Human Hemoglobin[1]

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ALTHOUGH DIFFERENCES IN THE PHYSICAL AND CHEMICAL PROPERTIES of human adult and fetal hemoglobins have been known for many years, the discovery of an electrophoretically abnormal hemoglobin in sickle cell disease provided the first positive evidence that adult human hemoglobin exists in more than one molecular form (*1, 2*). Two other species of adult human hemoglobin have now been described (*3, 4*), and an alkali-resistant hemoglobin component that has the properties of normal fetal hemoglobin has also been found in some anemic individuals (*5–11*). At least ten genetically distinct conditions that may be characterized by the hemoglobin composition of the erythrocytes have been observed, and it is of importance to the hemoglobin investigator to be able to recognize the presence of abnormal components in order to avoid the use of inhomogeneous preparations. To the geneticist and the hematologist the study of abnormal hemoglobins provides a method for differentiating inherited hematological abnormalities which other methods may fail to distinguish or even detect. One of the most significant conclusions based on these studies is that a molecular abnormality in a single protein may cause a sequence of events that characterizes a complex disease (*1*).

Fetal hemoglobin is produced in the human fetus and is the predominant form during prenatal life. At birth it comprises 55–98 per cent of the hemoglobin of infants (*8, 12, 13*). In the majority of healthy individuals this form of hemoglobin is no longer detectable after the first year of life, but in certain chronic anemias the production of fetal hemoglobin in varying amounts may continue indefinitely (Table 1).

Adult hemoglobin appears in the fetal blood early in prenatal life; in one 20-week fetus 6 per cent of the hemoglobin was of this form (*14*). It eventually replaces all the fetal hemoglobin in nonanemic individuals. *Normal adult hemoglobin* is the only form present in the great majority of adults. Three abnormal hemoglobins, all of which are rarer than normal adult or fetal hemoglobin and are associated with hematologic disorders, have been reported. From the biochemical and genetic evidence, which will be considered in this review, it will be evident that these are *abnormal adult hemoglobins* and not abnormal fetal hemoglobins that are being produced past fetal life.

TABLE 1

HEMOGLOBIN SPECIES PRESENT IN INDIVIDUALS WITH INHERITED AND ACQUIRED CONDITIONS

Condition	Species of hemoglobin present				
	a (normal adult)	*b* (sickle cell)	*c*	*d*	*f* (normal fetal)
Normal adult	+	−	−	−	−
" newborn	+	−	−	−	+
Sickle cell trait	+	+	−	−	−
" " anemia	−	+	−	−	+
Hemoglobin-*c* trait	+	−	+	−	−
Sickle cell–hemoglobin-*c* disease	−	+	+	−	±
Hemoglobin-*d* trait	+	−	−	+	−
Sickle cell–hemoglobin-*d* disease	−	+	−	+	+
Thalassemia minor	+	−	−	−	±
Thalassemia major	+	−	−	−	+
Sickle cell–thalassemia	+	+	−	−	+
Some acquired anemias	+	−	−	−	+

Sickle cell trait and sickle cell anemia are characterized by the presence of erythrocytes that are capable of undergoing changes in shape in response to changes in oxygen tension. When oxygenated, the cells are biconcave disks; when deoxygenated, they become sickle-shaped or multipointed. The erythrocytes in these two conditions differ in that a greater reduction in oxygen tension is required to induce complete sickling in sickle cell trait than in sickle cell anemia (*15*). The apparent relationship between oxygenation and sickling stimulated the initial studies in these laboratories of the hemoglobin in sickle cell disease (*1, 2*). The physical-chemical basis for the earlier observations was provided by the discovery that the hemoglobin in sickle cell anemia—a chronic, hemolytic anemia—consists mainly of a component having about three more net positive charges per molecule than normal adult hemoglobin in the pH range 5.7 to 8.0. In sickle cell trait, which is not associated with anemia, both this abnormal hemoglobin (named *sickle cell hemoglobin*) and normal adult hemoglobin were found in the same erythrocytes.

An early study of the inheritance of sickling resulted in the conclusion that a dominant gene is re-

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sponsible for the transmission of this erythrocyte property (*16*), but no distinction was made between the modes of inheritance of sickle cell anemia and sickle cell trait. More recently it has been postulated that individuals with sickle cell trait are heterozygous in the gene for sickling, and those with sickle cell anemia are homozygous in this gene (*17, 18*). In accordance with the latter hypothesis, each parent of an individual with sickle cell anemia must carrry at least one gene for sickling; indeed, this situation occurs in the great majority of families in which the inheritance of this disease has been investigated (*19*). There are, however, some families in which only one of the parents of a child having hemolytic anemia associated with sickling cells has sickle cell trait. In each such case investigation has revealed a hematologic abnormality differing from sickle cell trait. In some of these families the diseased child has sickle cell hemoglobin and a second abnormal hemoglobin (*3*), which differs electrophoretically from both normal and sickle cell hemoglobins. The nonsickling parent has both normal adult hemoglobin and the second abnormal hemoglobin, and the sickling parent has the sickle cell trait mixture of normal adult and sickle cell hemoglobins. Normal children, sickle cell trait children, and children with the normal adult-second abnormal hemoglobin combination have also been observed in such matings (*3, 20*). In one family the nonsickling parent and two of the children had normal adult hemoglobin and a third abnormal hemoglobin (*4*) having the same electrophoretic mobility as sickle cell hemoglobin but a higher solubility. This hemoglobin and sickle cell hemoglobin were present in the diseased children. A fourth genetic type of sickle cell disease, in which the nonsickling parent has the thalassemia gene, is known (*21*). The erythrocytes of the anemic children have characteristics that reflect the influence of both the sickling and thalassemia genes. Both normal adult and sickle cell hemoglobins are present in the erythrocytes, but their relative amounts are abnormal (*11, 22*). In addition to the hemoglobin components described above, normal fetal hemoglobin may be present in each of the four anemias (*23*).

Nomenclature

The first abnormal hemoglobin has invariably been found in sickling erythrocytes and is appropriately named sickle cell hemoglobin, but the other abnormal forms are unnamed. Different symbolic representations for the human hemoglobins have been proposed (*9, 24*), but none of them includes all the known forms. The system I have proposed (*4*), now extended to include fetal hemoglobin, appears to be the most adaptable to future developments in the field, and will be used throughout this account. The two normal forms of human hemoglobin, normal adult and normal fetal, may be represented by their initials, *a* and *f*. The abnormal adult forms, sickle cell hemoglobin and the second and third abnormal hemoglobins, may be designated by the letters *b, c,* and *d,* respectively, in the chronological order of their discovery.

Sickle cell anemia, or sickle cell disease, is characterized by the presence of erythrocytes that become sickle-shaped when deprived of oxygen, and by symptoms and signs that are ascribable to a chronic hemolytic anemia and vascular occlusions. Some observers have felt that there are shades of variation in the severity of the anemia in this condition. Investigations in recent years, as cited above, have disclosed the basis for these observations; namely, there are four biochemically and genetically distinct conditions in which sickling erythrocytes and some or all of the classical symptoms of sickle cell anemia are present. In some cases there is little or no anemia. All these conditions may be grouped under the heading of sickle cell disease, but specific names should be given to each of the genetically distinct conditions. The name sickle cell anemia may properly be assigned to the type in which sickle cell hemoglobin is the only form of adult hemoglobin present, since this condition is always associated with anemia and undoubtedly includes the great majority of cases described in the literature as sickle cell anemia. A second form of sickle cell disease results from the simultaneous presence of the sickling and thalassemia traits, and may be termed sickle cell–thalassemia. The other two forms may be conveniently named sickle cell–hemoglobin-*c* disease and sickle cell–hemoglobin-*d* disease, after the two adult hemoglobins present in each of these conditions.

The asymptomatic condition in which sickle cell hemoglobin and normal adult hemoglobin are present has long been known as sickle cell trait, and the analogous conditions involving hemoglobin-*c* and -*d* may be called hemoglobin-*c* trait and hemoglobin-*d* trait, respectively. Although thalassemia major and its relatively mild counterpart, thalassemia minor (*25*), are not known to be characterized by the production of an abnormal form of hemoglobin, they are manifestations of an inherited abnormality in hemoglobin metabolism (*10*) and must be included in any discussion of abnormal hemoglobin syndromes.

Methods of Investigation

The use of hematologic methods is essential in selecting cases to be examined for hemoglobin abnormalities. The sickling test is a reliable criterion for the presence of sickle cell hemoglobin. Microcytemia, hypochromia, increased osmotic resistance of erythrocytes to hypotonic saline, and target cells are observed in the presence of the thalassemia gene (*21, 25*). A high incidence of target cells has been reported in the presence of hemoglobin-*c* (*24*). The determination of the survival time of transfused erythrocytes, although too laborious to be useful as a routine diagnostic procedure, has yielded much information on the behavior of abnormal erythrocytes in the circulation (*26–28*). The importance of familial hematologic studies in detecting the rare forms of sickle

cell disease is apparent from the earlier discussion.

The hemoglobin composition of the erythrocytes is established by physical and chemical methods. Electrophoretic analyses not only determine the species of hemoglobin but also the ratios in which they are present in an individual, and familial studies of these ratios have yielded significant genetic data (*20, 29*). Fetal hemoglobin can be identified most rapidly by its high resistance to denaturation (*11*) in aqueous alkaline solutions, by a maximum in its absorption spectrum at 2898 A (*13, 30*), and by paper chromatography (*31*). A rapid method for determining the solubility of amorphous ferrohemoglobin (*4*) has contributed to the identification of hemoglobin-*d*, which is indistinguishable electrophoretically from sickle cell hemoglobin. The procedure has been refined to increase its reproducibility and to permit a tentative differentiation among other abnormal hemoglobin syndromes (*11*). The refinement consists of the use of exactly 50 mg of hemoglobin instead of the large excess previously specified. Also, instead of a series of precipitating systems, only two, containing 8.00 ml and 9.20 ml of 2.80 *M* phosphate buffer, respectively, in a system of 10.00 ml total volume, are used; 100 mg of sodium dithionite, $Na_2S_2O_4$, is added to each system to ensure complete conversion to ferrohemoglobin. The precipitation and equilibration are carried out at 25° C.

Results

The electrophoretic properties that we have observed in the differentiation of the normal and abnormal human hemoglobins are summarized in Table 2. The data for hemoglobin-*c* and -*d* have been derived

TABLE 2

Electrophoretic Properties of Human Hemoglobins

Name	Symbol	Isoelectric point*	Mobility at pH 6.5†	Relative mobility in .01 *M* Na_2HPO_4‡
Normal adult	*a*	6.87	2.4×10^{-5}	1
" fetal	*f*	—	—	2
Sickle-cell	*b*	7.09	2.9×10^{-5}	3
Second abnormal	*c*	—	3.2×10^{-5}	4
Third abnormal	*d*	7.09	2.9×10^{-5}	3

* Carbonmonoxyhemoglobin in potassium phosphate buffers of 0.1 ionic strength (*1*).

† Apparent mobilities of carbonmonoxyhemoglobin in $cm^2\ sec^{-1}\ volt^{-1}$, calculated from ascending boundaries in cacodylate buffer of 0.1 ionic strength (*3, 32*).

‡ In order of decreasing mobility of the carbonmonoxyhemoglobins. The numbers have no quantitative significance.

from analyses of mixtures, as neither form has been found free of either normal hemoglobin or sickle cell hemoglobin. In phosphate and cacodylate buffers of 0.1 ionic strength in the isoelectric region, normal adult, sickle cell, and hemoglobin-*c* have significantly different mobilities (*1–3*). Normal adult and normal fetal hemoglobin mobilities are nearly the same in these buffers, and mixtures of the two do not yield discrete boundaries on electrophoresis (*23*). In 0.01 *M* Na_2HPO_4, normal adult hemoglobin has a measurably higher mobility than fetal hemoglobin (*14*), and electrophoretic analysis in the Tiselius apparatus yields two-peak boundary diagrams. In this system the mobility of sickle cell hemoglobin is lower than that of fetal hemoglobin and that of hemoglobin-*c* is the lowest (*23*). These differences have been established by the examination of mixtures, since the poor buffering capacity of this system precludes precise determinations of absolute mobility. Sickle cell hemoglobin and hemoglobin-*d* have identical mobilities in all these buffers (*4, 23*), yet they are readily distinguished by ferrohemoglobin solubility determinations.

TABLE 3

Solubility of Naturally Occurring Hemoglobin Mixtures Containing Sickle Cell Hemoglobin

Inherited condition	No. of individuals	Solubility* in g/liter
Sickle cell trait	15	1.28–2.17
" " anemia	7	0.14–0.44
Sickle cell–hemoglobin-*c* disease	5	1.13–1.23
" " " -*d* "	1	0.66
" " thalassemia	3	0.44–0.90

* As amorphous ferrohemoglobin at 25° C in aqueous system of 10.00 ml total volume, containing 8.00 ml 2.80 *M* phosphate buffer (*4*), 100 mg of $Na_2S_2O_4$, and 50 mg of hemoglobin.

The electrophoretic results described above have been observed in carbonmonoxyhemoglobin solutions. Ferrohemoglobin solutions have been examined in phosphate buffer, and the normal adult and sickle cell ferrohemoglobins exhibit a difference in isoelectric points similar to that between the corresponding carbonmonoxyhemoglobins (*1*).

The conditions of the modified amorphous ferrohemoglobin solubility method were chosen so that, in the absence of sickle cell hemoglobin, precipitation does not occur in the system containing 8.00 ml of phosphate buffer. Mixtures that go completely into solution in this system are then examined in the system that contains 9.20 ml of buffer. Tables 3 and 4 summarize the results of this method, which will be

TABLE 4

Solubility of Naturally Occurring Hemoglobin Mixtures Not Containing Sickle Cell Hemoglobin

Inherited condition	No. of cases	Solubility* in g/liter
Normal adult	7	1.29–1.65
" newborn	12	1.95–2.55
Hemoglobin-*c* trait	3	1.80–2.07
" -*d* "	1	1.34
Thalassemia minor	2	1.54
" major	1	2.30

* As amorphous ferrohemoglobin at 25° C in aqueous system of 10.00 ml total volume containing 9.20 ml of 2.80 *M* phosphate buffer (*4*), 100 mg of $Na_2S_2O_4$, and 50 mg of hemoglobin.

considered in detail in a separate paper. The procedure not only offers a rapid method for detecting the presence of sickle cell hemoglobin but also yields characteristic solubilities for the different mixtures. Among the specimens containing sickle cell hemoglobin, those from sickle cell trait have the highest solubility, and those from sickle cell anemia the lowest. The other three forms of sickle cell disease are characterized by intermediate amorphous ferrohemoglobin solubilities. In sickle cell anemia the solubility is increased by the presence of fetal hemoglobin (*4, 11*). The wide range in sickle cell trait solubilities reflects the variations present in the normal adult–sickle cell hemoglobin ratios (*32*). The narrow range of values in sickle cell–hemoglobin-*c* disease is consistent with the nearly constant percentage of sickle cell hemoglobin among individuals with this disease (*23*). Among the highly soluble mixtures, the hemoglobin-*d* trait mixture has nearly the same solubility as normal adult hemoglobin (*4*), and the hemoglobin-*c* trait and normal newborn mixtures have higher solubilities as amorphous ferrohemoglobin.

Fetal hemoglobin differs from all the adult hemoglobins in its electrophoretic, spectrophotometric, and alkali-resistant properties (*11, 12, 14*). In thalassemia major an alkali-resistant hemoglobin having solubility, crystal form, electrophoretic mobility, and ultraviolet absorption spectrum identical with those of normal fetal hemoglobin is present, together with normal adult hemoglobin (*8, 10*). The alkali-resistant component in the sickle cell diseases has been found to be identical electrophoretically, spectrophotometrically, and immunologically with normal fetal hemoglobin (*11, 23, 33*).

The denatured globins of sickle cell and normal adult hemoglobins have the same electrophoretic mobilities and patterns. The native globins, however, show the same difference in electrophoretic mobilities as the hemoglobins from which they were derived (*34*). This confirms a previous deduction (*1*) based on the identity of the hemes in the two hemoglobins, that it is in their protein portions that these molecules differ.

Discussion

Differentiation of adult and fetal hemoglobin. In a series of sickle cell anemia individuals, 2–25 per cent of the hemoglobin was found to have a high resistance to alkali denaturation (*9*). A similar component has been observed in two individuals with sickle cell–hemoglobin-*c* disease (*23*) and in two with sickle cell–hemoglobin-*d* disease (*4*). This component has the same electrophoretic, spectrophotometric, and alkali-resistant properties as the fetal hemoglobin of normal newborn infants and is not found in the parents of the diseased individuals. Apparently, the same component appears in some individuals with acquired anemias (*9*). In one anemic individual with bone marrow fibrosis and myeloid metaplasia of the spleen, about 10 per cent of the hemoglobin was found to be the alkali-resistant type, and the rest normal adult hemoglobin (*23*). The same alkali-resistant hemoglobin has therefore been observed in the presence of all the other hemoglobins in anemic individuals but not in nonanemic adults (Table 1). Sickle cell hemoglobin and hemoglobin-*c* and -*d* occur together with normal adult hemoglobin in nonanemic individuals; whenever any of these four is present in an individual, the same form has been found in one or both of his parents. The classification of hemoglobin-*b*, -*c*, and -*d* as abnormal adult hemoglobins and of the alkali-resistant form associated with anemia as normal fetal hemoglobin is based on the foregoing considerations.

The genetic control of the hemoglobin synthetic mechanism that results in formation of fetal hemoglobin is thus assumed to be distinct from that involved in the production of adult hemoglobin; it is the latter that is modified in the elaboration of hemoglobin-*b*, -*c*, and -*d*. The presence of fetal hemoglobin long after infancy may be regarded as evidence of a continuance or a reactivation of an essentially embryonic mechanism in compensation for an anemia resulting from a block in adult hemoglobin synthesis (*10*) or from a chronic hemolytic process.

Genetic relationships among the adult hemoglobins. The qualitative inheritance of the adult hemoglobins has already been reviewed. The study of the hemoglobin ratios in heterozygotes has shed light on the quantitative aspects of the genetic control of adult hemoglobins. The relative proportions of sickle cell and normal adult hemoglobins in sickle cell trait differ widely among individuals. Among the families in which this ratio has been studied, three modal values of the ratio are present. The data can be explained by postulating the existence of three iso-alleles for normal adult hemoglobin which are responsible for the net synthesis within an erythrocyte of the same molecule at different rates, and a fourth allele, which results in the net synthesis of sickle cell hemoglobin at a constant rate (*18*). In contrast to the varying ratio of normal adult to sickle cell hemoglobin among individuals with the sickle cell trait, the ratio of sickle cell hemoglobin to hemoglobin-*c* in sickle cell–hemoglobin-*c* disease has been found to be nearly unity in each of eight individuals from seven different families (*23*).

Although the genetic data at hand are so limited as to render a positive conclusion largely speculative at this point, they are consistent with the assumption that a single, multiple-allelic series affects the variety of adult hemoglobins known to exist. This series would include a group of three iso-alleles of the normal type, effecting the synthesis of normal adult hemoglobin at distinctive rates, as well as three aberrant alleles effecting, respectively, the synthesis of hemoglobin-*b*, -*c*, and -*d* (*20*).

Thalassemia is manifested by a decrease in the amount of hemoglobin per erythrocyte, and it has been postulated that the action of the thalassemia allele is to interfere with the synthesis of normal adult hemoglobin (*10*). In thalassemia minor the presence of one

thalassemia allele interferes sufficiently with hemoglobin synthesis to result in a low mean corpuscular hemoglobin but not a severe anemia; a small fraction of alkali-resistant hemoglobin may be present (*9*). Thalassemia major results from the presence of two alleles for thalassemia, one from each parent, both of whom must therefore carry the thalassemia allele. Homozygosity for this allele is associated with a drastic reduction of normal adult hemoglobin production, and a severe, chronic anemia results. Fetal hemoglobin may comprise from 40 to nearly 100 per cent of the total hemoglobin (*8–10*), but even this apparent compensatory effort is usually inadequate. The assumption of iso-alleles at the sickle-cell locus, which result in different rates of production of normal adult hemoglobin, may account for the variations that are observed in the severity of thalassemia major (*20*).

In sickle cell–thalassemia one gene for sickle cell hemoglobin and one for thalassemia are present. In the light of genetic evidence that the sickle cell hemoglobin and the thalassemia genes are not allelic with each other (*21*), sickle cell–thalassemia must commonly be associated with double heterozygosity, the normal allele at each locus being present, but heterozygous with an aberrant allele. Hemoglobin studies in sickle cell–thalassemia show that normal adult, normal fetal, and sickle cell hemoglobins are present (*23*). Unlike sickle cell trait, in which the fraction of sickle cell hemoglobin is less than half the total (*1*), sickle cell–thalassemia is characterized by a preponderance of sickle cell hemoglobin (*22*). The thalassemia allele would therefore appear to provide a more effective block to normal adult than to sickle cell hemoglobin synthesis.

The molecular disease hypothesis. When sickle cell hemoglobin was discovered, the available evidence was reviewed in support of a hypothesis that sickle cell anemia is caused by a single inherited molecular abnormality—namely, a difference in the surface configurations of sickle cell hemoglobin and normal adult hemoglobin, which enables the former to form more stable aggregates when deoxygenated (*1, 35*). It was postulated that this property was responsible for the intravascular sickling that has been observed in sickle cell anemia (*15*), and that the intravascular sickling rendered the erythrocytes more susceptible to destruction. The presence of a large fraction of normal adult hemoglobin in sickle cell trait erythrocytes was believed to interfere with the sickling process and to prevent its occurrence at the oxygen tension of venous blood. Subsequent studies on the solubility and tactoid formation of hemoglobin solutions are in accord with this hypothesis. Increase in the relative amount of sickle cell ferrohemoglobin results in increased tactoid formation in concentrated hemoglobin solutions (*36*) and decreased solubility in concentrated salt solutions (*4, 37*).

Survival studies of transfused erythrocytes support the view that increased hemolysis results from intravascular sickling. The survival of sickle cell anemia erythrocytes in the circulation approximates an exponential decrease with time, with a half-life of 29 days or less, indicating that the cells are destroyed in a random manner (*26, 27, 38*). In other words, the cells in sickle cell anemia have a similar susceptibility to destruction, regardless of their age (*39*). The administration of a high concentration of oxygen to sickle cell anemia patients has resulted in a significant decrease in the number of sickled forms in the circulation (*40*). Although determinations of total urobilinogen excretion have failed to indicate a decreased rate of hemolysis, the more direct and sensitive method of transfused cell-survival determinations has shown a marked decrease in the rate of cell destruction during oxygen administration (*27*). In contrast, the concentration of transfused sickle cell trait erythrocytes, which show little or no sickling in venous blood (*15*), decreases linearly with time, and the mean life of the cells is about 110–120 days, a survival behavior which is identical with that of normal cells, and which suggests that sickle cell trait cells are destroyed as a function of their age; i.e., the older cells are more susceptible to destruction.

The available data on the other forms of sickle cell disease suggest a significant correlation among ferrohemoglobin solubility, intravascular sickling, and severity of anemia. The average fraction of sickle cell hemoglobin in sickle cell–thalassemia has been observed to be somewhat lower than in sickle cell anemia but higher than in sickle cell trait (*11*). A hemolytic anemia is present (*21*), and in one individual whose hemoglobin was 70 per cent of the sickle cell form, intravascular sickling has been observed (*22*). In sickle cell–hemoglobin-*c* disease the fraction of sickle cell hemoglobin is about 50 per cent, compared to the 24–45 per cent found in sickle cell trait, and the ferrohemoglobin solubility is but slightly less. The hemolytic anemia associated with this condition is mild (*24*), and in one adult a normal value of blood hemoglobin concentration, 15 g/100 ml, has been observed (*41*). The hemoglobin composition in sickle cell–hemoglobin-*d* disease cannot be determined electrophoretically; it has been observed, however, that the ferrohemoglobin solubility lies between that of sickle cell trait and that of sickle cell anemia (*4*). Of the two individuals known to have sickle cell–hemoglobin-*d* disease, the one who is the more anemic has a lower ferrohemoglobin solubility. It is not known whether intravascular sickling occurs in sickle cell–hemoglobin-*c* disease and sickle cell–hemoglobin-*d* disease.

The high erythrocyte lipid content (*42*), the high incidence of target cells (*21, 24, 25*), increased osmotic resistance to hypotonic saline (*21, 25*), and the decreased survival times of nonsickling cells (*24, 28*), which have been observed in some of these conditions, deserve further investigation. There can be little doubt that the stroma changes reflected in these abnormalities are associated with the abnormal hemoglobin metabolism in the erythrocytes and result from the same genetic aberration that alters the metabolism.

Their role in causing the hemolytic anemia of the sickle cell diseases is probably a secondary one. The accumulated evidence is strongly in favor of the hypothesis that intravascular sickling, which occurs in the presence of a high proportion of sickle cell hemoglobin, is the major factor in the hemolytic anemia of sickle cell disease.

Conclusion

Our investigations to date have been concerned primarily with the detection of abnormal hemoglobins and the characterization of the mixtures in which they occur. It may be seen from Table 1 that normal adult hemoglobin is the only form that occurs free of other components. Further characterization will depend upon the isolation in a homogeneous state of each of the molecular species. Normal adult and sickle cell hemoglobins illustrate how two of the common criteria for homogeneity may be inadequate. These proteins have the same oxygen dissociation curve (*43*) and the same oxyhemoglobin crystal form and solubility (*44*). Thus, two molecular species of the same protein, which are present in the same sickle cell trait individual, have the same physiological activity and probably the same phase rule behavior, although they differ markedly in their electrophoretic mobilities and ferrohemoglobin solubilities. These observations re-emphasize the importance of the application of several independent methods to check the homogeneity of protein specimens used in fundamental studies, such as the determination of amino acid composition and sequence. Whenever inhomogeneity is found, the possible presence of an inherited abnormality must be considered.

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News and Notes

Informal Meeting of Representatives of Associations for the Advancement of Science

A meeting of representatives of Associations for the Advancement of Science was called by Unesco at Belfast on Sept. 5, 1952, during the meeting of the British Association, in pursuance of the first recommendation of the International Meeting of Associations for the Advancement of Science held at Unesco House in Paris Sept. 8–9, 1950; namely, "that authorized representatives of the various associations attending the meetings of other associations take advantage of the occasion to discuss matters of common interest." In attendance were members of the British Association: A. V. Hill (president), Richard Southwell, George Taylor, M. G. Bennett, D. N. Lowe, J. M. Robertson; British Council: W. R. McAlpine, Mary L. Logan; American Association: Detlev W. Bronk (president), Jeffries Wyman; Australian and New Zealand Association: J. E. Cummins; French Association: Jeane Verne; Indian Association: S. R. Sen Gupta, C. L. Parricha; Pakistan Association: S. D. Muzaffar, M. O. Ghani; Swiss Association: A. von Muralt (president). The Unesco representative was Gerald Wendt.

Invited but unable to attend were: American Association: Paul Klopsteg, chairman of the Commit-