Study on a Hereditary Black Blood Disease
(Tamura and Takahashi’s Disease)

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INTRODUCTION

The presence of a hereditary disease which is characterized by black-brown blood and inherited dominantly, in a village of the Iwate prefecture and the results of biochemical studies of the disease were reported previously in this journal as the first1) and second2) reports. This paper presents the results of the further examinations.

METHODS AND RESULTS

1. Supplementary notes on the mode of inheritance of this disease
   a) Blood types

   The distribution of the ABO blood types of the patients of this disease was as follows: O, 34.8%; A, 20.92%; B, 25.58%; AB, 18.61%. This distribution is almost identical with that of the general Japanese, among whom type A is less and type O is more common, and agrees with the high incidence of type O in this North-Eastern district of Japan.

   The biological race index was 0.84 and lower than that of this prefecture which is 1.25. From this index this group may be considered as ‘Asiatic’. The inheritance of blood types follows the laws of heredity both in the healthy and in the diseased. No conspicuous feature was observed in the distributions of the Q and S blood types.

   b) Finger prints and palm patterns

   As to finger prints ulnar loops were found most frequently. Whorls were next, and arches and radial loops were rarely seen. These observations accord with the fact that whorls are common in the Pacific coast of the North-Eastern district.

   The index of finger prints $\frac{W}{R+U}$ was 50.8 and smaller than the average
Fig. 1.

0.6% HbO₂ . . . . . . . . . . . . . . 5.0 c.c.
M/15 phosphoric acid. . . . . . . . 4.0 c.c.
(1)  (as buffer keeping pH at 7.38)
Ascorbic acid 8.8×2 mg./c.c. . . . . 1.0 c.c.
(neutralized by N/10 NaOH)

Supernatant of the above mixture (I) after two hours' incubation at 38° was tested with Beckman's spectrophotometer.

Fig. 2.

(I)+Na₂S₂O₄

After reduction by adding Na₂S₂O₄ to the supernatant of (I).

of those of the Japanese.
Palm patterns were examined by Wilder's method. No peculiar changes were found. 7553 and 11975, which are most common in this prefecture, were most frequently seen among the patients of this disease.

In summary, no anthropological peculiarity was found in the studies of blood types, finger prints, palm patterns and biometry. The patients seemed to be innate in this district (Akashi⁹).

2. Biochemical examinations

The analysis of the blood of the patients of this disease revealed no
remarkable changes in the contents of inorganic phosphorous and inorganic copper. Blood enzymes such as catalase and phosphatase were normal and no change was found in the content of glutathion which is important in reduction of methemoglobin. The O₂-combining capacity, which was examined to find out the connecting mode of Hb-iron, was found to be normal.

3. Paper Electrophoresis of Hb

Electrophoretic analysis of 10% Hb solution was performed with Toyo filter paper No. 51 and veronal buffer (pH 8.6) at 500 V for 5–10 hours. A green, brown band was recognized by B.P.B. staining. This observation seems to be closely related with the previous finding of two abnormal (upper, dark green; lower, brown) absorptive layers in the hem solutions of the patients with this disease by the absorption chromato-
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Fig. 5. The sediment of cholehemochromogen dissolved in 4.0 c.c. of 1.5% NaOH. This sediment was obtained in the same condition as Fig. 1. The same absorptive curves were seen both in Fig. 1 and Fig. 5.

Fig. 6. After reduction of the sediment by Na$_2$S$_2$O$_4$, the both healthy subjects and the patients showed the same absorption.

4. Fluorescent substances in urine

Abnormal excretion of xanthurenic acid was found by PPC (Paper Partition Chromatography). Since the excretion of VB$_3$ and VB$_6$ into urine was normal, absence of Apoenzymes may be suspected in the process under xanthurenic acid in the metabolism of tryptophan.

5. Absorption spectrum of Hb

Almost no difference was noticed in Hb dispersion spectrum, and Hb and hem infrared absorption spectra between the patients with this disease and healthy subjects. It is an interesting subject to see what kind of decomposing processes and decomposed substances are responsible for the abnormal coloration of the Hb of the patients of this disease. To obtain green decomposed substances from Hb under nearly physiological conditions in vitro, HbO$_2$-Ascorbic acid-O$_2$ system was chosen and absorption spectrum was studied.
When oxygen saturated Hb water solution was incubated at 38°C with ascorbic acid neutralized with N/10 NaOH, the color turned to green as time elapsed and a green precipitant was also obtained at the same time. The following observations were made with a Beckman's spectrophotometer.

a) Supernatant

In Fig. 1, the absorption curve of the supernatant itself is shown. The optical densities were especially high in both sides of two absorptive bands around 500 mμ and 600 mμ. The top of absorption curve did not fall completely to the base line. The absorptions at 630 mμ and 670 mμ were obscure.

After reduction with Na₂S₂O₄, as shown in Fig. 2, the absorption at 670 mμ disappeared and that at 630 mμ was slightly intensified. However, since the absorptions around 500 mμ and 600 mμ were high, the absorption at 630 mμ was not so remarkable. When the supernatant was reduced with Na₂S₂O₄ after protein was hydrolized with NaOH, as shown in Fig. 3, the absorption in the green band at 558 mμ and 528 mμ corresponded to alkalihemochromogen.

The red band absorption at 630 mμ was shifted to 620 mμ and the absorption was so increased that no clear difference in absorption was recognized between the patients and healthy subjects.

When the supernatant was reduced with Na₂S₂O₄ and bubbled with CO₂, as shown in Fig. 4, the absorption at 630 mμ became marked as in the healthy subjects.

b) Precipitant

The green precipitant was denatured globin. When it was dissolved in NaOH, as shown in Fig. 5, the absorption was at 630 mμ. By reduction with Na₂S₂O₄, as shown in Fig. 6, it was shifted to 620 mμ and the absorption was increased just as in the healthy subjects.

CONCLUSION

The present studies failed to reveal any definite conclusions about pathogenesis of this disease. The one causality may be speculated among the green livores, the dark green abnormal absorption layers observed by chromatography, and the green-brown substance obtained by paper electrophoresis.

From the findings with spectrophotometer it is supposed that blood from the patients with this disease may be easily oxidized to form a complex compound of hematin group. Since no change is observed in the quantities of iron, copper and globin, it may be conceivable that the abnormal coloration is due to a specific structure of porphyrin nucleus. Further studies should be continued to clarify this point.
References

2) Tamura et al., Tohoku J. Exp. Med., 1956, 64, 333.