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MEDICAL GRAND ROUNDS

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THE THALASSEMIA SYNDROMES

Case #1. [redacted] This 15-year old [redacted] boy was referred to [redacted] with the diagnosis of severe anemia and congestive heart failure. The patient had been of small stature throughout his life and had had impairment of his physical capacity. Three months before he had noted increasing weakness, malaise and the onset of peripheral edema. He had seen a private physician who had found a hemoglobin of 2 grams% and cardiomegaly. The patient was transfused and referred for diagnosis.

An aunt was known to be anemic. Parents were unavailable for examination.

Physical examination: B/P 105/60, P 100, R 24.

An extremely small, [redacted] boy with large head with the typical thalassemic contour and facies. Weight 57 lbs. There was neck vein distension at 70°, cardiomegaly, S3 gallop, hepatosplenomegaly, and peripheral edema. Laboratory data: Hgb. 5.1 gm%, Hct. 16.5%; WBC and platelets were normal. Peripheral smear showed two cell populations (due to recent transfusions). The abnormal cells showed marked hypochromia, microcytosis, poikilocytosis and several nucleated RBC's were present. FBS 72 mgm%, Bilirubin 2.4 (0.2 direct) mgm%, Alkaline phosphatase 12 B.U., Albumin 4.6 gm%, Circ. time 11 seconds, Venous pressure 20 cm., Serum Iron, 213 microgm%; Hemoglobin electrophoresis showed no abnormal hemoglobin bands. Alkali denaturation for Hgb. F was not performed because of the recent transfusion therapy. Bone marrow was refused. Chest x-ray showed generalized cardiomegaly. Skull films were considered diagnostic of thalassemia major. Course: Patient was treated with bed rest, salt restriction and folic acid. Hgb. stabilized and the congestive failure became compensated. Comment: Despite the inability to confirm high hemoglobin F values due to recent transfusions, the physical examination, peripheral blood findings and radiologic abnormalities confirmed the diagnosis of thalassemia major. A maternal aunt was shown to have a mild hypochromic anemia consistent with thalassemia minor.

Case #2. 78-year old [redacted] woman was admitted to [redacted] for treatment of acute gastroenteritis. She gave a history of known anemia for at least 17 years. Liver therapy in past had been without response. There was a strong family history of anemia including the patient's mother, brother and two daughters.
Physical examination: There were no abnormalities except for some mental confusion.
Laboratory data: Hgb. 9.0 gm%. Peripheral smear revealed hypochromia, anisocytosis and poikilocytosis with target cells and basophilic stippling. Reticulocyte count was 2%; Serum Iron 260 micrograms% with TIBC 388 micrograms%. Erythrocytes showed increased resistance to osmotic lysis. Bone marrow revealed erythroid hyperplasia and increased iron stores. Hgb. electrophoresis revealed no abnormal hemoglobins. Hgb. F was 4%, Hgb. A2 was 4.5% (normal <3%).

Course: No specific therapy for the anemia was recommended. The patient was warned against taking iron therapy.
Comment: This patient had an asymptomatic, mild anemia with all of the features of thalassemia minor of the beta (high A2) type.

Definition

The thalassemias are a group of hereditary anemias of heterogeneous clinical expression resulting from the quantitative reduction in the synthesis of one or more of the normal globin polypeptide chains.

General References

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The structure of hemoglobin has been the most thoroughly elucidated of any protein. The exact amino acid sequences of the several normal polypeptide chains have been determined as well as the close three dimensional structural relationships of the chains to each other and the specific attachments of their heme groups(5). All of the normal human hemoglobins are composed of four polypeptide chains which make up the globin or protein portion of the molecule. Each chain is associated with a single heme molecule. Each of the polypeptide chains is composed of a series of typical alpha helices which fold in a characteristic shape at non-helical areas. The four chains form a definite three dimensional interaction with the shape of an irregular tetrahedron. The heme groups are situated in surface pockets in a specific structural relationship with each chain and to each other.

From fetal to adult life, four normal hemoglobins are recognized. These have been named Gower-II, fetal (F), A and A2. Each of these hemoglobins has in common a pair of polypeptide chains of identical structure called alpha (α) chains. The four hemoglobins differ by virtue of the non-alpha pair of chains which are always identical and have been named epsilon (ε), gamma (γ), beta (β) and delta (δ) respectively. Table I shows the structural composition and relative levels of these hemoglobins.

Table I Normal Hemoglobins

Hemoglobin	Structural Formula	% at birth	% after age 2 yrs.
Gower II*	$\alpha_2\varepsilon_2$	0	0
Fetal (F)	$\alpha_2\gamma_2$	60-90	<2
A **	$\alpha_2\beta_2$	10-40	>95%
A2 **	$\alpha_2\delta_2$	0-1.5	1.5-3.5

* chains disappear after 1st trimester.
** Values vary depending on technique employed for determination.

It is believed from comparison of the amino acid sequences of these polypeptide chains that all arose as gene duplications and further mutations from a single ancestral polypeptide structural gene. Thus, in terms of identical amino acids at the same locations, the α and β chains have a 42% correspondence; the α and γ 39%; the β and γ 71%; and the β and δ 93%.

It is apparent from the study of the abnormal hemoglobin states that the three dimensional integrity of the molecules and the function of the heme groups in oxygen transport and exchange are extremely dependent upon the specific amino acids present in certain key areas of the polypeptide chains(6). With only a few exceptions, all of the abnormal hemoglobins so far described differ from the normal hemoglobins in having a single amino acid substitution in one type of the globin polypeptide chains. In most instances, the substitution mainly results in a charge difference in the new molecule, but no significant functional difference. However, in certain cases, when the amino acid substitution site and type is proper, a critical abnormality in the structural integrity and/or function of the new molecule occurs.

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HEMOGLOBIN SYNTHESIS

Hemoglobin synthesis has been studied extensively in cell suspension and cell-free systems and in general follows the usual pattern demonstrated in unicellular and mammalian systems. Certain features are of importance and will be commented upon. DNA dependent messenger RNA synthesis for the structural polypeptide chains occurs almost entirely in the early nucleated red cell and ceases at about the polychromatophilic stage. However, globin synthesis continues into the reticulocyte stage where polysomes can still be demonstrated. Thus the mRNA for the polypeptide chains appears to be of much greater stability (hours to days) as opposed to that in most protein synthesizing systems (minutes)(7).

There is a very striking degree of balance demonstrated in normal hemoglobin synthesis which results in the prevention of excess heme or free polypeptide chains in the normal precursors and mature cells. In terms of the pathogenesis of thalassemia, the importance will become manifest. At all times the ratios of alpha and beta chain synthesis remain constant(8). In addition, it can be demonstrated that free globin can stimulate heme synthesis and that free heme can stimulate globin synthesis and also cause feedback inhibition of its own synthesis(9). The rate of transcription of the polypeptide chains on the polyribosomes is not linear, i.e., there are relative slow areas of extension of these chains(10). This may be due to the need for folding of these chains as non-helical regions are reached or because of rate limitation of one or more species of transfer RNA (tRNA) for certain amino acids. In addition, the area of the chains to which heme is attached shows a slow transcription and this suggests that the heme pocket may be "folded" while still on the ribosomes. Ingram has proposed that the reason for the low amounts of delta chain (and the Hgb A2) is due to the presence of one or more extremely slow areas of transcription which have occurred due to the amino acid differences between the beta and delta chains(11).

The result of these interrelationships is that a normal erythrocyte has a rather fixed amount of the normal hemoglobin types, no excess heme and no excess free globin polypeptide chains when maturity is reached.

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GENETICS OF THE HUMAN HEMOGLOBINS

The description of the abnormal hemoglobins has allowed a major understanding, not only of the genetic control of protein synthesis as a whole,

but more specifically the details of the genetics of the hemoglobin polypeptide chains. In 1949, Pauling showed that patients with sickle cell anemia had no hemoglobin A, but instead had an electrophoretically different hemoglobin, Hgb S(12). In addition he noted that parents of these patients generally had both types of hemoglobin, A and S. The discovery by Ingram that hemoglobin A and S differed only by a single amino acid substitution in the β polypeptide chain led to the "single gene-single polypeptide chain" hypothesis(13). Detailed studies of the inheritance pattern of many of the abnormal hemoglobins, the transmission of these abnormalities when more than one occur in the same family and the biochemical hemoglobin relationships when abnormal hemoglobins and thalassemia occur in the same family have led to the following conclusions regarding the genetics of hemoglobin(2). A pair of genes for each type of polypeptide chain are inherited, one from each parent. Each gene of an allelic pair controls the synthesis of half of the total quantity of its specific peptide chain. The δ and β chain genes are closely linked and the α chain genes are either widely separated from these genes or are present on a separate chromosome pair altogether. The precise location of the γ chain genes is unknown but no close linkage to any of the other genes has been demonstrated. In fact, there is recent evidence to suggest that multiple γ chain loci may actually exist, each producing γ chains differing by a single amino acid substitution(14). The abnormal hemoglobins which result from a single amino acid substitution have all been shown to be alleles of one of the normal peptide structural genes, mostly α or β . Figures 1 and 2 show diagramatically the genetic pattern for the normal individual and for the heterozygous form of the Hgb. S gene.

It is important to note that in this as in almost all heterozygous forms of abnormal hemoglobin states, the proportion of β^A chain is slightly greater than the proportion of β^S chain, thus resulting in slightly more Hgb. A (55-65%) than Hgb. S (35-45%). This actually is due to a more efficient rate of synthesis of β^A chains versus β^S chains(8). In the homozygous state, no Hgb. A can be formed.

As can be seen from this kind of model, in an α chain substitution, six kinds of hemoglobin would result because of the presence of α chains in all hemoglobins, i.e., $\alpha_2\beta_2$, $\alpha_2\delta_2$, $\alpha_2\gamma_2$, $\alpha^x_2\beta_2$, $\alpha^x_2\delta_2$, $\alpha^x_2\gamma_2$

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CLINICAL MANIFESTATIONS OF THE THALASSEMIA SYNDROMES

In the classical sense, the clinical patterns of the thalassemia syndromes have been subdivided in accordance with the severity of the disease. Two large groups of patients are well recognized which in the majority of instances correspond to the heterozygous and homozygous states of the common entity of beta thalassemia. These have been termed thalassemia minor and thalassemia major or Cooley's anemia. Though in general this division generally holds for a majority of patients, an increasing number of patients

have been noted to either fit clinically between these two extremes of disease (thalassemia intermedia) or have more or less severe forms of disease than would be predicted from their apparent genetic pattern. These observations have served to emphasize that the thalassemias are not only of multiple types, but that within any given biochemical/genetic class, a striking degree of heterogeneity exists. Nevertheless, for most clinical purposes, the major manifestations of thalassemia still are best described in these general categories.

Thalassemia Major (Cooley's Anemia)

As will be seen later, this disease results from a marked decrease to total absence of beta chain synthesis and thus Hgb. A. The initial diagnosis of this disease is often delayed six to eighteen months of life because of the normal preponderance of hemoglobin F at this age level. As the gradual switch from Hgb. F to A should normally be taking place, the lack of beta chain production becomes manifest. Progressive anemia becomes apparent associated with increasing splenomegaly and often hepatomegaly. As a consequence of the hematological abnormalities and the therapeutic manipulations necessary to maintain life, several other features of the disease appear at varying times.

Skin pigmentation becomes progressively apparent. This is manifested initially in skin creases but rapidly, particularly with transfusion therapy, becomes generalized and is indistinguishable clinically or morphologically from any other form of hemosiderosis or hemochromatosis. Icterus is almost invariably present as well.

Bone changes(15) are manifest early since they are essentially a result of progressive medullary expansion due to the massive degree of erythroid hyperplasia. Thus, bones of the head expand resulting in prominent frontal bosses, prominent occiput and a flattened, Mongoloid facial contour. Radiologically, bone density decreases resembling cystic changes, especially beginning in the distal extremities. The cranial tables become thickened due to a widening of the diploic space and coarse perpendicular trabeculae occur giving the traditional "hair on end" appearance.

Growth retardation invariably occurs so that all patients demonstrate both reduced stature and decreased bone age(16). Secondary sexual characteristics almost never develop, so that by adolescence the marked retardation in all areas of development, other than mental, are striking. The chronic effects of anoxia have been implicated as the mechanism although endocrine failure or growth hormone resistance has also been favored by some investigators(17).

Cardiovascular abnormalities are often demonstrated early and progressively increase such that heart disease is the major cause of death in these patients. Engle has noted that by the second decade of life 80% of these children have cardiac disease and two-thirds manifest serious complications(18). After the age of 8 years she noted cardiac enlargement and/or ECG abnormalities in 39/46 patients. Pericarditis, often refractory to treatment and occasionally resulting in death, may occur. Episodes indistinguishable from angina pectoris are not uncommon. Arrythmias and refractory congestive failure are the most serious manifestations and are associated with large, hemosiderin laden, fibrotic hearts. In Engle's series, 24/25 patients with congestive failure died of this complication, 14 within three months of onset of the decompensation.

Hepatobiliary disease is manifested by both microscopic and often clinically apparent cirrhosis(19). In addition, 10% of patients have

demonstrable bilirubin gallstones. At post mortem stones and/or sludge are seen in a majority of patients. Occasional symptomatic disease results.

Frequent infections are a problem in these children throughout their course and once ranked as a major cause of death. Now, with antibiotic therapy available this form of terminal event is second to cardiac disease.

Laboratory Findings

By definition the anemia(2,4) is always severe, frequently well below 5-6 grams of hemoglobin without transfusion therapy. The anemia morphologically is hypochromic microcytic and extremely marked anisocytosis, poikilocytosis and basophilic stippling and variation in hemoglobinization is present. Nucleated red cells are almost invariably present, often in large numbers. Even though the reticulocyte count is normally elevated, it is relatively low for the degree of anemia. Thrombocytopenia may occur when a component of hypersplenism ensues. The bone marrow shows marked erythroid hyperplasia with defective hemoglobinization of erythroid elements. Iron stains reveal moderate to marked degrees of iron overload as well as siderocytes and sideroblasts, some of the "ringed sideroblast" type.

Table II Percentage of Different Hemoglobins in Thalassemias

	A	A_2	F	Other
β Thalassemia				
Trait or Minor				
High A2	Decreased	> 2.5	< 5	--
High F	Decreased	Normal	8-36	--
Major	10-90	Nor ↑	10-90	--
Sickle-Thalassemia	< 50	> 2.5	> 2	>50 S
α Thalassemia				
Minor	Normal	Normal	Normal	--
Hgb. H Disease	70-90	Nor ↓	Normal	5-30 H
Major	0	0	0	100 Bart
Sickle-Thalassemia	> 50	Normal	Normal	< 50 S

Hemoglobin studies reveal no abnormal hemoglobins by routine electrophoretic techniques. The classical finding is the elevation of hemoglobin F, usually 30-60 per cent of the total hemoglobin mass(20). However, values from 3-100% have been reported. There is no relationship between the degree of anemia and hemoglobin F levels and a given patient tends to maintain the same percentage throughout the course(21). This fetal hemoglobin is identical chemically and functionally to normal Hgb. F(22). The hemoglobin F can be shown to be distributed in a heterogenous fashion in the red cell population which is true of the normal individual but is in contradistinction to the circumstance of hereditary persistence of fetal hemoglobin(23). Hemoglobin A2 levels may not be elevated when calculated as a percentage of the total hemoglobin mass, but when compared as a ratio with Hgb. A, they are usually elevated(24). Fessas has demonstrated that with supravital staining techniques nucleated red cells and reticulocytes may contain relatively specific inclusion bodies(25). These have been shown to consist of insoluble precipitates of free alpha chains(26).

Erythrokinetic studies reveal that there is a significant shortening of the red cell life span of an intrinsic type. Fecal urobilinogen excretion is elevated to a greater degree than the degree of hemolysis would indicate, however. Gabuzda, et al, have demonstrated that there is a differential survival of the thalassemia red cell population, those containing primarily Hgb. F having a longer survival(27). The plasma iron turnover rate is markedly increased but the incorporation of radioiron into erythrocytes is markedly reduced. Thus, a significant degree of ineffective erythropoiesis (intramedullary death) is present(28). In approximately 50% or more of these patients a paradoxical increase in iron absorption can be demonstrated(29).

In summary, the anemia in thalassemia major is a composite of defective hemoglobin synthesis, ineffective erythropoiesis and hemolysis. In addition, there is an abnormal ratio of the normal hemoglobins characterized by high levels of fetal hemoglobin, low levels of hemoglobin A and the presence of free alpha chains.

Investigation of the parents of patients with this disease reveals that they both usually have a condition of a mild nature which can be called thalassemia minor.

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Thalassemia Minor (30)

In this country, Wintrobe and Dameshek simultaneously described a clinical disorder which subsequently was shown to almost always be present in both parents of patients with Cooley's anemia. In general, the problem is usually identified when evaluating a patient with a mild hypochromic anemia which is unresponsive to therapy. Most patients are totally asymptomatic, although rarely splenomegaly and mild icterus may be present. None of the growth abnormalities or consequences of prolonged iron overload occurs in these patients unless prolonged unnecessary iron therapy is administered. However, many adults show mild radiologic abnormalities, especially in the skull.

Laboratory Findings

The anemia, if present at all is mild, rarely being below 9.5 grams of hemoglobin. The red cell indices show a hypochromic, microcytic picture and morphologically there is a wide variety of findings. Anything from minimally microcytic red cells to varying degrees of hypochromia, targeting and basophilic stippling can be seen. Those patients who have a normal hemoglobin level usually have a true erythrocytosis which compensates for the hypochromic erythrocyte population. Nucleated red cells are rare and reticulocytosis is usually absent. Osmotic fragility is usually decreased and has served as a means of screening large populations. The bone marrow shows mild to moderate erythroid hyperplasia, occasionally a slight increase in iron stores but no ringed sideroblasts.

Study of the hemoglobin patterns has been the major means of identifying these patients. Kunkel, et al, were the first to note that most parents of patients with Cooley's anemia have an increased percentage of the minor hemoglobin component A2(31). It is presently noted that about 80% of patients with the clinical picture and family hereditary pattern of thalassemia minor have an increase of hemoglobin A2 to about twice the normal percentage. This is mostly a relative increase due to a decrease in total hemoglobin A. This group of patients ("high A2 type") usually have a normal Hgb. F level although levels up to 5% are occasionally seen. About 8% of the patients with the picture of thalassemia minor have normal Hgb. A2 levels but demonstrate elevated levels of Hgb. F from 8-36% which is heterogenously distributed in the red cell population ("high F type")(32). As another reflection of the heterogeneity of these conditions, rare individuals who are parents of patients with typical Cooley's anemia may have elevations of both Hgb. A2 and F (high A2/F type)(33).

Another group of patients with a typical picture of thalassemia minor also have been described, particularly in black and oriental races, who have entirely normal proportions of Hgb. A, A2 and F. It is of interest that when two of these individuals mate they do not have offspring with Cooley's anemia. Instead, they have a high incidence of stillborns with hydrops fetalis(34). These fetuses, when examined, show almost entirely a new non-functional hemoglobin termed Bart's hemoglobin, which is composed of four gamma chains (γ_4). In addition, if the cord blood of infants born of this type mating is examined, some will demonstrate slight amounts (up to 10%) of Bart's hemoglobin which disappears as hemoglobin A synthesis increases. These children will then demonstrate the clinical picture of thalassemia minor(35). It is in families with this type of thalassemia minor that another disease (discussed below) appears -- so-called hemoglobin H disease.

Erythrokinetics in thalassemia minor are generally of the same type as in thalassemia major but much milder. In addition, the red cell life span is often normal.

An important, and often necessary, means of confirming the diagnosis of thalassemia minor is by studying family members. At least one parent of any patient with this suspected diagnosis should show a similar clinical and biochemical picture or evidence of its presence. In addition, approximately one-half of a large group of offspring of such a patient will also demonstrate evidence of inheritance of the genetic abnormality.

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Thalassemia Intermedia

As the knowledge of this group of diseases increased, it was recognized that other clinical syndromes existed which in terms of severity were intermediate between thalassemia major and minor, namely symptomatic anemia with evidence of the same erythrokinetic defects as Cooley's anemia but consistent with survival into adult life. The importance of this genetically mixed group of problems has been to help elucidate the genetic and biochemical defects of thalassemia and also to demonstrate the marked heterogeneity that exists in type and severity of the various subclasses. This group of patients have been found to consist of the following kinds of disease(36):

1. Inheritance in the doubly heterozygous state of two variants of thalassemia minor.
2. Apparent genetic pattern for classical Cooley's anemia but with a milder clinical course.
3. Double heterozygosity for a thalassemia gene and an abnormal hemoglobin gene (for example, sickle-thalassemia).
4. Hemoglobin H disease.

The latter two circumstances have been most important in elucidating some of the major concepts of the genetics and pathogenesis of thalassemia, and therefore, a few selected points will be made about them.

Sickle-Thalassemia(2)

This is a disease which consists of a spectrum of great variability clinically, ranging from no symptoms to a moderately severe sickle cell anemia syndrome. These patients demonstrate the presence of hemoglobin S, A and increased Hgb. F. However, unlike the patient with sickle cell

trait where the levels of Hgb. A exceed Hgb. S, there is a reversal of the ratios such that Hgb. S exceeds Hgb. A. This apparent "interaction" of the two genes occurs, however, only about 90-95% of the time when evidence for the inheritance of both a sickle gene and a thalassemia gene exists. This led to the concept of two general types of thalassemia genes, the interacting and non-interacting types(37). It was later shown that the interacting gene is usually the one which alone shows increased Hgb. A2 levels and the non-interacting gene is the one that results in normal ratios of Hgb. A, A2 and F.

Hemoglobin H Disease

Another group of patients with a moderately severe thalassemia picture occurs in which the presence of an unusual hemoglobin is demonstrable. This hemoglobin, called hemoglobin H, can be demonstrated electrophoretically, or by its tendency to precipitate in the form of inclusion bodies when the red cells are stained supravitally. This hemoglobin is non-functional and consists of four beta chains (β_4). This disease occurs sporadically in families in whom the gene for the normal A, A2, F thalassemia is present. Hemoglobin H disease usually appears more often in siblings (horizontally) than being transmitted in the usual pattern of parent to offspring(vertically)(38).

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GENETICS AND PATHOGENESIS OF THE THALASSEMIAS

The detailed study of large families with various forms of thalassemia and their association also with structural variants of alpha, beta and delta chains has permitted the development of a model of the genetic pattern of the thalassemias. In addition, the development of techniques for measuring the rates of synthesis of the various hemoglobin polypeptide chains has substantiated the validity of these models. It is now apparent that the clinical entities known collectively as the thalassemias result from a quantitative reduction in the synthesis of one or more of the normal polypeptide chains and that the consequences of the imbalance of polypeptide chain synthesis results in definable alterations in red cell structure and metabolism inducing the clinical abnormalities that are observed(39).

Ingram and Stretton first proposed that the thalassemias could be divided into two major genetic types as defined by their "interaction" or non-interaction" with structurally abnormal hemoglobin chains(37). They proposed that those "interacting" with Hgb. S to cause a reversal of the normal ratios of Hgb. A and S were due to a quantitative decrease in beta chain synthesis -- the beta thalassemias -- and the "non-interacting" type were alpha chain synthetic defects. Figures 3 through 6 diagramatically represent genetic models of some of the various thalassemias. Figure 1 demonstrates the normal genetic control of hemoglobin synthesis. Figure 2 represents the genetic scheme of sickle

cell trait. Because of the slightly less efficient synthesis of β^S chains as compared to β^A chains, hemoglobin A levels are always somewhat greater than hemoglobin S in this circumstance.

Beta Thalassemia

The gene for beta thalassemia is an allele of or closely linked to the beta chain structural gene. In the heterozygote, the abnormality results in an abnormally low amount of the beta chain synthesized under the control of the affected structural gene (Figure 3). There is a relative (and also absolute) increase of normal delta(8) chains, and therefore, the percentage of hemoglobin A2 is increased. This is the "high A2 type" of beta thalassemia. If a patient inherits this gene plus a gene for an abnormal beta chain (e.g. β^S) (Figure 4), then the amount of normal beta chain will be reduced (mild form) or absent (severe form) and a reversal of A to S ratios occurs ("interaction"). The heterogeneity within the high A2 type of thalassemia is apparent from this variation in beta chain synthesis. It can be demonstrated by chain synthesis studies to be a true phenomenon and genetically controlled since the amount of beta chain synthesized is quite constant by affected members of any given family (40).

Homozygous beta thalassemia (Figure 5) results in only small amounts or absent beta chain synthesis and thus little or no hemoglobin A. Except for the unexplained partial compensation by the continued proliferation of hemoglobin F containing cells, this disease would obviously be incompatible with life. It is apparent that a large pool of free alpha chains could exist in those cells which produce little or no beta or gamma chains. These can be demonstrated to be present as the characteristic inclusion bodies and result in intramedullary or early systemic destruction of the cells(41). In addition, those cells that produce significant amounts of gamma chains form hemoglobin F and these cells have a longer life span presumably because of the smaller pool of free alpha chains(27). Thus both increased synthesis of hemoglobin F in some cells and prolonged survival of these cells accounts for the high levels of Hgb. F in Cooley's anemia.

In summary, the pathogenesis of the anemia of thalassemia major, then, is a result of decreased Hgb. A synthesis because of severely inadequate beta chain synthesis resulting in: a) hypochromia; b) ineffective erythropoiesis (intramedullary death due to free alpha chain precipitation) and c) hemolysis (systemic death of mature cells induced by precipitation of free alpha chains).

Another form of beta thalassemia ("high F type") has now been shown to be due to complete suppression of chain synthesis controlled by the linked β and δ chains ($\delta\beta$ thalassemia). This explains the fact that this form of thalassemia does "interact" with beta structural variants but does not have high A2 levels(42).

Alpha Thalassemia

The genes for alpha thalassemia also appear to be alleles of or closely linked to the normal alpha chain structural loci. It can be seen from Figure 6 that since alpha chains are common to all of the normal hemoglobins, there will be a proportional reduction in each and thus no characteristic elevation of any one hemoglobin type in the heterozygote.

At birth, this circumstance might be expected to be associated with some excess gamma chains. This does occur, and the presence of Bart's hemoglobin (γ_4) can be demonstrated in the effected infant(35). Chain synthesis becomes balanced sufficiently in the adult, however, that no significant amounts of Hgb. H (β_4) occurs in the adult heterozygote. Figure 7 diagrams the concept of the "non-interaction" of alpha thalassemia and a beta structural variant that initially led Ingram and Stretton to propose this hypothesis. Subsequent synthesis studies have proven the absolute decrease in alpha chain production(43). It is of interest that the expected "interaction" does occur in the presence of coexistent alpha chain structural variants and alpha thalassemia(44).

A form of thalassemia intermedia, hemoglobin H disease (see page 11) is associated with the presence of free beta chains forming unstable Hgb. H (β_4) which results in an abnormally short life span of these cells. The genetics of this disorder are complicated, but family studies and hemoglobin synthesis studies suggest that these patients may be doubly heterozygous for a clinically "silent" alpha thalassemia gene (silent H gene) and the more severe classical alpha thalassemia gene(38,43).

The homozygous state of alpha thalassemia might be expected to be incompatible with life, since alpha chains are necessary for the formation of all functional hemoglobins. The high incidence of hydrops fetalis in families in which both spouses are alpha thalassemic heterozygotes is felt to be a result of this homozygote state. The dead fetuses show 80-100% of Bart's hemoglobin (γ_4) which substantiates this hypothesis.

It is also apparent that a wide variety of clinical pictures will result from the heterozygous, homozygous and doubly heterozygous forms of these genetic abnormalities and from their combinations with structurally abnormal hemoglobins(34,45).

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The Specific Defect

At the present time, the actual genetic fault resulting in these quantitative biochemical abnormalities is unknown. Many hypotheses have been proposed and some experimentally tested. As noted previously, hemoglobin synthesis appears to follow the classically defined steps of protein synthesis in other systems, with the exception that the messenger RNA appears to be more stable. Theoretically, several areas of the sequence from the gene to polypeptide chain could be altered and cause a thalassemia-like picture. The major proposed mechanisms include:

1) Substitution-rate hypothesis - Ingram and Stretton suggested that, as with the single amino acid substitutions in known abnormal hemoglobins where the rate of synthesis is decreased in the presence of the mutation, a similar mechanism might exist in thalassemia(37). In this circumstance, the amino acid substitution would be of like charge and thus result in an electrophoretically "silent" altered hemoglobin. However, structural studies have so far not shown any abnormality in the amino acid sequence of the hemoglobin chains in thalassemia.
2) Deletion of genetic material, e.g., by unequal crossover, might result in the absence of a functional structural gene for one or more linked polypeptide chains. This mechanism is probably the cause of Lepore hemoglobin which produces a thalassemia-like picture(46). Hereditary persistence of fetal hemoglobin could also result from this mechanism(47). In the thalassemias themselves, no non-functional fusion product has been identified. Such a deletion, however, might be responsible for the form of beta thalassemia with no polypeptide chain production and/or the ($\delta\beta$) thalassemia where neither polypeptide chain is coded for. This mechanism remains a strong possibility for some of these states.
3) Reduced transcription of DNA directed mRNA synthesis could result in decreased amounts of a specific mRNA and thus its polypeptide chain product. No data is yet available on this possibility and it remains plausible.
4) Unstable mRNA. Differences in stability of the mRNA for the various normal polypeptide chains has been suggested to occur naturally(10). Different relative rates of α , β and γ chain synthesis can be demonstrated at sequential stages in maturing cells from thalassemia patients (8). Either this is due to variability in mRNA stability or certain cells that produce one type of polypeptide chain (e.g., beta) are selectively destroyed resulting in a preponderance at later stages of maturation of another cell line which produces predominantly γ chains. A difference in cell survival in peripheral blood of hemoglobin A versus hemoglobin F containing cells in thalassemia has been demonstrated. Nevertheless, until mRNA for the polypeptide chains can be isolated, the instability hypothesis remains a possibility.
5) Availability of specific transfer RNA. If a mutation resulted in the coding for the same amino acid but a different tRNA for that amino acid (result of the known degeneracy of the nucleotide code), and if the other tRNA were in limited amount, then the rate of individual peptide chain transcription might be reduced. Weatherall has found, however, that the rate of transcription of individual beta polypeptide chains in β thalassemia appears normal(48).
6) Premature release of incomplete peptide fragments or failure of release of completed chains resulting in polysome blockade ("constipated ribosomes") has been proposed. Baglioni could find no experimental evidence for the former theory(49). The second mechanism might be expected to eventually lead to reduction of synthesis of other chains as well. Bank, et al have shown that alpha chain synthesis is probably normal in beta thalassemia (50).
7) Controller gene hypothesis. Ingram and Stretton also proposed a second mechanism of thalassemia which related to a controlling system for peptide chain synthesis. Following the classical work of Jacob and Monod(51) in bacterial protein synthesis, their model of the control of protein synthesis has been applied in several forms of thalassemia and related disorders. In simple terms, it is suggested that several genes are responsible for synthesis of a given polypeptide chain or protein. The structural gene, which determines the actual amino acid sequence, is closely linked to

another gene called the operator gene which is responsible for the activity of the structural gene. These genes together are termed the operon. In addition, a separate regulator gene is present elsewhere in the genetic material. Its gene product is termed a repressor which is theorized to combine, in its native form, with its specific operator gene and thus inhibit the activity of the structural gene(s). A smaller molecular weight material, which is capable of binding to the repressor and preventing its interaction with the operator gene, would allow the corresponding structural gene to be active. This material is termed an inducer or derepressor. In one scheme, proposed by Zuckerkandl(52), synthesis of beta, delta and gamma chains has been inserted in this model. As proposed, a second regulator gene for the γ chain structural gene would be present in the beta operon. Thus in the absence of inducer, beta and delta chain production would be suppressed and also γ chain repressor synthesis would be suppressed and the cell would make γ chains. With the exposure to an inducer substance, the repressor of the beta-delta operon would be inactivated, beta and delta chains would be produced as well as γ repressor. In turn, then γ chain synthesis would be "turned off". This would account for the fetal to adult hemoglobin switch that normally takes place. In addition, abnormal beta operator gene activity could explain the hereditary persistence of fetal hemoglobin. In terms of beta thalassemia, however, this hypothesis must take into account that hemoglobin F synthesis is not generally maintained in the affected cells and that the thalassemia defect acts only in cis (i.e., not on its allelic structural gene). The abnormality would necessarily lie in the beta operon because of the cis action of the gene. One proposal is that the beta structural gene is unable to respond to its operator gene. In this scheme, the beta operon would be turned on, little or no beta chain would be produced, delta chain synthesis would occur and δ repressor would be produced and thus γ chain synthesis would be suppressed as well, resulting in the classically affected low A, low F cell. Other models are possible. It must be cautioned that no evidence has yet definitely shown that this kind of controlling mechanism for protein synthesis exists in multicellular organisms and remains only another theory to explain the basic defect in thalassemia.

Other theories can and have been proposed as well. At the present time, with the knowledge we have available regarding known chain synthetic defects in the thalassemias and the variations in compensatory chain production, different thalassemias may actually result from various types of these abnormalities. Thus the controller gene hypothesis, genetic deletions and formation of unstable mRNA are all strong possibilities and each may be the definable abnormality in one or more of these syndromes.

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THERAPY

For the most part, it is only patients with thalassemia major in whom treatment is required. The patient with thalassemia minor is generally asymptomatic and the major emphasis in these patients is to avoid the unnecessary administration of iron or transfusions which could result in preventable iron overload states and occasional cases of viral hepatitis. Although a modest degree of anemia exists in thalassemia intermedia, there is as yet no evidence that chronic transfusion therapy or other measures have any beneficial effect, and they carry the same possible detrimental consequences.

Thalassemia major, however, is a severe disease which results in the multiple growth and development problems, recurrent infections and progressive iron overload states, described above, with death usually occuring by age 20. The hallmark of therapy has been and remains transfusion. A host of problems exists utilizing this form of chronic therapy and the standard approach in these patients has been very unsatisfactory. The general practice has been to give transfusions at a point at which the patient cannot carry out any significant normal functions or to maintain a hemoglobin level just above that which is accompanied by this degree of functional impairment. Most frequently this means maintaining hemoglobin levels above 6.5-7 grams % and necessitates red cell administration every 2-4 months. This regimen does not prevent any of the growth abnormalities and may actually enhance the development of significant organ dysfunction due to the progressive hemosiderosis since chronic severe hypoxia still exists. Experimental data is available which suggests that the hypoxia is a most important parameter for the development of the pathological effects of iron overload in the heart and liver(53). Thus, in recent years, several groups have been evaluating the feasibility of maintaining higher hemoglobin levels on a chronic basis. These "hypertransfusion regimens" have not clearly delineated an answer but several promising results have been reported(54). When these programs have been instituted at an age when many of the bony defects, growth problems and organ dysfunction problems have already been present, the results have been less clear cut than when the programs have been initiated at the time of diagnosis in infancy. However, even then significant improvements have occurred. Patients with cardiac enlargement and failure have had impressive responses. Heart, spleen and liver sizes are markedly reduced. Recurrent infections become almost no problem and the children manifest an almost normal record for school attendance. Variable increases in growth percentiles have been observed. As of yet, no reversal of the problem of sexual development has been noted.

On the other hand, when started in infancy, normal growth and development patterns have been documented during the first two to three years, no bone changes have appeared, and the children have not yet manifested any evidence of cardiac or hepatic dysfunction. None of these studies have yet gone on long enough to say anything about survival data.

These programs have generally been designed to maintain hemoglobin levels above 10 grams% at all times. This requires transfusions approximately one time per month. When the excess iron load compared to the previous standard regimens is calculated, it is found this results in approximately a 50% increase in total iron administered per year. Of major significance, however, may be the fact that due to the depression of

intrinsic erythropoiesis, the striking increase in G. I. iron absorption is probably shut off and may actually make the total iron load much less than this figure. The long term effects of this increased iron overload with less anemia remains the major question. There is no doubt, however, that the patient has an almost normal life during this time and none of the psychologically devastating cosmetic problems of bone growth have ensued since intrinsic erythrocyte production is almost totally suppressed.

Splenectomy has been performed on a selective basis for hypersplenism in Cooley's anemia for many years. The indications are not precise, but certain patients demonstrate significant benefit. If one demonstrates a clinically obvious increasing transfusion requirement and can show that the survival of Cr^{51} tagged normal donor erythrocytes is significantly shortened and accompanied by abnormal splenic sequestration, this procedure should be considered. Other consequences of this hypersplenic state may be pain and eating difficulties from the massive size of the spleen and thrombocytopenia with a bleeding diathesis. The major drawback to this procedure is the well recognized incidence of often fatal bacterial sepsis in the splenectomized thalassemic individual. In one series, 9 of 35 such patients have experienced severe bacterial septic episodes (usually pneumococcal or E. coli) and six have been fatal(55). The major threat seems to be during the first two post-splenectomy years. Antibiotic prophylaxis is being evaluated in this circumstance. Of significance is that this procedure probably will not become necessary in the hypertransfused children since significant splenic enlargement is reversed or prevented due to the suppression of extra medullary hematopoiesis and the relatively normal survival of transfused donor cells.

Iron chelating agents have been evaluated in thalassemia, but the quantity of iron that it has been possible to remove on a chronic basis has for the most part been quantitatively insignificant compared to the transfusion iron load.

General supportive care is required and the recognition of the occasional development of worsening anemia due to relative folic acid deficiency should be kept in mind as a possible therapeutically reversible complication.

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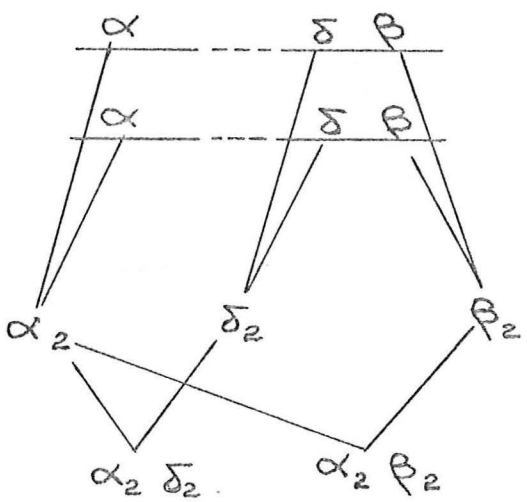
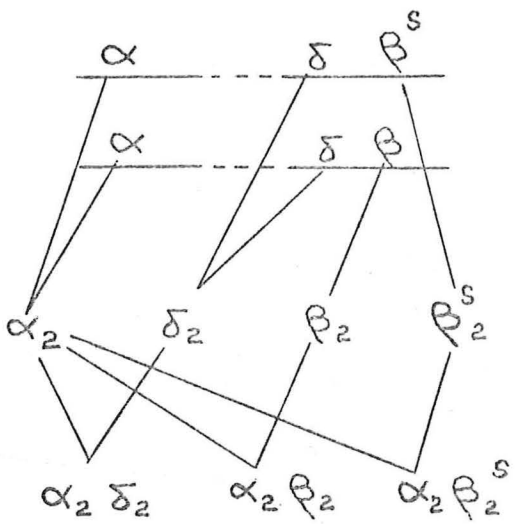


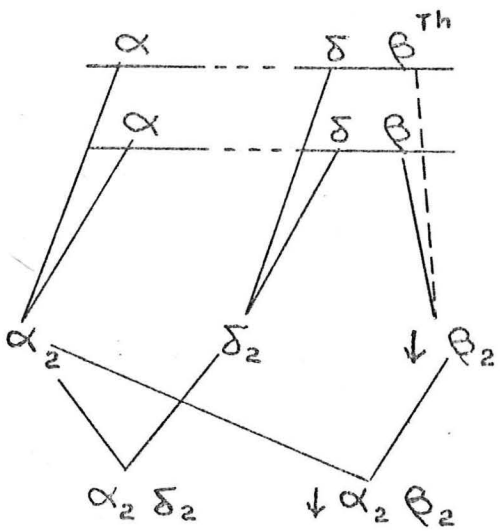
A2 A

Fig. 1 Normal



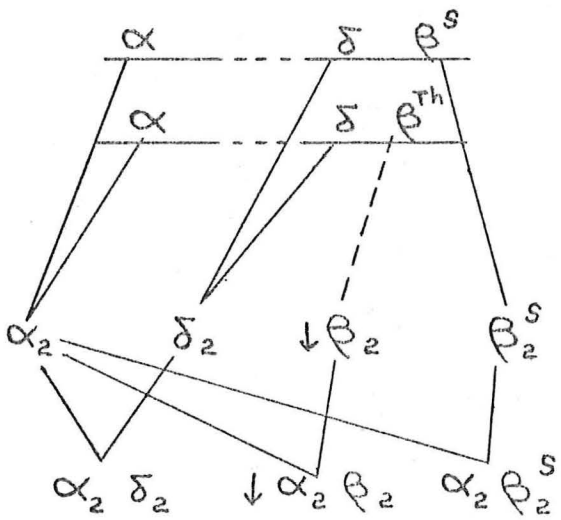
A2 A S

Fig. 2 Sickle Trait



A2 ↓ A

Fig. 3 Heterozygous Beta Thalassemia



A2 ↓ A S

Fig. 4 Sickle-Beta Thalassemia

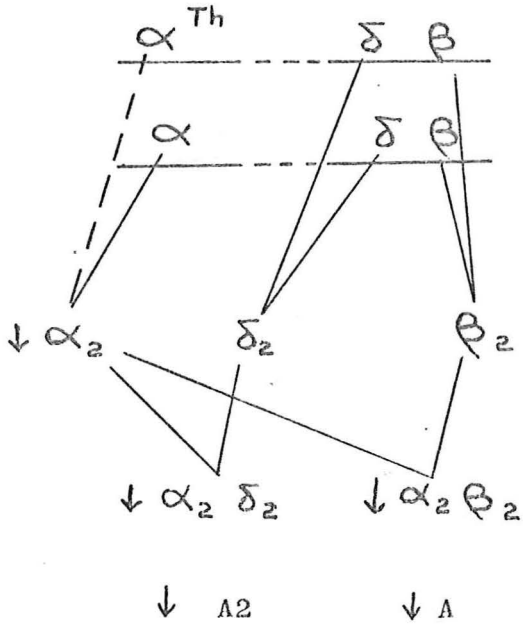


Fig. 5 Heterozygous Alpha Thalassemia

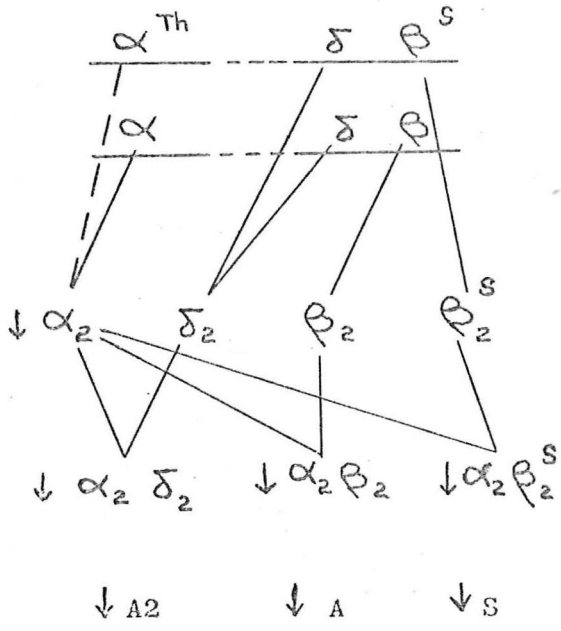


Fig. 6 Sickle-Alpha Thalassemia