Reciprocal Relationship of Hemoglobins Aδ and F in Beta Chain Thalassemias, a Key to the Genetic Control of Hemoglobin F

By Wolf W. Zuelzer, Abner R. Robinson and Clifford R. Booker

The existence of more than one kind of gene capable of producing the stigmata of thalassemia was first suggested as a possible explanation for the surprisingly wide spectrum of hematologic phenotypes observed in syndromes thought to result from the combination of a gene for thalassemia with a gene for one of the abnormal hemoglobins such as hemoglobin S or hemoglobin C. The idea seemed plausible that several different genes might bring about the picture of thalassemia when present in the simple heterozygous state but might act in quite different ways in the presence of another abnormal gene affecting the composition or rate of production of hemoglobin.

This hypothesis was carried a significant step farther by Ingram and Stretton, who proposed the concept that thalassemia is a molecular abnormality of hemoglobin comparable to other hemoglobinopathies in that the mutant gene causes an amino acid substitution within the molecule but more difficult to recognize because the substitution, in the case of thalassemia, does not happen to produce a change in the net electrical charge and is therefore not manifested by an altered electrophoretic behavior.

The hemoglobin molecule is now thought to be composed of two pairs of polypeptide chains, in the case of hemoglobin A two α and two β chains whose production is assumed to be under the control of two corresponding pairs of α and β genes. The hypothesis of Ingram and Stretton accordingly visualizes two general classes of thalassemias, those in which the α chains are affected, with numerous potential variants in each class corresponding to the many possible substitutions.

Pending the demonstration of abnormal amino acid sequences in the major component (“Hemoglobin A”) of the hemoglobin of persons heterozygous for a thalassemia gene, the study of the so-called minor components, hemoglobin Aδ and hemoglobin F, in such persons provides clues for the existence of...
multiple variants and furnishes strong indirect evidence in support of the new genetic and biochemical theory. Both components are altered quantitatively in thalassemia with such frequency as to suggest that their behavior should yield important genetic information, especially if one or another quantitative pattern of either or both fractions should be found to be consistently associated with a particular thalassemia gene. Variations in these patterns can be used to distinguish variant genes and trace them within a given pedigree.

The study of the two pedigrees to be described demonstrated the presence of two distinct thalassemia genes within each family, both producing the same hematologic manifestations but differing with respect to their effect on the two minor components. Of special interest is the fact that the combination of the two different mutant genes produced the picture of classical thalassemia major in the propositus of the second pedigree. This fact and the observation of a reciprocal relationship in the quantitative behavior of the two minor fractions between the two mutants support the hypothesis that these two particular genes are allelic and both represent $\beta$ chain mutations.

Pedigree R.

The propositus was a 5-year old boy who had recently been found to have a mild unexplained anemia. Except for some degree of pallor, said to have been present all his life, he had no symptoms referable to the anemia. Physical examination disclosed no significant abnormalities. The hematologic findings, summarized in table 1, were consistent with the diagnosis of thalassemia minor. Briefly, there was a mild hypochromic-microcytic polycythemia, the morphology of the erythrocytes was definitely altered, target cells, ovalocytes and other poikilocytes were present, the osmotic resistance of the red corpuscles was increased, and the serum iron level was within the normal range. On this basis and in view of the fact that the patient was of Mediterranean descent, the hematologic diagnosis of thalassemia minor was established.

Studies of the patient's hemoglobin by means of electrophoresis on paper, starch block, and agar gel, and by alkali denaturation, disclosed no abnormal components but showed the presence of 10 per cent alkali-resistant hemoglobin. By contrast, the $A_2$ hemoglobin was within the normal range, 1.8 per cent. Both these values being outside the range usually encountered in uncomplicated thalassemia minor, the parents and as many other members of the family as were available to us were studied with respect to the composition of their hemoglobin as well as the presence of the stigmata of thalassemia minor (fig. 1).

Both sides of the family were of Mediterranean origin. The father's family had emigrated from Sicily within the preceding generation, that of the mother from the Italian mainland in the vicinity of Naples. There was no known history of anemia on either side. Both parents, however, proved to have thalassemia minor with blood pictures which were indistinguishable from that of the propositus (table 1). The mother's serum iron level initially proved to be low and the iron-binding capacity elevated, but after three months of sustained iron therapy these values became normal while the blood picture remained unaffected.

Analysis of the hemoglobin pattern of the parents' blood showed that while the father exhibited the usual finding of an elevated $A_2$ fraction and only a minimal increase of the alkali-resistant hemoglobin, the mother's hemoglobin had essentially the same composition.

*The starch block electrophoresis was carried out according to the method of Kunkel and Wallenius. Other methods used in this laboratory have been referred to in previous communications."
### Table 1.—Pertinent Hematologic and Biochemical Findings—R. Family

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<th>Pedigree Number</th>
<th>Age</th>
<th>Hb.</th>
<th>RBC</th>
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<th>MCV</th>
<th>Retic.</th>
<th>Target Cells</th>
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*Equivocal.
as that of the propositus, namely an A₂ fraction within the normal range but a much greater proportion of fetal hemoglobin than is ordinarily seen in thalassemia minor.

The further investigation showed these differences between the two sides of the family to be consistent. On the maternal side the grandfather (I 3), an aunt (II 9) and three of her children (III 4, III 5, III 6), were found to have thalassemia, and in each instance the pattern was the same, a normal A₂ fraction and an elevation of fetal hemoglobin well beyond the expected range. By contrast, the paternal grandmother (I 2), the father himself (II 5), and one sibling (III 1), showed the usual pattern of thalassemia minor. The remaining sibling (III 3) likewise had thalassemia minor with a pattern resembling that of the maternal side and of the propositus. No abnormal patterns were observed in any of the individuals who did not show evidence of thalassemia.

**Comment**

The hematologic diagnosis of thalassemia presented no problem on either side of this pedigree. In each of the 11 affected individuals the morphologic stigmata, the corpuscular constants and the increased osmotic resistance of the erythrocytes were typical of thalassemia minor and the values for serum iron and iron-binding capacity furnished confirmatory evidence.

The hereditary pattern expected for thalassemia was likewise obvious and constituted an additional diagnostic feature, but it is clear that two different genes were involved, each clearly traceable by its consistent association with a different pattern in the composition of hemoglobin. Clearly the propositus himself and one of the siblings (III 3) had inherited the maternal gene while the remaining sibling carried the paternal gene for thalassemia. Judging from the hematologic as well as the biochemical findings, none of the three children inherited both of the thalassemia genes and it was thus not possible to learn whether the combination of the two particular genes present in this family

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**Fig. 1.**

PEDIGREE OF R. FAMILY

- [Diagram showing family relationships and hemoglobin patterns]

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HEMOGLOBIN RELATIONSHIPS IN THALASSEMIA

would produce the usual consequences of homozygosity, i.e. thalassemia major, or would interact in a different way.

Pedigree L.

The propositus was an 8-year old white girl who was referred to the Hematology Department of the Children's Hospital of Michigan because of severe anemia requiring frequent transfusions. The diagnosis of thalassemia major had been made by an osteopathic physician (!) four years earlier and was confirmed at that time by the Department of Pediatrics of the University of Michigan. The patient subsequently developed ulcerative colitis. From the age of 4 years she received numerous transfusions. At first these were spaced at two to three month intervals but recently the response had been unsatisfactory even when blood was given as often as once a week.

Physical examination showed a pale, undersized, female child with a typical mongoloid facies, protuberant abdomen, marked splenomegaly, tachycardia and a systolic heart murmur.

The first hematologic data collected in this laboratory and obtained only five days after the last transfusion are summarized in table 2. Apart from the severe anemia itself, the noteworthy features were the presence of a dual cell population, a minority of normochromic, normocytic cells representing presumably donor cells, and severely hypochromic and microcytic erythrocytes showing the classical picture of Cooley's anemia with poikilocytosis, schizocytes, target cells and many nucleated red cells.

A finding of additional interest was the positive direct antiglobulin test and the presence of antibodies of Rh specificity in the patient's serum which indicated that the reason for the recent failures of transfusion therapy was the fact, subsequently verified, that she was Rh negative and had been receiving Rh positive donor blood. Because of the recent transfusions, quantitation of the hemoglobin components of this patient was not expected to give a true picture. Only hemoglobin A and hemoglobin F were demonstrable, the latter in a concentration of 13.7 per cent.

The family was of Sicilian origin on both sides. The parents denied consanguinity. Neither parents, nor the patient's sister, nor other close relatives (fig. 2) were known to have anemia. The subsequent hematologic studies showed, however, that the typical stigmata of thalassemia minor were present in both parents, in the sister (III 2) and in a paternal aunt and uncle and in two of the latter's children (table 2). The mother was found to have an elevated hemoglobin A₂ fraction in her blood. The hemoglobin of the father, the sister, and the four thalassemic relatives on the paternal side, showed A₂ values well within the normal range but in all but one of these individuals the percentage of hemoglobin F was increased beyond the level seen in this laboratory in the vast majority of Caucasians heterozygous for thalassemia (see below).

COMMENT

The pattern of thalassemia genes in this pedigree is the same as in Pedigree R and the presence of the two dissimilar genes, one characterized by high A₂, the other by high F values, needs no further comment. The significant contribution of this pedigree lies in the fact that the propositus had thalassemia major, evidently resulting from the combination of these two distinct genes.

DISCUSSION

The observations presented here indicate that in thalassemic pedigrees the quantitative behavior of fetal hemoglobin, hitherto generally regarded as irregular and unpredictable, can have a rather precise genetic meaning comparable to that of the A₂ fraction to which it is clearly related. It is now evident that the characterization of a particular thalassemia gene requires the determin-
Table 2.—Pertinent Hematologic and Biochemical Findings—L. Family

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<th>Pedigree Number</th>
<th>Age</th>
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</tbody>
</table>
nation of both these minor components of hemoglobin. In this manner the existence of two distinct genes whose combination in one person had produced the classical picture of thalassemia major could be demonstrated in two pedigrees belonging to the same ethnic group. One gene was associated with the familiar pattern consisting in an elevation of the A₂ fraction with at most minimal increase in the proportion of hemoglobin F. The other had the reverse effect of causing rather substantial increases in the amount of fetal hemoglobin in the absence of elevations of the A₂ fraction.

Early reports had indicated an increased percentage of the A₂ fraction in thalassemic heterozygotes of Mediterranean extraction with such frequency that it bade fair to become a diagnostic sine qua non and seemed likely to be the expression of an essential mechanism. Sporadic exceptions such as reported in the original study by Kunkel and his associates and later by Carcassi et al. might conceivably be interpreted as due to technical limitations or, in view of the narrow range of values separating the normal from the thalassemic state, to statistical overlap. It is clear that such explanations are not applicable where a consistent pattern can be traced through an entire pedigree in association with a particular gene. Here at least, the failure to produce an elevation of the A₂ fraction is a characteristic of a gene with otherwise typical manifestations.

Ceppellini likewise observed hereditary transmission of microcytosis with normal levels of the A₂ fraction in an Italian population and designated this variant of thalassemia as type “Ma” (microcytosis, A₂ not increased) as opposed to the commoner type “MA” (microcytosis, A₂ increased). His report, however, made no mention of the behavior of hemoglobin F in the two variants.
This is true of the majority of studies dealing with the A₂ fraction. Marinone and Bernasconi \(^{13}\) who investigated both components in a series of thalassemic persons in an Italian population in which, however, the type "Ma" was not represented, failed to notice a relationship. Yet our findings suggest that a highly significant relationship does in fact exist between the quantitative behavior of hemoglobin F and that of the A₂ fraction in the heterozygous state for thalassemia, a relationship moreover, which furnishes a basic clue to the understanding of biochemical and genetic mechanisms involved in the disorder.

Heretofore no serious attempt seems to have been made to explain the rather large variations in the percentage of hemoglobin F reported in thalassemic heterozygotes. In the majority of cases studied after infancy, a period obviously unsuitable for establishing standards, the values have ranged from less than one to about three per cent,\(^{14}\) but an upper limit of approximately ten per cent seems to have been widely accepted.\(^{15}\) Disregarding the much higher percentages reported in alleged heterozygotes by Aksoy\(^{16}\) because of features such as severe anemia, splenomegaly and osseous changes not generally accepted as manifestations of simple heterozygosity, a spread from one to ten per cent is still considerable for a rather well defined genetic disorder and is not readily explained as mere statistical scatter.

Our observations furnish at least one explanation for these apparent vagaries, namely the existence of particular thalassemia genes associated with low or normal levels of hemoglobin A₂ for which a higher than usual range of hemoglobin F is characteristic. In all but one of the cases in the two families described here in which the A₂ fraction was not elevated, the proportion of fetal hemoglobin was exceptionally high. Moreover, an intrafamilial pattern was suggested by the fact that the figures ranged from six to twelve per cent in the R. pedigree and from 3.3-7.4 per cent in the L. pedigree. The two genes are therefore similar but not necessarily identical.

In order to document more fully the relationship between the values for hemoglobin F and those for the A₂ fraction, the figures obtained in our laboratory in thalassemic heterozygotes were examined. Only cases of Italian and Greek ancestry were included for this purpose. Not counting the members of the R. and L. families, a total of 35 cases of typical thalassemia minor was available, for the most part parents or other immediate relatives of propositi with thalassemia major and representing 15 additional kindreds. In this unselected group the A₂ fraction was uniformly increased above the figure regarded as normal in this laboratory, the values ranging from 3.1 to approximately 6.0 per cent. Together with the four members of the two families described in whom the A₂ fraction was elevated (the paternal relatives I-2, II-5, and III-1 of pedigree R. and the mother, II-3, of pedigree L.), there were thus 39 "high A₂" thalassemias which could be compared with 14 "low or normal A₂" thalassemias.

In the group with high A₂ levels none showed levels of hemoglobin F as high as 4.0 per cent and in only four instances or about ten per cent of the entire group did the level exceed 3.0 per cent, the highest value being 3.8 per cent (fig. 3). These figures are in good agreement with those generally reported
HEMOGLOBIN RELATIONSHIPS IN THALASSEMIA

Fig. 3.—Graphic comparison of the hemoglobin levels in 39 cases of thalassemia minor with elevated A₂ fraction from 17 unselected kindreds with those in 14 cases of thalassemia minor with normal A₂ values in the two pedigrees described.

in the literature and indicate that levels of fetal hemoglobin of 3.0 to 3.5 per cent represent the approximate upper limit for this type of thalassemia.

By contrast, 13 of the 14 persons in the two families who possessed a "low or normal A₂" thalassemia gene showed levels of hemoglobin F in excess of 4.0 per cent (fig. 3), the single exception being the uncle (II-6) of the propositus in the L. pedigree whose level, however, still exceeded 3.0 per cent.

Thus, despite a small overlap, a significant difference between the two groups is apparent, and the range approximately between 3.5 and 4.0 per cent emerges as having a critical significance. It is of interest that the exceptional cases of thalassemic heterozygotes reported by Kunkel et al. in their original study as showing no elevation of the A₂ values likewise had relatively high levels of fetal hemoglobin and the same was true of the four such cases included in a recent report by Wolff. It appears therefore that low levels of hemoglobin A₂ are consistently associated with high levels of hemoglobin F and that this relationship expresses an important mechanism. The converse is less consistently true though in general elevations of the A₂ fraction are accompanied by no or at best minimal increases of hemoglobin F. Our case III-1 of pedigree R. (table 1) represents an exception and the table presented by Wolff likewise includes three cases in which an elevated A₂ level was associated with relatively high levels of fetal hemoglobin. These exceptions will be discussed in the light of the hypothesis presented below. In general, however, the trend is unmistakable and the relationship of the two minor components in thalassemia tends to be a reciprocal one.
The significance of these findings derives from the fact that both components are now known to contain $\alpha$ polypeptide chains, paired in the case of hemoglobin F with $\gamma$ chains\textsuperscript{18} and in that of the A\textsubscript{2} fraction with $\delta$ chains\textsuperscript{19} and their behavior can therefore be interpreted in relation to the hypothesis of Ingram and Stretton.\textsuperscript{4} This hypothesis assumes that a "silent" amino acid substitution in the hemoglobin molecule is the essential lesion in thalassemia, that such a substitution may involve either an $\alpha$ or a $\beta$ chain and that the rate of synthesis of the abnormal polypeptide is depressed. If the percentage of a component possessing $\alpha$ chains is increased it is clear that the rate of $\alpha$ chain synthesis is not impaired. Assuming that the hypothesis of Ingram and Stretton is valid, neither of the two variants of thalassemia described here can therefore represent an $\alpha$ chain mutation since each is associated with an increase in the proportion of a component possessing $\alpha$ chains, either hemoglobin A\textsubscript{2} or F. Both kinds of thalassemias can thus be classified as $\beta$ thalassemias and represent distinct variants of $\beta$ genes.

While in this manner the location of the defect can be placed in a $\beta$ chain, the question is not answered why in one type the excess $\alpha$ chains are paired with $\delta$ chains to produce the increase in the A\textsubscript{2} fraction while in the other no excess $\delta$ chains are manufactured and their rate of synthesis may actually be decreased but instead the production of $\gamma$ chains is vastly increased. Such differential effects cannot be dismissed as simple compensatory phenomena. But how can a $\beta$ gene whose primary effect should be limited to a $\beta$ chain substitution with consequent depression of $\beta$ chain synthesis determine whether an excess of either $\gamma$ or $\delta$ chains accumulates? Yet there can be no doubt that in each case such effect is directly controlled by a single gene.

The dilemma can be resolved by the assumption that the nature of the substitution within the anomalous $\beta$ chain itself differs in the two types and in each case leads to a different kind of metabolic block involving a different step in the synthesis of the polypeptide, and thus resulting in the accumulation of a different by-product, depending on the metabolic pathway which is left intact. In this manner $\delta$ or $\gamma$ chains would be formed preferentially as determined by the character of the $\beta$ chain substitution, and either the A\textsubscript{2} fraction or the hemoglobin F would increase accordingly. The polypeptides of the $\gamma$ and $\delta$ type might thus be regarded as primitive, incomplete or altered $\beta$ chains.

This hypothesis implies that up to a certain point the synthesis of these three polypeptides entails the use of a common pathway and consequently, that the molecules are built up in a step-wise manner. Apart from the common steps, the synthesis of $\gamma$ chains would appear to represent the most primitive pathway, which remains intact even when steps required for the formation of both $\beta$ and $\delta$ chains are affected by a block depressing their rate of synthesis. Thus, in the variants of $\beta$ thalassemia without elevation of the A\textsubscript{2} fraction, in which the substitution seems to interfere with a step required for $\beta$ and $\delta$ chain synthesis, $\gamma$ chains can still accumulate in consequence of the block. If, on the other hand, $\delta$ chain synthesis is not interfered with but a step required only for the specific completion of the $\beta$ chain is blocked, $\delta$ production will increase first, the process being as it were, closer to the "site" of the block. But since in this case the more primitive $\gamma$ chain pathway is likewise intact, a block of sufficient
severity or the presence of unusual metabolic demands for the synthesis of hemoglobin may force the increased utilization of this pathway as well, and γ chains as well as δ chains may accumulate, i.e. both the minor components F and A₂ may be elevated. In this manner the occasional exceptions to the reciprocal relationship between the two components in which hemoglobin F as well as hemoglobin A₂ is increased can be accounted for, and the hypothesis can thus reconcile the existence of the various types of β thalassemias observed.

The concept of a step-wise build-up in the formation of the polypeptides of hemoglobin for which our observations furnish support is not necessarily in conflict with current ideas regarding the template action of the genetic DNA material in the synthesis of proteins as specific substances. It is possible to conceive of such a process in one of two ways: If one wishes to postulate completely separate genes for each of the three polypeptides, β, γ, and δ, three templates with partially identical structure would have to be visualized, playing competitively as it were, upon a common substrate of amino-acids and creating partially identical polypeptides. Normally in extrauterine life the β template predominates and forces nearly all of the substrate into the ultimate β pathway, but in the presence of a mutant β gene this pathway may be rendered so difficult that the δ and γ templates become relatively more effective.

The difficulty with the assumption of three complete and independent genes which seems to be required by the "one gene—one enzyme" hypothesis lies in the fact that the synthesis of δ chains may also be depressed or at least not enhanced when β chain synthesis is affected as in the case of the "low or normal A₂" thalassemias described here. Such an effect suggests the concept of a "secondary" genetic unit which can operate only when the "primary" genetic unit furnishes an already partially organized polypeptide substrate since δ chain production seems to be dependent on an intact pathway controlled by a β gene.

In the case of the γ chains of hemoglobin F another alternative is available which indeed requires a modification of the strict "one gene—one enzyme" theory but does not appear to us to be basically incompatible with that theory. This assumption is based on the interpretation of the "switch-over" from the production of fetal to adult hemoglobin in the normal fetus as a maturation process in which the same gene successively produces different effects. Analogies involving the activity of enzymes and hormones will readily come to mind. It is conceivable that the β genes, i.e., the genes controlling the production of the polypeptide pair complementary to the α chains, are not expressing their ultimate "β" effect until some essential metabolic system is activated. Until then the effect of these genes channels the synthesis of the "non-α" polypeptides into a more primitive channel leading to the formation of the more primitive γ chains. With the completion of the ultimate ontogenetic maturation process, e.g. the activation of an enzyme system, the ultimate and complete expression of these same genes becomes manifest in the production of the more "advanced" β chains and the pathway of β chain synthesis henceforth predominates. However, the utilization of the original primitive γ pathway is never completely abandoned as shown by the persistence of traces of fetal hemoglobin in adult life. It is enhanced when a mutation makes β chain synthesis, i.e. the utilization
of the ultimate $ pathway, the mature expression of a $ gene, more difficult, as in thalassemia.

It is evident that the genetic mechanisms involved are highly complex and not yet fully described by the terms of current genetic theory. Certain other, less speculative deductions can be made from our observations. The occurrence of thalassemia major in the propositus of the L. pedigree strengthens the view that the two different genes combined in this individual are allelic and both depress $ chain synthesis, since they interact in the same manner as identical thalassemia genes of the ordinary type. Using the terminology of Ingram and Stretton in a suitably modified form, the genes may be written $\alpha^s\beta^a$ for the high $A_2$ type and $\beta^\gamma$ for the variants with normal or low $A_2$ but increased fetal hemoglobin. The genotype of ordinary thalassemia major would then be $\alpha^s\beta^a$ and that of the propositus of the L. family $\alpha^s\beta^a\beta^\gamma$. Both types of "homozygotes" lack a mechanism for the production of normal $\beta$ chains, a fact which adequately explains the severe anemia and microcytosis in either case.

Gerald and Diamond have described a remarkably similar situation in a family in which, however, one of the thalassemia genes was consistently associated with an identifiably abnormal hemoglobin component designated as Hb Lepore. The combination of this gene with an "ordinary" thalassemia gene with elevation of the $A_2$ fraction likewise produced the classical picture of thalassemia major and the Lepore trait is therefore likely to be another variant of $\beta$ thalassemia, resulting from still another type of substitution in a $\beta$ chain. Interestingly enough, the $A_2$ fraction was not elevated in the heterozygotes. The genotype of the propositus described by Gerald and Diamond might be written $\alpha^s\beta^a\beta^\gamma\text{Lepore}$.

Evidence that all these variants are multiple alleles affecting the locus for $\beta$ chain synthesis can be derived from the fact that, in addition to mutual interaction, at least one type, the "high $A_2$" variant appears to interact consistently with the sickling gene, a mutant already known to be at the $\gamma$ locus. On purely genetic grounds, Ceppellini has furnished statistical evidence for allelism between sickling and a common form of thalassemia.

It remains to discuss those hereditary conditions in which fetal hemoglobin may be elevated and which must be distinguished from the thalassemia minor types with relatively high hemoglobin F reported here. In our case material, thalassemia major can be excluded (except for the propositus of the L. family) on obvious clinical and hematologic grounds. Moreover, in this condition the elevations of hemoglobin F are generally of a much higher order. But it may be pertinent to comment on the large variations in the amounts of fetal hemoglobin recorded in thalassemia major. Allowing for differences in the age of the subject and specific situations in which the metabolic requirements might be altered, it appears likely that here, as in thalassemia minor, the character of the particular $\beta$ chain substitutions plays a role in determining the rate of $\gamma$ chain synthesis. If the gene of the R. family is not identical with that of the L. family, as seems likely in view of the interfamilial differences in the levels of hemoglobin F, our observations embrace three variants of thalassemia genes,

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each affecting the rate of synthesis of fetal hemoglobin in a somewhat different manner, and at least one additional variant is that of the Lepore trait. Assuming a minimum of four distinct thalassemia genes of the $ type—and it may be confidently predicted that other variants will come to light—ten combinations are possible in what are called "homozygotes," and variations in the composition of hemoglobin in thalassemia major are indeed to be expected.

These considerations do not include the so-called "high F" gene which requires separate discussion. The carriers of this gene do not show the stigmata of thalassemia, either minor or major, but exhibit elevations of hemoglobin F in the range of 20–40 per cent. The trait is inherited in a simple Mendelian manner and appears to interact with the sickling gene and with at least certain types of thalassemia genes. It appear likely, in view of these facts and the hypothesis presented above, that the high F gene is a mutant $ gene which conveys the structure of $ chains on the non-$ polypeptide under its control, or perhaps a throwback to an earlier evolutionary stage in which the $ genes had not yet developed the structure necessary to make the step from $ chain to $ chain synthesis possible, a molecular atavism as it were. In any case, while the high F gene must be considered in all situations where fetal hemoglobin shows a permanent and substantial elevation, it is evident that this gene is not responsible for any of the cases we have described.

**SUMMARY**

Two pedigrees, each exhibiting the presence of two distinct thalassemia genes are described. Though indistinguishable by their hematologic stigmata, the genes in each pedigree differed in their effect on the quantitative behavior of the minor hemoglobin components A2 and F. One gene produced the usual pattern of elevated values for the A2 fraction with minimal if any increases in the proportion of hemoglobin F. Another gene failed to produce increases in the A2 fraction but gave rise to substantial increments in the amounts of fetal hemoglobin. Still another gene produced a similar pattern but with less marked elevations of hemoglobin F though still well above the usual levels for thalassemic heterozygotes. There were intrafamilial similarities with respect to these features. In one family, the combination of two dissimilar genes had produced the picture of thalassemia major in the propositus.

For the particular thalassemia genes observed a reciprocal relationship in the quantitative behavior of the two minor components could be shown on the basis of comparisons including a larger case material studied by the authors. The apparent vagaries in the quantitative behavior of fetal hemoglobin in thalassemic heterozygotes could thus be explained as related to the presence or absence of an elevated A2 fraction. In general, levels of fetal hemoglobin in the former type rarely exceed 3.5 per cent, in the latter they are rarely below 4.0 per cent.

*Since this paper was submitted, further evidence in favor of allelism of the high F gene and known $ genes has become available. However apparent non-allelism has been observed in a family studied by Huisman which, if confirmed, would make a revision of this concept unavoidable.
In accordance with other genetic information the findings are compatible with the assumption that both types of thalassemia genes represent mutations of β genes affecting the synthesis of β chains and thus represent a series of multiple alleles at the β chain locus. This interpretation fits the hypothesis of Ingram and Stretton regarding the nature of the defect in thalassemia.

The factor determining whether predominantly δ or γ chains and consequently hemoglobin A₂ or hemoglobin F (or other fractions) are formed appears to be the nature of the particular amino-acid substitutions in the anomalous β chain, resulting in the utilization of one or another metabolic pathway leading to the formation of different by-products of a metabolic block. The genetic implications of this hypothesis are presented with a view to establishing that current genetic theory does not yet fully account for the observations.

The interpretation of γ chains of fetal hemoglobin as primitive β chains produced under the control of the same pair of genes which in normal adult life regulate the synthesis of β chains is offered as the basis for a unifying concept concerning the nature and genetic control of fetal hemoglobin. If valid, this concept appears applicable to all conditions thus far known, normal or abnormal, in which fetal hemoglobin occurs.

**SUMMARIO IN INTERLINGUA**

Es describite duo consanguiineitates que ambes exhibi le presentia de duo distincte genes de thalassemia. Ben que iste genes esseva indistinguibile in lor stigmas hematologic, illos differeva—in ambe consanguiineitates—in lor efecto super le comportamento quantitative del minor componentes hemoglobinic A₂ e F. Un del genes produceva le usual combination de elevate valores pro le fraction A₂ con minime o nulle augmentos del proportion de hemoglobina F. Un secunde gen non causava augmentos in le fraction A₂ sed resultava in accrescimentos appreciabile de hemoglobina fetal. Ancora un altere gen produceva un simile configuration sed con minus marcate elevationes de hemoglobina F, attingente—nonobstante—nivello ben in supra de lo que es usual in heterozygoticos thalassemic. Esseva notate similitudes intrafamilial con respecto a iste caracteristicas. In un del familias le combination de duo dissimile genes habeva producete le tableau de thalassemia major in le probando.

Pro le genes thalassemic observate in particular, un relation reciproc in le comportamento quantitative del duo componentes minor poteva esser demonstrate super le base de comparations con un plus extensc casuistica studiate per le autores. Le apparente excentricitates in le comportamento quantitative de hemoglobina fetal in heterozygoticos thalassemic poter era assi explicar se como un phenomeno que es relationate con le presentia o le absentia de un elevation del fraction A₂. A generalmente parlar, in le prime del duo mentionate typos le nivello de hemoglobina fetal excede rarmente 3,5 pro cento; in le secunde, ille nivello es rarmente infra 4,0 pro cento.

De accordo con aliter informaciones genetic, le constatationes del presente studio se trova in compatibilitate con le supposition que ambe typos de gen de thalassemia representa mutationes de genes beta, afficiere le synthese de catenas beta, e que assi illos representa un serie de alleles multiple al loco de
catena beta. Iste interpretation es in harmonia con le hypothese de Ingram e Streton con respecto al natura del defecto in thalassemia.

Le factor determinante si catenas delta o catenas gamma es formate predominantemente—e consequentemente hemoglobina A₂ o hemoglobina F (o ancora altere fractiones)—pare esser le character del substitutiones aminoacidic particular in le catena beta anormal, con le resultato del utilisation del un o del altere circuito metabolic e le consequentia del formation del un o del altere producto lateral de un bloco metabolic. Le signification inherente in iste hypothese es presentate con le objectivo de establir que le theoria genetic in su stato currente es non ancora completemente qualificate a explicar le observations.

Le interpretation que catenas gamma de hemoglobina fetal es catenas beta primitive produite sub le governantia del mesme par de genes que in le vita adulte normal determina le synthese de catenas beta es presentate como base de un conception unificatori del natura e del regulation de hemoglobina fetal. Si illo se prova valide, iste conception pare esser applicable a omne le conditions usque nunc cognoscite—normal e anormal—in le quales hemoglobina fetal es incontrate.

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Reciprocal Relationship of Hemoglobins Aα and F in Beta Chain Thalassemias, a Key to the Genetic Control of Hemoglobin F

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