Chapter 4

DISCUSSION

Seven healthy subjects with normal hematologic data and normal Hb A_2 and alkali resistant hemoglobin representing normal controls revealed a mean of total radioactivity β/α globin chain ratio of 0.92 ± 0.05 or in otherwords the mean of α/β ratio = 1.08 ± 0.05 . The result was agreeable to the previous study by Pootrakul et al. (1973), in which 6 normal control showed the α/β of 1.08 ± 0.04 . This suggested that the rate of globin chain synthesis in α chain was approximately equal to β-chain.

As already mentioned in Chapter 1, the β-thalassemia (A_2-thalassemia) are divided into two subtypes designated as β-$thal_1$-classical and β-$thal_2$- mild on the basis of interaction with Hb E. The precise genotype designation of the heterozygotes were difficult or impossible because the hematologic data as shown in Tables 2 and 3, of the two groups were very similar including the quantitative Hb A_2 except that the mean of MCH and alkali resistant hemoglobin of the β-$thal_1$ trait were statistically less than that of the β-$thal_2$ trait (Table 4, p.30). The globin chain synthesis studies in reticulocytes of 9 cases of obligatory β-$thal_2$ trait revealed a mean of total radioactivity β/α ratio of 0.50 ± 0.02 (Table 3, p.28). Nine cases of obligatory $thal_1$ trait were also studied and the distribution of the total radioactivity β/α ratio of 7 cases apparently segregated in one group with a mean of 0.44 ±

0.014 . The ratios of P. Do. and K. Pi. were 0.76 and 0.56 which significantly different from that of the first group. This will be discussed latter.

Although the radioactivity β/α ratio of the β-thal$_1$ heterozygote were close to that of β-thal$_2$ they were significantly different with $P < 0.001$. This suggests that the measurement of globin chain synthesis is a sensitive mean to make a precise diagnosis of the heterozygote for β-thal$_1$ or β-thal$_2$, inspite of the similar hematologic findings.

The means of specific activity β/α ratio of the β-thal$_1$ and β-thal$_2$ trait were 0.50 ± 0.03 and 0.57 ± 0.05 respectively. Both radio-activity and specific activity β/α ratio of the two groups were markedly decreased. This results are agreeable to the previous studies of hemoglobin synthesis in β-thalassemia trait by Bank and Marks, (1966) and Friedman et al. (1972). This indicated that β globin chain in peripheral blood of β-thalassemia trait is decreased in synthesis. Since the specific activity β/α ratio in bone marrow studies (the bone marrow contains mostly nucleated red cells) of heterozygous β-thalassemia was equal to one inspite of the specific β/α ratio of 0.4 - 0.5 in reticulocytes (Schwartz, 1970; Kan et al. 1972). This implied that there was compensatory synthesis of β chain in the nucleated red cells in bone marrow in order to response to the presence of the β-thalassemia defect by means of an increased synthesis of mRNA. The decreased β chain synthesis in reticulocytes may be due to a rapid break down of

unstable and defective mRNA. The final verification of the hypothesis regarding the rates of production and destruction of mRNA awaits the development of methods for accurate quantitation of hemoglobin mRNA in red cells.

It has been described that the hematologic data including Hb A_2 of an obligatory double heterozygote for α-thalassemia and β-thalassemia was similar to that of a simple β-thalassemia trait (Wasi et al. 1969). Thus the precise diagnosis must depends on the genetic evidence and the globin chain synthetic studies. The specific activity β/α chain ratio in a double heterozygote for α-thalassemia and β-thalassemia was evidently increased and near to one (Knox-Macanley et al. 1972). The two obligatory β-thal$_1$ traits, P. Do. and K. Pi. revealed the radioactivity β/α of 0.76 and 0.59, which were higher than that of the β-thal$_1$ trait alone. It is most likely that both probably inherit an extra α-thalassemic gene. This would readily explain the decreased α-chain synthesis resulting the increase in β/α ratio. The specific activity β/α ratio in P. Do. was 0.98 which was similar to the normal control (Table 1, p.23). This was consistant with the double heterozygote for α-thal and β-thal.

Although the radioactivity β/α of K. Pi. being 0.59 which was excluded from the mean of β-thal$_1$ trait but its specific activity β/α ratio of 0.56 was appearently less than that of P. Do . However the inheritance of thalassemia gene in K. Pi. was not entirely ruled out since the α-thalassemic gene was found to segregate in

one of his brother, subject P. Di. (Table 5, p.32).

Five heterozygous Hb E from the relatives of the families of both β-thal$_1$/Hb E disease and β-thal$_2$/Hb E disease revealed a mean of hemoglobin concentration of 12.38 $\pm$ 1.37 . The hematologic data appeared to be within normal limits except the MCH was slightly decreased. A mean of quantitative Hb E was 27.5 % which was agreeable to the previous studies (Wasi et al. 1969). The means of radioactivity β/α and β^E/α were 0.60 $\pm$ 0.05 and 0.37 $\pm$ 0.03 respectively, thus the total radioactivity $\beta+\beta^E/\alpha$ was 0.97 $\pm$ 0.04 that was close to one like the value of the control. Although the β^E was less synthesized than the β^A, the specific activity β^A/α and β^E/α were 0.98, and 0.99 (Table 5, p.32). This suggested that the relative non radioactive pool of either β^A or β^E was approximately equal to that of the α chain. The subject P. Di. (Table 5), a brother of subject K. Pi (Table 2, p.26) had hemoglobin level 9.9 gm% which was less than the five heterozygous Hb E. Furthermore the quantitative Hb E of P. Di. was 21 %. This strongly indicated that P. Di had an extra α-thal$_1$ gene besides the abnormal Hb E trait since it has been known that the α-thal$_1$ partially suppresses the β^E synthesis (Tuchinda et al. 1964; Wasi et al. 1967). The radioactivity β/α and β^E/α of the patient P. Di. were also consistent with the presence of α-thalassemia$_1$ gene. Therefore it is reasonable to assume that the P. Di was a double heterozygous state for α-thal$_1$ and Hb E.

As previously described, either β-thal$_1$ or β-thal$_2$ trait will give rise to clinical disorders of chronic hemolytic anemia upon the

interaction with Hb E. In general, the β-thal$_1$/Hb E disease with hemoglobin types of E+F presents clinical manifestations more severe than that of the β-thal$_2$/Hb E disease (Hb types of E+F+A). Since it was evident by this investigation that β-thal$_2$ gene suppressed normal β chain synthesis less than β-thal$_1$ trait, this would, at least in part, explain the presence of Hb A and less severity in clinical manifestation of the β-thal$_2$/Hb E disease. The means of hematologic data of 5 patients with β-thal$_2$/Hb E disease (Table 7 p.38) showed that only the MCH and quantitative Hb E were significantly higher than the values of the 6 patients with β-thal$_1$/Hb E disease (Tables 6 p.34 and 8 p.39). The mean of radioactivity total β chain/α chain; $\beta+\beta^E/\alpha$ in β-thal$_2$/Hb E, and β^E/α (no β chain) in β-thal$_1$/Hb E disease revealed statistic difference ($P < 0.05$) (Table 8).

Hemoglobin E ($\alpha_2\beta_2^{26Glu\rightarrow Lys}$) has been known to be prevalent in South East Asia esspecially in Thailand. The practical designation of the variant is based on the characteristics on the starch gel electrophoresis only. However, a mutant known as Hb E Saskatoon ($\alpha_2\beta_2^{22Glu\rightarrow Lys}$) found in a Scottish family is very similar to the Hb E ($\alpha_2\beta_2^{26Glu\rightarrow Lys}$) in terms of the mobility on starch gel electrophoresis, the amino acid alteration and mutation located at β TpIII, (Tryptic peptide No.3 of β chain) (Vella et al. 1967). Since the mutation Hb E Saskatoon might possibly occur among the South East Asia population and might possibly interact with β-thalassemia resulting in different clinical manifestations

from Hb E ($\alpha_2\beta_2^{26Glu\rightarrow Lys}$), the characterization of Hb E ($\alpha_2\beta_2^{26Glu\rightarrow Lys}$), based solely on electrophoretic mobility and statistic evidence could give a spurious diagnosis in some cases unless the biochemical investigation was carried out. For these reasons, the slow β chain corresponding to the β^E from both β-thal$_1$/Hb E disease and β-thal$_2$/Hb E disease were isolated and studied the peptide mapping (Figure 12 p.42). The results suggested that they were identical to the Hb E ($\alpha_2\beta_2^{26Glu\rightarrow Lys}$) which were common in Thailand. The result also indirectly supported the hypothesis that the different clinical manifestations of β-thal$_1$/Hb E disease and β-thal$_2$/Hb E disease was most likely due to the different expressivity of the β-thal$_1$ and β-thal$_2$ gene.

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