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HEMOGLOBIN BIOSYNTHESIS IN RETICULOCYTES OF A HEMOGLOBIN M HYDE PARK PATIENT

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Abstract

Reticulocytes were collected from the venous blood of a Hb M Hyde Park (Hb M HP: $\alpha_2{}^A\beta_2{}^M$; β^M: β 92 His→Tyr) patient, and they were incubated with Lingrel-Borsook culture medium containing ^{3}H-Leu for hemoglobin biosynthesis. Hemolysate containing whole biosynthesized hemoglobins was prepared from these reticulocytes. Hemoglobins were separated individually by cellulose acetate membrane electrophoresis of metHb type hemolysate and by DEAE-cellulose column chromatography of oxyHb type hemolysate. Globins were prepared from hemoglobins and they were divided into the α and the non-α chains by urea dissociation cellulose acetate membrane electrophoresis and urea CM-cellulose chromatography. The isolated fractions of hemoglobins and globin chains thus collected were measured for their radioactivities in a liquid scintillation counter.

It was shown that both metHb M HP and oxyHb HP (oxyHb M Hyde Park with deletion of one molecule of heme from its abnormal β^M chains) incorporated ^{3}H-Leu 2.3 times as much as the relevant metHb A and oxyHb A did. The specific radioactivity ratio of globin chains (β/α) was only 0.45 : 1.00 (β^M/α^A) in metHb M HP, whereas it was 1.00 : 1.00 (β^A/α^A) in metHb A.

In the case of globin chains prepared from the oxyHbs separated by urea CM-cellulose column chromatography of the hemolysate the radioactivity ratio β^M/β^A was 1.22 : 1.00, being approximately equal to 1 : 1. This is interpreted that the abnormal β^M chain is produced at a rate nearly the same as or slightly higher than that of the β^A chain. On the contrary, the radioactivity ratio of non-α chain/α chain ($[\beta^A+\beta^M]/\alpha^A$) was significantly smaller than unity, namely 0.45 : 1.00. This suggests that production of the α chain is enhanced over the level of the non-α chain ($\beta^A+\beta^M$), and there is an excess of the α chain over the non-α chains, resulting in formation

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of free α chain pool in the cytosol of reticulocytes of the Hb M HP patient. Exchange of α chain between the molecules of pre-existing Hb M HP~Hb HP and the free α chain pool is thought to be significantly large on account of spontaneous dissociation of the molecules of the abnormal unstable hemoglobins (Hb M HP~Hb HP; probably, $\alpha_2{}^A\beta_2{}^M \rightarrow \alpha_2{}^A + \beta_2{}^M$).

INTRODUCTION

In 1966, Shibata and his associates[1] found a family with congenital cyanosis living in Akita Prefecture, Japan, and discovered a new abnormal hemoglobin belonging to Hb M which they designated Hb M Akita. However, the structural analysis of this hemoglobin disclosed that it was identical with Hb M Hyde Park (Hb M HP ; $\alpha_2{}^A\beta_2{}^M$; β^M : β 92 His $\rightarrow$ Tyr)*, which had been described by Heller et al.[2] in the United States of America shortly earlier in the same year.

The hemolysate from the cyanostic patients of this family contained two abnormal hemoglobins, Hb M HP which was chocolate-colored and Hb Hyde Park (Hb HP) which was red in color[3].

Cellulose acetate membrane electrophoresis (pH 8.6) of oxyHb type hemolysate yielded three bands, the thickest one which was composed of Hb A and Hb M HP, the medium one which concerned with Hb HP and the narrowest one which was Hb A_2, lining up from the anode to the cathode. These were all alike in color, being red, although the thickest band appeared to be a little blackish[3].

When the metHb type hemolysate was electrophoresed on cellulose acetate membrane (pH 7.3), metHb M HP was separable as a chocolate-colored band discretely to the anode side of the metHb A band, and it constituted 23~32 per cent of the whole hemoglobins of the hemolysate[3].

Shibata and Iuchi[4] showed that Hb M HP, which was by itself unstable and chocolate brown in color, was spontaneously transformed into another unstable reddish hemoglobin, namely Hb HP or $\alpha_2{}^{A(1h)}\ \beta^{M(1h)}\ \beta^{M(0h)}$**, by deletion of the heme from one of its abnormal β^M chains.

As perceived from the molecular instability of these hemoglobins (Hb M HP and Hb HP) the cyanosis inherent in Hb M Hyde Park disease is associated with a mild to moderate anemia which is hemolytic in character. Substantial evidence for accelerated hemolysis in this hemoglobinopathy was afforded by

*Notations α^A and β^A refer to the α and the β chains of normal adult hemoglobin ($\alpha_2{}^A\beta_2{}^A$). Notation β^M indicates the abnormal β chain of Hb M Hyde Park.

**1h shows that the relevant chain holds its heme in its proper site, and 0h represents that the chain has lost its heme.

Shibata and his associates who studied ferrokinetics, measurement of erythrocyte life span by ^{51}Cr labelling and ^{99m}Tc myeloscintigraphy[5]. They demonstrated ineffective erythropoiesis in the enlarged spleen[5].

The propositus (aged 59, at present) of the Hb M Akita family has been visiting the Kawasaki Medical School Hospital repeatedly for his health examination for these 14 years. On the occasion of his last visit in this year, we collected his venous blood for the purpose of studying hemoglobin biosynthesis in reticulocytes, and got a result which was unexpected to us. There was biosynthetic unbalance between the α^A chain and the non-α chains ($\beta^A + \beta^M$). It is, therefore, thought that this altered pattern of hemoglobin biosynthesis may be contributing to the acceleration of hemolysis in Hb M HP hemoglobinopathy to a certain extent. The present paper aims to describe the results obtained in our hemoglobin biosynthesis experiments.

MATERIALS AND METHODS

1) Peripheral blood of the patient was examined by standard hematological method[6].

2) Hemoglobin biosynthesis[7] : Venous blood of the patient was washed with physiological saline and all the red cells were collected. Two ml of the red cells were ultracentrifuged (15,600 × G, Eppendorf Model 5412) in a cold room for 15 minutes and the erythrocyte layer rich in reticulocytes (0.2 ml, upper layer of the red cells) was aspirated, mixed with the Lingrel-Borsook culture medium (1.3 ml) containing ^3H-Leu (100 μCi) and incubated at 37°C for 2 hours for hemoglobin biosynthesis.

3) Preparation of hemolysate[7] : The whole reticulocytes layer holding biosynthesized hemoglobins was repeatedly washed with physiological saline to remove the culture medium.

To the washed reticulocyte layer was added 0.1% saponin solution in proportion of 1.0 : 2.0, and mixed. The hemolyzed solution thus obtained was passed through a Millipore filter (Millipore Co.; pore size, 1.2 μm) to obtain a clear hemolysate.

4) Separation of hemoglobins : (a) Separation of metHb A from metHb M HP……MetHb type hemolysate was prepared by addition of a small amount of concentrated (5%) potassium ferricyanide solution to the hemolysate and it was electrophoresed on cellulose acetate membrane (pH 7.3)[8,9]. (b) Separation of oxyHb A, oxyHb HP and Hb A_2 …… DEAE-cellulose (DE 52, Whatman Ltd.) column chromatography (Abraham et al.[10]) (Na^+ ion gradient, 5∼10 mM → 50 mM ; NaCl-glycine-KCN buffer solution [pH 7.8] ; flow rate, 0.2 ml/min ; fraction size, 1.2 ml/tube) was employed for this purpose.

5) Hemoglobin components in the eluates from the electrophoresed cellulose acetate membrane and those of eluates fractionated by column chromatography were concentrated to levels appropriate for the relevant tests by vacuum aspiration method using collodion bags[11].

6) Globin chain separation of biosynthesized hemoglobin : Urea dissociation CM-cellulose chromatography (Clegg et al.[12]) and urea dissociation cellulose acetate membrane electrophoresis (Ueda et al.[13]) were employed.

7) Measurement of the concentrations of hemoglobins and globin chains : The absorbances of the solutions containing the respective fractions were measured in a spectrophotometer at 415 nm for hemoglobin concentration (A_{415}) and at 280 nm for globin chain peptide concentration (A_{280}).

8) Determination of radioactivities (dpm) of hemoglobins and globin chains[14] : (a) Solution of metHb eluted and concentrated by the procedure described in 4-(a) (1 ml) or fractionated solutions of a globin chain obtained by the procedure of 6) (1 ml) were poured into individual vials, into which 6 ml aliquots of Scintisol (Dojin Chemical Co.) had been introduced. One ml of distilled water was added to each, mixed, and shaken sufficiently. The radioactivities (dpm) of the mixture were read in a liquid scintillation counter. (b) A half ml fractions of oxyHb solutions obtained by DEAE-cellulose chromatography (described in 4-(b)) were poured into individual vials. To each of them were added 0.2 ml of 30% of H_2O_2 and 0.4 ml of 60% perchloric acid and mixed. The mixtures were warmed in an oven at 60°C. After 30 minutes, 8 ml of scintisol was added to the vials and their radioactivies (dpm) were measured in a liquid scintillation counter.

RESULTS

The patient was, allegedly, enjoying healthy daily life but cyanosis was apparent in his countenance. Hematological examination revealed moderate hemolytic anemia (RBC 2.96×10^{12}/l, Hb 9.1 g/dl, VPRC 28.3 % MCV 95.6 fl, MCH 30.7 pg, reticulocyte count 1.8%, isopropanol precipitation test of the hemolysate positive within 20 min, and hemoglobin composition Hb A : Hb M HP : Hb A_2 : Hb F = 59.6 : 38.0 : 2.6 : 0.8).

MetHb M HP was separated discretely from metHb A by cellulose acetate membrane electrophoresis (pH 7.3) of metHb type hemolysate as shown in Fig. 1. The specific radioactivity (dpm/A_{280}) ratio of metHb A : metHb M HP was 0.44 : 1.00.

Dissociation of the globins prepared from the metHbs isolated by this electrophoresis into their non-α and α chains disclosed a specific radioactivity ratio of β^A/α^A = 1.00 : 1.00 in metHb A, indicating equal incorporations of

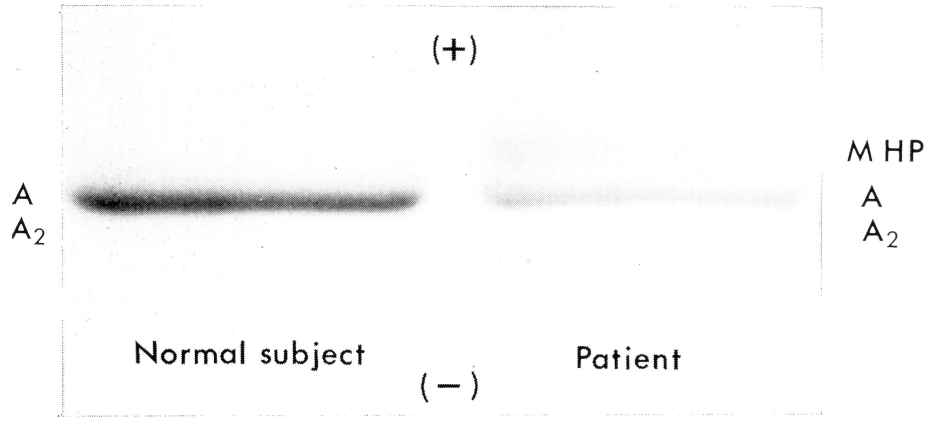

Fig 1. Cellulose acetate membrane electrophoresis (pH 7. 3) of the met-hemoglobinized hemolysate prepared from reticulocytes subjected to hemoglobin biosynthesis (in ^{3}H-Leu containing culture medium). A : metHb A ; A_2 : metHb A_2 ; M HP : metHb M Hyde Park.
Radioactivities (dpm/A_{280})……A 26111, M HP 59983 (A : M HP=0.44 : 1.00). Radioactivities (specific activities) of the globin chains prepared by urea dissociation cellulose acetate membrane electrophoresis from metHb A (α^A and β^A) and metHb M HP (α^A and β^M).
α^A 20632 ; β^A 20643 (β^A : α^A =1.00 : 1.00).
α^A 52565 ; β^M 23567 (β^M : α^A =0.45 : 1.00).

^{3}H-Leu into the α^A and the β^A chains. On the contrary, the radioactivity ratio β^M/α^A was 0.45 : 1.00 in metHb M HP. This suggested that distinctly larger amount of ^{3}H-Leu was present in the α^A chain than in the β^M chain.

As shown in Fig. 2, chromatographic separation of the oxyHb type hemolysate on DEAE-cellulose column in Na^+-glycine-KCN gradient buffer solution (pH 7.8) yielded Hb A_2 (peak P_1, Na^+ 10～12 mM), Hb HP (peak P_2, Na^+ 15 mM) and Hb A (peak P_3, Na^+ 30 mM) in accordance with the concentrations of Na^+ ion. However, Hb M HP was not eluted, but remained on its top layer, being adsorbed to the DEAE-cellulose.

Total radioactivity incorporated into Hb HP (peak P_2) was larger than that taken into Hb A (peak P_3), showing a ratio of P_3 : P_2=0.44 : 1.00. The evidence for the identity of peak P_2 with Hb HP was afforded by the estimation of heme content. The heme content of the hemoglobin of peak P_2 was 75 per cent of Hb A, being just at the same level as that of Hb HP=$\alpha_2{}^A\beta^{M(1h)}\beta^{M(0h)}$, which lost one out of four hemes per molecule.

Fig. 3 illustrates the result of urea dissociation CM-cellulose chromatography of whole globins prepared from the hemolysate containing biosynthesized hemoglobins (mixture of Hb A, Hb M HP, Hb HP etc.). It is apparent from

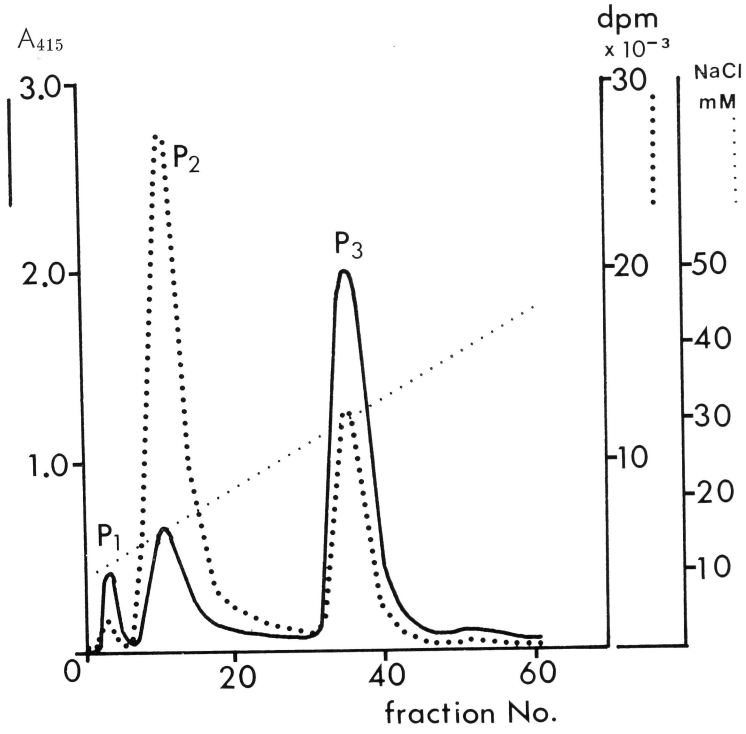

Fig. 2. DEAE-cellulose (DE 52) column chromatography (Na^+ 10 mM~50 mM gradient) of the fresh hemolysate (oxyHb) prepared from reticulocytes subjected to hemoglobin biosynthesis (in ^{3}H-Leu containing culture medium). P_1 (Na^+ 10~12 mM)·····Hb A_2 ; P_2 (Na^+ 15 mM)····· Hb HP ; P_3 (Na^+30mM)·····Hb A. (Hb M HP remains on the column being adsorbed to DE 52).
Radioactivities (total counts) : P_2 146600 ; P_3 65200. (P_3 : P_2 =0.44 : 1.00)
Heme contents : P_2 75% ; P_3 100%.
Aged hemolysate (allowed to stand at 8°C for 14 days after preparation) yield 4 effluent peaks due to degradation of Hb HP : p (Na^+ 5 mM), probably Hb HP undergoing degeneration whose heme content was 61%, P_1' (Hb A_2), P_2' (Hb HP) and P_3' (Hb A). Their total radioactivities were 14206(p) ; 76600 (P_2') ; 56400(P_3'). P_3' : (p+P_2') was 0.62 : 1.00.

this figure that the α chain and the non-α chains, namely α^A, β^A and β^M, are individually separated, and the α^A chain is higher in total radioactivity than the sum of β chains (β^A and β^M), giving a value of 0.45 : 1.00 for $(\beta^A+\beta^M)/\alpha^A$ ratio. The β^M/β^A specific radioactivity ratio was 1.22 : 1.00.

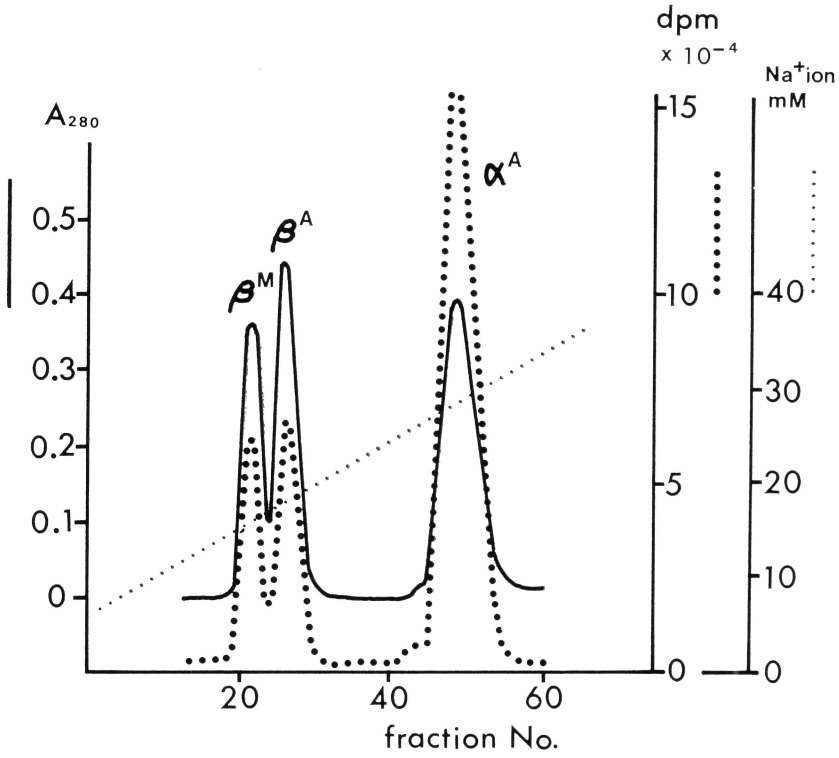

Fig. 3. Urea dissociation CM-cellulose chromatography of the hemolysate prepared from reticulocytes subjected to hemoglobin biosynthesis (in ^{3}H-Leu containing culture medium), possessing Hb A ($\alpha_2^A\beta_2^A$) and Hb M HP ($\alpha_2^A\beta_2^M$) together. β^M (Na^+ 16 mM), β^A (Na^+ 19.2 mM) and α^A (Na^+ 28~32 mM). Radioactivities (dpm/A_{280}) : α^A 420557 ; β^A 218475 ; β^M 266859.
α^A : β^A : β^M=1.58 : 0.82 : 1.00, β^A : α^A=0.52 : 1.00, β^M : α^A=0.63 : 1.00, β^M : β^A=1.22 : 1.00.
Total radioactivities (dpm) : α^A 768357 ; β^A 216395 ; β^M 130939.
α^A : β^A : β^M=5.87 : 1.65 : 1.00.

DISCUSSION

The experimental results described above will be analyzed and interpreted in the following way.

In the biosynthesis experiment all the globin chains composing the molecules of Hb A, Hb M HP and Hb HP (α^A, β^A and β^M) are alike in the demand of ^{3}H-Leu for building up their own molecules. Generation of one molecule is

completed by every incorporation of 18 residues of ^{3}H-Leu from the culture medium. Accordingly, the total ^{3}H-Leu radioactivity count (dpm) of each of the separated chain fractions represents the amount of the relevant chain which has been produced for the two hour period of incubation, and the specific radioactivity count (dpm/A_{280}) will indicate the quantity of the freshly produced chain per unit amount of pre-existing relevant hemoglobin, or relative velocity of incorporation into the chain if the former (freshly produced) is distinctly small in amount as compared with the latter (pre-existing).

1° The specific and total radioactivities of the abnormal hemoglobin fractions (metHb M HP [$\alpha_2^A\beta_2^M$], oxyHb HP [$\alpha_2^A\beta^{M(1h)}\beta^{M(0h)}$]) were larger than those of the normal hemoglobin fractions (metHb A [$\alpha_2^A\beta_2^A$], oxyHb A [$\alpha_2^A\beta_2^A$]) (Figs. 1 and 2). This indicates that ^{3}H-Leu was incorporated into the abnormal hemoglobins at higher rate than into the normal hemoglobin. Impressively, the ^{3}H-Leu incorporation ratios of Hb M HP : Hb A and Hb HP : Hb A were the same (1.00 : 0.44).

2° The specific radioactivity ratio β^A/α^A was 1.00 : 1.00 in metHb A fraction, but the radioactivity ratio β^M/α^A in metHb M HP (and oxyHb HP) was 0.45 : 1.00. This shows a well balanced utilization of freshly generated globin chains, α^A and β^A, in the formation of Hb A molecules. By contrast, there is an unbalanced association of the newly created globin chains, α^A and β^M, for building the molecules of the abnormal hemoglobin (Hb M HP). Freshly produced α^A chains are included in the molecules of Hb M HP more abundantly than the newly generated β^M chains.

3° The analysis of labelled globin chains in the hemolysate containing all of the biosynthesized hemoglobins gave specific radioactivity ratio $R=\alpha^A:\beta^A:\beta^M$ was 1.58 : 0.82 : 1.00 (Fig. 3). From this figure the β^M chain is produced 1.22 times as rapidly as the β^A chain. It is therefore conceived that Hb M HP ($\alpha_2^A\beta_2^M$) is generated with production rate 1.22 fold as fast as Hb A ($\alpha_2^A\beta_2^A$). If hemoglobin composition of the hemolysate is guessed from this production rates on the assumption that all the freshly generated β chains are used for the production of normal and abnormal hemoglobins effectively and completely, a ratio of Hb A ($\alpha_2^A\beta_2^A$) : Hb M HP ($\alpha_2^A\beta_2^M$) = 0.82 : 1.00 or Hb A : Hb M HP = 1.00 : 1.22 will be obtained. Actually, the ratio of Hb A : Hb M HP in hemolysate by direct estimation was 59.6 : 38.0 or 1.00 : 0.64. The difference between the values of these ratios, the guessed and the actually estimated, will be suggestive of loss of the abnormal hemoglobin after its production due to its molecular instability.

4° The total radioactivity ratio $R'=\alpha^A:\beta^A:\beta^M$ was 5.87 : 1.65 : 1.00 (Fig. 3). This ratio refers to the quantities of the relevant globin chains which

was produced and really existed in the reticulocyte at the end of 2 hours' incubation in culture medium for hemoglobin biosynthesis. The amount of α^A chain (5.87) is larger than the sum of those of the β^A chain (1.65) and the β^M chain (1.00). There was α^A chain 2.22 times as much as the non-α chains, for $\alpha^A : (\beta^A + \beta^M)$ was $5.87 : (1.0 + 1.65) = 2.22 : 1.00$.

It is apparent that the α^A chain was present in excess of the non-α chain. The excess of the α^A chain which is free and not in association with its counterpart (the non-α chains) is therefore expected to form a pool of free α chain.

5° α chain exchange between the free α chain pool and the molecules of the pre-existing abnormal hemoglobins (Hb M HP, Hb HP).

Let the hemoglobin chains without radioactivity which were contained in reticulocyes prior to the commencement of biosynthesis experiment be denoted by α^A, β^A and β^M, and the freshly biosynthesized chains with radioactivity which were made of ^{3}H-Leu by α^{A*}, β^{A*} and β^{M*}. It is presumed that, in hemoglobin biosynthesis experiment, as soon as new globin chains, α^{A*}, β^{A*} and β^{M*}, are produced with ^{3}H-Leu in reticulocytes of a Hb M HP patient they are spontaneously united to form Hb A ($\alpha_2^{A*}\beta_2^{A*}$) and Hb M HP ($\alpha_2^{A*}\beta_2^{M*}$) by associations of α^{A*} with β^{A*} and α^{A*} with β^{M*}, respectively. However, in this instance, the α^{A*} chain is supplied in excess of the β^{A*} and the β^{M*} chains, if an intracellular surplus pool of α^{A*} has been existing. It is supposed that the association of α chain with β chain in a Hb A ($\alpha_2^A\beta_2^A$) molecule is more stable than that of α chain with abnormal β^M chain in a Hb M HP ($\alpha_2^A\beta_2^M$) molecule. If this is truly the case, the α^{A*} chain which has been produced anew and put into the surplus α^{A*} chain pool will preferentially attack the pre-existing non-radioactive hemoglobin molecules $\alpha_2^A\beta_2^M$ and will intrude into them in substitution for the non-radioactive α^A chain. If the molecules of the pre-existing non-radioactive abnormal hemoglobins are dissociated in a way as $\alpha_2^A\beta_2^M \rightarrow \alpha_2^A + \beta_2^M$, and free β_2^M chains is present in free state, the association of α_2^{A*} with β_2^M will be easier. The radioactive Hb M HP ($\alpha_2^{A*}\beta_2^M$) molecules which are produced in these ways will increase in number. The unbalancedly larger accumulation of radioactivity in the α^A chains than in the β^M chains that was seen by the analysis of Hb M HP ($\alpha_2^A\beta_2^M$) anb Hb HP ($\alpha_2^A\beta^{M(1h)}\beta^{M(0h)}$), as evidenced by the radioactivity ratio of $\beta^M : \alpha^A = 0.45 : 1.0$ (Fig. 2 and 3) will, thus, be understood as one of the results of the α chain exchange between the non-radioactive pre-existing Hb M HP ~ Hb HP molecules and the radioactive surplus α chain pool.

In addition, it may be germane to suppose that increase in production of $\alpha_2^{A*}\beta^M$ will cause consumption of α_2^{A*}, and the consumption of α_2^{A*} will induce accelerated production of the α^A chain. Excessive generation of the α^A

chain over that of the sum of β chains ($\beta^A+\beta^M$) may be accounted for in this way.

White and Brain[4] made a representative investigation on *in vitro* biosynthesis of an unstable hemoglobin in 1970. They collected reticulocytes from the peripheral blood of a patient heterozygous for Hb Köln $\alpha_2{}^A\beta_2{}^{Köln}$ (β 98 Val →Met), an unstable hemoglobin of β chain anomaly and incubated them in a culture medium containing ^{3}H-Leu for hemoglobin synthesis.

They demonstrated that (1) the abnormal $\beta^{Köln}$ chain was produced at slower rate than the normal β^A chain, (2) the α^A chain was generated in excess of the total sum of the non-α chains ($\beta^A+\beta^{Köln}$), (3) there was a considerably large pool of free α chain in the cytosol of reticulocytes, and (4), in consequence of the expansion of free α chain pool, exchange took place between the free radioactive (labelled) α chain pool and the non-radioactive (unlabelled) α chains of the unstable abnormal hemoglobin due to molecular dissociation after the lysis of the cell and during subsequent separative procedure, resulting in the α chain radioactivity disproportionately large to the radioactivity of the abnormal $\beta^{Köln}$ chain in the abnormal hemoglobin fraction. By contrast, such an exchange of the α chain was not demonstrable in the normal hemoglobin fraction, because it was more stable in molecular structure than the abnormal hemoglobin.

The same aspects were observed also by other investigators. Relatively excessive synthesis of α chain (expansion of free α chain pool) and exchange of the α chain between the molecules of the pre-existing abnormal hemoglobin and the free α chain pool containing the labelled (freshly produced) α chains were observed not only in the unstable hemoglobins of β chain anomaly of normal synthetic rate (Hb Köln[14] β 98 Val→Met, Hb Hammersmith[15] β 42 Phe→Ser, Hb Bristol[16] β 67 Val→Asp, Hb Shepherd's Bush[17] β 74 Gly→Asp, Hb Sabine[18] β 91 Leu→Pro), but also found in those of subnormal synthetic rate (Hb Riverdale-Bronx[19] β24 Gly→Arg, Hb Borås[17] β 88 Leu→Arg) and of supernormal synthetic rate (Hb Gun Hill[20] β 91→95 deleted).

Entirely the same feature of hemoglobin biosynthesis was noted in β-thalassemia which is characterized by suppression of the β^A chain and absence of the production of abnormal hemoglobins.[21,22,23,24]

As to the hemoglobin biosynthesis in the reticulocytes of patients with Hb M diseases few communications are available from the literature. Hb M Saskatoon (β 63 His→Tyr) was studied by Baine and his associates[25] recently. This abnormal hemoglobin was slightly unstable, and its abnormal β^M chain was synthesized a little more abundantly than the normal β^A chain (specific radioactivity ratio was 1.12～1.18). The production of α^A chain was proportionate

to or slightly more than that of the non-α chains ($\alpha^A/[\beta^A+^M]$ was 0.97～1.14). The free α chain pool was not mentioned by them.

In conclusion it is stated that hemoglobin synthesis in our Hb M Hyde Park patient was identical in pattern with that seen in hemoglobinopathies of unstable hemoglobins of β chain anomaly with supernormal synthetic activity. It was different from the hemoglobin synthesis in β-thalassemia in spite of the presence of some analogous features between them (excessive production of the α^A chain over the β^A chain, expansion of free α chain pool and decreased non-α chain/α chain synthetic ratio), because in Hb M HP disease, unlike in β-thalassemia, (1) the production of β chains (β^A and β^M) is not suppressed to subnormal level, and (2) the α chain exchange takes place only between the free α chain pool and the abnormal hemoglobin Hb M HP ($\alpha_2^A\beta_2^M$), and normal hemoglobin Hb A ($\alpha_2^A\beta_2^A$) is not involved in it.

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