Studies on Abnormal Hemoglobins

VI. Electrophoretic Demonstration of Type S (Sickle Cell) Hemoglobin in Erythrocytes Incapable of Showing the Sickle Cell Phenomenon

By Karl Singer, M.D. and Ben Fisher, M.D.

It has been postulated\(^1,2\) that sickle cell anemia occurs only in individuals who are homozygous for the gene controlling the appearance of type S (sickle cell) hemoglobin, whereas the sickle cell trait indicates a heterozygous condition. This well documented theory has, however, encountered the following difficulties. (1) The amount of S hemoglobin in sickle cell trait carriers has been found to vary from 22 to 45 per cent.\(^3,4\) According to Neel, Wells and Itano,\(^5\) the expression of the S hemoglobin gene may be under the modifying influence of other genetic factors, since the lower or higher proportions of S hemoglobin are a familial characteristic. (2) The quantity of S hemoglobin in sickle cell anemia patients also shows variations from 76 to 100 per cent, with reciprocal values for F hemoglobin.\(^5,6\) No satisfactory genetic explanation of this phenomenon has yet been put forward. (3) Occasionally, a family is encountered in which the children have sickle cell anemia, but the red cells of one of the parents do not exhibit the sickle cell phenomenon, although the sickling tests are performed with the most refined technics.\(^7\) This apparent exception to the genetic theory has tentatively been explained\(^6\) by the assumption that the S hemoglobin concentration within the red cells of such a nonsickling parent may be insufficient to elicit sickling.

This paper deals with a family in which the erythrocytes of the mother's blood are incapable of sickling, the father has a typical sickle cell trait, and 2 of their 3 children have unmistakable, severe sickle cell anemia. An electrophoretic study of the hemoglobins of all members of this family was undertaken in an attempt to elucidate this apparent aberration from the genetic concept of sickle cell disease.

**Methods**

1. The sickle cell test was performed by the sodium metabisulfite method,\(^8\) and also by exposing oxalated blood samples to CO\(_2\) for \(\frac{1}{2}\) hour and then examining several preparations microscopically.

2. The alkali resistant (type F) hemoglobin was determined by the method previously described.\(^5\)

3. Electrophoresis of the hemoglobin solutions was carried out in a Tiselius apparatus (American Instrument Company), utilizing a cylindrical lens-diagonal slit optical system. The hemoglobin solutions were first prepared in a manner identical with that used for the

From the Department of Hematologic Research, Medical Research Institute, Michael Reese Hospital, Chicago, Ill.

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alkali denaturation technic. The pigment was then converted to CO hemoglobin and diluted to a concentration of 1 Gm. per cent with a cacodylic acid, sodium cacodylate buffer of pH 6.5 and ionic strength 0.1, as suggested in the original paper of Pauling et al. Dialysis of the hemoglobin solution against this buffer was carried out in a mechanical dialysis outfit. The electrophoresis was done in a standard Tiselius cell at 1.5 C., under a potential gradient of 5.3 volts/cm. Only the ascending boundary was used for interpretation. Planimetric quantitations of the various components were made according to the method of Tiselius and Kabat.

**CLINICAL OBSERVATIONS**

The pertinent clinical and hematologic data of family W. are summarized in Table 1. Both parents have always been in good health. The father's blood gives a positive sickling test, but the mother's erythrocytes could never be made to sickle on numerous occasions. Of the 3 children, 2 have had well established sickle cell anemia since early childhood. The blood of the third child shows the sickling tendency, but no significant anemia is present, and she is in a state of good health.

**ELECTROPHORETIC STUDIES**

Figure 1 contains photographs of the electrophoretic patterns obtained with the hemoglobin solutions of the members of family W. The pattern of the father's hemoglobin (fig. 1, a) is that of a typical trait, containing 33 per cent S and 67 per cent N hemoglobin. The alkali denaturation value of his hemoglobin was within normal limits.

The youngest child, Sharon (fig. 1, e), was also confirmed to be a trait by electrophoresis, with 41.4 per cent S hemoglobin. No F hemoglobin was demonstrable.

The bloods of the other 2 children, Sylvia and Ronald (fig. 1, c and d), showed...
Fig. 1—Pedigree and electrophoretic patterns of hemoglobin of Family W.

Fig. 2—Demonstration of S fraction in Mrs. W.'s hemoglobin by means of addition experiments.
the expected patterns of sickle cell anemia with S hemoglobin per cent concentrations of 88 and 85, respectively, and a smaller component which had the mobility of N hemoglobin. Under the conditions of our experiments, types N and F hemoglobin migrate with the same apparent mobility and, therefore, cannot be distinguished by electrophoresis. The concentrations of the slower moving component, as determined by planimetry, amounted to 12 per cent in Sylvia's blood and to 15 per cent in Ronald's, which is in reasonably good agreement with the values of 11.5 and 10.4 per cent, respectively, as obtained by the alkali denaturation technic.*

Electrophoretic analysis of the mother's hemoglobin showed that the curve was skewed in the direction of the migration; this indicates the presence of a small component of faster mobility besides her main pigment compound. This small fraction (5 per cent of the total) was identified as S hemoglobin by its faster mobility ($2.9 \times 10^{-5}$ cm$^2$/volt/sec., versus the mobility for the N hemoglobin which was $2.6 \times 10^{-5}$ cm$^2$/volt/sec.), and by the following addition experiments (fig. 2).

When the hemoglobin solution prepared from the red cells of Mrs. W. was mixed with a known sickle cell anemia hemoglobin solution (containing no F pigment) in the proportion of 95 to 5 per cent respectively, an increase to 10 per cent in the abnormal fraction could be consistently demonstrated, confirming that this fraction represented S hemoglobin. As controls, type N hemoglobin solutions, mixed in the same relationship with the S hemoglobin preparation, resulted in the appearance of two components (the smaller amounting to 5 per cent) and then gave an electrophoretic pattern similar to that seen with the undiluted original specimen of Mrs. W. (fig. 2).

These experiments demonstrate that Mrs. W. is a carrier of the sickle cell trait but that the expression of the S hemoglobin gene is very low (about 5 per cent). Attempts were also made to examine siblings of Mrs. W., but, unfortunately, her 2 sisters refused to cooperate because they are Christian Scientists.

**DISCUSSION**

Electrophoretic analysis of the hemoglobin of Mrs. W. revealed that even in the absence of a positive sickle cell test, erythrocytes may still contain small amounts of S hemoglobin.

Sickling of erythrocytes occurs in vivo or in vitro only when the S hemoglobin is in the reduced state.* Perutz and Mitchison$^{13}$ have shown that the solubility of reduced S hemoglobin is considerably less than that of reduced N hemoglobin. This low solubility is responsible for the “crystallization” of the reduced pigment which, in turn, causes the characteristic distortion of the red cell shape. A similar explanation of the sickle cell phenomenon was entertained by Ponder$^{14}$ and by Granick$^{14}$ prior to the discovery of S hemoglobin.

Harris$^{15}$ demonstrated that in concentrated solutions of reduced S hemoglobin “tactoids” form which can be visualized with the phase microscope. Tactoids are composed of long, thin, rod-like particles which have a parallel and equidis-

* The technical problem of correlating the values for the non-S hemoglobin fraction of sickle cell anemia blood, as obtained by electrophoresis and alkali denaturation, will be discussed elsewhere.
tant arrangement.\textsuperscript{16} Tactoids found in concentrates of S hemoglobin reveal a striking resemblance to sickled red cells.\textsuperscript{15, 17} Harris\textsuperscript{15} also found that tactoid formation will not occur when the S hemoglobin concentration is below 10 Gm. per cent. Since the mean corpuscular hemoglobin concentration of the average red cell is $34 \pm 2$ per cent,\textsuperscript{18} it is obvious that a certain minimal amount of S hemoglobin is required to elicit the sickle cell phenomenon.

The minimal concentration of S hemoglobin in solutions prepared from readily identified trait erythrocytes has been found to be approximately 22 per cent.\textsuperscript{3, 4} With a mean corpuscular hemoglobin concentration of 34 per cent, one may conclude that about one-fifth (7 per cent) is the minimal intra-erythrocytic concentration of S pigment necessary for eliciting the sickle cell phenomenon. This figure of 7 per cent is thus smaller than the minimum concentration of at least 10 per cent S hemoglobin found by Harris\textsuperscript{19} as essential for tactoid formation. This discrepancy may lead one to assume that other intra-erythrocytic constituents may possibly influence the solubility and enhance precipitation of S hemoglobin. Recently it has been possible to demonstrate in our laboratory that N or F hemoglobin, as well as other proteins such as albumin, have such an enhancing action.\textsuperscript{19} Although the factors determining the solubility of reduced S hemoglobin within the red cell are probably very complex, it is clear that a minimum quantity of S hemoglobin must be available to produce sickling. When the amount is below this minimum, the presence of S hemoglobin can only be detected by electrophoresis. Therefore, a negative sickling test does not preclude the possible existence of a small amount of S hemoglobin within the erythrocytes; this finding carries with it obvious genetic and possible medico-legal implications.

The demonstration that nonsickling individuals may still be carriers of the sickle cell trait confirms the basic concept of Neel\textsuperscript{1} regarding the hereditary transmission of sickle cell disease. Thus, the nonsickling parents of children with sickle cell anemia are only apparent, but not real, exceptions to this theory. Why some individuals have such a low expression of the S hemoglobin gene is not clear. Modifying influences of other genes, as assumed for the readily recognizable sickle cell trait,\textsuperscript{4} are most likely implicated. Unfortunately, our experiments to prove this aspect of the problem on siblings of Mrs. W. could not be carried out. Such studies should be undertaken in similar cases.

The fact that erythrocytes incapable of sickling may still carry S hemoglobin, may also be of value in instances of disputed parentage of children with well established sickle cell anemia. Only when S hemoglobin is found to be entirely absent by electrophoretic analysis of the blood of one of the presumed parents, is the conclusion valid that this individual cannot be involved in the creation of such a child. Further observations similar to those presented in this communication are, of course, necessary to support this statement.

SUMMARY

1. A family is reported in which 2 children have unmistakable sickle cell anemia, but the mother's red cells do not sickle; the father and a third child exhibit the typical sickle cell trait. Electrophoretic studies of the hemoglobin solution prepared from the mother's erythrocytes demonstrated, however, the
presence of a small amount (5 per cent) of type S hemoglobin. This abnormal component was identified by its mobility and by addition experiments.

2. The finding that the mother's erythrocytes contain small quantities of S hemoglobin, but are incapable of sickling, confirms the genetic concept that sickle cell anemia will only develop when both parents transmit the gene for S hemoglobin.

3. The physico-chemical and possible medico-legal aspects of the fact that S hemoglobin is sometimes only detectable by means of electrophoresis, are discussed.

REFERENCES

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