

## HEMOGLOBINOPATHY, WITH SPECIAL REFERENCE TO THE ABNORMAL HEMOGLOBINS FOUND IN JAPAN\*

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Hemoglobin containing a globin moiety of abnormal properties is referred to as abnormal hemoglobin. There are a number of hereditary diseases in which synthesis of normal hemoglobin is partially or completely displaced by the production of abnormal hemoglobin. They are called hemoglobinopathies. Sickle cell anemia and thalassemia are the typical of them.

Since 1949 when Pauling, Itano, Singer and Wells<sup>1)</sup> discovered the first abnormal hemoglobin, Hemoglobin S, in sickle cell anemia, a wealth of knowledge has been accumulated on hemoglobinopathy. A variety of abnormal hemoglobins, totaling no less than 30, have been reported from various parts of the world during the past 12 years. They were designated with alphabets or after the names of the places where they were discovered or after the origins of propositus<sup>2)</sup>.

In the Far East, hemoglobinopathies are prevalent in the countries adjoining Japan.<sup>3)</sup> China is now regarded to be a cradle of thalassemia and thalassemia-Hb H disease. Thailand is known for the endemicity of thalassemia-Hb E disease, and hemoglobin E is not uncommonly found among the people of Indonesia and the Philippines.

Present anthropology postulates that the ancestors of Japanese come to the four islands of Japan in ancient times either from the South Sea Islands or from the northern continent of the Far East. No matter which direction they came from, it is reasonable to assume that they had brought and handed down to their descendants some abnormal hemoglobin primarily of the land where they originated. There is also some possibility that hemoglobin S and hemoglobin C, the abnormal hemoglobins among negroes, may be found in the mixed-blood children born to Japanese mothers and American Negro fathers who were in service and came to Japan after the end of the Pacific War.

Since the title of this speech "Hemoglobinopathy" involves problems that are too numerous to be adequately covered within the limited period of time, it is wise for the author to concentrate on a few important themes which would be of interest to

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the hematologists of this country: (1) Is there any hemoglobinopathy at all in Japan? If so, what kind of hemoglobinopathy? (2) What is the most practical technique for its detection? (3) How to characterize a new abnormal hemoglobin?

### (1) Hemoglobinopathies in Japan

In this country a systematic survey of hemoglobinopathy dated from 1957, when several hematologists began the study with the conventional techniques (paper electrophoresis, solubility test, alkali denaturation test etc.) in Tokyo, Kyoto, Ube and Fukuoka. The author who established a survey center in Ube, Yamaguchi Prefecture, examined approximately 1700 Japanese people during the period from 1957 to the end of 1959. Our survey failed to detect even a single case of hemoglobinopathy, only to support the then predominant view among the Japanese hematologists who vaguely anticipated its absence in this country.

In spite of negative results, the surveys were carried on. The reward came shortly after the end of 1959 when several abnormal hemoglobins were reported all at once from different parts of this country. They were Hb Tokyo (associated with hemolytic anemia and splenomegaly)<sup>4</sup>, Hb Shimonoseki<sup>5</sup>, Hb Kokura<sup>6</sup> and Hb Ube 1 (associated with Heinz body formation and splenomegaly)<sup>7</sup>, all of which belong to slow moving hemoglobins (Figures 1 and 2). A fast-moving hemoglobin which is

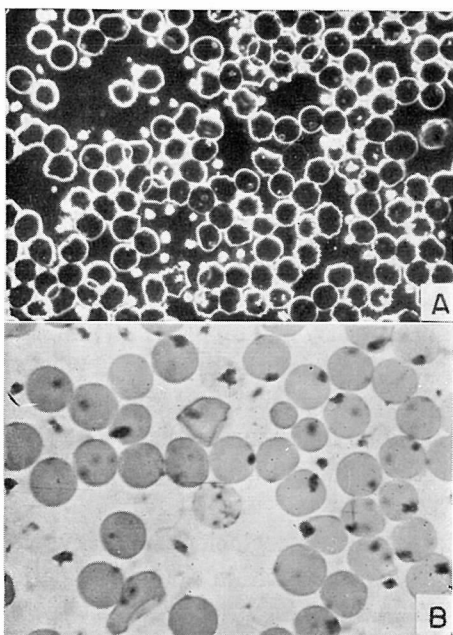


Fig. 1 Heinz bodies of Hb Ube 1 trait. A: Phase contrast microscopy. B: Supravital stain with brilliant-cresyl blue.

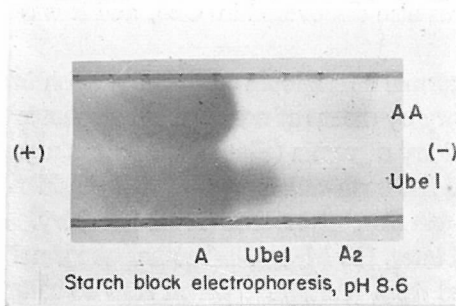


Fig. 2 Starch block electrophoresis of the hemolysate of Hb Ube 1 trait. AA: Normal subject (control). Ube 1: Hb Ube 1 trait. A: Hb A<sub>1</sub>, Ube 1: Hb Ube 1, A<sub>2</sub>: Hb A<sub>2</sub>.

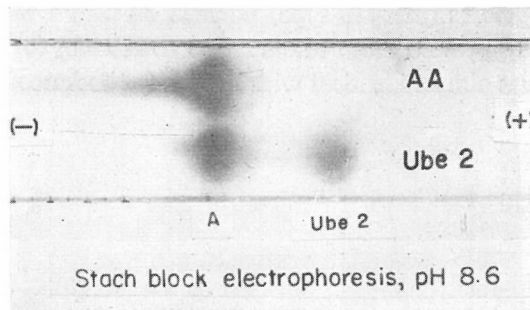


Fig. 3 Starch block electrophoresis of the hemolysate of Hb Ube 2 trait. AA: Normal subject (control). Ube 2: Hb Ube 2 trait. A: Hb A, Ube 2: Hb Ube 2.

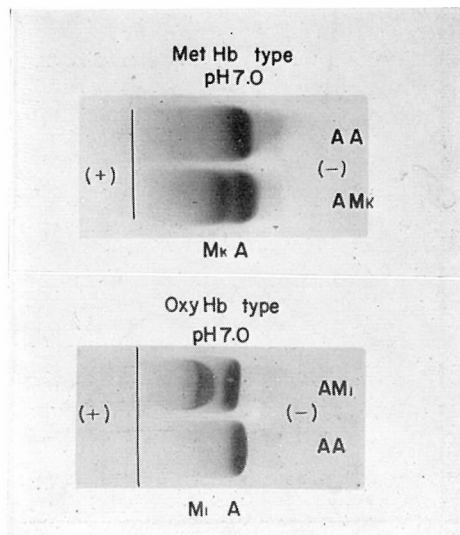


Fig. 4 Agar-gel electrophoresis of the hemolysates of hereditary nigremia and Hb *M<sub>Kurume</sub>* trait. AA: Normal subject. AM<sub>I</sub>: Hereditary nigremia. AM<sub>K</sub>: Hb *M<sub>Kurume</sub>* trait. A: Hb A, M<sub>I</sub>: Hb M<sub>Iwate</sub>, M<sub>K</sub>: Hb *M<sub>Kurume</sub>* trait. A: Hb A, M<sub>J</sub>: Hb M<sub>Iwate</sub>, M<sub>K</sub>: Hb *M<sub>Kurume</sub>*. Electrophoretic separation of Hb *M<sub>Kurume</sub>* (oxy Hb type) from Hb A is impossible.

different from Hb H was also discovered in Ube, and it was tentatively named Hb Ube 2 (Figure 3)<sup>8</sup>.

Early in 1960 an abnormal hemoglobin with dark greyish brown color was isolated in the author's laboratory by means of agar gel electrophoresis from a blood sample of a patient with hereditary nigremia (Figure 4)<sup>9</sup>. This nigremia is seen in an area in Iwate Prefecture and has been called Tamura-Takahashi's disease. This was the first abnormal hemoglobin ever discovered in this country. This hemoglobin was designated Hb M<sub>I</sub>, and later, Hb M<sub>Iwate</sub>. Another abnormal hemoglobin similar to Hb M<sub>Iwate</sub> was reported from Kurume<sup>5</sup>, which is to be called Hb M<sub>Kurume</sub>. Both Hb M<sub>Iwate</sub> and Hb M<sub>Kurume</sub> are characterized by the properties which completely conform to those of the well documented Hb M. Detailed spectroscopic examination of these hemoglobins (met-Hb type) purified on starch block electrophoresis revealed that Hb M<sub>Iwate</sub> bore close resemblance to Hb M<sub>B</sub> (of Boston, U. S. A.), while Hb M<sub>Kurume</sub> was almost identical with Hb M<sub>S</sub> (of Saskatoon, Canada) (Figures 5 and 6).

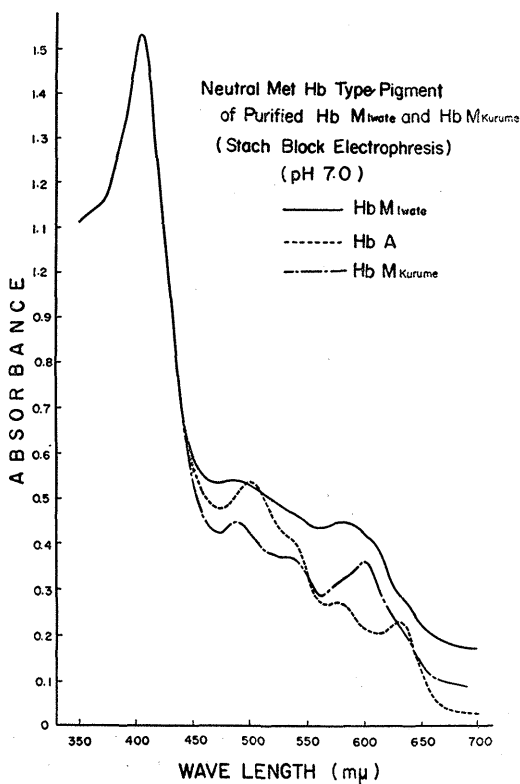


Fig. 5 The absorption curves of methemoglobins of Hb M<sub>Iwate</sub> and Hb M<sub>Kurume</sub>.

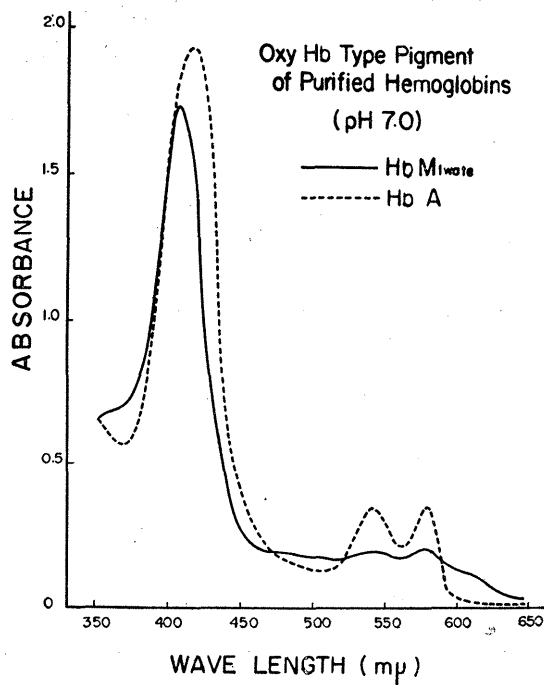


Fig. 6 The absorption curve of the oxyhemoglobin type pigment of Hb M<sub>Iwate</sub>.

Thalassemia minor<sup>10)</sup> and thalassemia-like diseases (including high F gene<sup>11)</sup>) were also encountered in Tokyo and other districts. It is estimated from the results of our survey that the incidence of hemoglobinopathy in Japan will be approximately one per 2,000 population.

A list of abnormal hemoglobins which have hitherto been detected in this country is presented in Table 1.

Table 1. List of abnormal Hemoglobins discovered in Japan

A. Slow-moving hemoglobins

1. *Hb Tokyo*:— Isolated from a patient with hemolytic anemia and splenomegaly (Fukutake, 1960 in Tokyo).<sup>1)</sup> Heredity has been established by family study. This hemoglobin migrates between Hb A and Hb F on paper electrophoresis (pH 8.6). Solubility and alkali denaturation are normal. Beta-chain anomaly is suspected.
2. *Hb Ube 1*:— Found in a patient (aged 11, female) with hemolytic anemia who received splenectomy about a year ago and was examined by Shibata and his associates in 1960.<sup>1)</sup> Heinz bodies in numerous erythrocytes of the peripheral blood is the outstanding feature of abnormality. The hemoglobin resembles closely Hb S in migration on paper and agar gel electrophoreses (pH 8.6 and 6.5). It is divided into three fractions on agar (pH 7.0). Can be isolated from Hb A on Amberlite IRC 50 chromatography. Negative sickling test. Solubility and alkali denaturation are normal. Heredity is not demonstrable. Probably identical with the hemoglobin found in Salt-Lake City (Scott et al, 1960<sup>11)</sup>).
3. *Hb Shimonoseki*:— Detected from a patient with paralytic in-sane and some members of his

family who are living in Shimonoseki (Yamaoka and his coworkers, 1960).<sup>5)</sup> This hemoglobin migrates to the position of Hb S on paper electrophoresis (pH 8.6), and splits into two fractions on starch block electrophoresis (pH 8.6). Sickling test is negative. Normal in solubility and alkali denaturation. Produces no clinical symptoms.

4. *Hb Kokura*:—Demonstrated in a man (Korean?) living in Kokura (Yamaoka and his coworkers, 1960).<sup>6)</sup> This hemoglobin is faster than Hb Shimonoseki on paper electrophoresis (pH 8.6)
5. *Hb S and Hb C*:—Morita<sup>18)</sup> and Shirai (1960) obtained abnormal hemoglobins reminiscent of Hb S and Hb C from mixed blood children with negro fathers living in Yokohama.

B. Hemoglobin M, namely Hb  $M_{Iwate}$  and Hb  $M_{Kurume}$  (Syn. Hb  $M_{Hida}$ )

C. Fast-moving hemoglobins

*Hb Ube 2*:—Detected from a patient (aged 44) with uterine cancer and her daughter (aged 20), by Shibata and his associated in Ube (1960).<sup>8)</sup> Fast-moving on paper and agar gel electrophoreses (pH 8.6), but obviously slower than Hb H. No separation from Hb A on electrophoresis at pH 6.5. Amberlite IRC 50 chromatography is equivocal. Sickling test is negative. Normal in solubility and alkali denaturation. Causes no clinical symptoms.

This is a hemoglobin of  $\alpha$ -chain anomaly, resembling Hb K. Polypeptide 3 is abnormal on fingerprinting.

## (2) Practical techniques for the detection of abnormal hemoglobins

Paper electrophoresis, sickle cell preparation, solubility test of ferrohemoglobin and alkali-denaturation test have been conventionally employed for the detection of abnormal hemoglobins. However, the author has been dissatisfied with the efficiency of paper electrophoresis. A search for a better technique led us to the development of a new procedure of agar gel electrophoresis which features a small electrophoretic tank containing petroleum ether as cooling agent.<sup>8)</sup>

This system (Figure 7) is very simple in manipulation and enabled us to discriminate Hb A<sub>1</sub>, Hb A<sub>2</sub> and other kind of abnormal hemoglobin in acid or alkaline media within as short a time as thirty minutes. As long as we depended on paper electrophoresis, there was no success to separate Hb  $M_{Iwate}$  from Hb A in the hemolysate of hereditary nigremia, but as soon as the technique was switched to the agar gel electrophoresis, Hb  $M_{Iwate}$  was clearly separated as a fast-moving dark greyish brown band discrete from the red Hb A band.

Hb Ube 1 and Hb Ube 2 were for the first time demonstrated by the screening test using this agar gel electrophoresis, and subsequently confirmed by paper electrophoresis, and chromatography with Amberlite IRC 50 (Figure 8).

Agar gel electrophoresis is therefore recommended as a useful addition to the conventional techniques for the detection of abnormal hemoglobins. In the author's laboratory the agar gel electrophoresis has displaced the paper electrophoresis as the routine for the past year.

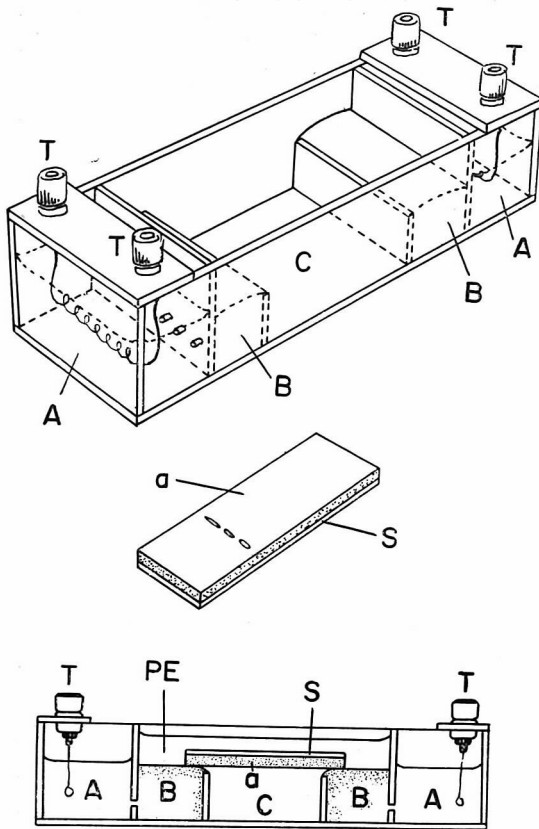


Fig. 7 Electrophoresis tank. Top: A sketch of the tank. Middle: Slide glass with agar layer. Bottom: Side view of the tank. A: electrode vessel. B: accessory compartment filled with agar. C: space in which cooling agent is placed. S: slide glass with agar layer (a). T: electric terminal.

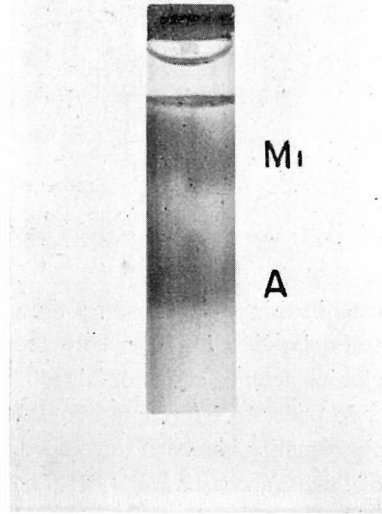


Fig. 8 Amberlite IRC 50 chromatography of the hemolysate of hereditary nigremia. A: Hb A.  $M_1$ : Hb  $M_{1water}$ .

### (3) Simple method for the detection of peptide chain anomaly.

Until recently chemical characterization of hemoglobin was made by electrophoresis (Tiselius, paper, starch and agar), alkali denaturation test, solubility test of ferrohemoglobin, chromatography and spectroscopy are all in all to the exact description<sup>3)</sup>. However, they are no longer satisfactory, because recently Schroeder and others<sup>12)</sup> demonstrated that hemoglobin molecule is composed of  $\alpha$  and  $\beta$  peptide chains, which are inherited independently. Description of chain anomaly has therefore become necessary to characterize a new hemoglobin.

Detection of chain anomaly requires advanced techniques of protein chemistry,

for which hematologists are not well trained. The hybridization technique<sup>13)</sup>, a recent technique which has replaced the previous chemical methods, involves in vitro combination of a known and an unknown peptide chains and thus provides information for identification of the unknown (Figure 9). This procedure has been

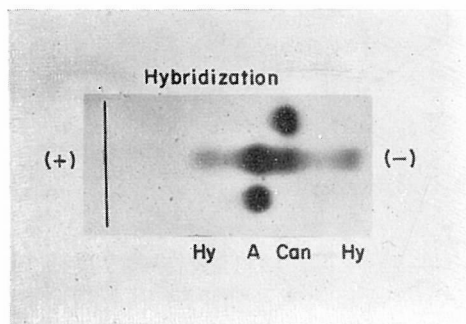


Fig. 9 Hybridization of normal human hemoglobin (A) and canine hemoglobin (Can). The hybrids formed (Hy) are demonstrated by agar gel electrophoresis.

used in this field for some time. Unfortunately, however, it is not feasible in the countries like Japan, where the control hemolysates of standard abnormal hemoglobins are not easily obtained.

In order to overcome this difficulty, a simple technique for the detection of chain abnormality has been developed in the author's laboratory. This is an improvement of Satake's procedure<sup>14)</sup> for the study of animal hemoglobins. First, the globin of hemoglobin A was prepared by Anson-Mirsky's technique<sup>15)</sup>, and it was then dissolved in a concentrated solution of urea (pH 8.0). In this solution the globin is dissociated spontaneously into  $\alpha$  and  $\beta$ -chains, so that they are readily demonstrable by paper electrophoresis.

An experiment with the globins of Hb A and Hb S revealed that  $\beta$ -chain produces a spot which migrates more closely to the anode than  $\alpha$  chain. This difference in electrophoretic migration between  $\alpha$  and  $\beta$  chains has been useful for the detection of chain anomaly of the hemoglobins discovered in Ube. It was this technique that  $\alpha$ -chain anomaly in Hb  $M_{Iwate}$  was disclosed. The  $\alpha$ -chain of Hb  $M_{Iwate}$  migrated to the anode more rapidly than that of Hb A. No difference in electrophoretic migration was seen with  $\beta$ -chain (Figure 10).

If more detailed study of chain anomaly is desired, the  $\alpha$ -chain of Hb  $M_{Iwate}$  should be isolated and purified so that it might be subjected to the "fingerprinting", the technique devised by Ingram<sup>16)</sup> which will disclose the abnormality of the amino acid sequence.

Satake's technique<sup>14)</sup> to be described below will be the simplest for the preparation of  $\alpha$ -chain of hemoglobins, as exemplified by the study on Hb  $M_{Iwate}$ . The



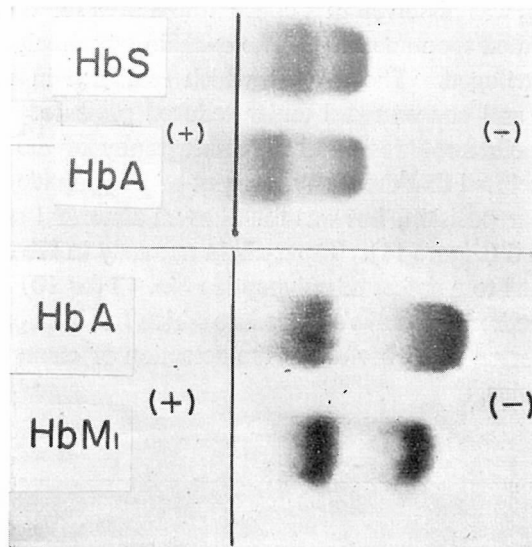


Fig. 10 Chain anomaly of Hb  $M_{Iwate}$ . Top: Urea-dissociation paper electrophoresis of Hb A and Hb S reveals that bands proximal to the anode refer to  $\beta$  chains. Bottom: The  $\alpha$  chain of Hb  $M_{Iwate}$  ( $M_I$ ) is distinctly faster (situating closer to the anode) than that of Hb A.

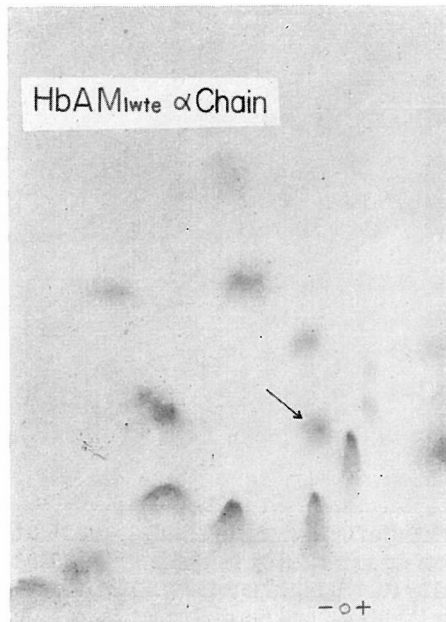


Fig. 11 Fingerprinting of the mixed  $\alpha$  chains of Hb A and Hb  $M_{Iwate}$ . Arrow points to the abnormal spot.

globin of Hb  $M_{Iwate}$  was dissolved in a concentrated urea solution in which  $\alpha$  and  $\beta$  chains were dissociated spontaneously. The  $\alpha$ -chain was precipitated by trichloroacetic acid and centrifuged. The  $\alpha$ -chain which remained in the supernatant was purified by dialysis and concentrated under reduced pressure. The fingerprinting, namely, the paper electrophoresis and chromatography of the trypsinized solution of this  $\alpha$ -chain, disclosed that the ninhydrin spot of polypeptide 13 (or 10) could not be seen at its proper position, but was found as an abnormal spot in area between polypeptides 3 and 6 (Figure 11). The  $\alpha$ -chain anomaly in Hb  $M_{Iwate}$  was therefore thought to be related to a defect of polypeptide No. 13 (or 10) (Figure 12).

The improved method of Satake's procedure which has been presented is relatively simple, and is expected to be useful for the detection of chain anomaly of various abnormal hemoglobins.

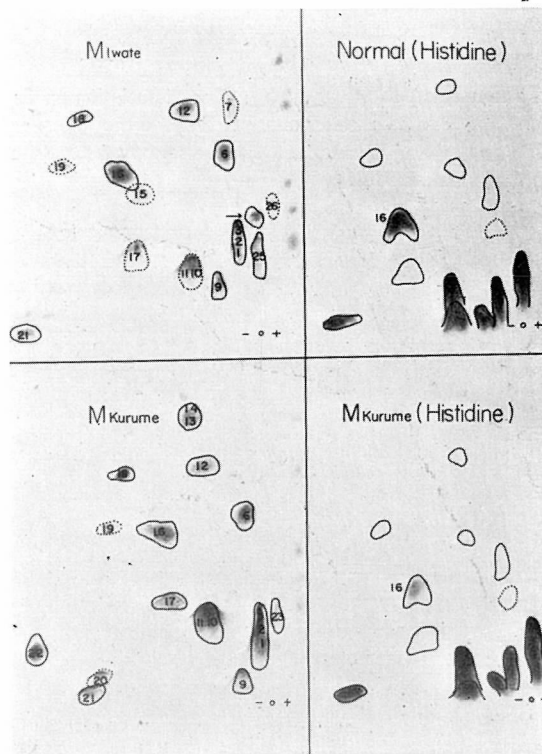


Fig. 12 Fingerprinting of the  $\alpha$  chains of Hb  $M_{Iwate}$  and Hb  $M_{Kurume}$ . Left half: Polypeptide spots demonstrated by ninhydrin spray. Right half: Polypeptide spots developed by Pauly's histidine reaction. Note the abnormal spot shown by the arrow in Hb  $M_{Iwate}$  and the distinctly faint coloration of polypeptide No. 16 in Hb  $M_{Kurume}$ .

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