Paper Electrophoresis of Abnormal Hemoglobins and Its Clinical Applications

A Simple Semiquantitative Method for the Study of the Hereditary Hemoglobinopathies

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Study of hemoglobin by physicochemical techniques has become a valuable tool in the investigation and diagnosis of hereditary hemoglobin abnormalities. The significant stimulus to carry out such studies was the demonstration that hemoglobin in sickle cell anemia is electrophoretically different from normal adult hemoglobin.\(^1\) Another abnormal hemoglobin, hemoglobin C, which differs electrophoretically from both sickle cell and normal adult hemoglobin was discovered soon afterward.\(^2\) Filter paper electrophoresis has furnished a simple tool for electrophoretic studies of hemoglobin,\(^3\)\(^4\)\(^5\) since it does not require the complex apparatus and special skills necessary for the Tiselius moving boundary method. Another advantage is that paper electrophoresis can be used for mass screening purposes since multiple samples can be separated simultaneously.

The investigations to be described below deal with a simple technic of paper electrophoresis which allows semiquantitative analysis of the proportions of the various hemoglobins present. Clinical results obtained with this method are presented.

Materials and Methods

1. Hemoglobin Solution

Hemoglobin solutions were prepared using 5 to 10 ml of oxalated or heparinized blood (clotted blood may be used, but is less convenient). Red cells were washed three to four times with physiologic saline after removal of the plasma. The red cells were hemolyzed with distilled water (1.2 to 1.8 volumes) and toluene (0.4 volume), using the technic outlined by Singer, et al.\(^6\) The resulting hemoglobin solution has a concentration of about 8 to 12 Gm. per 100 ml. The actual concentration was verified colorimetrically. The alkali denaturation test for fetal hemoglobin\(^6\) was usually performed on this sample.

The portion of the hemoglobin solution used for paper electrophoresis was diluted to about 3 (±0.5) per cent. Oxyhemoglobin solutions may be kept for several weeks without deterioration if stored at 4°C. Dialysis of the hemoglobin solutions and conversion into carboxyhemoglobin were not necessary for paper electrophoretic separation.

2. Paper Electrophoresis Cell

The electrophoresis cell used was a slightly modified version of that described by one of us\(^7\) elsewhere (fig. 1). The lucite cell was divided by a center partition into anode and cathode compartments as shown in the figure. A rubber or plastic tube connected the two compartments.

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compartments to allow initial volume equilibration of buffer solution levels in the two sections. The electrodes were introduced into their respective compartments through two small holes which were bored into each compartment in the wall opposite the connecting tube. The pyramid cover closed the chamber.

The following modification of the apparatus used in our experiments is not shown in figure 1. A baffle, consisting of a $\frac{1}{4}$ inch lucite strip $33 \times 4.8$ cm. was placed in each compartment $1.3$ cm. from the outer wall. This partition was attached to the base and to the wall which holds the electrode leaving a $1$ cm. open space at the opposite end. Thus each electrode compartment was subdivided into two unequal sections. This baffle prevented diffusion of electrode products to the ends of the strips.

3. Electrodes

The two electrodes were made of thin pieces of platinum foil, $1 \times 1$ cm., spot-welded to the platinum wire.

4. Supporting Rack

The supporting rack for suspension of the filter paper strips was made of glass rods (fig. 1). A more sturdy rack may be constructed of bakelite, metal rods, and glass tubing (fig. 2). This rack withstands temperatures of from $120$ to $150$ C. in the drying oven.

5. Power Supply

A regulated voltage power supply may be used but is not necessary.*

6. Buffer

One liter of barbital buffer pH $8.6$, ionic strength $0.05$, was used for each electrophoresis run. (A stock solution of $20.6$ Gm. sodium barbital, $3.68$ Gm. di-ethyl barbituric acid, made up to $1$ liter was diluted $1:1$ with distilled water to prepare this solution.)

* An inexpensive unit which has given satisfactory results is the Heath Kit Model PS2 (Heath Company, Benton Harbor, Mich.). It must be assembled by the buyer.
Brass rods in glass tubing

Fig. 2.—Supporting frame for suspensions of filter paper strips.

7. Preparation of Filter Paper

Whatman filter paper, no. 3 MM, has been used exclusively. Sheets were cut into strips 30 cm. X 29 mm. The dry strips were carefully bent over a rounded surface of at least \( \frac{1}{2} \) inch diameter (such as a pipet) to avoid producing a crease. A small pencil mark was made at the midpoint or apex of the strip, to provide a reference point for application of solution.

8. Application

Three percent hemoglobin solution was measured into a 0.01 ml. pipet.\(^*\) The solution was streaked evenly in a thin line across the center of the dry strip, leaving a small margin (about 2 mm.) on each side. As soon as the solution had been applied, the strip was placed on the rack which was in the buffer-filled cell. Buffer solution from the compartments was evenly applied to both sides of the strip with a medicine dropper, maintaining the two ascending buffer fronts equidistant from the apex and allowing the final coalescence of the fronts to occur spontaneously by capillarity. This technique of buffer application “gathers up” hemoglobin to result in a sharp fine starting zone at the apex. Otherwise, hemoglobin will be carried to one side of the apex and poor results due to blurring will be obtained. Eight filter paper strips (29 mm. wide) ordinarily were used in this cell. After application of the buffer to the final filter paper strip the cover was placed on the cell and the levelling connection between the two buffer compartments was closed.

9. Electrophoresis Run

The power supply was adjusted to 260 to 280 volts. This potential was applied for three to five hours. The current varied between 1.2 to 1.6 milliamps per strip. As many as three electrophoresis cells were used in parallel from the same power supply. All analyses were performed at room temperature. Separation of different hemoglobins could be recognized within 2 to 3 hours. Optimum separation was usually obtained between 4 to 4.5 hours. Longer runs (over 6 hours) usually produced blurred and diffuse patterns.

10. Drying

After completion of the electrophoresis, the rack was removed from the cell and immediately placed in the drying oven, maintained at a temperature of about 140 C. for 10 to 15 minutes. If lower temperatures were used for drying, the resulting patterns tended to be less sharp. Preliminary reading of the patterns was made before placing them in the drying oven since a slight amount of blurring seemed to be unavoidable on drying even at 150 C.

\(^*\) Breed & Brew, Blue line, Exax (Fisher) have been found convenient. In order to avoid abrasions of the filter paper the tips were smoothed with emery paper.
11. Staining

If quantitative analysis was desired, the strips were stained overnight in a solution of bromphenol blue (0.01 per cent bromphenol blue, 5 per cent acetic acid, 5 per cent mercuric chloride). After staining, the strips were washed three times by immersion in 2 per cent acetic acid for 10 minutes each. After blotting, the blue-stained strips were dried on non-absorbent paper or in an oven at 60 C.

Elution of the dye which quantitatively stained the protein moiety of hemoglobin was performed by cutting out the desired spots and immersing them in a solution of 0.01 N sodium hydroxide. They were left for 60 minutes in the eluting solution. The resulting blue eluate was placed into appropriate colorimeter tubes and read at 575 micrometres wavelengths in a photoelectric colorimeter or spectrophotometer.

12. Photoelectric Scanner

A self-recording photoelectric scanning device, to be described elsewhere was used for scanning hemoglobin patterns. Since hemoglobin produces a colored spot, strips were scanned (at 520 micrometres) without staining. It was not found necessary for these studies to make the strips translucent with oil.

13. Technical Considerations

A small amount of protein usually trailed behind the spots or, in separation of different hemoglobins, could be seen between the characteristic spots. Part of this material apparently consisted of small amounts of hemoglobin which were adsorbed on the filter paper. Other portions consisted of contaminating proteins other than hemoglobin, since it is not necessary to prepare pure hemoglobin solutions for these analyses. With increasing time of storage of hemoglobin solution, trailing became more pronounced. These trails were disregarded in quantitative work on the assumption that the various adsorbed hemoglobin types trail in approximately equal proportions to each other. The artificial mixture experiments reported below demonstrated that this assumption may be correct. Further work is required to elucidate this problem.

For each run on an unknown sample it is advisable to place a known S-A hemoglobin mixture (for instance, from a patient with the sickling trait) or a sample of S and A hemoglobin each on the strips. This helps to define the relative mobility of the two fractions in that particular run and facilitates diagnosis of the unknown sample. This precaution is advisable since variation of a few millimeters in the distance of migration of a given hemoglobin may be encountered from strip to strip and from run to run. Since hemoglobin C migrates only a very small distance from the apex toward the anode, confusion with other hemoglobin types is usually no problem.

If any question exists whether an unknown sample that resolves in one component only is an electrophoretically normal or abnormal hemoglobin, it should be mixed with normal adult hemoglobin in equal proportions. Failure to separate the mixture establishes the unknown to have the same electrophoretic mobility as normal adult hemoglobin (or F hemoglobin) (see below).

In comparing both streaking and spotting techniques for application of hemoglobin solutions, it was found that more reliable quantitative results could be obtained with the streaking technic. For photoelectric scanning only streaking is permissible. Rough semiquantitative separations, however, are possible with the spotting technic (e.g. all specimens in figure 3 except c and h were spotted). Only .005 ml. of 3 per cent hemoglobin should be used if spotting is done.

Although we used several buffers in our initial experiments, we found that the barbital buffer chosen gave best results. Further systematic experimentation with a variety of buffers at varying pH may uncover better conditions for the separation of the various hemoglobins.

* By spotting we understand that no effort is made to place the hemoglobin solution in a thin streak across the apex of the strip.
Results

1. Electrophoretic Mobility of Various Hemoglobins

Paper electrophoresis under the conditions of our study clearly separated normal (hemoglobin A), sickle (hemoglobin S), and hemoglobin C. Fetal hemoglobin (hemoglobin F) could not be separated from adult hemoglobin in the standard 4 hour runs employed. Seven to 16-hour runs with cord blood hemolysates, showed that fetal hemoglobin had less mobility than adult hemoglobin under the conditions of our study. Since long runs produced a very diffuse pattern, quantitative separation of hemoglobin F became impossible. For this reason, the alkali denaturation test was always used for the determination of fetal hemoglobin.

All varieties of hemoglobin moved to the anode with varying degrees of mobility. Adult hemoglobin migrated with greater mobility than any other hemoglobin.

Since sickle cell hemoglobin has fewer positive charges than hemoglobin A but more positive charges than hemoglobin C, its mobility was found to be intermediate between that of hemoglobin C and hemoglobin A. The relative mobilities of hemoglobins under the conditions of our experiments may therefore be expressed as A > F > S > C. Hemoglobin D was not encountered in our experience, but has been found to migrate with the same mobility as sickle cell (S) hemoglobin. However, hemoglobin D does not give a positive sickling test with reducing agents. Figure 3 shows the various hemoglobin combinations encountered in patients.

It was found that about 10 per cent of normal adult hemoglobin or fetal hemoglobin could be fairly accurately detected in combination with the sickling hemoglobin (S). Still smaller amounts of hemoglobin A showed up in combination with hemoglobin C. On the other hand, if the major component was normal adult hemoglobin, amounts of less than about 15 per cent of hemoglobin S were easily overlooked, even with technically perfect runs. Photoelectric scanning sometimes detected even these small quantities of S hemoglobin. Fortunately, the usual hemoglobin combinations existing in the hereditary hemoglobinopathies are of the easily recognizable type. The combination of very small amounts of abnormal hemoglobin with normal hemoglobin so far has been reported only once (5 per cent sickle cell and 95 per cent A hemoglobin in a sickle

Fig. 3.—Hemoglobin types detected by paper electrophoresis: (a) normal adult hemoglobin (100 per cent A); (b) sickling trait (35 per cent S, 65 per cent A); (c) sickle-thalassemia disease (82 per cent S, 18 per cent A) (the paper pattern is indistinguishable from that seen in sickle cell anemia with a high hemoglobin F value); (d) sickle cell anemia (100 per cent S); (e) homozygous Hb C disease (100 per cent C); (f) homozygous Hb C disease after transfusion (82 per cent C, 18 per cent A); (g) hemoglobin C trait (30 per cent C, 70 per cent A); (h) sickle-hemoglobin C disease (50 per cent C, 50 per cent S); (i) cord blood (note no significant separation between Hb A and F).
Such a combination could not be detected by this method under the conditions described.

2. Artificial Mixture Experiments and Quantitation

It has been established\(^1\) that the hemoglobin of sickle cell trait patients consists of 24 to 45 per cent sickle (S) hemoglobin and 55 to 76 per cent normal adult (A) hemoglobin. Our initial experiments with hemoglobin solutions from such patients yielded the expected paper patterns which consisted of a denser hemoglobin A than hemoglobin S spot. It was felt that with some practice it would be possible to predict rather accurately the amount of the various hemoglobins present by simple inspection of the density of the spots of the paper strips. Since hemoglobin is colored, staining is not required for this purpose. In order to work out a more quantitative approach to the separation of the mixtures, hemoglobin solutions were obtained from a patient with sickle cell anemia with practically 100 per cent S hemoglobin and from a normal subject (100 per cent hemoglobin A). The two hemoglobin solutions were carefully adjusted to exactly the same hemoglobin concentration, and artificial mixtures were prepared in varying proportions to each other. After electrophoresis, the hemoglobin fractions were cut apart and eluted. Figure 4 shows expected values plotted against results obtained by elution analysis. Quite good agreement could be obtained when relatively large amounts of the two fractions were present. If one fraction was 20 per cent or less of the other component, elution was less exact. Control experiments eluting hemoglobin from uncut paper strips yielded values which

![Graph showing quantitative recovery of Hb S and A fractions from artificial mixtures by dye elution.](Bromphenol blue.)
were practically identical with the sum of the amounts of hemoglobins of the eluted two fractions.

The results of the elution analysis and the scanning technique are shown in Figures 6 and 7. Figure 5 illustrates separation by paper electrophoresis of a set of artificial mixtures prepared with 100 per cent C hemoglobin and from a normal subject. In both instances good recovery could be obtained although small amounts of hemoglobin C tend to be overestimated by the scanning technique. This apparently is caused by the trailing phenomenon discussed above.

For clinical purposes, quantitation by elution analysis or photoelectric scan-
Diagnosis of the hereditary hemoglobinopathies

Sickleemia—sickling trait. Ordinarily, electrophoresis is not required for the diagnosis of the sickling trait. Occasionally, however, electrophoresis becomes necessary. Thus we encountered several instances of low hemoglobin values in patients with a positive sickling test, caused by a variety of anemias superimposed on the sickling trait. An illustrative case was a 24 year old man with fever, mild hemolytic anemia, and a positive sickling test. The electrophoretic hemoglobin pattern was that of the sickling trait (fig. 3 b) and clarified the clinical picture. Further developments revealed that the patient suffered from transient hemolysis due to a pyogenic infection. After the infection was treated, hemolysis ceased. In such cases, paper electrophoresis is a better differential diagnostic tool than the alkali denaturation test since 3 per cent of patients with sickle cell anemia have a normal alkali denaturation test.14

Sickleemia—sickle cell anemia. Electrophoresis of hemolysates of patients with sickle cell anemia produced a characteristic pattern which consisted of a major component of hemoglobin S (75 to 100 per cent) and usually of a minor component of hemoglobin F. Since in many patients with the disease, the minor component was less than 10 per cent or even absent,14 quantitation of the fetal fraction from paper electrophoresis proved difficult or impossible because of poor resolution of very small amounts of fetal (or normal A) hemoglobin from
the main component. The alkali denaturation test was used to estimate the amount of fetal hemoglobin in such cases. The validity of this procedure was recently confirmed by combined alkali denaturation and Tiselius electrophoresis of hemolysates from untransfused sickle cell anemia patients. The amount of the minor electrophoretic component of such samples was identical with the alkali-resistant fraction (hemoglobin F) as estimated by the alkali denaturation test.

**Sickleemia—sickle cell–hemoglobin C disease.** We were able to find five patients with sickle cell–hemoglobin C disease (fig. 3 h) by electrophoretic analysis of hemolysates of cases that had been previously diagnosed as sickle cell anemia, but were not found to be quite typical. In conformance with reports by other workers, sickle cell–hemoglobin C disease was found to be a milder anemia with fewer symptoms than classical sickle cell anemia. Electrophoresis is essential for diagnosis of this type of sickleemia. The alkali denaturation test usually reveals normal to slightly elevated values of fetal (F) hemoglobin.14

**Sickleemia—sickle cell–thalassemia disease.** The transmission of the sickling gene from one parent and that of thalassemia from the other parent results in another variant of sickleemia known as sickle cell-thalassemia disease or microdrepamocytosis. Most cases of this condition have occurred in patients of Mediterranean ancestry. Only one Negro patient with the documented diagnosis of this disease has been reported previously. In our series, another Negro patient with this disease was discovered.

A 21 year old Negro woman who had been diagnosed to have sickle cell anemia was found to have splenomegaly and hypochromic anemia with a high F Hb value. No transfusion had been given. Paper electrophoresis yielded a pattern of 82 per cent sicklehemoglobin and 18 per cent A (or F) hemoglobin (fig. 3 c). This would have been indistinguishable from the usual sickle cell anemia pattern. The alkali denaturation test, however, revealed only 1 per cent F hemoglobin. The presence of normal (A) hemoglobin in combination with more than 50 per cent S hemoglobin so far has only been encountered in sickle cell-thalassemia disease. This diagnosis was made and confirmed by family studies. Five cases of thalassemia minor were uncovered in this Negro family. The patient's 11 month son had a positive sickling test and mild hypochromic anemia. Sickle cell-thalassemia disease was suspected but ruled out by the absence of reticulocytosis and by an electrophoretic pattern of 70 per cent A and 30 per cent S hemoglobin which is typical of the sickling trait. The hypochromic anemia was believed to be due to iron deficiency.*

Practical use may be made of the fact that varying amounts of the S hemoglobin component are combined with other hemoglobin types in the different manifestations of sickle cell disease. Thus, the amount of sickle cell hemoglobin in patients with the sickling trait varies between 25 to 45 per cent; in those with sickle cell-hemoglobin C disease it is usually about 50 per cent; in sickle cell-thalassemia disease, 65 to 80 per cent; and in sickle cell anemia usually more than 85 per cent, rarely as low as 75 per cent. Estimating the amount of sickle hemoglobin from the density of the streak or spot often will make the correct diagnosis of the type of sickle cell disease present.

* A detailed study of this family will be published elsewhere.
Hemoglobin C abnormalities. Hemoglobin C was first recognized in combination with S hemoglobin in patients with a mild variety of sicklemia (see above—sickle cell-hemoglobin C disease). The combination of hemoglobin C with hemoglobin A ("C" trait or heterozygous "Hb C") has always been detected in one of the parents of patients with sickle cell—hemoglobin C disease. The general incidence of the C trait was found to be 1.8 per cent among five hundred Negroes studied at random in a clinic population. Hemoglobin C so far has not been encountered in white people. Mating of two C trait carriers should produce a certain number of offspring with the homozygous state for hemoglobin C—such a case was found.

The hemoglobin of a 43 year old Negro woman with mild normochromic microcytic anemia with many target cells, splenomegaly, and arthralgia, was found to consist of 100 per cent hemoglobin C (fig. 3 c). This patient thus represented an example of homozygous hemoglobin C disease—a new hematologic entity. Family study of this patient revealed that her siblings and both of her children were heterozygous carriers of the hemoglobin C trait (fig. 3 g) (30 to 40 per cent C hemoglobin, 60 to 70 per cent A hemoglobin). Genetically, study of this family and other published data suggested that the gene responsible for the production of hemoglobin C probably is a multiple allele of the genes governing production of sickling as well as of normal hemoglobin. The double dose of hemoglobin C, just as that of hemoglobin S, produces a hemolytic disease which, however, is much milder than sickle cell anemia.

Heterozygous hemoglobin C carriers showed no evidence of hematologic disease although many target cells and increased osmotic resistance were always seen. This agrees with findings of other workers. Red cell survival time studies have revealed a normal life span of erythrocytes from such patients. The amount of hemoglobin in C trait carriers has varied between 34 to 40 per cent in our experience. Diffuse bony aches and joint pains were observed in three C trait carriers. Further studies are required to clarify this finding.

Table 1 is modified after Itano and summarizes the quantitative distribution of various hemoglobins in different conditions as detected electrophoretically and by alkali denaturation. Our results are pooled with those of Itano and of Singer for percentage distribution of the various abnormal hemoglobins, and with those of Singer for percentage distribution of F hemoglobin. Fairly good agreement could be obtained in all our cases.

Use of method for life span determinations

Paper electrophoresis may be used for life span determinations of the red cells in some of the hereditary hemoglobinopathies. Thus, the decay of normal adult hemoglobin from transfused normal red cells may be followed in patients with homozygous hemoglobin C disease or with sickle cell anemia (with low F hemoglobin values) by quantitative determination of the disappearance of A hemoglobin at various intervals. This was done in the patient with 100 per cent hemoglobin C. Normal red cells had a life span of 106 days. No extracorpuscular hemolytic mechanism existed.

Similarly, pure S or pure C hemoglobin from appropriate patients could be given to recipients with A hemoglobin, and the disappearance curve of the
A S

DC

Not actually demonstrated yet.

These conditions reveal a "normal" S-pattern under our conditions and are indistinguishable in conventional electrophoresis.

Best characterized by alkaline denaturation test for F hemoglobin.

Indistinguishable by electrophoresis but D-sickle cell disease has higher solubility.

3% of sickle cell anemia patients have no F hemoglobins.

Most common pattern is 50% C-50% S.

TABLE 1.—Quantitative Distribution of Abnormal Hemoglobins in Various Diseases

| Normal (homozygous A) adult† | 98.4–100% | — | — | — | 15–50% |
| Normal newborn‡ | 15–50% | — | — | — | 50–85% |
| Normal child up to 2½ yrs.‡ | 97–100% | — | — | — | up to 3% |
| Sickle cell trait§ | 55–76% | 24–45% | — | — | Traces |
| Sickle cell anemia¶ | — | 75%–100%* | — | — | 0–25% ‡ |
| C-trait | 60–75% | — | 25–40% | — | Traces |
| Sickle-C disease | — | 33–50%** | 50–67%*** | — | Usually less than 2% |

| Homozygous C | — | — | 100% | — | Traces |
| D-trait§ | 51–65% | — | — | 35–49% | ? |
| Sickle-D disease¶ | — | — | — | ? | ? |
| Homozygous D*| — | — | — | — | ? |
| Thal. minor† | 90–100% | — | — | — | 0–10% |
| Thal. major‡ | 0–60% | — | — | — | 40–100% |
| Sickle-thal. disease | 18–33% | 67–82% | — | — | 1–17% |
| Some seq. anemias† | 90–98% | — | — | — | 2%–10% |

* Not actually demonstrated yet.
† These conditions reveal a "normal" spot on paper electrophoresis under our conditions and are indistinguishable in conventional electrophoresis.
‡ Best characterized by alkaline denaturation test for F hemoglobin.
§ Indistinguishable by electrophoresis but D trait does not sickle.
¶ Indistinguishable by electrophoresis but D-sickle cell disease has higher solubility.
* Most common pattern is 50% C-50% S.
** Usually less than 2%.
*** Slightly increased amounts of fetal hemoglobin were found in several but not all patients with these disorders.

TABLE 2.—Anemias Where Paper Electrophoresis Has Demonstrated Normal Adult Hemoglobin

<table>
<thead>
<tr>
<th>Type of anemia</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Hereditary spherocytosis</td>
<td>4</td>
</tr>
<tr>
<td>2. Hereditary nonspherocytic hemolytic disease</td>
<td>5*</td>
</tr>
<tr>
<td>3. Paroxysmal nocturnal hemoglobinuria</td>
<td>1</td>
</tr>
<tr>
<td>4. Hereditary nonhemolytic elliptocytosis</td>
<td>2</td>
</tr>
<tr>
<td>5. Thalassemia minor</td>
<td>5</td>
</tr>
<tr>
<td>6. Thrombotic thrombocytopenic purpura</td>
<td>1</td>
</tr>
<tr>
<td>7. Idiopathic acquired hemolytic anemia</td>
<td>2</td>
</tr>
<tr>
<td>8. Symptomatic acquired hemolytic anemia</td>
<td>4</td>
</tr>
<tr>
<td>9. Hemolytic anemia secondary to renal disease</td>
<td>1</td>
</tr>
<tr>
<td>10. Idiopathic acute hemolytic anemia (probably drug-induced)</td>
<td>1</td>
</tr>
<tr>
<td>11. Aplastic anemia</td>
<td>3*</td>
</tr>
<tr>
<td>12. Anemia of chronic infection</td>
<td>1</td>
</tr>
</tbody>
</table>

*Slightly increased amounts of fetal hemoglobin were found in several but not all patients with these disorders.

abnormal fraction followed. In view of the above mentioned technical difficulties which prevent good separation of small amounts of S hemoglobin from normal hemoglobin under our conditions, a rather large amount of S hemoglobin would have to be administered for life span determination of red cells containing this
abnormal hemoglobin. Because of their low blood volume, the use of children as recipients might make such a procedure practicable.

**Use of the method in work-up of miscellaneous anemias**

Paper electrophoresis in conjunction with the alkali denaturation test for fetal hemoglobin has been used in the work-up of a variety of hemolytic and other anemias. In all cases, except in the hereditary hemoglobinopathies discussed above, normal adult hemoglobin was found. Table 2 summarizes the various anemias investigated where normal hemoglobin was found. Slightly increased amounts of fetal hemoglobin were found in two patients with hereditary nonspherocytic hemolytic disease and in one patient with aplastic anemia.

**Discussion**

Because of their simplicity it is likely that paper electrophoretic technique ultimately will replace Tiselius electrophoresis in most studies of hemoglobin abnormalities. Tiselius electrophoresis will continue to be of great value for the determination of mobility constants and for very exact quantitative work.

Since semiquantitative readings of the patterns may be performed by simple inspection of strips, and since quantitative analysis may be obtained by photoelectric scanning as well as by elution techniques, the free paper strip method as described offers distinct advantages. Paper electrophoresis of serum proteins may be performed with the same apparatus and with an identical buffer solution. Some skill is required to learn the proper streaking technique of application and to adjust the buffer fronts so that they reach the hemoglobin streak at the same time. With some practice this technique can be easily mastered.

We feel that the technique is simple enough for introduction into routine hospital laboratories that deal with a large Negro population. Hemoglobin electrophoresis is required in cases of unexplained anemia in a Negro patient. The method offers a satisfactory approach for studies dealing with the incidence of abnormal hemoglobin traits in various populations. Hemoglobin abnormalities add another genetic marker to the human chromosome. Linkage relations remain to be elucidated. Medicolegal applications for paternity cases may be suggested. The method presumably may be of value for clinical and anthropologic studies of sickle cell disease in Africa and elsewhere, where the genetic relationship of the various manifestations of sicklemia await definitive study.

**Summary**

1. Paper electrophoresis of abnormal hemoglobins is a simple and convenient technique for the study of the hereditary hemoglobinopathies.

2. A semiquantitative paper electrophoretic technique is described, which allows rather accurate quantitation of the various hemoglobin components by inspection alone.

3. For exact results, the more elaborate techniques of elution or photoelectric scanning may be employed. The accuracy of these quantitative techniques is illustrated by artificial mixture experiments.

4. The clinical applications of the method in the study of sickle cell disease and hemoglobin C abnormalities are discussed. Apart from the more common
hemoglobin abnormalities (such as sickle cell trait, sickle cell anemia, C trait, sickle cell-hemoglobin C disease), a patient with 100 per cent hemoglobin C (homozygous hemoglobin C disease) and a Negro patient with sickle cell-thalassemia disease were discovered. Normal adult hemoglobin (hemoglobin A) was found in all other hereditary and acquired anemias studied. Slightly increased amounts of fetal hemoglobin were detected in cases of hereditary nonspherocytic hemolytic disease and aregenerative anemia.

5. This technic may be used for red cell life span determinations by serially following the disappearance of a certain hemoglobin type transfused into a patient with a different hemoglobin variety. Further applications of the technic are suggested.

6. The combination of the technics of paper electrophoresis and alkali denaturation offer an adequate, simple, and practical tool for diagnosis and investigation of hereditary hemoglobinopathies.

7. Identical apparatus and buffer may be used for serum protein electrophoresis.

**Summario in Interlingua**

1. Electrophorese a papiro de hemoglobininas anormal es un simple e commode methodo pro le studio de hemoglobinopathias hereditari.

2. Es describite un technica semiquantitative de electrophorese a papiro, le qual permitte, per inspection directe, un satis exacte quantificatiomi del varie componentes de hemoglobina.

3. Pro obtenet resultatos plus exacte, on pote usar le technicas plus complicate de elution e scrutiniom photoelectric. Le precision de iste technicas quantitatives es illustrate per experimentos con mixturas artificial.

4. Es discutite le application del technica in le studio del morbo de cellulas falciforme e del anormalitates de hemoglobina C. Esseva diagnosticate non solo le plus commun anormalitates de hemoglobina (per exemplo cellulas falciforme, anemia a cellulas falciforme, characteristica C, morbo de cellulas falciforme a hemoglobina C) sed etiam le caso de un patiente con 100 pro cento hemoglobina C (morbo a hemoglobina C homozygotic) e un patiente negro con thalassemia a cellulas falciforme. In omne le altere casos studiate de anemias hereditate o acquirite, hemoglobina adulte normal (hemoglobina A) esseva constatate. Quantitates aliquo augmentate de hemoglobina fetal esseva constatate in casos de morbo hemolytic nonspherocytic hereditari e de anemia aregenerative.

5. Le technica pote esser usate in determinar le duration de vita de erythrocytos per observationes in serie del disparition de un certe typo de hemoglobina que ha essite injicite in un patiente con hemoglobina de un typo differente. Varie altere applicationes del technica es etiam proponite.

8. Le combination del technica de electrophorese a papiro con le technica de disnaturation a alcali representa un adequate, simple, e practic procedura pro diagnosticar e investigar hemoglobinopathias hereditari.

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