopathy in this country, but only the uneven distribution of enthusiastic students of abnormal hemoglobins in Japan. The active centers of the study of hemoglobinopathy are unfortunately localized in Chugoku and Kyushu, the Western part of this country.

CONCLUSION

About five years ago, it was the consensus of the opinion of hematologists that hemoglobinopathy was not distributed in Japan. However, on the day of this writing in 1966, as many as 26 variants of abnormal hemoglobins have been recorded in this country. There are, at present, about 120 variants of abnormal hemoglobins known throughout the world. Accordingly, Japan constitutes an area where a large number of variants of hemoglobins are encountered despite

that hemoglobinopathy is a rarity in this country.

Hemoglobinopathy is concerned with mutation of the amino acid sequence in the α or the β chain of hemoglobin. Such mutation is estimated to occur only once in 10,000,000 births[57].

So far, the disease has been regarded as curiosity in this country, in spite of the fact that the study of hemoglobinopathy made in the United States and in England contributed greatly to the advance of our knowledge in hematology, human genetics and biochemistry of hemoglobin molecules[58].

Japan is now at the dawn of the study of abnormal hemoglobins. New abnormal hemoglobins discoverd in this country have been increasing in number year after year. These hemoglobins are becoming a useful tool for the elucidation of chemistry and physics of hemoglobin as well as for the study of triplet code theory[58,59] in human genetics.

REFERENCES

1) Pauling, L., Itano, H. A., Singer, S. J., and Wells, I. C.: Sickle cell anemia, a molecular disease. *Science*, **110** : 543-548, 1949.
2) Fukutake, K.: Clinical investigation of abnormal hemoglobins (Hb F and Hb S). *Jap. J. Clin. Path.*, **1**: 164-174, 1953.
3) Shibata, S.: Hemoglobinopathy, with special references to the abnormal hemoglobins found in Japan. *Acta Haem. Jap.*, **24** : 141-155, 1961.
4) Takahara, S.: Acatalasemia (Lack of catalase in blood) and an oral progressive gangrene. *Proc. Japan Acad.*, **27** : 295-301, 1951.
5) Tamura, A., and Takahashi, M.: Study on the hereditary black blood disease (Tamura and Takahashi's disease). *Tohoku J. Exp. Med.*, **54**: 209-213, 1951.
6) Tamura, A., Takahashi, M., Ishii, H., and Akashi, S.: A study on the hereditary black blood disease (Tamura and Takahashi's disease or nigremia hereditaria). *Jap. J. Human Genet.*, **4** : 180-195, 1959.
7) Shibata, S., and Ichi, I.: A simple technique of agar gel electrophoresis for rapid separation of hemoglobins. *Acta Haem. Jap.*, **24** : 51-58, 1961.
8) Shibata, S., Tamura, A., Iuchi, I., and Takahashi, H.: Hemoglobin M_I : Demonstration of a new abnormal hemoglobin in hereditary nigremia. *Acta Haem. Jap.*, **23** : 96-105, 1960.
9) Shibata, S.: Hemoglobin M_I—Demonstration of a new abnormal hemoglobin in hereditary nigremia. *Proc. 8th Internation. Congr.*, vol. 2, Pan-Pacific Press (Tokyo), 1962 (pp. 1068-1073).
10) Kimura, N., Nishimoto, S., Nawata, Y., Mori, F., Kodama, S., and Nakakura, S.: Hemoglobin M Disease. A case report. *Jap. Heart J.*, **1** : 456-465, 1960.
11) Yamaoka, K.: Studies on hemoglobinopathies observed in Japan. *Proc. 8th Internation. Congr. Hemat.* (Tokyo, 1960), vol 2, Pan-Pacific Press (Tokyo), 1962 (pp. 1056-1063).
12) Fukutake, K., and Kato, K.: Hemolytic anemia due to a new abnormal hemoglobin. *Proc. 8th Internation. Congr. Hemat.* (Tokyo, 1960), vol. 2, Pan-Pacific Press, 1962 (pp. 1220-1223). Shida, K.: Studies on hemoglobin Tokyo. *Tokyo-Ika-Daigaku Zasshi*, **21** : 89-103, 1963.
13) Lehmann, H.: The hemoglobin molecule. *Proc. 8th Internation. Congr. Hemat.* (Tokyo, 1960), vol. 2, Pan-Pacific Press, 1962 (pp. 1023-1033); Lehmann, H.: Haemoglobinopathies. Proc. Kyoto Symposium on Hematology. *Acta Haem. Jap.*, **23**: Supple., 1960 (pp. 20-35).

14) Ingram, V. M.: Abnormal human hemoglobins. I. The comparison of normal human and sickle cell hemoglobins by "Fingerprinting" *Biochim. Biophys. Acta*, **28**: 539-545, 1958.

15) Wieme, R. J.: An improved technique of agar gel electrophoresis on microscope slides. *Clin. Chim. Acta*, **4**: 317-321, 1959.

16) Take, T.: On the dissociation of hemoglobin under the action of urea. *J. Biochem.*, **49**: 206-210, 1961.

17) Shibata, S., Miyaji, T., Iuchi, I., and Ueda, S.: A comparative study of hemoglobin M_{Iwate} and hemoglobin M_{Kurume} by means of electrophoresis, chromatography and analysis of peptide chains. *Acta Haem. Jap.*, **24**: 486-494, 1961.

18) Itano, H. A., and Robinson, E.: Formation of normal and doubly abnormal haemoglobins by recombination of haemoglobin I with A and C. *Nature*, **183**: 1799-1800, 1959.

19) Robinson, E. A., and Itano H. A.: Identification of the recombinant products of canine and human haemoglobins. *Nature*, **188**: 798-800, 1960.

20) Shibata, S., Iuchi, I., Ueda, S., Miyaji, T., and Takeda, I.: Agar gel electrophoresis of the hybrid of canine and human hemoglobins: A simple convenient means for the determination of chain anomaly. *Acta Haem. Jap.*, **25**: 675-681, 1962.

21) Shibata, S., Miyaji, T., Iuchi, I., Ueda, S., Takeda, I., Kimura, N., and Kodama, S.: Hemo globin M_{Kurume}: Its identity with hemoglobin $M_{Saskatoon}$. *Acta Haem. Jap.*, **25**: 690-694, 1962.

22) Chernoff, A. I., and Liu, J. C.: The amino acid composition of hemoglobin. II. Analytical technics. *Blood*, **16**: 54-70, 1961.

23) Hill, R. J., Konigsberg, W., Guidotti, G., and Craig, L.: The structure of human hemoglobin. I. The separation of the α and β chains and their amino acid composition. *J. Biol. Chem.*, **237**: 1549-1554, 1962.

24) Hayashi, H.: A simple method for the fractionation of globins into their α- and β chains. *J. Biochem.*, **50**: 70-71, 1961.

25) Miyaji, T., Iuchi, I., and Shibata, S.: Hemoglobin Shimonoseki ($\alpha_2^{54Arg}\beta_2^{A}$), a slow moving hemoglobin found in a Japanese family, with special reference to its chemistry. *Acta Haem. Jap.*, **26**: 531-537, 1963.

26) Frick, P. G., Hitzig, W. H., and Betke, K: Hemoglobin Zürich. I. A new hemoglobin anomaly associated with acute hemolytic episodes with inclusion bodies after sulfonamide therapy. *Blood*, **20**: 261-271, 1962.

27) Bachmann, F., and Marti, H. R.: Hemoglobin Zürich. II. Physicochemical properties of the abnormal hemoglobin. *Blood*, **20**: 272-286, 1962.

28) Shibata, S., Iuchi, I., and Miyaji, T.: L'hemoglobinose M au Japon. *Nouv. Rev. Fr. D'Hemat.*, **5**: 49-61, 1965.

29) Maruya, T., Kono, M., Harada, M., Miyazaki, T., Sinzaki, R., Nagano, K, Aoyama, H., and Siraishi, T.: Studies on the hereditary nigremia in Hokkaido. *Acta Haem. Jap.*, **25**: 331-331, 1962.

30) Hörlein, H., und Weber, G.: Über chronische familiäre Methämoglobinämie, und eine Modifikation des Methämoglobins. *Deut. Med. Wschr.*, **73**: 476-478, 1948.

31) Singer, K.: Hereditary hemolytic disorders associated with abnormal hemoglobins. *Amer. J. Med.*, **18**: 633-652, 1955.

32) Tamura, A.: Hereditary nigremia. Shinryo, 16: 212-216, 1963.

33) Iuchi, I., Takeda, I., Miyaji, T., and Shibata, S.: Artificial synthesis and hybridization of Hb M_{Iwate}. *Acta Haem. Jap.*, **27**: 425-430, 1964.

34) Shibata, S., Iuchi, I., Miyaji, T., and Tamura, A.: Molar extinction coefficients of hemoglobin M_{Iwate}, with a note on the spectrophotometric determination of this hemoglobin in the hemolysate of hereditary nigremia. *Acta Haem. Jap.*, **26**: 641-649, 1963.

35) Shibata, S., Iuchi, I., Miyaji, T., and Ueda, S.: Spectroscopic characterization of Hb M_{Iwate}, and Hb M_{Kurume}, the two variants of hemoglobin found iu Japan. *Acta Haem. Jap.*, **24** : 477-485, 1961.

36) Shibata, S , Miyaji, T., Iuchi, I., and Tamura, A.: Substitution of tyrosine for histidine (87) in the α chain of hemoglobin M_{Iwate}. *Acta Haem. Jap.*, **27** : 13-18, 1964.

37) Shibata, S., Tamura, A., Iuchi, I., and Miyaji, T.: Hereditary nigremia and hemoglobin M_{Iwate}. *Proc. Jap. Acad.*, **40** : 220-225, 1964.

38) Heller, P., Weinstein, H. G., Jakulis, V. J., and Rosenthal, I. M.: Hemoglobin $M_{Kankakee}$, a new variant of hemoglobin M. *Blood*, **20** : 287-301, 1962.

39) Jaffe, E. R., and Heller, P.: Methemoglobinemia in man. Moore, C. V., and Brown, E. B. *Progress in Hematology*. vol. 4. Grune & Stratton (New York, London), 1964 (pp. 48-71).

40) Ramot, B.: Discussion about Shibata's speech (hereditary nigremia) at *the third congress of the Asian and Pacific Society of Hematology* (Jerusalem, 1964).

41) Kikuchi, G., Hayashi, N., and Tamura, A.: Oxygen equilibrium of hemoglobin M_{Iwate}. *Biochim. Biophis. Acta*, **90** : 199-201, 1964.

42) Motokawa, Y., Hayashi, N., and Kikuchi, G.: On the reactivity of hemoglobin M_{Iwate}. *Arch. Biochem & Biophys.*, **105** : 612-619, 1964.

43) Hayashi, A., Yamamura, Y., Ogita, S. and Kikkawa, H.: Hemoglobin M_{Osaka}, a new variant of hemoglobin M. *Jap. J. Human Genet.*, **9** : 87-94, 1964.

44) Shimizu, A., Hayashi, A., Yamamura, Y., Tsugita, A., and Kitayama, K.: The structural study on a new hemoglobin variant, Hb M_{Osaka}. *Biochim. Biophys. Acta*, **97** : 472-482, 1965.

45) Gerald, P. S., and Efron, M. L.: Chemical studies of several varieties of Hb M. *Proc. Nation. Acad. Sci.*, **47** : 1758-1769, 1961.

46) Suzuki, T., Hayashi, A., Yamamura, Y., Enoki., Y., aud Tyuma, I : Functional abnormality of hemoglobiu M_{Osaka} *Biochem. & Biophys. Res. Comm.*, **19** : 691-695, 1965.

47) Shibata, S., Iuchi, I., Miyaji, T., Ueda, S., aud Takeda, I.: Hemolytic disease associated with the production of abnormal hemoglobin aud intraerythrocytic Heinz bodies. *Acta Haem. Jap.*, **26** : 164-173, 1963.

48) Morita, H., et al.: Discussion in the symposium (Hemoglobinopathy). *The 25th general meeting of the Japan Society of Human Genetics* (Kyoto, 1962).

49) Tamai, A.: *Personal communication.* The hemoglobins were examined in the author's laboratory.

50) Shibata, S., Miyaji, T., Iuchi, I., Ueda, S., and Takeda, I.: Hemoglobin Hikari (α_2^A $\beta_2 61^{AspNH2}$): A fast-moving hemoglobin found in two unrelated Japanese families. *Clin. Chim. Acta*, **10**: 101-105, 1964.

51) Shibata, S., Iuchi, I., and Miyaji, T.: Unpublished data.

52) Hanada, M : and Rucknagel, D L.: The characterization of hemoglobin Shimonoseki. Blood, 24 : 624-635, 1964.

53) Hanada, M.: Abnormal hemoglobin found in Shimonoseki and Kyushu district. *Jap. J Clin. Hemat.*, **6**: 21-28, 1965.

54) Ager, J. A. M., Lehmann, H., and Vella, F.: Haemoglobin "Norfolk" a new hemoglobin found in an English family *Brit. Med. J.*, **2**: 539-541, 1958; Baglioni, C.: A chemical study of hemoglobin Norfolk, *J. Biol. Chem.*, **237** : 69-74, 1962.

55) Shibata, S , Iuchi, I., and Hamilton, H.: The first instance of hemoglobin E in a Japanese family *Proc. Japan Acad.*, **40** : 846-851, 1964.

56) Okura, K., and Shibata, S.: Unpublished data.

57) Neel, J. V.: The geography of hemoglobinopathies in *Proceedings of the Conference on Genetic polymorphism and Geographic Variation in Disease*, ed. by Blumberg, B. S., Grune & Stratton, (New-York) 1961 (p. 105).

58) Ingram, V. M.: *The Hemoglobins in Genetics and Evolution.* Columbia Univ. Press (New

York, London), 1963.
59) Beale, D., and Lehmann, H.: Abnormal haemoglobins and the genetic code. *Nature*, **207**: 259-261, 1965.
60) Shibata, S., Iuchi, I., Miyaji, T., and Takeda, I.: Hemoglobinopathy in Japan. *Bull. Yamaguchi Med. School*, **10**: 1-9, 1963.
61) Shibata, S.: Hemoglobinopathies of the Japanese. *Proc. 16th General Assembly of the Japan Medical Congress* (Osaka, 1963), vol. 1, Nippon-Isho-Shupan-Kyokai (Tokyo) 1963 (pp. 605-619).
62) Yamaoka, K.: Hemoglobinopathy in Kyushu. *Shinryô*, **16**: 181-197, 1963.
63) Miyake, T., Takekuma, N., and Kawakita, Y.: Abnormal hemoglobins found in Kumamoto district. *Jap. J. Clin. Hemat.*, **6**: 28-29, 1965.
64) Iuchi, I.: Abnormal hemoglobins found in Chûgoku district. *Jap. J. Clin. Hemat.*, **6**: 31-35, 1965.
65) Pik, C., and Raine, D. N.: The chemical identification of two cases of Hb M (Hb M_{Boston} and Hb $M_{Saskatoon}$) occuring in England. *Clin. Chim. Acta*, **10**: 90-92, 1964.
66) Konigsberg, W., and Lehmann. H.: The amino acid substittution ln hemoglobin M_{Iwate}. *Biochim. Biophys. Acta*, **107**: 266-269, 1965.
67) Shimizu, A., Tsugita, A., Hayashi, A., and Yamamura, Y.: The primary structure of hemoglobin M_{Iwate}. *Biochim. Biophys. Acta*, **107**: 270-277, 1965.
68) Shibata, S., Miyaji, T. and Iuchi, I.: Unpublished data.
69) Shibata, S., Miyaji, T. and Iuchi, I.: Unpublished data.