ANALYTICAL REVIEW

Perspectives in the Genetics of Sickle Cell Disease

By JAMES V. NEEL, M.D., PH.D.

ALL TOO OFTEN medical genetics is regarded as a largely descriptive science devoted to the collection of pedigrees with the deductive aspects confined to the determination of the most probable genetic mechanisms responsible for certain numerical relationships. It is not generally recognized that the methodology of genetics can on occasion offer investigators a valuable tool in the definition and clarification of clinical entities. Recent developments pertaining to sickle cell disease serve to illustrate the possible application of genetic technics to the synthesis of clinical observations.

For many years after the discovery of the sickling phenomenon, confusion existed concerning the clinical relationship between persons whose blood cells could be induced to sickle by appropriate tests but who were apparently healthy, and persons with a severe hemolytic anemia and protean symptomatology who sickled more readily, and, in fact, whose peripheral blood film often contained sickle cells. Gradually, however, it became clear that the apparently healthy persons who sickled did not merely represent a remission of the diseased state, nor did those with active disease ever return completely to normal. Especial credit for this distinction between the sickle cell trait and sickle cell anemia is due Diggs[1] and Sherman.[2]

The medical literature abounds with reports of studies of the various manifestations of sickle cell disease not only in Negroes but in Caucasians not known to have any Negro ancestry whatsoever. A re-examination of certain of this literature is now indicated in the light of recent developments which make it clear that from the genetic standpoint it is necessary to recognize three types of sickle cell disease, with the possibility of still further subdivision to come.

When the families of a random series of Negro patients with sickle cell anemia are investigated with modern technics for eliciting the sickling phenomenon, it is observed that in the great majority of cases the blood of both parents can be induced to sickle. This observation implies that sickle cell anemia usually develops on the background of a genetic contribution from both parents. The simplest genetic postulate which will account for this observation, and also the numerical relationships between children with sickle cell anemia, the sickle cell trait and no hematologic abnormality, in families in which one child with sickle cell anemia has been born, is that there is a gene which in the simplex (heterozygous) state is responsible for the sickle cell trait, and in the duplex (homozygous) state is responsible for sickle cell anemia. A child who receives from each parent the gene responsible for the sickling phenomenon is destined to develop sickle cell anemia.[3–5] As yet there is no clear evidence that more than one gene is involved in the presence or absence of the sickling phenomenon.

From 20 to 45 per cent of the hemoglobin of persons with the sickle cell trait,

From the University of Michigan Heredity Clinic, Ann Arbor, Mich.

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and 80 to 100 per cent of the hemoglobin of persons with sickle cell anemia, has been demonstrated by the technics of electrophoresis to be of an abnormal type.[6, 7] Studies on families in which the sickle cell trait is segregating have demonstrated significant intrafamily correlations in the proportion of abnormal hemoglobin present.[8] That is to say, in some families in which the sickle cell trait is present, the proportion of hemoglobin which is abnormal tends to be lower than in others. The present most probable explanation of this observation appears to be that there are modifying genes which, although incapable alone of eliciting sickling, can in the presence of a sickle cell gene influence significantly the production of abnormal hemoglobin. This is in keeping with a genetic truism that the action of most if not all genes is powerfully influenced by the genetic milieu in which they find themselves. It has recently been shown that the formation of tactoids which can be demonstrated in solutions of sickle cell trait and sickle cell hemoglobin, and which is thought to provide the molecular basis for the sickling phenomenon, does not occur below certain critical concentrations of the sickle cell type of hemoglobin.[9] The theoretical possibility emerges that some of the genetic modifiers referred to above may depress the production of the sickle cell type of hemoglobin to the point where tactoid formation cannot take place.

Although both parents of a child with sickle cell anemia usually exhibit the sickle cell trait, there are occasional clear exceptions to this generalization. Such exceptions were relatively more frequent in the older literature, before the technics for eliciting sickling had reached their present level of dependability. These unusual families permit of at least five explanations: (1) discrepancy between the legal and the biological father; (2) suppression of the action of the sickle cell gene by a genetic modifier; (3) technical failures in eliciting sickling; (4) mutation; and (5) contribution by the apparently normal parent of a gene or genes which, in conjunction with a single sickle cell gene, produces sickle cell anemia.

Insufficient data are at hand to determine the relative frequencies with which each of these five alternatives obtains. However, hematologic and biochemical studies of these apparently "exceptional" parents have made it clear that where nonpaternity cannot be demonstrated, the fifth alternative deserves especial consideration before the other three possibilities be entertained. There is now clear evidence for the occurrence of at least two other genetic backgrounds on the basis of which there may develop a clinical syndrome frequently indistinguishable from classical sickle cell anemia:

1. The nonsickling parent may be heterozygous for the gene responsible for thalassemia (Mediterranean anemia), with transmission of this gene to a child who receives a sickle cell gene from the other parent; sickle cell anemia develops on the background of heterozygosity for both the sickle cell and thalassemia genes.[10–12] This type of sickle cell anemia approximates in severity the more usually encountered type. All of the reported cases where this situation exists have involved individuals nominally of Italian or Greek derivation, and it seems probable that this is the explanation for the majority of cases of sickle cell disease reported in Caucasians since, given the existence of a "Caucasian" gamete containing a sickle cell gene, the probability that it meet another such "Caucasian" gamete is in most communities less than that it meet a gamete containing

a thalassemia gene, because of the higher frequency of thalassemia in Caucasians. Yet there have recently been described non-Negroid communities where the frequency of sickling is such as to render the usual (homozygous) genetic explanation of sickle cell anemia the more probable even in Caucasians.[13, 14]

2. Recent electrophoretic studies of the nonsickling parent have demonstrated that in some instances there is present a previously unrecognized type of abnormal hemoglobin, inherited as if due to a dominant gene, with sickle cell anemia developing as above on the background of heterozygosity for both the sickle cell gene and the gene responsible for the new abnormality.[15, 16] In terms of electrophoretic mobility, this newly recognized abnormality differs from normal hemoglobin even more than sickle cell hemoglobin. On the basis of a very limited experience to date, it appears that this particular genetic entity tends to be milder than either of the other two types of sickle cell disease, and may have contributed to the concept of symptoms in the absence of anemia.[17]

We must recognize, then, on the basis of genetic studies, three types of sickle cell anemia, one much more common than the other two. Of the two less frequent types, the one accounts for most of the cases in Caucasians, and the other for some (or possibly all) of the "mild" cases of sickle cell anemia, such as have in the past contributed to confusion regarding the validity of the distinction between the sickle cell trait and sickle cell anemia.

The question of the desirability of using the term "sickle cell anemia" to include the end product of three different genetic situations is certain to evoke discussion. There appears to the author to be ample precedent for this. For instance, the term retinitis pigmentosa designates a clinical entity which may be inherited in at least four different ways.

It seems probable that with the passage of time, all gradations between individuals with a small proportion of the sickle cell type of hemoglobin and individuals with 100 per cent of this type of hemoglobin will be described, the lower portion of the range related to the presence of a single sickle cell gene, the upper portion of the range related to the presence of two sickle cell genes, and the intermediate values for the most part due to the interaction of a single sickle cell gene with other specific genes such as have been described above. The majority of individuals exhibiting the sickling phenomenon will fall into two well defined categories, corresponding to the two ends of the range, with relatively few intermediates, analagous to the situation in thalassemia. Nevertheless, the possible occurrence of such intermediates raises a problem as to the dividing line between the sickle cell trait and sickle cell anemia. It is suggested that the term sickle cell anemia be reserved for those individuals in whom the sickling phenomenon is associated with a significantly increased rate of blood destruction.

The recent report that a fraction of the hemoglobin present in sickle cell anemia resists denaturation at a pH of 12.7 for one minute, whereas this is not the case in the sickle cell trait or in normal individuals, would appear to offer a ready means of distinction between the trait and the disease.[18, 19] However, there is a wide range in the proportion of "resistant" hemoglobin, and it appears possible that "intermediates" may in time be recognized here. Furthermore, it remains to be seen whether this difference is qualitative, quantitative, or both, i.e., hemoglobin resistant to alkaline denaturation may be present in both trait

and anemia, with a fraction of that present in the disease usually *more* resistant than any present in the trait. In other words, the present test conditions may, in sickle cell anemia, "cut the tail off" a distribution of alkali resistant hemoglobin molecules which in part overlaps with a distribution present in the sickle cell trait. The relation of the "alkali resistant" fraction to the "electrophoretically abnormal" fraction remains unclear. The possibility that a single gene may be responsible for the presence of two distinct abnormal types of hemoglobin is of especial interest to the geneticist.

The sickling phenomenon has been observed in between 15 and 20 per cent of all African natives living south of the Sahara tested to date.[20] On the above genetic hypothesis, this implies that approximately 5 to 10 out of each 1,000 children born should develop sickle cell anemia. With some 180,000,000 Africans living south of the Sahara, one would expect this disease to be a well defined medical problem. Such does not appear to be the case. Although sickle cell anemia has been described in native Africans, these reports are relatively few in number. This situation admits of two chief explanations. It is, on the one hand, possible that the disease occurs but has not been recognized, perhaps because of the early death of affected persons. It is, on the other hand, possible that in native Africans genetic modifiers such as have been referred to above as possibly affecting the proportion of abnormal hemoglobin in the sickle cell trait, and or unknown environmental factors, tend to mitigate the effects of homozygosity for the sickling gene. Essentially this latter possibility has been broached by several clinicians with wide experience in Africa.[21, 22] According to this latter view, "the appearance of sickle-cell anemia depends, not only on the extent to which the trait is present in a community, but also on the extent to which admixture with other genetic strains has occurred."[21] The "high" incidence in America is presumably in some way a reflection of Negro-Caucasian-Indian admixture.

The differentiation between these two possible explanations of the African findings should not be difficult. Four lines of attack are indicated. (1) The developments in the field of electrophoresis referred to above permit one to quantitate in very objective terms the results of heterozygosity and homozygosity for the sickling gene. Should the second alternative prove correct, then the percentage of abnormal hemoglobin should on the average be less in pure Negro heterozygotes or homozygotes than in Negro-Caucasian-Indian admixtures. (2) According to theory, one-quarter of the children of marriages involving two persons with the sickle cell trait should at birth possess disease potentialities not present in the offspring of normal x normal or normal x sickle cell trait marriages. A comparison in Africa of the issue of these three types of marriage, such as has recently been carried out in Italy with thalassemia,[23] should, if conducted on a sufficiently large scale, permit a definite decision. (3) As the general level of medical practice among the African native improves, the accumulation of further casuistic data of help in reaching a decision will be inevitable. Particular interest attaches, of course, to the occurrence of the disease in infancy and childhood. Recent data suggest that the disease is more common in Africa than the scattered case reports which have appeared thus far would indicate.[24–26] (4) Finally, in the United States the relationship in "Negroes" between the apparent

amount of white admixture and the severity of the sickle cell disease needs further evaluation.

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