Chapter 3

# RESULTS

Repeated experiments for determining the globin chain synthesis of either different or the same incorporative studied of the patient have been carried out in several subjects. The results showed very reproducibility.

## Hematologic and globin synthesis studies in normal control

The hematologic data of seven healthy controls are summarized in Table 1. The hematologic mean values of hemoglobin concentration (Hb)(14.17 ± 1.25), red cell count (RBC) (89.37 ± 0.59), MCH (30.07 ± 1.74), MCHC (33.01 ± 1.27), alkali resistant hemoglobin (alk. resist. Hb) (0.72 ± 0.11) were apparently with in normal range. The osmotic fragility of red cell as determined by Donnon's fragiligraph (Donnon, 1963) showed mostly normal. A mean of $\beta/\alpha$ radioactivity ratio is 0.92 ± 0.05 indicating of total balance chain synthesis of $\beta$ and $\alpha$ chain in reticulocyte study. The specific activity of $\beta/\alpha$ ratio is 0.95 ± 0.09, this allows a comparison to be made of amount of newly synthesized chains to that of chains already extent.

## Hematologic and globin synthesis studies in classical β-thalassemia trait ($\beta$-thal$_1$ trait)

Summarization of hematologic and globin synthesis data of

Table 1. Hematologic and globin synthesis data of seven subjects as a normal control.

| Subjects | Age | Hb g/100ml | RBC $x10^6/\mu l^3$ | PCV % | MCV* $\mu^3$ | MCH** pg | MCHC*** % | Hb $A_2$ % | Alk. Resist. Hb (%) | Osmotic fragi-lity | Radioactivity | | Specific activity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | $\beta/\alpha$ | $\alpha/\beta$ | $\beta/\alpha$ | $\alpha/\beta$ |
| Vi. Sp. | 28 | 12.50 | 4.20 | 38.80 | 97.40 | 29.80 | 32.20 | 3.07 | 0.56 | N | 0.88 | 1.13 | 0.89 | 1.16 |
| Au. Sp. | 26 | 15.15 | 4.75 | 44.50 | 93.60 | 31.90 | 34.00 | 2.68 | 0.54 | N | 0.88 | 1.13 | 1.00 | 1.00 |
| C. Sr. | 30 | 14.40 | 4.60 | 44.20 | 96.10 | 31.30 | 32.50 | 3.10 | 0.61 | N | 0.88 | 1.13 | 0.92 | 1.09 |
| Si. Sp. | 20 | 13.00 | 4.48 | 37.80 | 84.00 | 29.00 | 34.40 | 2.84 | 0.57 | D | 1.00 | 1.00 | 0.99 | 1.01 |
| Ti. Vu. | 26 | 13.40 | 4.29 | 39.00 | 90.90 | 31.20 | 34.30 | 2.74 | 0.90 | N | 0.93 | 1.07 | 0.93 | 1.07 |
| B. C. | 26 | 14.75 | 4.84 | 41.00 | 84.70 | 30.50 | 36.00 | 2.34 | 0.82 | N | 0.98 | 1.03 | 0.90 | 1.10 |
| Su. St. | 26 | 16.00 | 5.96 | 47.00 | 78.90 | 26.80 | 34.00 | 2.25 | 1.03 | N | 0.91 | 1.09 | 0.92 | 1.09 |
| Mean | | 14.17 | 4.73 | 41.76 | 89.37 | 30.07 | 33.91 | 2.72 | 0.72 | - | 0.92 | 1.08 | 0.95 | 1.06 |
| S.D. | | 1.25 | 0.59 | 3.50 | 6.96 | 1.74 | 1.27 | 0.33 | 0.20 | - | 0.05 | 0.05 | 0.09 | 0.09 |

N = Normal

D = Decrease

$$MCV^* = \frac{PCV}{RBC} \times 10 \quad \mu^3$$

$$MCH^{**} = \frac{Hb}{RBC} \times 10 \quad pg$$

$$MCHC^{***} = \frac{Hb}{PCV} \times 100 \quad \%$$

nine cases obligatory β-thalassemia$_1$ trait are shown in Table 2. Base on the distribution of β/α ratio of the nine subjects shown in Figure 10 (p.41), which will be discussed later. Seven apparently segregated in one group with a mean of β/α being 0.44 ± 0.014 which was believed to represent a β-thal$_1$ trait. But the two subjects P.Do and P.Ki showed the β/α ratio significantly increased which presumably represent the double heterozygote for β-thal$_1$ and α-thalassemia. The hematologic mean value of seven cases of β-thal$_1$ were shown in Table 2. The subjects are slightly anemia, with a mean of hemoglobin concentration of 10.91 ± 0.73. Hematologic means of MCV and MCH indicated hypochromic microcytic red cells. This was substantiated by decreased osmotic fragility studies. The quantitative Hb A$_2$ was elevated with a mean of 5.35 ± 0.87, while the alkali resistant hemoglobin was slightly increased, with a mean of 0.99 ± 0.28.

The incorporative studies in reticulocytes of the subjects were carried out, the globin chains were fractionated by CMC chromatography and the actual optical density at 280 nm and incorporative radioactivity of each chain were determined as shown in Figure 6.(p.27). The means of β/α radioactivity ratio and specific activity ratio were 0.44 ± 0.014 and 0.50 ± 0.03 respectively.

Although the subjects P.Do, and P.Ki had hematological data, (as shown in Table 2) including the quantitative Hb A$_2$ similar to the seven heterozygous β-thal$_1$, the β/α total radioactivity ratio and specific activity of these two subjects were significantly different. The increase of β/α ratio of the two subject were

believed to be the effect of inheritance of $\alpha$-thalassemia gene which will be discussed later.

Hematologic and globin synthesis studies in mild β-thalassemia trait ($\beta$-thal$_2$ trait).

A summary of hematologic and globin chain studies of nine cases of obligatory $\beta$-thal$_2$ trait is shown in Table 3. The mean of hematologic values of hemoglobin concentration, MCV, and MCH, which were 11.32 ± 1.09, 72.06 ± 5.08, and 22.71 ± 1.32 respectively, indicated mild degree of thalassemic red cell changes. The Hb $A_2$ and alkali resistant hemoglobin of the subjects were measured, with a mean of 5.25 ± 0.59, and 2.06 ± 1.16 . The CMC chromatography of an incorporative study in a case of $\beta$-thal$_2$ trait is shown in Figure 7. The mean of radioactivity $\beta/\alpha$ ratio was 0.50 ± 0.02 while that of specific activity was 0.57 ± 0.05 .

Comparison of hematologic mean values of normal, $\beta$-thal$_1$ and $\beta$-thal$_2$

A summary of t-tests for hematologic mean values between $\beta$-thal$_1$ trait (Table 2, p.26) and normal controls (Table 1, p.23), and between $\beta$-thal$_2$ trait (Table 3, p.28) and normal control, and between $\beta$-thal$_1$ and $\beta$-thal$_2$ trait are shown in Table 4. It can be seen that all hematologic mean values of both $\beta$-thal$_1$ trait and $\beta$-thal$_2$ trait significantly differ from those of the normal control group. Although the mean of radioactivity ratio of $\beta$-thal$_1$ trait (0.44 ± 0.014) seems to be close to that of $\beta$-thal$_2$ (0.50 ± 0.02), they were

Table 2. Hematologic and globin synthesis data of seven subjects with heterozygous classical β-thalassemia in the upper part and two subjects with double heterozygote $\beta$-thal$_1$ and α-thalassemia in the lower part.

| Subjects | Age | Hb g/100ml | RBC $\times 10^6/\mu l^3$ | PCV % | MCV $\mu^3$ | MCH pg | MCHC % | Hb $A_2$ % | Alk. Resist. Hb (%) | Osmotic fragi-lity | Radioactivity | | Specific activity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | β/α | α/β | β/α | α/β |
| Py. Sp. | 37 | 10.65 | 5.74 | 38.00 | 66.20 | 18.90 | 28.00 | 6.72 | 0.79 | D | 0.43 | 2.33 | 0.47 | 2.12 |
| Py. Sm. | 14 | 11.90 | 5.87 | 40.00 | 68.10 | 20.30 | 29.70 | 6.06 | 0.89 | D | 0.45 | 2.21 | 0.54 | 1.85 |
| Sj.Tni. | 30 | 9.50 | 5.45 | 29.50 | 54.10 | 17.40 | 32.20 | 5.10 | 0.98 | D | 0.42 | 2.38 | 0.51 | 1.96 |
| Sj. Ln. | 51 | 10.50 | 5.68 | 34.50 | 60.70 | 18.50 | 30.40 | 4.30 | 1.60 | D | 0.44 | 2.26 | 0.49 | 2.04 |
| Ta. M. | 27 | 11.30 | 5.48 | 35.00 | 63.90 | 20.60 | 32.30 | 5.36 | 0.77 | D | 0.43 | 2.31 | 0.54 | 1.85 |
| K. Po. | 15 | 11.10 | 5.46 | 36.00 | 65.90 | 20.30 | 30.80 | 5.39 | 1.19 | D | 0.42 | 2.36 | 0.45 | 2.22 |
| J. T. | 20 | 11.00 | 5.58 | 37.80 | 67.70 | 19.70 | 29.10 | 5.79 | 0.82 | D | 0.46 | 2.17 | 0.52 | 1.92 |
| Mean | - | 10.91 | 5.58 | 35.85 | 64.23 | 19.65 | 30.55 | 5.35 | 0.99 | D | 0.44 | 2.29 | 0.50 | 1.99 |
| S.D. | - | 0.73 | 0.17 | 3.13 | 4.74 | 1.32 | 1.56 | 0.87 | 0.28 | - | 0.01 | 0.08 | 0.03 | 0.14 |
| P. Do.* | 13 | 11.90 | 5.68 | 38.00 | 66.90 | 20.95 | 31.10 | 5.26 | 1.53 | D | 0.76 | 1.30 | 0.98 | 1.05 |
| K. Pi.* | 14 | 11.10 | 5.46 | 36.00 | 67.20 | 21.50 | 31.90 | 4.11 | 0.85 | D | 0.51 | 1.96 | 0.56 | 1.79 |

* subject with double heterozygote $\beta$-thal$_1$ and α-thalassemia.

CLASSICAL β—THALASSEMIA TRAIT

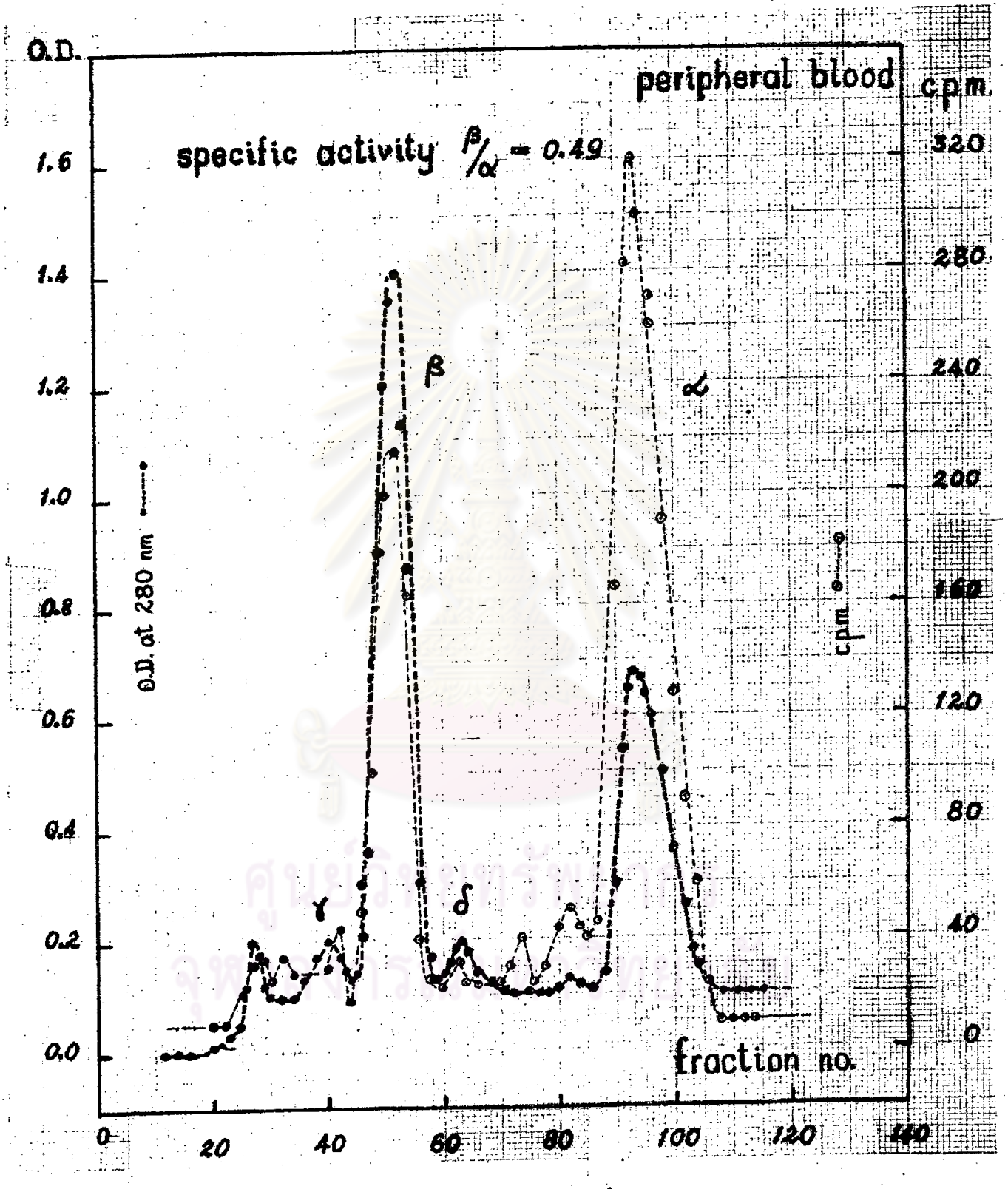



Figure 6. CMC chromatography of globin from Sj. Ln. subject with classical β-thalassemia trait.

Table 3. Hematologic and globin synthesis data of nine subjects with heterozygous mild β-thalassemia.

| Subjects | Age | Hb g/100ml | RBC $x10^6/\mu l^3$ | PCV % | MCV $\mu^3$ | MCH pg | MCHC % | Hb $A_2$ % | Alk. Resist. Hb (%) | Osmotic fragi-lity | Radioactivity | | Specific activity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | β/α | α/β | β/α | α/β |
| Ys. V. | 8 | 11.10 | 5.23 | 34.10 | 65.20 | 21.20 | 32.55 | 5.58 | 1.28 | D | 0.50 | 2.19 | 0.59 | 1.69 |
| Ys. J. | 37 | 12.60 | 5.38 | 39.70 | 73.80 | 23.40 | 31.73 | 5.70 | 3.78 | D | 0.54 | 1.84 | 0.69 | 1.45 |
| Si. Ud. | 24 | 13.60 | 6.29 | 41.50 | 66.00 | 21.60 | 32.77 | 5.78 | 1.13 | D | 0.48 | 2.08 | 0.53 | 1.89 |
| Si. Yp. | 47 | 10.20 | 4.91 | 32.90 | 67.00 | 20.80 | 31.00 | 5.42 | 1.23 | D | 0.49 | 2.03 | 0.54 | 1.85 |
| Kn. Su. | 35 | 10.60 | 4.62 | 34.50 | 74.70 | 22.90 | 30.72 | 4.19 | 0.84 | D | 0.49 | 2.06 | 0.52 | 1.90 |
| Kn. Vj. | 38 | 11.40 | 4.63 | 37.10 | 80.10 | 24.60 | 30.72 | 5.63 | 2.13 | D | 0.47 | 2.14 | 0.54 | 1.86 |
| Kn. Rn. | 30 | 10.80 | 4.68 | 35.70 | 76.30 | 23.10 | 30.25 | 4.54 | 2.76 | D | 0.50 | 2.00 | 0.57 | 1.75 |
| T. Vr. | 15 | 11.00 | 4.88 | 34.90 | 71.50 | 22.50 | 31.51 | 5.43 | 3.89 | D | 0.50 | 2.00 | 0.57 | 1.25 |
| To. Br. | 20 | 10.60 | 4.36 | 32.30 | 74.00 | 24.30 | 32.81 | 5.00 | 1.47 | D | 0.51 | 1.25 | 0.56 | 1.29 |
| Mean | | 11.32 | 5.00 | 35.86 | 72.06 | 22.71 | 31.56 | 5.25 | 2.06 | D | 0.50 | 2.02 | 0.57 | 1.77 |
| S.D. | | 1.09 | 0.58 | 3.07 | 5.08 | 1.32 | 0.96 | 0.56 | 1.16 | D | 0.02 | 0.09 | 0.05 | 0.14 |

D = decrease

MILD β–THALASSEMIA TRAIT

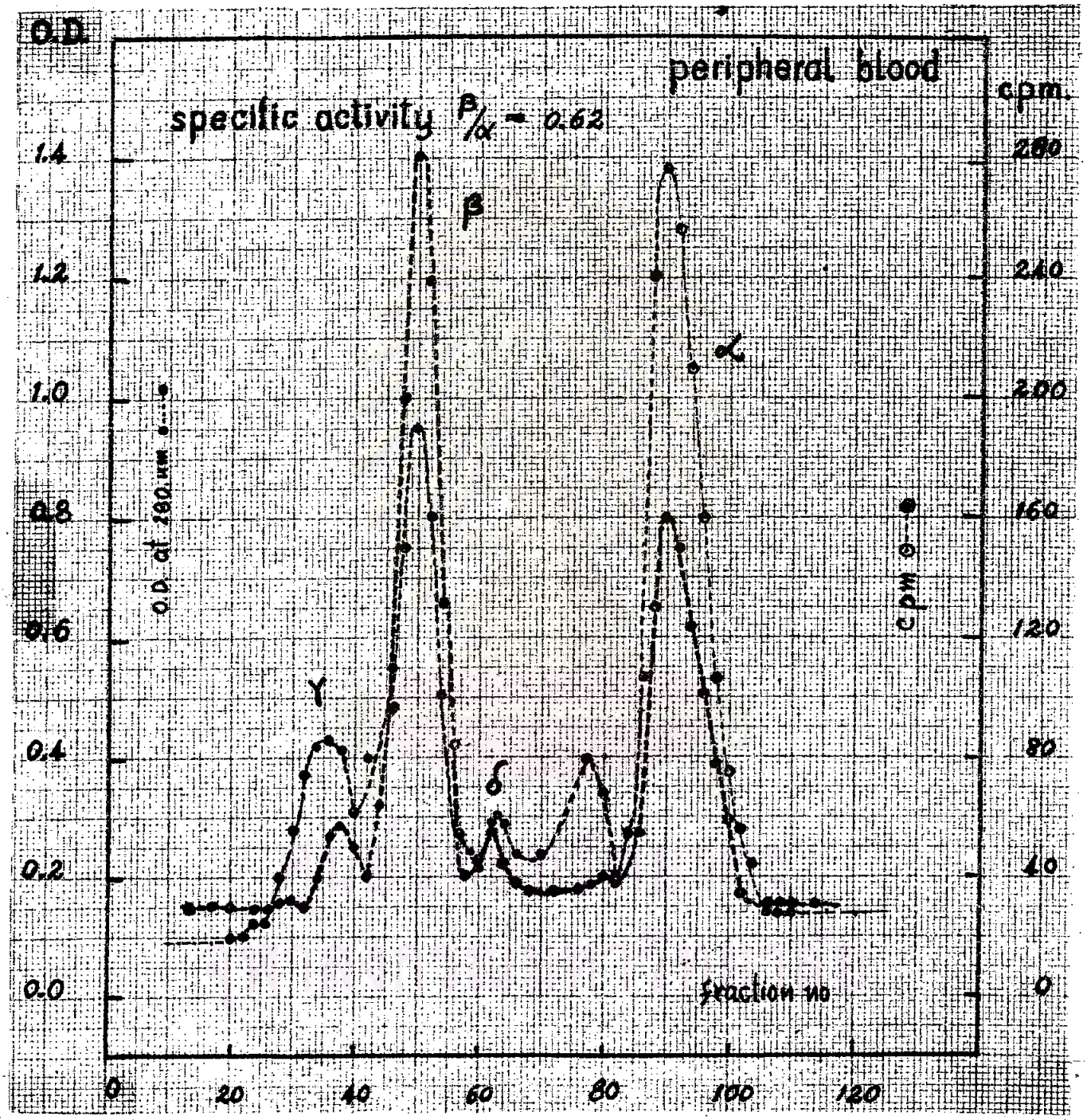



Figure 7. CMC chromatography of globin from Ys. V. subject with mild β-thalassemia trait.

Table 4. Summary of t-test of hematologic globin synthesis mean value.

| Genotypes | No. of cases | | Hb g/100ml | RBC $x10^6/\mu l^3$ | PCV % | MCV $\mu^3$ | MCH pg | MCHC % | Hb $A_2$ % | Alk. Resist. Hb % | Radio-activity $\beta/\alpha$ | Specific activity $\beta/\alpha$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Normal | 7 | Mean | 14.17 | 4.73 | 41.76 | 89.37 | 30.07 | 33.91 | 2.72 | 0.72 | 0.92 | 0.95 |
| | | S.D. | 1.25 | 0.59 | 3.50 | 6.96 | 1.74 | 1.27 | 0.33 | 0.20 | 0.05 | 0.09 |
| $\beta$-thal$_1$ | 7 | Mean | 10.91 | 5.58 | 35.85 | 64.23 | 19.65 | 30.55 | 5.35 | 0.99 | 0.44 | 0.50 |
| | | S.D. | 0.73 | 0.17 | 3.13 | 4.75 | 1.32 | 1.56 | 0.87 | 0.28 | 0.01 | 0.03 |
| $\beta$-thal$_2$ | 9 | Mean | 11.32 | 5.00 | 35.86 | 72.06 | 22.71 | 31.56 | 5.25 | 2.06 | 0.50 | 0.57 |
| | | S.D. | 1.09 | 0.58 | 3.07 | 5.08 | 1.32 | 0.96 | 0.56 | 1.16 | 0.02 | 0.05 |
| $\beta$-thal$_1$/ 7 Normal | 7/7 | P | $<.001$ | $<.005$ | $<.005$ | $<.005$ | $<.001$ | $<.001$ | $<.001$ | $<.050$ | $<.001$ | $<.001$ |
| $\beta$-thal$_2$/ 7 Normal | 9/7 | P | $<.001$ | $<.400$ | $<.005$ | $<.001$ | $<.001$ | $<.001$ | $<.001$ | $<.005$ | $<.001$ | $<.001$ |
| $\beta$-thal$_1$/ $\beta$-thal$_2$ | 7/9 | P | $<.400$ | $<0.020$ | $>90$ | $<0.005$ | $<.001$ | $<.200$ | $<.800$ | $<0.020$ | $<0.001$ | $<0.010$ |

statistically different ($P < 0.001$) and the means of MCV and MCH of these two groups were apparently different with $P < .005$ and $< .001$ respectively.

Heterozygous Hb E

Hematologic and globin chain synthesis studies of five cases of Hb E trait were summarized on the upper part of Table 5. After hematologic studies, subject P.Di. was found to be a double heterozygote for hemoglobin E and $\alpha$-thalassemia. Besides the subject P.Di who will be discussed later, the heterozygous Hb E revealed slightly low MCH. And the other hematologic mean values: PCV, MCH, MCHC and alkali resistant hemoglobin appeared to be within normal limits. A mean of quantitative Hb E was 27.48 ± 1.53. The means of $\beta/\alpha$, $\beta^E/\alpha$ and $\beta+\beta^E/\alpha$ radioactivity ratio were, in sequence, 0.60 ± 0.05, 0.37 ± 0.03 and 0.97 ± 0.036 . The specific activity of $\beta/\alpha$ and of $\beta^E/\alpha$ ratio were very close to one (0.98 ± 0.05 and 1.00 ± 0.06 respectively).

The subject P.Di. had hemoglobin concentration of 9.9 gm% and 21 % Hb E, instead of around 27 % as in a heterozygote. The radioactivity $\beta/\alpha$ ratio, and the specific activity $\beta/\alpha$ ratio of the subject; 0.8 and 1.29 respectively, revealed obvious difference from the other heterozygotes (Table 5). The subject was most likely to inherit an extra $\alpha$-thalassemic gene which would be consistent with the reduction of Hb E and with the radioactivity $\beta/\alpha$ ratio of 0.8 .

Table 5. Hematologic and globin synthesis data of five subjects with heterozygous hemoglobin E, one subject with double heterozygous of hemoglobin E and $\alpha$-thalassemia.

| Subjects | Age | Hb g/100ml | RBC $x10^6/\mu l^3$ | PCV % | MCV $\mu^3$ | MCH pg | MCHC % | Hb E % | Alk. Resist. Hb (%) | Radioactivity | | | Specific activity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | $\beta/\alpha$ | $\beta^E/\alpha$ | $\beta+\beta^E/\alpha$ | $\beta/\alpha$ | $\beta^E/\alpha$ |
| P. Pd. | 37 | 10.00 | 4.20 | 32.00 | 76.20 | 23.80 | 31.30 | 25.07 | 0.40 | 0.69 | 0.33 | 1.02 | 1.05 | 1.10 |
| Sj. B. | 52 | 12.90 | 5.87 | 59.00 | 100.50 | 21.90 | 21.80 | 29.10 | 0.74 | 0.55 | 0.40 | 0.95 | 0.96 | 0.94 |
| Py. Pm. | 36 | 12.80 | 4.71 | 40.00 | 84.90 | 27.60 | 32.50 | 28.06 | 0.59 | 0.59 | 0.36 | 0.95 | 1.00 | 0.99 |
| Si.Chj. | 54 | 12.70 | 5.07 | 38.70 | 75.00 | 25.00 | 33.30 | 27.04 | 1.42 | 0.59 | 0.35 | 0.24 | 0.90 | 0.99 |
| To. Ti. | 59 | 13.50 | 5.63 | 42.00 | 74.60 | 24.60 | 32.10 | 28.12 | 0.49 | 0.60 | 0.40 | 1.00 | 0.99 | 0.97 |
| Mean | | 12.39 | 5.09 | 42.34 | 82.24 | 24.58 | 30.20 | 27.48 | 0.73 | 0.60 | 0.37 | 0.97 | 0.98 | 1.00 |
| S.D. | | 1.37 | 0.68 | 10.04 | 11.04 | 2.07 | 4.75 | 1.53 | 0.41 | 0.05 | 0.03 | 0.04 | 0.56 | 0.06 |
| P. Di* | 11 | 9.90 | 2.80 | 24.00 | 87.50 | 35.40 | 41.00 | 21.00 | 1.16 | 0.80 | 0.28 | 1.08 | 0.96 | 0.94 |

* = double heterozygote of hemoglobin E and $\alpha$-thalassemia

Hematologic and globin synthesis studies in $\beta$-thal$_1$/Hb E disease

Hematologic data and globin chain synthesis studies of six cases of $\beta$-thal$_1$/Hb E disease are summarized in Table 6, except the patient B. Tv. The data indicated marked anemia with a mean of hemoglobin concentration of 7.5 $\pm$ 1.98 . The mean corpuscular volume, red blood cell changes and marked decrease in red cell osmotic fragility indicated the marked hypochromic microcytic red cells. The hemoglobin types of all cases had only two components, Hb E + Hb F. The mean of quantitative measurements of Hb E by DEAE sephadex chromatography was 51.65 $\pm$ 11.79 , and the alkali resistant of the patients had a mean of 27.13 $\pm$ 11.99. The CMC chromatography of globin from $\beta$-thal$_1$/Hb E disease as shown in Figure 8, revealed $\gamma$, $\delta$, $\beta^A$ and $\alpha$ chains. No peak corresponding to $\beta^A$ was observed. This suggested that $\beta$-thal$_1$ gene, in the presence of $\beta^E$ gene and their interaction, would completely suppress the $\beta^A$ globin chain synthesis. A mean of radioactivity of $\beta^E/\alpha$ was 0.40 $\pm$ 0.08 .

Hematologic and globin synthesis studies in $\beta$-thal$_2$/Hb E disease

A summary of hematologic data and globin chain ratio of five $\beta$-thal$_2$/Hb E disease is shown in Table 7. The hematologic mean values, appearently less severe in terms of hypochromic microcytic anemia, than that of the $\beta$-thal$_1$/Hb E disease. All had hemoglobin types of E+F+A. (Figure 5, p.16). The presence of Hb A was entirely ruled out a history of previous blood transfusion. The mean of quantitative Hb E and alkali resistant hemoglobin were

Table 6. Hematologic and globin synthesis data of six subjects with $\beta$-thal$_1$/Hb E disease

| Subjects | Age | Hb g/100ml | RBC $x10^6/\mu l^3$ | PCV % | MCV $\mu^3$ | MCH pg | MCHC % | Hb E % | Alk. Resist. Hb (%) | Osmotic fragili -ty | Radioac- -tivity $\beta^E/\alpha$ | Specific activity $\beta^E/\alpha$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Yt. K. | 25 | 5.85 | 3.90 | 23.00 | 58.90 | 15.00 | 22.40 | 45.60 | 27.76 | VD | 0.37 | 0.69 |
| B. Tv. | 20 | 10.80 | 5.94 | 34.00 | 57.20 | 18.00 | 31.70 | 42.70 | 42.44 | VD | 0.31 | 0.69 |
| J. R. | 23 | 5.10 | 2.63 | 18.00 | 69.00 | 19.40 | 28.30 | 58.04 | 17.73 | VD | 0.47 | 0.68 |
| Sj. Tn. | 29 | 7.70 | 4.99 | 27.00 | 54.10 | 15.40 | 28.50 | 36.62 | 38.76 | VD | 0.32 | 0.57 |
| Dp. Sr. | 25 | 7.70 | 4.06 | 26.00 | 63.00 | 19.00 | 29.60 | 59.37 | 24.92 | VD | 0.46 | 0.70 |
| Au. Bs. | 22 | 7.90 | 4.03 | 25.90 | 67.00 | 18.50 | 30.50 | 67.54 | 11.06 | VD | 0.48 | 0.80 |
| Mean | | 7.51 | 4.26 | 25.65 | 61.53 | 17.60 | 28.50 | 51.65 | 27.13 | | 0.40 | 0.69 |
| S.D. | | 1.98 | 1.12 | 5.24 | 5.81 | 1.92 | 3.25 | 11.80 | 11.99 | | 0.08 | 0.07 |

VD = very decrease

CLASSICAL β–THALASSEMIA/Hb E DISEASE

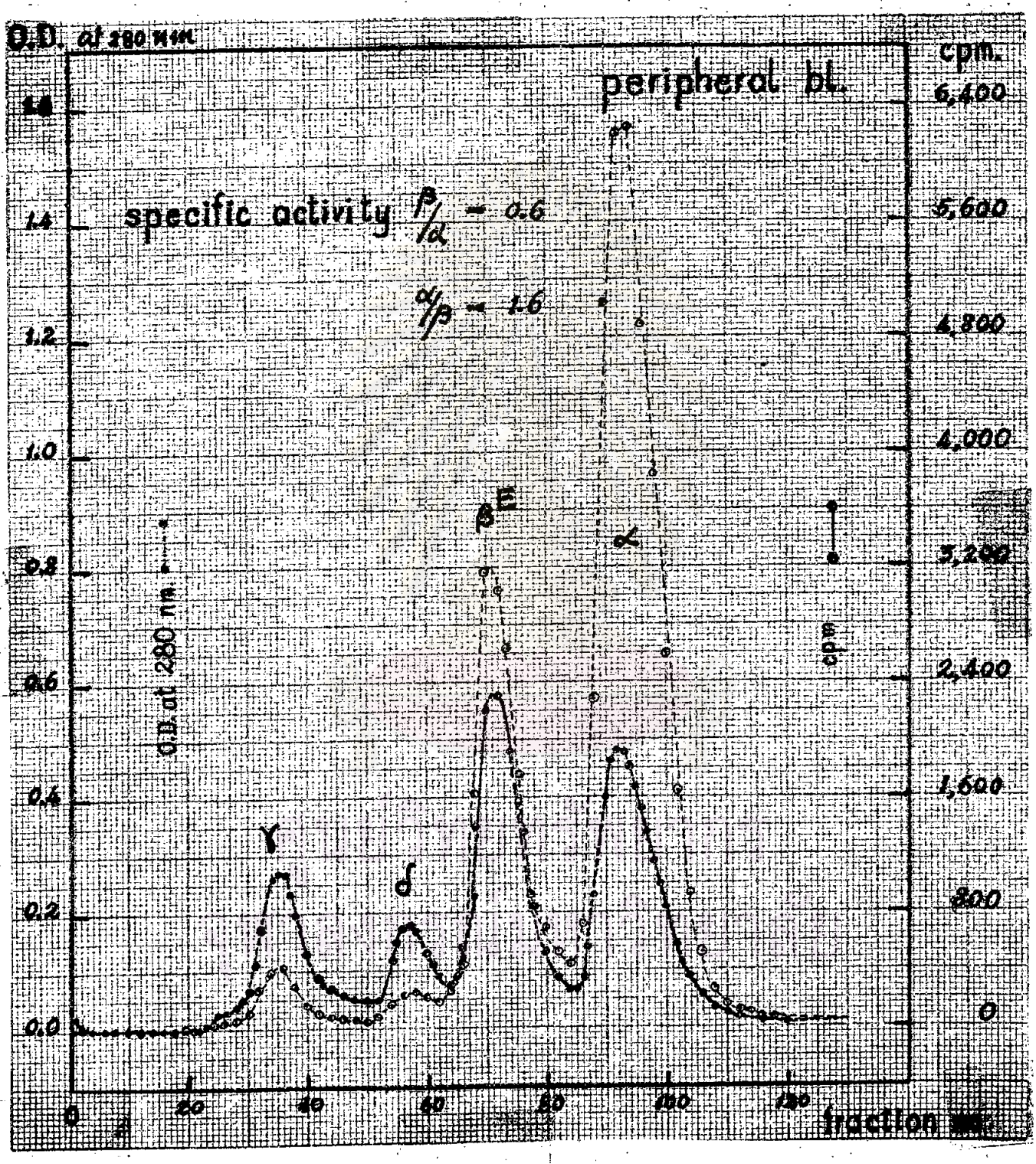



Figure 8. Typical CMC chromatograms of globin from patient J.P. with β-thal$_1$/Hb E disease.

59.99 ± 8.04 and 9.68 ± 5.03 respectively. The CMC chromatography of globin from β-thal$_2$/Hb E disease is shown in Figure 9. It was evident that a peak corresponding to $\beta^A$ chain was noticed. The mean of radioactivity $\beta^A + \beta^E/\alpha$ ratio was 0.51 ± 0.07.

Comparison of hematologic and globin synthesis mean values between β-thal$_1$/Hb E and β-thal$_2$/Hb E disease

A summary of t-tests for hematologic and globin synthesis mean values between β-thal$_1$/Hb E (Table 6, p.34) and β-thal$_2$/Hb E disease (Table 7, p.38) is shown in Table 8. Only quantitative amount of Hb E of the former is significantly higher than that of the latter ($P < .01$).

A summary histogram of globin synthesis studies in various genotypes

Summary results of globin synthesis in control, Hb E trait, β-thal$_1$ trait, β-thal$_2$ trait, β-thal$_1$/Hb E disease, and β-thal$_2$/Hb E disease are shown in Figure 10. It can be seen that the distribution of the radioactivity of β/α ratio of β-thal$_2$ trait was not overlapped, separated from that of β-thal$_1$ trait. The two β-thal$_1$ trait have the ratio 0.76 and 0.51 which were higher values than that of the simple β-thal$_1$ trait, are believed to inherited an extra thalassemia trait leading to the increase β/α ratio.

Tryptic peptide mapping of β and $\beta^E$ globin chains

This was to confirm the identity of the slow variants from

MILD β–THALASSEMIA/Hb E DISEASE

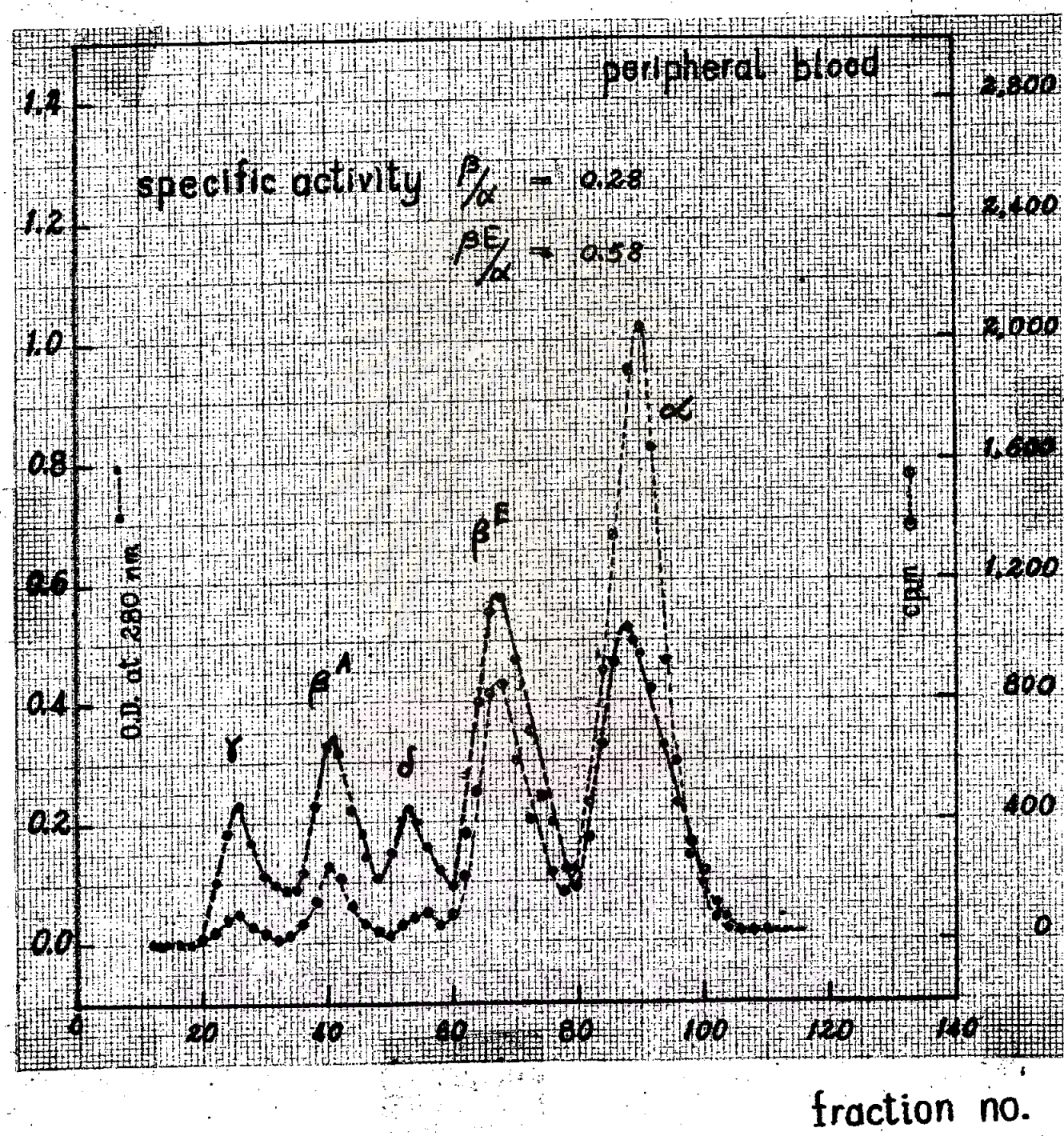



Figure 9. Typical CMC chromatograms of globin from patient Ys. Ar. with β-thal$_2$/Hb E disease.

Table 7. Hematologic and globin synthesis data of five subjects with β-thalassemia$_2$/Hb E disease

| Subjects | Age | Hb g/100ml | RBC $x10^6/\mu l^3$ | PCV % | MCV $\mu^3$ | MCH pg | MCHC % | Hb E % | Alk. Resist. Hb (%) | Osmotic fragi-lity | Radioactivity | | | Specific activity | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | $\beta/\alpha$ | $\beta^E/\alpha$ | $\beta+\beta^E/\alpha$ | $\beta/\alpha$ | $\beta^E/\alpha$ |
| Si. Pc. | 26 | 7.20 | 3.08 | 24.90 | 80.80 | 23.30 | 28.90 | 63.16 | 14.07 | VD | 0.03 | 0.37 | 0.41 | 0.33 | 0.50 |
| Ys. Ar. | 40 | 10.60 | 5.28 | 34.60 | 65.50 | 20.00 | 31.40 | 52.50 | 6.26 | VD | 0.11 | 0.36 | 0.47 | 0.36 | 0.64 |
| Sr. Jr. | 25 | 11.70 | 5.40 | 35.00 | 64.80 | 21.70 | 33.40 | 50.31 | 7.72 | VD | 0.12 | 0.41 | 0.53 | 0.37 | 0.69 |
| To. Ss. | 22 | 7.80 | 4.04 | 27.30 | 67.50 | 19.30 | 28.50 | 67.54 | 11.06 | VD | 0.40 | 0.55 | 0.59 | 0.31 | 0.78 |
| To. Bc. | 24 | 7.90 | 4.03 | 25.90 | 64.30 | 19.60 | 30.50 | 66.48 | 9.31 | VD | 0.40 | 0.53 | 0.57 | 0.33 | 0.73 |
| Mean | | 9.04 | 4.37 | 29.54 | 68.58 | 20.78 | 30.54 | 60.00 | 9.68 | | 0.07 | 0.44 | 0.51 | 0.34 | 0.67 |
| S.D. | | 1.98 | 0.97 | 4.88 | 6.94 | 1.69 | 1.99 | 8.05 | 5.04 | | 0.04 | 0.09 | 0.07 | 0.02 | 0.11 |

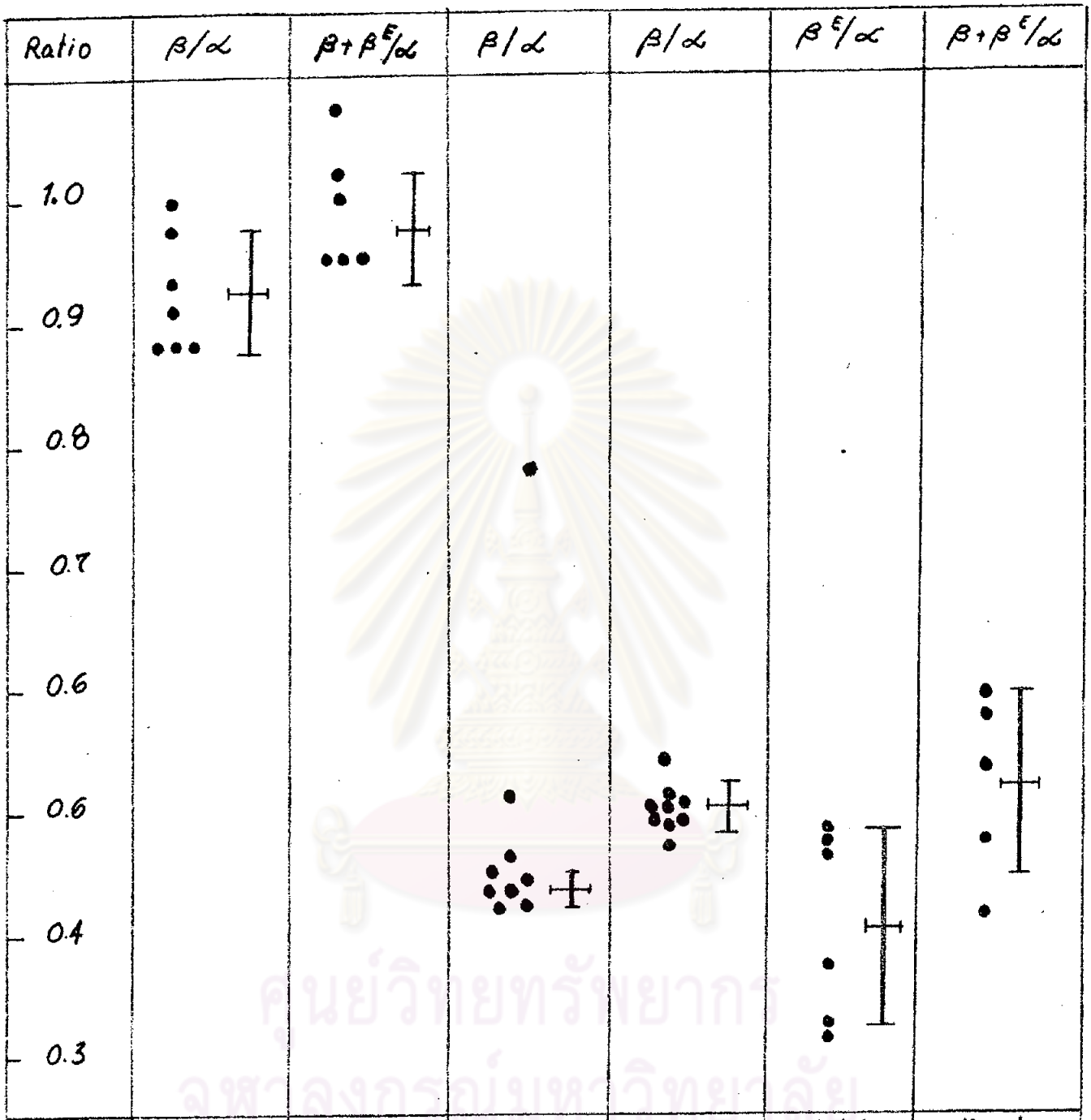
VD = very decrease

Table 8. Summary of t-test of hematologic and globin synthesis mean values between $\beta$-thal$_1$-Hb E disease and $\beta$-thal$_2$-Hb E disease.

| Genotypes | No. of cases | | Hb g/100ml | RBC $x10^6/\mu l^3$ | PCV % | MCV $\mu^3$ | MCH pg | MCHC % | Hb E % | Alk. Resist. Hb (%) | Radioactivity $\beta^E/\alpha$ | Radioactivity $\beta+\beta^E/\alpha$ | Specific activity $\beta^E/\alpha$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\beta$-thal$_2$-Hb E | 5 | Mean | 9.04 | 4.37 | 29.54 | 68.58 | 20.78 | 30.54 | 60.00 | 9.68 | 0.44 | 0.51 | 0.07 |
| | | S.D. | 1.98 | 0.97 | 4.88 | 6.94 | 1.69 | 1.99 | 8.05 | 5.04 | 0.09 | 0.07 | 0.04 |
| $\beta$-thal$_1$-Hb E | 6 | Mean | 7.51 | 4.26 | 25.65 | 61.53 | 17.60 | 28.50 | 51.65 | 27.13 | 0.40 | 0.40 | 0.69 |
| | | S.D. | 1.98 | 1.12 | 5.24 | 5.81 | 1.92 | 3.25 | 11.79 | 11.99 | 0.08 | 0.08 | 0.07 |
| $\beta$-thal$_1$-Hb E/ $\beta$-thal$_2$-Hb E | 6/5 | P | $<.25$ | $<.90$ | $<.25$ | $<.20$ | $<.02$ | $<.25$ | $<.01$ | $<.20$ | $<.50$ | $<.05$ | $<.80$ |

both $\beta$-thal$_1$/Hb E disease and $\beta$-thal$_2$/Hb E disease as being $\alpha_2\beta_2^{26Lys}$ by peptide mapping.

After the globin chain separation on CMC chromatography, the slow $\beta$ abnormal chain corresponding to the $\beta^E$ from both types of $\beta$-thal/Hb E disease were desalted, lyophilized, and digested with trypsin. The tryptic peptide mapping was carried out by high voltage electrophoresis pH 6.5 followed by descending chromatography and compared with a peptide map of $\beta^A$ (Figure 11). Tryptic peptide maps of $\beta^E$ from both types of $\beta$-thal/Hb E disease were identical (Figure 12). The corresponding TpIII (tryptic peptide No.III) of the slow variant was missing but two new peptides: a basic and acidic peptides were observed on the peptide of the slow variant. The tryptic peptide maps were similar to the known $\beta^E$ which glutamic acid residue 26 is replaced by lysine. In summary, the slow variant -Hb E of both types of the disease is identical to Hb E ($\alpha_2\beta_2^{26Lys}$) according to the tryptic peptide mapping.




| Designation | control | HbE trait | β-thal, trait | β-thal, trait | β-thal, /HbE | β-thal, /HbE |
|---|---|---|---|---|---|---|
| Mean | 0.92 | 0.97 | 0.44 | 0.50 | 0.40 | 0.51 |
| S.D. | 0.05 | 0.04 | 0.014 | 0.02 | 0.08 | 0.07 |
| No | 7 | 5 | 7 | 9 | 6 | 5 |

Figure 10. β/α ratio of radioactivity in various genotypes. The mean of each group is indicated by a horizontal line.

Figure 11. Tryptic peptide map of normal β chain
a = Tryptic peptide No.III (TpIII)


Figure 12. Tryptic peptide map of $\beta^E$ chain from both types of β-thal/Hb E disease.
b = Position of missing TpIII
c = acidic peptide
d = basic peptide