The Inheritance of Sickle Cell Anemia[1]

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IF A DROP OF BLOOD is collected from each member of a randomly assembled series of American Negroes and sealed under a cover slip with vaseline, to be observed at intervals up to 72 hours, in the case of about 8 percent of the individuals composing the series a high proportion of the erythrocytes will be observed to assume various bizarre oat, sickle, or holly leaf shapes. This ability of the erythrocytes to "sickle," as the phenomenon is commonly described, appears to be attended by no pathological consequences in the majority of these individuals, and they are spoken of as having sicklemia, or the sickle cell trait. However, a certain proportion of the individuals who sickle are the victims of a severe, chronic, hemolytic type of anemia known as sickle cell anemia. This proportion has been variously estimated at between 1:1.4 (*8*) and 1:40 (*4*). The essential difference between sicklemia and sickle cell anemia appears at present to depend at least in part upon the relative ease with which sickling takes place. In sickle cell anemia the erythrocytes may frequently sickle under the conditions encountered in the circulating blood, whereas in sicklemia sickling does not usually occur under these conditions (*12*). This difference has been attributed to a greater tendency of the erythrocytes of sickle cell anemia to sickle when the O_2-tension is reduced, although recently this viewpoint has been challenged (*13*). Perhaps because of this difference—although there may be other factors involved, such as the aniso- and poikilocytosis to be observed in some individuals with the disease, and a greater resistance to hemolysis of trait cells when sickled than sickle cell anemia cells when sickled—the erythrocytes of a patient with sickle cell anemia have a greatly shortened life span, both in the individuals with the disease and in normal persons who have been transfused with the cells of sickle cell anemia patients, whereas sicklemia erythrocytes have an normal life span (*3, 14*).

The ability of the red cells to sickle was observed to have a genetic basis not long after sickle cell anemia was recognized as a clinical entity (*5*). On the basis of a study of one large family, Taliaferro and Huck (*15*) postulated that the ability to sickle was due to a single dominant gene. At that time the clinical distinction between sicklemia and sickle cell anemia had not been clearly drawn, and the inference was that this gene was more strongly expressed in some individuals (sickle cell anemia) than in others (sicklemia). This has remained the accepted hypothesis up to the present time. Several years ago the author, in a review on the clinical detection of the genetic carriers of inherited disease (*9*), was led to suggest an alternative hypothesis—namely, that there existed in Negro populations a gene which in heterozygous condition results in sicklemia, and in homozygous condition in sickle cell anemia. This hypothesis has a counterpart in the relationship which has been demonstrated to exist between thalassemia major and minor (*10, 16*). Recently the opportunity has arisen to give this hypothesis a thorough test.

There exist a number of arguments permitting a critical decision between the two hypotheses. The present preliminary note will consider only one of these arguments. If the homozygous-heterozygous hypothesis is correct, then both the parents of any patient with sickle cell anemia should always sickle (barring the occasional role of mutation; see below). If, on the other hand, the disease is due to a dominant gene with variable expression, only one parent need sickle, although occasionally, due to the chance marriage of two sicklers, both parents may sickle. In calculating the exact proportion of sicklemia to be expected among the parents of individuals with sickle cell anemia according to the dominant hypothesis, certain assumptions must be made. To the best of the author's knowledge, the question of the phenotype of the homozygote has never been raised by those who have accepted the variable dominant hypothesis of sickle cell anemia. For purposes of calculation we shall assume that under the variable dominant hypothesis all homozygotes have sickle cell anemia—alternative assumptions, such as intra-uterine lethality, are possible. We shall further assume that one in fifty heterozygotes also develops sickle cell anemia. Finally, we shall assume on the basis of the clinical data that the fertility of those with sickle cell anemia approximates 20 percent of normal, with the result that only a few individuals with this disease—so few

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that they may be disregarded in so rough a calculation—have one or both parents who are likewise affected. With these assumptions we may calculate, as shown in Table 1, that the proportion of sickling among the parents of individuals with sickle cell anemia should be 0.765. If one assumes that more than one in fifty of the heterozygotes develop sickle cell anemia, or that the homozygote is lethal, then the proportion of sickling parents should be even lower.

TABLE 1

CALCULATION OF THE PROPORTION OF SICKLING TO BE EXPECTED AMONG THE PARENTS OF INDIVIDUALS WITH SICKLE CELL ANEMIA ACCORDING TO THE VARIBLE DOMINANT HYPOTHESIS*

Type of marriage	Frequency of marriage	Frequency of offspring of the indicated genotype			Sickle cell anemia patients	
		Sk Sk	Sk sk	sk sk	Proportion in general population	Proportion among total anemia patients
One sickler parent (Sk sk × sk sk)	2 × 0.08 × 0.92 = 0.1472	.....	0.0736	0.0736	0.02 × 0.0736 = 0.001472	0.4693
Two sickler parents (Sk sk × Sk sk)	0.08 × 0.08 = 0.0064	0.0016	0.0032	0.0016	0.0016 + (0.02 × 0.0032) = 0.001664	0.5307
Total					0.003136	1.0000

* The assumption is made that all individuals homozygous for the sickling gene (Sk) develop sickle cell anemia, as do 1 in 50 persons heterozygous for the gene, and further that individuals with sickle cell anemia reproduce so infrequently that no significant error is introduced by their omission.

Expected proportion of sickling parents = (proportion of patients having *one* parent sickler) × (proportion of sicklers among these parents) + (proportion of patients having *both* parents sicklers) × (proportion of sicklers among these parents) = 0.4693 × ½ + 0.5307 × 1 = 0.765.

Thus far we have tested 42 parents of 29 patients with sickle cell anemia for the occurrence of sickling. In 13 instances both parents were studied and in 16, only one. Tests have been conducted in a variety of ways; especial reliance has been placed on a combination of the techniques described by Scriver and Waugh (*11*) and Hansen-Pruss (*7*), whereby a tourniquet is applied to a finger for 3–5 minutes, and then a drop of static blood from the finger is placed on a slide to which a small amount of Janus green or methylene blue has been added, and it is quickly covered with a cover slip which is sealed with vaseline. Observations are made at intervals up to 72 hours. Five preparations have been made for each individual. Every parent tested to date has sickled. This is the result expected from the homozygous-heterozygous hypothesis outlined above. On the other hand, the probability of the occurrence of such a number of positive parents under the variable dominant hypothesis is $(0.765)^{42}$, or 0.000013.

There are to be found in the literature a number of reports where one or both parents of a child with sickle cell anemia have been tested and found not to sickle (review in reference *9*). The results of tests for the sickling phenomenon are known to be variable; it is felt that the experience quoted may be explained in terms of lack of familiarity with the techniques necessary to elicit sickling.

The approximate frequency of the gene responsible for sickling in the American Negro (p) may be determined from the equation $2p\ (1-p) = 0.08$. Solution of this equation yields a p value of 0.042, from which the incidence at birth of this chronic, disabling, and fatal disease among American Negroes may be placed at $(0.042)^2 = 1.8$ per 1000.[2] The ratio among Negro births in the United States of those with sicklemia to those who will develop sickle cell anemia should therefore be approximately 80 : 1.8 = 44 : 1; in the Negro population as a whole the ratio of sicklemia : sickle cell anemia is significantly higher because of the greater mortality among those with sickle cell anemia. In Africa, the incidence of sickling has been reported to vary from approximately 12 percent in Northern Rhodesia (*1*) to 17 percent in the Gold Coast Negroes and 19 percent in natives of Nigeria and the Camaroons (*6*). This would correspond to a gene frequency of approximately 0.064–0.106, and a frequency of the homozygote of 4.1–11.2/1000. The complex and fascinating problems in gene dynamics raised by frequencies of this order will be dealt with in another paper.

In a genetic situation such as appears to obtain here, where the heterozygote, who may be termed the genetic carrier of the disease, may be readily distin-

[2] The correct formula is $y = \alpha\ p + (1-\alpha)p^2$, where α = the mean coefficient of inbreeding. The value of α for the American Negro is unknown, but probably quite small, in the neighborhood of 0.0005. For present purposes the value of p^2 is a sufficiently close approximation to y.

guished from normal and from the homozygote, it is possible to predict with a high degree of accuracy which marriages should result in homozygous individuals—in this case, children with sickle cell anemia. Since (homozygous) individuals with sickle cell anemia either die young or, if they reach maturity, have a greatly lowered fertility, the vast majority of cases of the disease are the issue of marriages between two (heterozygous) persons with the sickle cell trait. In the absence of marriage between individuals whose erythrocytes exhibit the sickling phenomenon, the frequency of the homozygote would greatly decrease, and sickle cell anemia would tend to disappear, with only a very rare case arising as a result of mutation in a normal individual married to a person homozygous or heterozygous for the sickling gene.

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TECHNICAL PAPERS

New Sectioning Techniques for Light and Electron Microscopy

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The application of the electron microscope to many biological problems has been seriously hampered by the lack of a rapid practical method of cutting uniformly thin sections having adequate area and integrity of structure. Because of the very slight penetrating power of the beam in commercial electron microscopes and the great relative depth of field involved, specimen structure is difficult to interpret when sections are over a fraction of a micron in thickness.

Although various solutions to this problem have been described in the literature, their success and general application have been limited. A departure from classical approaches to the problem has been the high speed microtome (*2*, *3*). However, this precision equipment is not only expensive and complicated but produces a low percentage of usable sections. Moreover, the sections are cut so rapidly and abundantly that selection is time-consuming and uncertain. Several workers (*1*, *5*) have described a technique which uses the thinnest portions of wedge-shaped sections for electron microscopy. Their methods, however, have been laborious and difficult to reproduce.

The most recent effort has been that of Pease and Baker (*4*), who have used standard histological techniques to embed tissue in collodion and paraffin. For sectioning, they altered a Spencer rotary microtome so that the unit of advance was reduced to approximately one-tenth the calibrated value. The microtome was then reported to produce sections as thin as 0.1 μ. Many workers, however, have had trouble in using their technique, mainly because of the exacting demands made on the microtome-advancing mechanism. Another disadvantage has been the difficulty of making very thin sections with the standard embedding media, such as paraffin and collodion.

In recognition of this problem, a promising new development in ultramicrotomy is presented. It consists of a method for obtaining extremely thin sections, involving the use of a methacrylic resin as an embedding medium, a thermal expansion device for advancing the specimen in a commercial microtome, and metallic shadow-casting for increasing observable detail in some of the sections. These techniques form the basis of a new method for producing numerous thin sections suitable for obtaining transmission images at the higher magnifications in the conventional light, phase-contrast, and electron microscopes. Polymerization of *n*-butyl methacrylate provides a rapid and simple means for embedding the fixed biological material in a solid resin. This gives an optically clear matrix from which the sections are cut, one at a time. Smooth, continuous advance of the embedded