*The Grand Rapids, Mich., school system uses a method derived from molecular information as part of its health service to pupils. The U.S. Army has been screening troops for sickle cell hemoglobin at Fort Knox, Ky., on an investigative basis since 1970.*

# Automated Mass Screening for Hemoglobin S

## *A Rational Method*

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SICKLE cell disease has suddenly captured the attention of the general public and officials at all levels of government. Substantial funding for the detection, treatment, and research of sickle cell disease will become available very shortly from Congress. Federal money will also be spent for counseling and education.

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Recent diagnostic innovations (*1–22*) have yielded rapid screening methods. Some of these tests have a known molecular basis, being predicated on the submolecular pathology of sickled hemoglobin S, that is, the existence of hydrophobic bonds between interacting tetramers of hemoglobin S (*4, 5, 22*). Automated versions of the dithionite test process whole blood specimens rapidly (one every 30 seconds) at a reagent cost of 2 cents each and with high specificity. The consideration of intricate and sophisticated molecular information to achieve the highest diagnostic accuracy at the lowest possible cost has led to the development of a two-phase mass screening method which has been proved by extensive field trials in both civilian and military populations (*23–25*) totaling in excess of 84,000 tests as shown in the table.

The data in the table represent the status of work in progress at four centers using our diagnostic techniques. When the studies are completed, the investigators will have them published separately. Note that in addition to S hemoglobin, hemoglobins C, F, and thalassemia have been diagnosed.

Differences among these studies are minor. For example, Bemis has coupled the automated dithionate test with an automated method for hemoglobin determination. Thus, using blood speci-

mens originally intended to be screened for hemoglobin S, his approach also detects anemias from any cause and substantially increases the value of the screening effort at very little extra cost. The study by Cawley includes periodic spot checking of all negative automated dithionite tests by hemoglobin electrophoresis.

Unless the goals of mass screening programs, the implicit responsibilities in such programs, and the principles and implications of diagnostic methods are clearly understood, a boon may tragically be converted into a boondoggle. Diagnostic errors and misinformation will burden some of the 23 million black Americans who represent the potential population at risk for this disease. Approximately 10 percent of the black population may have the S hemoglobin gene in one form or another. No mass screening program should be undertaken unless the screeners are able to offer a set of options to newly detected victims of the disease for further diagnosis, treatment, and genetic counseling.

## Screening Versus Marital Counseling

The purpose of screening for sickle cell disease is to detect persons (hence, afflicted families) with S hemoglobin in any of its many forms and combinations and to direct such patients to facilities for additional diagnosis, treatment, and counseling at the lowest possible cost. By our methods, interacting hemoglobinopathies such as C and thalassemia will always be found *when it is medically important:* in persons, mating couples, or families previously shown to have S hemoglobin. The attention should be on the person most at risk—the individual or family with S hemoglobin. The C trait and even the CC state by itself is essentially innocuous. Hence screeners for sickle cell hemoglobin are not concerned with the general incidence of C hemoglobin in the entire black population.

The goal of marital counseling, on the other hand, is to arrest the perpetuation in the genetic pool of S hemoglobin, and especially the SC combination, which is almost as serious as homozygous S. In pregnancy, SC hemoglobinopathy is particularly hazardous to both neonate and mother. Hence, for purposes of marital counseling we recommend that the blood of both mates be studied by the dithionite test and hemoglobin electrophoresis. These tests will yield information related to the presence of not only S but C, thalassemia, and other disadvantageous hemoglobinopathies such as M. Also pertinent data on zygosity will be obtained and sound recommendations on mating would follow.

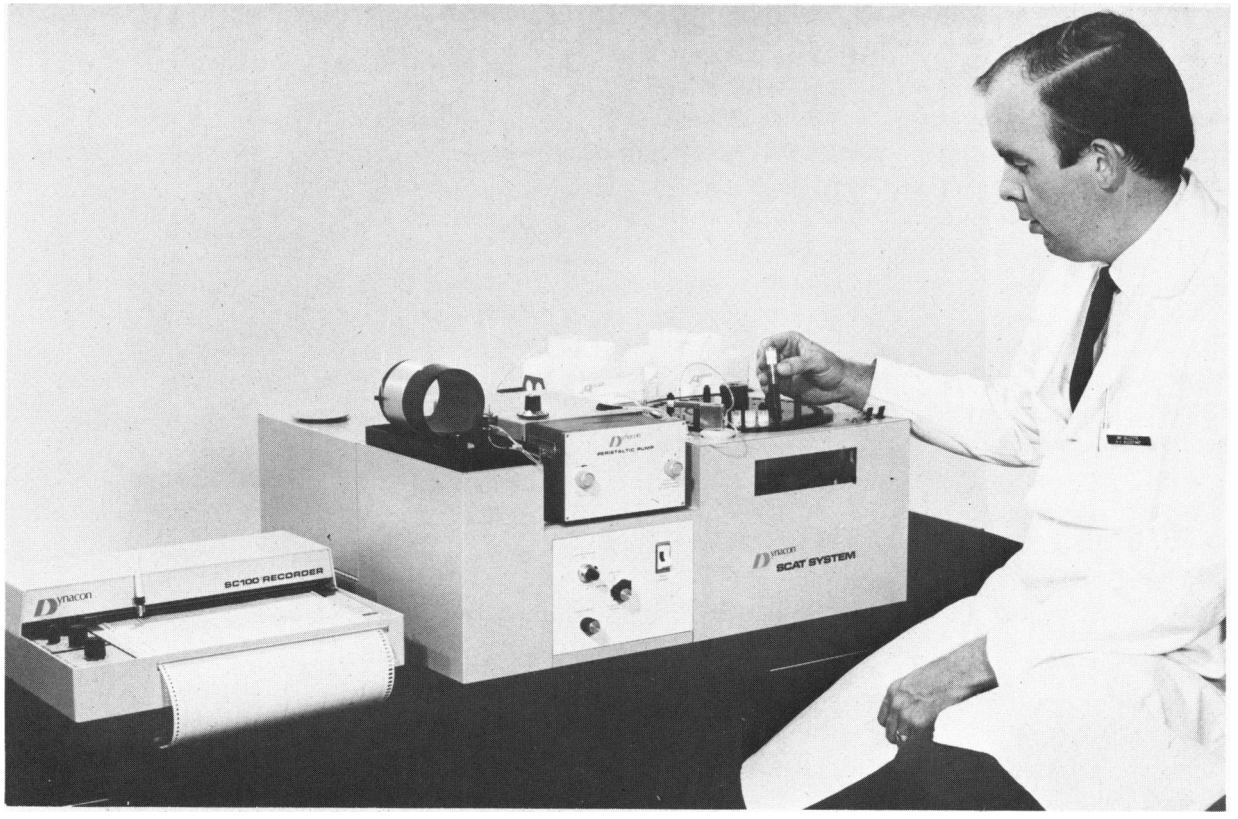
Mass screening is retrospective and therapeutic in outlook; marital counseling is prospective and preventive. Since the purposes of mass screening and marital counseling are different, the methods which serve their distinct ends will be different. The best ratio of cost to effectiveness in mass screening is achieved from an initial study of blood specimens by the automated dithionite test. Those that are positive by the dithionite test are additionally studied by hemoglobin electrophoresis.

The reason for using these particular tests in

**Status of four concurrent sickle cell testing programs, December 1972**

| Investigator and study center | Persons tested | | | Hemoglobinopathy | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total | Black | White | SS | AS | AC | SC | AD | AF | Sickle-thalassemia |
| Col. Frank R. Camp, Jr., U.S. Army Medical Research Laboratory, Fort Knox, Ky. | 61,485 | 8,606 | 52,879 | 4 | 769 | 0 | 2 | 0 | 0 | 0 |
| Edwin L. Bemis, MD, Deaconess Hospital, Milwaukee, Wis. | 12,880 | (1) | (1) | 13 | 569 | 0 | 27 | 0 | 0 | 1 |
| Phillip E. Runkel and Robert M. Nalbandian, MD, Board of Education, Grand Rapids, Mich. | [2] 8,698 | [3] 8,634 | [3] 64 | 1 | 305 | 0 | 2 | 0 | 0 | 0 |
| Leo P. Cawley, MD, Wesley Medical Center, Wichita, Kans. | 1,480 | (1) | (1) | 3 | 136 | 2 | 2 | 1 | 20 | 0 |
| Total | 84,543 | (1) | (1) | 21 | 1,779 | 2 | 33 | 1 | 20 | 1 |

[1] Data by race not available. [2] Includes 319 black pupils whose specimens showed a positive reaction to the dithionite test. We do not know how many of these pupils and their families are getting diagnostic workups or how many are not having additional studies. [3] Close estimates from data of study in progress.




**Sequential multiple analyzer and recorder used in the accelerated automated dithionite test**

Source: Reference 22.

this precise sequence derive from logical considerations of (*a*) specific molecular pathology of an ever-growing list of hemoglobinopathies (now in excess of 150 molecular species), (*b*) the principle and limitations of the test used at the molecular level of reference, and (*c*) the economics and logistics of the screening techniques. There are several intricate and subtle molecular reasons which support the proposed mass screening method.

## Nonspecificity of Earlier Tests

It is appropriate to examine the principles and limitations of testing for S hemoglobin and to consider the advantages of recent innovative techniques. The objective is to find an eclectic method or combination of methods which provide the most accurate diagnostic information at the lowest possible cost.

Before the development of the Murayama test (*8, 22, 26–28*) and the dithionite test, methods in common use did not have a known molecular basis for the detection of hemoglobin S. These nonspecific tests have been classified into three convenient categories (*2, 22*): sickling tests, electrophoresis, and so-called solubility (dithionite) tests.

*Sickling tests.* The eight human hemoglobinopathies and the seven animals that yield hemoglobinopathies currently known to sickle *(2, 7, 8, 22, 26, 29)* are listed as follows.

| *Human hemoglobinopathies* | *Animal hemoglobins* |
|---|---|
| S | Deer |
| C (Harlem) | Sheep |
| C (Georgetown) | Goat |
| I | Mongoose |
| Bart's | Raccoon |
| Alexandra | Hamster |
| Memphis/S | Squirrel |
| Pôrto Alegre | |

So far as we know, the molecular mechanism for sickling in these hemoglobinopathies, except S and C (Harlem), are distinct and different.

There is no appropriate animal hemoglobin model for the study of sickling in hemoglobin S erythrocytes *(8, 22)*. Isaacs and Schneider and associates have shown that, under appropriate

conditions, any human hemoglobin may sickle *(30, 31)*.

Sickling tests are inadequate for detecting hemoglobin S. The widely used 2 percent sodium metabisulfite test is manual, subjective, nonspecific, relatively expensive, and may require up to 24 hours for a determination. Schneider and associates *(32)* and Raper *(33)* have discussed the several technical flaws and unreliability of the metabisulfite sickling test. It is evident that the sickling tests are not adequate for the diagnosis of S hemoglobin. Now that the molecular mechanism of sickling is better understood, sickling tests must be replaced by more specific, reliable, and convenient procedures.

*Hemoglobin electrophoresis.* Hemoglobin electrophoresis is a nonspecific method of identifying hemoglobinopathies in general and hemoglobin S in particular *(2, 7, 8, 22, 26)*. Several hemoglobin variants share the same alphabetic designation, although molecular structures in any such set of hemoglobins may be different. Hemoglobinopathies such as C, D, G, and M serve as appropriate examples. This nonspecificity and lack of discrimination with hemoglobin electrophoresis determinations occur because most hemoglobinopathies are named as a function of their electrophoretic mobility at specified pH and other conditions.

Under standard conditions at a given pH, the mobility depends primarily on the algebraic sum of the electrical charges of the polar groups protruding from the surface of the quaternary structure of a particular hemoglobin molecular species. The number of amino acid substitutions and permutations in 574 positions among 19 amino acids giving the same net molecular charge overall and, hence, the same electrophoretic designation is astronomical.

For hemoglobin S at the usual pH ranges, there are at least 20 other known hemoglobinopathies which move electrophoretically like S *(22)*, and under other specified conditions, there are an additional 14 which have mobilities identical with hemoglobin S *(22)*. These hemoglobinopathies are shown in the following list.

| *At pH 8.0 to 8.9* | *Under other specified conditions* |
|---|---|
| S | Russ |
| D | Shimonoseki |
| Flatbush | Köln |
| Zurich | G (Philadelphia) |
| Stanleyville, II | G (Port Arthur) |
| Sealy (Sinai, Hasharon) | G (Galveston) |
| P | G (Texas) |
| Etobicoke | Alexandra |
| Sabine | Zurich |
| Gun Hill | E |
| Shimonoseki | Ocho Rios |
| Kokura | Kokura |
| Umi | Umi |
| L Ferrara | St. Luke's |
| Leiden | |
| Lepore | |
| G (Coushatta) | |
| Memphis/S | |
| Korle-Bu | |
| Osu-Christianborg | |
| Ocho Rios | |

With the passage of time and the discovery of new hemoglobinopathies, this list will continue to increase as it already has *(2, 7, 8, 22, 26)*.

As a demonstration of the manner in which the identities and distinctions of clinically important molecular hemoglobin species may be obscured by electrophoresis, we shall examine hemoglobin C in some detail. At a pH from 8.2 to 8.6, hemoglobin C is one of the more slowly moving hemoglobins. The hemoglobin C of Hunt and Ingram has lysine instead of the normally occurring number six glutamic acid residue in each beta globin chain. This is a nonsickling hemoglobin which by itself is quite benign clinically, but it can interact with hemoglobin S and be involved in the molecular polymerization of sickling.

However, two other hemoglobinopathies, C (Georgetown) and C (Harlem), are non-S sickling variants. There is a minor disagreement about the nature of these two molecular species, and the interested reader is referred to other sources for the details *(4, 5, 8, 22, 34)*. Both of these hemoglobinopathies will cause clinically evident disease which is milder than the usual sickle cell disease. C (Georgetown) is characterized by a lysine substitution for the number seven glutamic acid position in the beta globin chain. C (Harlem) is caused by the crossing over of hemoglobins Korle-Bu and S, substituting valine for glutamic acid in the number six position and asparagine for aspartic acid in the number 73 position in the beta globin chain.

Hemoglobins E and $O_{Arab}$ also move electrophoretically like C. Thus, hemoglobins with substantial, distinctly different molecular constructions and clinical manifestations appear identical when electrophoresis is the only method of identification.

Hemoglobin electrophoresis, furthermore, is a

more expensive, slow, manual method, requires subjective interpretation, and cannot be automated. At best, not more than 300 specimens per technician-day can be studied by electrophoresis using mass production methods. To process a comparable number of blood specimens, 2½ to 6 times the cost of reagents and three to four times as many technician-days are required by the hemoglobin electrophoresis method than by the automated dithionite test.

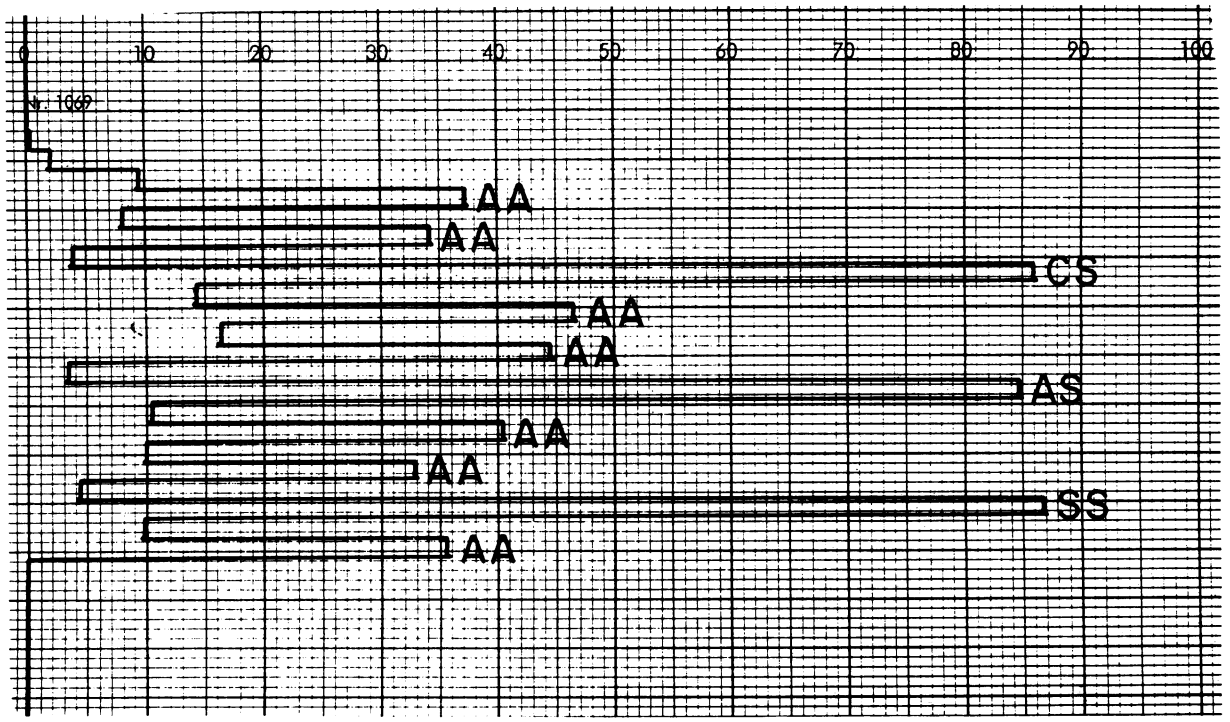
Hemoglobin electrophoresis is a useful additional determination because it characterizes molecular parameters different from those determined by the dithionite test and is important for the determination of zygosity. With present methods, the electrophoretic technique allows the simultaneous determination of hemoglobins $A_2$ and F, making it particularly useful in the diagnosis of interacting thalassemias when associated with sickle cell disease. Clinically significant thalassemia can be detected very inexpensively by automated techniques which measure the hematocrit or the mean corpuscular volume. This method for the discovery of thalassemia in the population is far less expensive than electrophoresis. Hemoglobin electrophoresis is emphatically *not* the method of choice in the initial screening technique and should never be used as the only diagnostic test (*8, 22, 25*).

*Solubility tests.* Itano and Pauling (*35*) and Itano (*36*) established the groundwork for the dithionite test. The molecular basis for the dithionite test, discussed in detail elsewhere (*4, 5, 22*), detects the presence of deoxygenated or sickled hemoglobin S in aqueous systems when it is present as a nematic liquid crystal system. The test reagents consist of potassium phosphate, sodium dithionite, and saponin. When hemoglobin S containing erythrocytes are introduced into such a solution, the following events occur.

1. The erythrocytes undergo immediate lysis.
2. The hemoglobin molecules deoxygenate and undergo configurational changes.
3. Tetramer-tetramer interaction proceeds, mediated by hydrophobic bonding.
4. Nematic liquid crystal systems are formed, obscuring the transmittance of light.

The existence of a nematic liquid crystal system may be objectively detected by instrumentation (*A, B*) as a markedly reduced percent of transmittance of light, in sharp contrast to systems of hemoglobin A which transmit light without im-

**Typical curves produced every 24 seconds in the accelerated automated dithionite test from anticoagulated whole blood specimens at a reagent cost of 0.1 to 0.3 cent each**




Source: Reference 22.

pairment (*4, 5, 22*). Furthermore, with the use of urea, it can be determined whether such nematic liquid crystal systems are hydrophobic-bond dependent (*4, 5, 22*). This optional, additional capability allows one to make a judgment concerning whether a given specimen which is positive to the dithionite test is an "S" or a "non-S sickling" hemoglobin. By using the dithionite test with urea, differences among the three molecular variants of hemoglobin C are easily observed. This technique differentiates among the three C hemoglobinopathies and other sickling hemoglobinopathies. The details have been discussed elsewhere (*4, 5, 22*).

Our views on the Sickledex test (*27*) and our modifications of it have been published in detail (*2, 4, 6–8, 37*). A U.S. patent issued in 1970 to Tillem (*38*) confirms our earlier conclusion (*2*) that the reagents of the dithionite test and the Sickledex test are the same.

The dithionite test in either of the two tube versions (*4, 22*), or preferably in either of the two automated versions (*5, 22*), provides an ideal technique for the initial mass screening of large populations because of its high specificity and extremely low cost. The automated dithionite test processes 960 whole blood specimens per technician-day at a chemical reagent cost of 2 cents per determination in a sequential multiple analyzer (*A*).

Currently we are conducting accelerated automated dithionite tests in the field trials of a new instrument (*B*) which processes 1,200 whole blood specimens per technician-day at a chemical reagent cost of 0.1 to 0.3 cent each (see chart). A positive dithionite test necessitates immediate hemoglobin electrophoresis of part of the remaining specimen, which may be taken directly from the same specimen cup on the instrument turntable.

The following two lists summarize and contrast the salient features of hemoglobin electrophoresis and the solubility (dithionite) test.

HEMOGLOBIN ELECTROPHORESIS

1. Based on the net electrical charge on the surface of the hemoglobin molecule; a function of the algebraic sum of the polarity of side groups; nonspecific.
2. Subjective, manual, must be interpreted.
3. About 15 minutes are required to get specimen patterns; cannot be automated; mass production methods cannot exceed 300 per technician-day.
4. Twenty other known hemoglobinopathies have electrophoretic patterns identical with S hemoglobin under the usual conditions. At least 14 more under specified conditions are indistinguishable from S.
5. Hemoglobins C, C (Harlem), C (Georgetown), E, and $O_{Arab}$ are all grouped as "C"; sickling and nonsickling C hemoglobinopathies are not distinguished.
6. As new hemoglobinopathies are discovered, some, in addition to the 29 known examples, will have electrophoretic mobilities like S. Therefore, with time, the method will become even more nonspecific.
7. To process a given load of blood specimens, 2½ to 6 times the cost of reagents and supplies and at least three to four times more technician-days are required by the hemoglobin electrophoresis method than by the automated dithionite test.

AUTOMATED DITHIONITE TEST

1. Based on the specific molecular pathology of hemoglobin S.
2. Objective, mechanized.
3. Processes specimens every 24–30 seconds; 960–1,200 specimens processed per technician-day.
4. Highly specific because of molecular basis of test; specificity can be increased even more with urea.
5. Discriminates between C (Harlem) and C (Georgetown); both are clinically important non-S sickling hemoglobinopathies.
6. Specificity for S hemoglobin remains constant.
7. Economical at a reagent cost of 2 cents per test.

## Eclectic Method for Mass Screening

The highest degree of diagnostic accuracy at the lowest possible cost for the detection of hemoglobin S is obtained with a two-phase system: initial testing by the highly specific, inexpensive, rapid, automated dithionite test and subsequent electrophoresis of only those specimens which are positive by that test. This sequence assures the most favorable ratio of cost to effectiveness with optimal diagnostic accuracy. Hence, this method has been adopted as the central diagnostic technique of our successful mass screening programs because it serves the goals of a comprehensive screening program. In addition, its low cost assures continuity of the program year after year.

## Comprehensive Citywide Program

Our approach (*22, 23, 25*) offers a comprehensive, total health care service which includes screening, diagnosis, treatment, and genetic counseling—all at extremely low cost. Therefore, a program of this scope can be carried out within the normal operating budget of most local health agencies. Our program is operated through the Grand Rapids, Mich., public school system. It is voluntary, administered without charge, and pre-

sents a set of options to the afflicted black students and their families at every step of the program. Recently, the details have been published elsewhere (*22, 23, 25*), but a brief description of the program follows.

The program is school centered and uses the administrative services of the school health system. After coordinated information about sickle cell disease and the testing program is disseminated via the local news media for 3 or 4 weeks, a mass screening program is conducted. Although the testing program is offered to all students, black students are encouraged to participate.

Blood specimens are processed rapidly at a cooperating central hospital laboratory employing the automated dithionite test. Specimens with a positive reaction to this technique are immediately studied by hemoglobin electrophoresis, using the remainder of the blood specimen while specimens from other students are being processed simultaneously by the automated method.

Afflicted students identify their afflicted families. When a student yields a blood specimen positive to the dithionite test, an appropriate letter is mailed from the school superintendent's office to his family. The letter says that all members of the family should be tested at one of a number of specified facilities. Options for further diagnosis, treatment, and genetic counseling are also described in the letter. Blood specimens from all cooperating family members are studied by the dithionite test and hemoglobin electrophoresis.

New students entering the school system since the last screening are tested just after the semiannual grade change when, the local news media again presents an intensive, coordinated informational campaign. Testing new students in each school is done by the school nurse. She employs the dithionite tube test which entails a reagent cost of 2 cents each. Alternatively, such specimens may be processed by the automated dithionite test (same reagent cost) at a local hospital laboratory. No student is tested more than once. No fees are charged to the students or families.

Simple, adequate, but critically essential records of the test results are maintained confidentially in a central board of education office. The front of the form used in the Grand Rapids sickle cell anemia testing (SCAT) program provides identification, family information, permission and authorization for the sickle sell screenng test, and prenumbered specimen identification labels. This information is not copied again, thereby eliminating clerical errors. The labels are printed along the right-hand margin and are perforated for easy detachment so they can be used to identify the blood specimen provided by the registrant. The numbers on the labels are coordinated with those on the registration card.

In the Grand Rapids SCAT program each number is unique to only one student in the entire school system, and members of his family each have their own number. The first two digits of the number always correspond with the current year: for example 72–501 means the 501st specimen taken in 1972, 73–501 will mean the 501st specimen taken in 1973.

The results of the dithionite test are recorded on the back of the card and if the test is positive, the results of hemoglobin electrophoresis are also recorded. A record of the ultimate referral of the patient is kept by the school nurse.

A wallet-card form is issued to all persons, both students and family members, who take the dithionite test. Each person tested receives such a wallet card showing the test results, whether positive or negative. Card holders are further advised that they should show the card to the physician when they seek medical attention or hospitalization. Moreover, the card is invaluable in forewarning physicians of the patient's sickle cell hemoglobin. Thus diagnostic problems may be averted, and vigilant care in the course of possible anesthesia anticipated.

The SCAT program is funded under the annual operating budget of the Grand Rapids Board of Education as an official health service. This approach through the school system initially obviates the needless testing of large numbers of sickle-cell-negative individuals.

## Benefits From Automated Screening

We share the view of Dr. Lemuel W. Diggs, Goodman Professor of Medicine, Emeritus, and consultant to the sickle cell center, University of Tennessee College of Medicine, who, for many years, has noted that the statistics on the prevalence of sickle cell disease are notoriously inaccurate (personal discussion, January 1972). By using automated mass screening techniques in metropolitan areas across the country, a population data base consisting of persons afflicted with a specifically defined inheritable disease is generated. By appropriate techniques geneticists can establish, for the first time, accurate statistics for this debilitating and eventually lethal disease.

Previously, tests were nonspecific, manual, and subjective; consequently, statistics were parochial and variable. With the advent of a mechanized, highly precise, and accurate technique, an efficient diagnostic tool is available to the biostatistician and the geneticist.

Use of the automated dithionite test makes possible, for the first time, the compilation of national statistics on this disease. Data from geographically separate areas can be integrated so that national statistics on incidence, morbidity, mortality, gene frequency, body weights, birth weights, and susceptibility to other diseases will become available on sickle cell disease for the first time in the United States.

## Prospective Mass Screening Programs

On the basis of our experience with the SCAT mass screening program in Grand Rapids, we emphasized certain operational points (*22, 23, 25*) which may be useful in similar studies.

1. Techniques and methods of dissemination of information and education.

2. The use of a documentary educational film. (The TV documentary, "Sickle Cell Disease; Paradox of Neglect," is available as a 16-mm sound and color film from TV-13, WZZM, Box Z, Grand Rapids, Mich. 49501. On April 10, 1972, this documentary was awarded the "EMMY" by the National Academy of Television Arts and Sciences.)

3. The use of student "truth squads" and educational school projects.

4. The identification and communication value of an acronym, SCAT.

5. The critical importance of simple but adequate multipurpose data forms with unique, preprinted identification numbers to eliminate clerical error.

6. The importance of a free and voluntary screening program to avoid medicolegal entanglements.

7. The value and significance of a wallet identification card for all persons tested.

A comprehensive, two-phase sickle cell mass screening program is in successful operation in Grand Rapids. The promise of this model is that, coupled with genetic counseling, successive generations of black Americans will become progressively healthier by eliminating the sickle cell hemoglobin gene.

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**EQUIPMENT REFERENCES**

(A) Dual Channel AutoAnalyzer, Technicon Corporation, Tarrytown, N.Y. 10591.
(B) Dynacon Automatic Analyzer, Precision Technology, Inc., 375 Oak Tree Road, Palisades, N.Y. 10964.

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**NALBANDIAN, ROBERT M. (Wayne State University, Detroit, Mich.), CAMP, FRANK R., Jr., CONTE, NICHOLAS F., and PROTHRO, WINSTON B.:** ***Automated mass screening for hemoglobin S. A rational method. Health Services Reports, Vol. 88, February 1973, pp. 165–173.***

Mass screening programs, coupled with genetic counseling, hold promise of eliminating sickle cell disease, leaving successive generations of black Americans progressively healthier. A rational method, based on an understanding of the principles and limitations of currently used diagnostic tests and an analytical appreciation of the molecular pathology of hemoglobin S, consists of a two-phase system.

Initial screening of the population at risk is undertaken by the automated dithionite test of blood specimens, and only positive specimens are studied further by hemoglobin electrophoresis. This technique yields all necessary and pertinent data for diagnosis and genetic counseling at the lowest possible cost. Interacting hemoglobinopathies such as thalassemia and C are always detected by this method when they are medically important, that is, in an individual, a mating couple, or a family previously shown to have S hemoglobin in some form.

Eight human hemoglobins sickle. Because of the principle of electrophoresis, the method is a highly nonspecific technique for the detection of hemoglobin S. At pH 8.0–8.9, at least 20 other hemoglobins move electrophoretically like S. Under other specified conditions an additional 14 hemoglobins have mobilities identical with hemoglobin S.

Electrophoresis is slow, nonspecific, manual, subjective, and relatively expensive. The principle of the dithionite test is based on the easy detection of hydrophobic-bond-dependent nematic liquid crystal systems formed by hemoglobin S.

Using the automated dithionite test, 960 whole blood specimens per technician-day can be processed at a reagent cost of 2 cents each. An accelerated version, currently undergoing extensive field trials, processes 1,200 whole blood specimens per technician-day at a reagent cost of 0.1 to 0.3 cent each. When the sequence of this mass screening technique is reversed, the same data are still obtained but at far greater cost in time, effort, and money.

An ongoing, comprehensive school-centered mass screening program, offering options for screening, diagnosis, education, treatment, and counseling, has been based on screening by the two-phase system. An aggregate experience of more than 84,000 tests at four study centers supports our recommendations.