INCIDENCE AND AGE RELATED FREQUENCIES OF HEMOGLOBIN S IN SELECTED RANDOM AND NONRANDOM POPULATIONS

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ABSTRACT

INCIDENCE AND AGE RELATED FREQUENCIES OF HEMOGLOBIN S IN SELECTED RANDOM AND NONRANDOM POPULATIONS

By

Frankie J. Brown

Blood samples from 4208 black Americans were analyzed for the presence of hemoglobin S by use of cellulose acetate electrophoresis and by solubility determinations in a 2.3 molar phosphate buffer solution. The individuals tested were derived from five sample groupings, one of which was randomly selected from the Lansing, Michigan black population, two which represented the results of nonrandom screening programs from the same general area, one from a college testing program, and one from a state institution for the mentally retarded.

The gene frequency for the hemoglobin S allele in the random sample was found to be 0.043. When compared to this value, no significant frequency differences for the allele were found in the nonrandom populations. (Variation 0.031 to 0.047.)

Comparisons of the observed and expected genotypic frequencies for the AA, AS, AC, SS, CC and SC genotypes failed to produce any significant differences in any of the samples studied.

The age related frequencies for hemoglobin S carriers were calculated for four samples (the random sample and three of the nonrandom groups). Results of these calculations showed no significant differences in the percent of AS individuals with age. Frequency fluctuations with age did occur, however, as well as fluctuations in the mean values for the percent of red cell hemoglobin S. These fluctuations were not statistically significant.

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Frankie J. Brown

A THESIS

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To C.B. and Chuckie
and my parents

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INTRODUCTION

Specific screening and testing for the presence of hemoglobin S have been carried out on numerous populations in several countries. Increased incidences have closely correlated with the prevalence of tropical conditions conducive to the abundance of the malarial parasite, Plasmodium falciparum; thus the heterozygote is thought to have increased fitness over both homozygotes and thereby constituting a balanced polymorphism.

In the United States, the gene occurs predominantly in the black population by virtue of their African ancestry, but has also been reported with low frequency in other groups. Frequency estimates for the black population have varied from 5 to 20 percent. This variation appears due to the variety of testing methods and the geographic area of the country from which the sampling is derived. Most of these determinations have been carried out on clinic or hospital populations and are thus considered biased frequency estimates.

The present study proposes to examine the frequency of hemoglobin S and its age related frequencies in a random sample of black residents of the Lansing, Michigan area.

The results are then compared to the frequencies obtained from a nonrandom screening program carried out on four other populations: a clinic and a general population from the same area requesting evaluation, a college population and the black residents in a State Home and Training School.

LITERATURE REVIEW

Hemoglobin S, the defect in sickle cell disease, is an abnormal variant of adult hemoglobin A and is inherited as an autosomal codominant. It differs from its normal prototype by a single amino acid substitution in which glutamic acid is replaced by valine in the sixth position of the beta chain. (Ingram, 1956). This molecular lesion is thought to have arisen by a point mutation (Pauling, 1964) which resulted in a change in the mRNA codon triplet from GAA or GAG for glutamic acid to GUA or GUG for valine. This substitution has conferred upon the hemoglobin S molecule a variety of chemical and physical properties which allow its isolation from other hemoglobin types.

Specific chemical and physical peculiarities of hemoglobin S have been well documented. In 1917, Emmel was the first to note that after placing a drop of blood obtained from anemic patients under a sealed cover slip, there was a progressive increase in the number of sickled cells with the passage of time. Hahn and Gillespie (1927) were the first to show that the unique distortion of the sickled cell was primarily dependent upon an alteration of the hemoglobin from oxygen-saturated to the unsaturated state. Later

Scriver and Waugh (1930) found that the number of sickled cells increased as oxygen decreased and carbon dioxide accumulated during venous stasis of an extremity. Pauling et. al., (1949) demonstrated by free electrophoresis of carbon monoxyhemoglobin in a phosphate buffer of 0.1 ionic strength and at a pH of 6.9, that the hemoglobin from patients with sickle cell anemia differed from the normal in its electrophoretic mobility. In 1950, Perutz and Michison showed that deoxygenated S hemoglobin was much less soluble than deoxygenated normal hemoglobin. This led to the development of a test for sickle hemoglobin based on its relative insolubility by Itano in 1953. During that same year, Havinga and Itano (1953) showed that the abnormality in sickle cell hemoglobin resided in the globin portion of the molecule and Singer and Singer (1953) showed that sickling, the formation of tactoids and the tendency toward gelation were specific characteristics of reduced hemoglobin S. Later, Murayama (1956) found that hemoglobin S had a negative coefficient of gelation since the gel of deoxygenated hemoglobin S liquifies when placed at 0°C while at 20°C it is transformed into a gel which is further facilitated by an increase to 37°C. In attempts to explain the basis for the sickling phenomenon, Pauling (1952) proposed that hemoglobin S molecules might be able to aggregate or stack together in long chains because of having complementary surface conformations. Later experimental observations by Murayama (1967) suggested that the

substitution of valine for glutamic acid at the sixth position in the beta chains allowed an intramolecular hydrophobic bond to form. This changed the conformation in such a way as to allow molecular stacking and subsequent filament formation.

It has long been known that the possession of hemoglobin S is an inherited phenomenon. In 1949 Neel and Beet independently proposed that it is inherited as a simple Mendelian recessive gene to the dominant gene A for normal adult hemoglobin. Later observations have led to the modification of this inheritance pattern to one of codominance since the expression of both genes can be seen in heterozygous individuals. Nevertheless, there remains a disproportionate predominance of the A gene product since in such persons, hemoglobin A is more readily produced than is hemoglobin S, their relative proportions typically being 60 percent A to 40 percent S.

In a given individual, hemoglobin S may be found with one of a number of other hemoglobin types. McKusick (1971) lists 59 mutants of the beta chain alone. Additionally, there are 33 alpha chain substitutions. Thus the variety of S- genotypes are many. Generally however, in any population, only a few are common. In the American black population for example, only the AS, SS, SC, SD, and Sβ-thalassemia types are seen with relative frequency while others are extremely rare.

The occurrence of hemoglobin S has been reported in all parts of the world but is most common in Africa, Asia Minor and India (Livingston 1967). Significant incidences have been reported in the Negroes of North and South America. In almost all instances where elevated frequencies have been noted, they have coincided with the prevalence of malaria, particularly the type produced by the parasite, Plasmodium falciparum. Several studies have indicated that the individuals heterozygous (AS) for hemoglobin S may be at a selective advantage over their AA and SS counterparts in these malarial areas due to differential survival and/or increased fertility. Allison (1954) found that among school children in Uganda, those with sickle cell trait had lower malaria parasite rates. On examination of adults of the Luo tribe inoculated with malaria, he found that those with the sickle cell trait showed parasites in their blood far fewer times than nonsicklers. Allison also showed that the frequency of the trait in many East African tribes is closely correlated with endemicity of malaria. Raper (1955) found a lower parasite rate in AS individuals and was also able to show that they did not have as many infections as nonsicklers. He then gave conclusive evidence that those with the sickle trait do not die as frequently from cerebral malaria which is often a lethal complication of falciparum malaria. Several other studies have indicated greater fertility for both male and female AS individuals (Delbrouck, 1958; Allison,

1964; Roberts and Boyo, 1960; Fercheins, 1961; and Eaton and Mucha, 1971). Such studies would indicate that individuals heterozygous for hemoglobin S have increased fitness over both AA and SS homozygotes in malarial area. This may therefore give rise to a stable equilibrium between the two alleles and constitute a balanced polymorphism.

The exact origin of the sickling gene remains unclear. While it is always possible that a hemoglobin may have arisen in different parts of the world independently, according to Lehmann and Huntsman (1966), it is possible to interpret the present day distribution of sickling in the world as a result of a single mutation. They indicate that descendants of a population who occupied the ancient fertile Arabia before the present day Semites have been recognized. This group is thought to have been ancestors of the present aboriginal tribes of India who may have made their way to Africa via the former land bridge between Asia and Africa. It is suggested that the sickling gene arose among these people in Neolithic times and it was distributed by them eastward into India and westward into Africa. The finding of high incidences among the primitive hill tribes of the negroid Veddoids of southern India and southern Arabia has led to this theory.

Despite the uncertainty of its origin, the sickling gene presently has widespread distribution. Table 1 represents a modification of data compiled by Livingstone (1967)

Table 1. Incidence of hemoglobin S.

Country	Frequency Ranges-%	Remarks
Polynesia		Excludes Hawaii
Puerto Ricans	0	
Negroes	2.9	
Hawaii		
Caucasians	0	
Orientals	0.5	
Micronesia	-	No reports
Melanesia	0	
Australia	0	Only 2 small reports
New Guinea	0	Two small samples
Phillippines	0	Only one small study
Indonesia		
Central Sumatra	0.4	One small study
Djakarta	0.9	One small study
Borneo	-	No reports
Malaya	0	
Thailand	-	No reports
Laos	-	No reports
Cambodia	-	No reports
Vietnam	-	No reports
China	-	No reports
Taiwan	-	No reports
Korea	0	One small study; n=50
Japan	-	No reports
Ceylon	0	One small study; n=800
Andaman Islands	0	Two small samples

Table 1. Continued.

Country	Frequency Ranges-%	Remarks
India	0 - 40	
Pakistan	1.3	One small study; W. Pakis.
Iran	0	One report
Afghanistan	-	No reports
Iraq	-	No reports
Turkey	0.2 - 27.3	
Lebanon	0.1 - 4.0	Avg.=0.1 - 0.8
Syria	0 - 50.0	Three small samples
Israel	0 - 0.8	
Jordan	0 - 0.3	
Saudi Arabia	1.1 - 25.1	
Kuwait	-	No reports
Aden		
Arabs	1.8	
Zabidis	22.8	
Yemen	0	One sample, n=104
Cyprus	1.4 - 8.0	Two small studies
Greece	0 - 32.2	Most reports from Macedonia. Negligible frequencies elsewhere
Yugoslavia	-	No reports
Italy	0.2 - 4.3	
Portugal	0 - 0.1	Two large samples
Czechoslovakia	-	No reports

Table 1. Continued.

Country	Frequency Ranges-%	Remarks
Sweden	-	No reports
W. Germany	-	No reports
Switzerland	-	No reports
Netherlands	-	No reports
Great Britain		
British soldiers	0	
Preg. females of W. Indian, Afr. Medit. ancestry	11.4	
Egypt	0 - 0.2	Two samples
Libya	0.8 - 10.4	
Tunisia	2.0 - 3.3	
Morocco	0.5 - 2.0	
Algeria	0 - 5.8	One sample: 10.3% at El Golea Oasis
Mauretanie	3.9 - 12.6	
Cape Verde Islands	2.8 - 7.3	
Senegal	0 - 33.3	Avg. freq.=about 15%
Guinea	0 - 33.8	
Portuguese Guinea	0.2 - 25.1	
Gambia	6.1 - 28.4	
Sierra Leone	23.8 - 30.6	
Liberia	0.5 - 26.1	
Ivory Coast	2.9 - 14.2	
Upper Volta	2.0 - 33.8	

Table 1. Continued.

Country	Frequency Ranges-%	Remarks
Ghana	4.2 - 23.5	
Togo	7.9 - 10.9	
Dahomey	12.5 - 14.7	
Niger	5.4 - 22.4	
Nigeria	9.7 - 32.6	
Cameroons	1.5 - 28.2	
Sao Tome	4.2	
Pricipe Islands	22.1	
Central African Republic Babinga - Pygmies	16.8	
Congo - Brazzaville	24.5	Small sample, n=53
Gabon	8.2 - 19.2	
Congo (Kinshasa)	5.3 - 44.0	1.0% for Tutsi at Itombwa Plateau
Rwanda and Burundi	0 - 25.9	0% in Tutsi, highest in Bamosso
Angola	0 - 36.7	0% in bushmen, highest in Bangala
Botswana	0 - 2.0	
South Africa	0 - 1.9	
Mozambique	0 - 40.0	Avg. about 2%, highest on N. Coast, Nampula and Porto Amelia
Rhodesia, Zambia, Malawi	0 - 27.0	Average 10-15%
Tanzania	7.4 - 40.5	

Table 1. Continued.

Country	Frequency Ranges-%	Remarks
Kenya	0 - 34.2	
Uganda	1.0 - 39.4	Sm. Sample in Lutomi Kraal had 46 of 53= 86.8%
Sudan	0 - 30.4	Sm. samples, avg.=5-10%
Ethiopia	0	One sm. study at Addis Ababa
Somalia	0	
Seychelles	-	No reports
Comoro Islands	0 - 4.7	
Madagasca	0 - 23.2	
Canada	1 in 6300	
United States		
Whites	0 - 0.7	
Negroes	4.5 - 20.6	
Indian	1.1 - 1.3	Small samples
Mexico	1.0 - 10.0	Avg.=3%
Guatemala	-	No reports
British Honduras	22.2 - 25.4	
Honduras	8.0	One study on Caribs of San Juan
El Salvador	1.1 - 1.5	Two small studies
Nicaragua	-	No reports
Costa Rica	3.0	One sm. study at Terrabas
Panama	0 - 20.5	Avg. about 10%

Table 1. Continued.

Country	Frequency Ranges-%	Remarks
Cuba	5.3 - 6.5	Two sm. samples of blacks
Haiti	6.9 - 12.3	
Dominican Republic	5.8	One sm. sample of Creoles
Jamaica	3.6 - 10.8	
Puerto Rico		
Whites	0.8	
Negroes	8.4	Avg.=3.6
Lesser Antilles	1.6 - 14.0	
Trinidad (Negroes)	9.3	One study, n=204
Curacao (W. Indies)	5.1 - 9.2	
West Indies	9.6	One study, n=998
Colombia	9.4 - 14.7	Negroes mainly
Venezuela		
Non-Negroes	0 - 9.4	
Negroes	2.5 - 6.5	
Guyana	-	No reports
Surinam (Negroes)	10.4 - 25.0	
French Guinana	1.3 - 17.9	Avg.=10%
Brazil		
Whites	0 - 1.4	
Mulattoes	3.0 - 15.7	
Negroes	3.6 - 11.7	
Chile	0 - 0.1	
Bolivia	-	No reports
Peru	-	No reports
Ecuador	-	No reports
Burma	-	No reports
Iran	5.3	In patients with anemia

and reflects the frequency ranges reported from different areas of the world other than the U.S. Unfortunately, some populations have not been sampled for this defect while others are represented by small samples. It is possible to discuss, however, general trends and locate areas of frequency concentrations.

Of eastern Asia and the Pacific, Livingstone notes that there are few hemoglobin variants found in these areas, however, the occurrence of hemoglobin S has been reported in Polynesia and Indonesia. It is interesting to note that Plasmodium falciparum is common in Southeast Asia without the occurrence of hemoglobin S.

As indicated in Table 1, the sickle cell gene has a wide but irregular distribution in Southern Asia, the Middle East and Europe. In India, for example, the incidence varies from 0 percent in certain tribes of the Nilgiri Hills to estimates in excess of 30 percent in the Bestar area. In the arid areas, the occurrences are correlated with oases and river valleys and is thus found in oasis populations of Saudi Arabia but not among the Bedouin desert nomads. It has been reported sparingly in Jordon, Lebanon and Syria while in Southern Turkey, among the Eti-Turks, frequencies exceeding 20 percent are not uncommon. Highly elevated frequencies have also been reported in Macedonia and other areas of Northern Greece while much lower incidences occur in Italy and Portugal. Very few studies have been carried

out in other European countries; however, those sampled reported no hemoglobin S present in their indigenous populations.

Studies in Africa have revealed a broad belt of high incidence of the sickling trait extending roughly across the middle third of the continent, including Madagascar (Lehmann et al., 1966; see Figure 1). This area represents the most concentrated incidence of hemoglobin S but actual frequencies found here can vary quite markedly from less than one percent to 46 percent and appears to be dependent on the tribe sampled. Most groups show an incidence of between 12 and 30 percent. An incidence of 46 percent was found among the pigmoids of East Africa, 33 percent in certain groups in Senagal, Guinea and Upper Volta and over 35 percent for areas of the Congo, Angola and Uganda. Generally there is a rapid decrease in the gene as one proceeds either north or south hence sickling is very rare in Egypt, Algeria and South Africa.

The majority of sickle cell genes came to the New World with the Eighteenth century slave ships. It is generally believed that most of the slaves were drawn from the costal and adjacent areas of West Africa and Gambia to the Niger River with the Congo basin making the next greatest contribution and lesser numbers drawn from such places as Madagascar and Mozambique (Neel, 1951). Thus the North and South American frequencies should reflect frequencies from

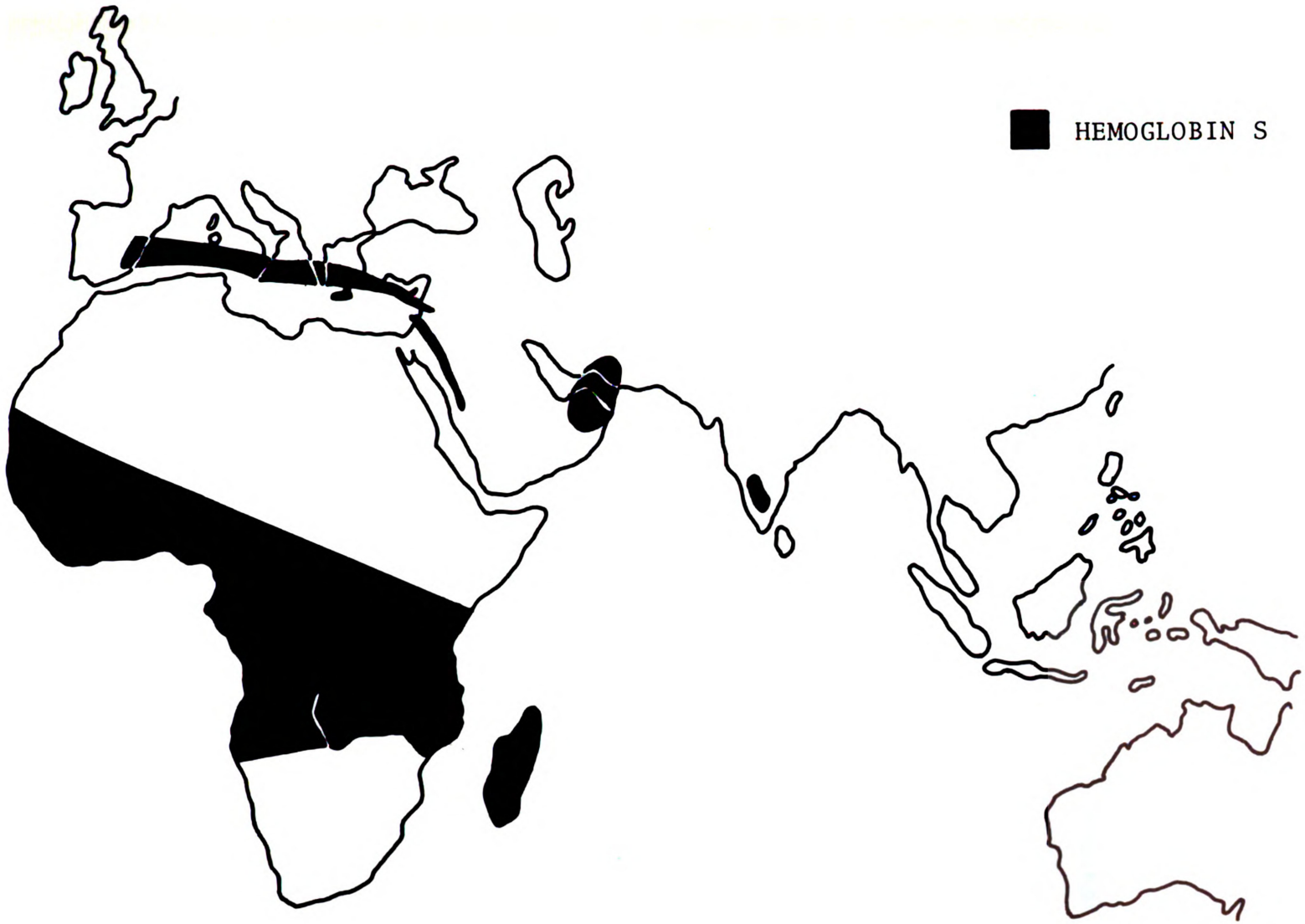

Figure 1. Geographic distribution of hemoglobin S. (Modified from Lehmann and Anger, 1966)

these African areas. Indeed, in the absence of malaria and in the presence of other hemoglobin genes, a decline in frequency should be realized due to elimination by natural selection. Presently in Mexico and Central America, high frequencies have been maintained in British Honduras (25 percent) and Panama. In the Caribbean Islands, frequencies vary on the average between 5 and 10 percent. Figures for South America include 10.7 percent among Negroes in Caracas, 15.7 percent in Mulattoes in Brazil and up to 25 percent in Surinam. In Puerto Rico and Brazil, frequencies of hemoglobin S in the white population were 0.8 percent and 1.4 percent respectively.

Sickle cell anemia was first described and characterized in the United States by Herrick in 1910 in a black West Indian male. Following this initial description, a few isolated cases were reported. In 1923 and 1924, Sydenstricker undertook the testing of 300 hospital patients and 1800 Negro school children and thus reported the first frequencies of sickling in the American black population. Since that time, numerous studies have been conducted to determine the etiology and prevalence of the disease and the trait. Such studies have indicated that the incidence varies in different areas of the U.S. and may thus reflect the areas of African origin and the degree of white admixture. The Appendix documents the reported incidences of hemoglobin S in the

U.S. Livingstone (1967) has summarized part of the data up to 1967. Modifications and additions have been made which include more recent studies and reflect the various testing methods used.

Unlike much of the data from other areas of the world, in the U.S., in several instances, larger samples have been obtained. Reported sampling has been done in twenty-four states and the District of Columbia. Most of these, however, have been generated from hospital and clinic populations. A few of the more recent samples have been derived from sickle cell screening programs in the public schools or at community clinics. Two samplings of military populations have also been made. Additionally, screening of enrollees in the Job Corps program has been done.

In this country, the average reported incidences of hemoglobin S in the black population have ranged from 5.8 percent in Georgia to 12.1 percent in South Carolina with an overall mean average for all states of 8.5 percent (Table 2). The highest frequency reported from general sampling of the American black population (20.6 percent) was noted in a small study by Pollitzer and associates (1966) in James Island, South Carolina. Nalbandian et al. (1972) found 105 hemoglobin S positives of 380 emergency room patients in Childrens Hospital in Detroit for an incidence of 27.6 percent. This represents the greatest frequency in a clinic population. Review of studies from the literature indicates

Table 2. Incidence of hemoglobin S in the American black population.

State or Population sampled	Number tested	Number with HgbS	Frequency percent
California	4,028	351	8.7
Connecticut	1,358	115	8.5
District of Columbia	9,503	640	6.7
Florida	1,618	131	8.1
Georgia	2,347	135	5.8
Illinois	7,930	648	8.2
Indiana	2,355	202	8.6
Iowa	3,000	267	8.9
Kansas	1,449	132	9.1
Louisianna	2,910	246	8.5
Maryland	1,772	121	6.8
Massachusetts	650	47	7.3
Michigan	6,245	492	7.9
Mississippi	1,310	155	11.8
Missouri	2,249	203	9.0
New York	6,828	451	6.6
North Carolina	490	41	8.4
Ohio	988	79	8.0
Pennsylvania	6,101	524	8.6
South Carolina	8,197	993	12.1
Tennessee	11,473	966	8.4
Texas	24,496	2,221	9.1
Virginia	3,822	236	6.2
West Virginia	275	18	6.5
Wisconsin	9,881	931	9.4
Military	1,000	75	7.5
Job Corps	11,182	967	8.6
TOTALS	133,457	11,387	8.5

that a total of 133,457 blacks have been tested for this defect. This figure is probably much lower than the actual numbers tested since the results of many local screening programs have not been published. Additionally, some studies have included blacks but racial distributions were not reported.

While the majority of studies in this country have been carried out in the Negro population, a few samples have included other racial groups. Rucknagel (1964) found an incidence of 20.2 percent in the Wesorts of Maryland. He also reported a frequency of 1.7 percent in the Oklahoma Indians. Lawrence (1927) found a 3.0 percent incidence in white students of Nashville. Pollitzer, et al. (1959) in studies of the Lumbee Indians and an Indian triracial isolate in North Carolina found a frequency of 1.7 percent while in 1966 Pollitzer and associates found in another triracial isolate in South Carolina and the Seminole Indians of Florida figures of 12.2 percent and 9.6 percent respectively. Thompson, et al. (1964) in a sampling of 1045 Caucasians found one AS individual while Fielding, et. al. (1972) found five whites with sickle cell trait in 3426 white job corpsmen. Other workers have reported no hemoglobin S among the Caucasians tested in their samples. (Moffitt and McDowell, 1959; Killingsworth and Wallace, 1936; Cardoza, 1957.) In a small series by Killingsworth and Wallace (1956), an incidence of 1.2 percent was found

among the Mexicans of Dallas, Texas while Fielding and co-workers (1972) reported 0.22 percent among Mexican and Latin American job corpsmen. These latter workers also found a 3.8 percent incidence among Puerto Ricans in their program.

From the foregoing review of published studies of hemoglobin S frequencies in the U.S., it may be noted that variations in incidence may have resulted from several factors. Earlier estimates have tended to be low, perhaps due in large measure to inadequate testing procedures. Many of these investigators employed the simple moist preparations or sealed wet smear techniques. This resulted in a fair percentage of false negative reactions. The later inclusion of two percent sodium metabisulfite as a reducing agent gave more reliable results but still produced a number of false negatives and false positives. Other methods such as supravital staining (Tomlison, 1941) and the test tube method used by Neel (1951) lacked consistency of results. Furthermore, none of these testing procedures could distinguish between the various hemoglobin S disorders. It was not until 1953 that the development of the solubility test by Itano and the introduction of filter paper electrophoresis by Spaet that test results became more accurate and reproducible. Modifications of these two procedures used concurrently, remain the methods of choice for current sickle cell testing.

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A second factor contributing to the frequency variation in the U.S. appears to be due to the geographical location of the population sampled as well as ethnic origin and admixture with other groups. Thus the 20.6 percent incidence found by Pollitzer, et al. (1966) seems due to the relative isolation of this population from other groups resulting in a fairly homogenous racial mixture. Similar reasoning would account for the elevated frequencies in other triracial isolates sampled while a markedly decreased frequency would be noted in many nonblack populations and in black populations where much admixing has occurred.

It may be argued that perhaps another reason for variation could result from the methods of sampling employed. Since the reported samples are derived from hospital and clinic populations or nonrandom screening programs, biased frequencies would be obtained. It seems clear, therefore, that random sampling of populations at risk would be necessary to provide a more accurate estimate of the incidence of this gene. To this end, the present study was conducted.

MATERIALS AND METHODS

Sampling Procedures

This study was composed of five samples. Sample #1 consisted of a randomly selected sample of blacks drawn from the Lansing, Michigan black population of 12,234 according to cluster sampling procedures and based on the 1970 census data. The individual blocks sampled were selected in proportion to the number of blacks residing there, thus, the greater number of blacks in a particular block, the greater the probability of selection. A total of forty-three blocks were chosen, representing forty-three clusters of persons and a potential sample size of 3,073.

After ascertaining the location of each of the blocks in the sample frame, addresses were obtained and each household was sent letters of introduction to the program and our expected visit to the residence. Each household was approached on an individual basis, requesting their participation in the program by completion of a family health survey interview and having their blood samples drawn for subsequent testing.

All families were informed by letter of the results of their tests. Those families found to contain any positive individuals were invited to genetic counseling sessions.

Sample #2 was obtained from a mobile screening program in the Lansing area during the month of March 1972. This testing was performed at or near five school sites around the community and included mainly school children and a smaller number of adults. Sample #3 also originated from the same general area but represents those individuals requesting the sickle cell test through a city prenatal and general health services clinic. Sample #4 is composed of black students (American blacks only) enrolled at Michigan State University during Spring quarter 1971 during the campus sickle cell campaign. Sample #5 indicates the results of testing of all the black residents in the Lapeer State Home and Training School, a residential facility for the mentally retarded.

Testing Procedures

Blood samples of 5 to 10 cc. were collected by venipuncture into evacuated tubes to which EDTA (ethylene diamine tetraacetic acid) had been added. Other samples were obtained by fingerprick into heparinized capillary tubes. All samples were stored at 4°C, usually for one to three days until electrophoresis was performed. Each sample was divided into two parts. One aliquot was retained as whole blood for performance of the solubility test; the other was prepared for hemoglobin electrophoresis.

The solubility test used was a modification of the method of Itano (1953) which makes use of the fact that reduced hemoglobin S is insoluble in a high molecular phosphate buffer and was prepared in the following manner: A 2.3 molar phosphate buffer was made using 165.6 gm. monobasic Sodium Phosphate (NaH_2PO_4) and 156.2 gm. Dibasic Sodium Phosphate (Na_2HPO_4) per liter. To this was added 30 gm. Sodium Dithionite (reducing agent) and 30 gm. Saponin (erythrolytic agent). To 2 ml. of this reagent was added 0.02 ml. whole blood. The mixture was shaken and turbidity noted after 5 minutes incubation at room temperature. Sufficient turbidity to obscure a printed background was judged as a positive test. Both positive (hemoglobin S) and negative (hemoglobin A) controls were used with each series of testing.

The aliquot of blood for electrophoresis was centrifuged at 2,000 x g for separation of plasma from the red blood cells. After extraction of the plasma layer, the resultant packed red cells were washed with five volumes of 0.85 percent saline and again centrifuged at 2,500 x g for 10 minutes. The saline was aspirated and hemolysates were prepared by adding 0.1 ml. packed red cells to 0.5 ml. Hemolysate Reagent (Helena Labs) then shaken vigorously to complete lysis.

For capillary tube samples, centrifugation of the tubes was carried out for 5 minutes for plasma-red cell

separation. The tubes were then cut at the plasma-red cell interface. Hemolysates were made by adding the packed red cells to five volumes of Hemolysate Reagent and shaken.

A linear application of 0.5 - 1.0 microliter of hemolysate was placed on the cathode side, one inch from the end of a blotted cellulose acetate plate which had been presoaked in the electrophoretic buffer solution for 20 minutes. The plates were then placed in the electrophoretic chamber (Thomas Model 20) filled with Supre-Heme buffer, pH 8.6 (Helena Labs) and supported by two fixed bridges which were covered with cheese cloth layers to insure proper wicking action. Electrophoresis was carried out by the application of a current of 2mAmps per plate for 20-25 minutes. The plates were then removed and placed in Ponceau S stain for 10 minutes. Excess stain was removed by two consecutive washes in 5 percent acetic acid followed by a 2 minute dehydration in 95 percent ethanol. Clearing was accomplished by soaking each plate for at least 2 minutes in a solution of glacial acetic acid in ethanol (25 ml. acetic acid in 75 ml. ethanol) and then allowed to dry in air, before interpretations were made.

Upon obtaining the dry, cleared cellulose acetate plate, determination of the hemoglobin type was made by its comparison with electrophoresed hemoglobin controls (Helena Labs) and the results of the solubility test. For all those presenting hemoglobin S patterns, further densitometric

readings were made to determine the percentages of the various hemoglobins present (S, A_1 and A_3, A_2 and F), by use of a Densicord densitometer (Photovolt, Inc.). These determinations were performed on the random sample population only.

RESULTS

All sampling and testing of the five black populations were carried out between March 1971 and June of 1972 and resulted in a total of 4,208 individuals screened. Table 3 summarizes the numbers obtained from each of the samples studied and shows that approximately 44 percent of those tested were in the randomly selected group while 22 percent were from the Mobile Unit screen, 21 percent from the campus testing with the Clinic and State Home Screens contributing smaller percentages of 8 percent and 5 percent respectively. Since participation in the random group was by consent, the 1841 individuals in this group represented close to 60 percent of the total anticipated. Both refusals

Table 3. Summary of samples studied.

Sample Number	Origin of Sample	Number Tested	Percent of Total
1	Random Selection	1841	43.75
2	Mobile Screen	943	22.41
3	Clinic Screen	343	8.15
4	Campus Screen	883	20.98
5	State Home and Training School	198	4.71
	TOTAL	4208	100.00

of individuals to consent to be tested and inaccuracy of census data to provide correct locations of black residents contributed to the decreased size of this sample.

From the results obtained in the random sample, calculations of the frequencies of the hemoglobin A, S, and C alleles were made directly (see Table 4). The gene frequency for hemoglobin A was found to be 0.945 while the S and C alleles have values of 0.042 and 0.012 respectively. Frequencies of these three alleles were calculated for samples 2 thru 5 in the same manner. These values are recorded in Table 4a.

Table 5 indicates the actual hemoglobin genotype frequencies obtained from each population studied. The combined percentages of those with S in the samples varied from 6.2 in the mobile screen to 9.0 in the clinic population. The randomly selected group was 8.42 while the campus and Training School was 6.7 and 7.6 respectively. Table 5 also shows that the incidence of hemoglobin C was 2.4 in the random sampling, 2.97 in the mobile screen, 4.08 in the clinic population, 2.71 in the campus sample and 3.03 in the Training School group. The combined C frequency was 3.87 percent and includes the AC, SC and CC genotypes. The combined incidence of individuals with elevated levels of fetal hemoglobin (F) was 1.12 percent for all groups and six individuals were found to have hemoglobin types other than the A, S, C and elevated F. None of the

Table 4. Frequencies of the hemoglobin A, S and C alleles in a randomly selected population.

Genotype	No. Individuals	A	S	C
AA	1641*	3282	0	0
AS	152	152	152	0
SS	3	0	6	0
AC	44	44	0	44
CC	1	0	0	2
TOTALS	1841	3478	158	46
Frequency of alleles		.945	.043	.012

*Includes both AA and AAF↑ individuals.

Table 4a. Frequencies of the hemoglobin A, S and C alleles in nonrandomly selected populations.*

Sample Number	A	S	C
2	.953	.031	.015
3	.933	.047	.020
4	.965	.034	.001
5	.944	.041	.015

*Calculations were made directly by same method used in Table 4.

Table 5. Hemoglobin genotype frequencies for samples studied.

Sample No.	AA		AS		SS		SC		AC	
	#	%	#	%	#	%	#	%	#	%
1	1623	88.16	152	8.26	3	.16	0	0	44	2.39
2	848	89.73	57	6.04	0	0	0	0	28	2.97
3	277	80.76	30	8.71	1	.29	0	0	14	4.08
4	798	90.37	58	6.57	0	0	1	.11	23	2.60
5	175	88.38	14	7.07	1	.51	0	0	6	3.03
Totals	3721	88.43	311	7.39	6	.14	1	.02	115	2.73

CC		AAF↑		Others		S-thal		Totals	
#	%	#	%	#	%	#	%	#	%
1	.05	18	.98	0	0	0	0	1841	100
0	0	6	.64	3	.32	1	.11	943	100
0	0	21	6.12	0	0	0	0	343	100
0	0	1	.11	2	.23	0	0	883	100
0	0	1	.51	1	.51	0	0	198	100
1	.02	47	1.12	6	.14	1	.02	4208	100

Table 5a. Comparison of observed and expected genotype frequencies for samples studied.

	N	AA	AS	AC	SS	CC	SC	X^{2**}
Calculated Expected Frequencies*	1841	.893	.081	.024	.002	.0002	.0005	-
Obs. Sample #1	1841	.891	.083	.024	.0016	.0005	000	2.21
Obs. Sample #2	940	.899	.062	.0297	0000	0000	000	6.147
Obs. Sample #3	343	.869	.088	.003	.003	0000	000	5.28
Obs. Sample #4	881	.907	.066	.026	000	0000	.001	5.58
Obs. Sample #5	197	.888	.071	.030	.005	0000	000	1.496

*Computed from A, S and C allele frequencies derived from randomly selected population.

**X^2 (five degrees of freedom)-no significant differences for any values.

genotypic frequencies obtained were found to be significantly differently from those expected from Table 4. (See Table 5a.)

The random sampling was derived from family groupings. A frequency distribution was made, therefore, in Table 6 for unrelated males and/or female heads of families to compare with overall results or to note any effects of combining family group data. It was found that the incidence of the AS genotype was 8.39 percent for males and a slightly lower frequency of 7.36 percent for females for a combined average of 7.7 percent. The occurrence of the AC genotype in this group was 2.2 percent and one male was found to have elevated F. No other genotypes were found.

Table 6. Frequency distributions for unrelated persons (Male and/or female heads of families).

	AA	%	AS	%	AC	%	AAF↑	%	Total	%
♂	255	89.16	24	8.39	6	2.09	1	0.35	286	100
♀	393	90.34	32	7.36	10	2.29	0	0	435	100
Total	648	89.86	56	7.77	16	2.22	1	0.14	721	100

Total Sample = 1841
Number of Families = 537
Average Family Size = 3.43

In Table 7 the mean ages for each sample with respect to hemoglobin types were tabulated. This was done for

Table 7. Mean ages for samples studied.*

Sample No.	AA #	AA Mean Age	AS #	AS Mean Age	SS #	SS Mean Age	SC #	SC Mean Age
1	1603	21.19	151	22.67	3	14.0	0	0
2	844	15.05	57	15.33	1	38***	0	0
3	265	16.9	30	14.8	1	1**	0	0
4	783	20.4	58	19.7	0	0	1	20**
Totals	3495	19.21	296	19.87	5	16.2	1	20.0

*Ages were not recorded for sample #5.

**Only one individual in the category.

*** = S-thalassemia.

AC		CC		AAF↑		Others		Total	
#	Mean Age	#	Mean Age	#	Mean Age	#	Mean Age	#	Mean Age
44	18.0	1	4**	18	28.17	0	0	1820	21.24
28	17.43	0	0	6	9.83	2	22.0	938	15.14
12	18.3	0	0	21	7.1	0	0	329	16.1
22	20.1	0	0	1	26**	3	24.7	868	20.37
106	18.31	1	4.0	46	16.11	5	23.6	3755	

samples 1-4 only since no ages were recorded for sample #5. As can be seen, the total mean ages of all individuals in these samples were 21.29, 15.14, 16.1 and 20.37, thus the clinic and mobile screens included more younger individuals. Comparisons of ages of AA persons to those with AS genotypes showed little variability in that two samples showed a higher mean age for AS individuals, while in two groups the mean ages were higher in AA individuals. Differences in mean ages for both genotypes were not significant in any of the samples and varied between 0.7 and 2.1 years with an average of 1.14 years. For the SS, SC and S-thalassemia persons, no strict comparisons could be made due to the small number of individuals in these categories. The same is true for the single CC person and those with elevated fetal hemoglobin as well as those who were found to possess other undetermined hemoglobin types. It was also noted that the total mean age for all persons with the AC genotype was 18.31. This compared favorably with the 19.21 years for the total mean age for all AA persons.

Table 8 shows the age related frequencies of individuals with S genotypes in samples 1-4. The subjects were divided into ten age groups for comparison purposes. From these data, it can be seen that the percentage of S affected individuals is variable within each sample group. There is also no consistency with respect to age between samples. Generally, however, there seem to be some random fluctuations

Table 8. Age related frequencies of hemoglobin S.

Age Groups (yrs)	Sample #1				Sample #2			
	Total #	of S	% of Sample	% with S*	Total #	of S	% of Sample	% with S
0-4	230	16	12.64	7.0	85	7	9.0	8.2
5-8	244	14	13.41	5.7	194	8	20.7	4.1
9-12	221	16	12.15	7.2	236	15	25.1	6.4
13-17	255	30	14.01	11.8	181	12	19.3	6.6
18-25	296	27	16.3	9.1	87	6	9.3	6.9
26-35	210	18	11.5	8.6	91	5	9.7	5.5
36-45	161	16	8.9	9.9	43	3	4.6	6.8
46-55	99	7	5.4	7.1	16	2	1.7	12.5
56-65	77	7	4.2	9.1	3	0	0.3	0
66 ↑	24	3	1.3	12.5	3	0	0.3	0

*% with S includes all S genotypes.

Sample #3				Sample #4			
Total	# of S	% of Sample	% with S	Total	# of S	% of Sample	% with S
98	11	29.8	11.2	-	-	-	-
36	4	10.9	11.1	-	-	-	-
26	1	7.9	3.8	-	-	-	-
25	5	7.6	20.0	4	0	0	0
68	4	20.7	5.9	812	38	93.7	7.1
48	3	14.6	6.3	40	1	4.6	2.5
18	2	5.8	11.1	11	0	0	0
8	1	2.4	12.5	1	0	0	0
1	0	0.3	0	-	-	-	-
1	0	0.3	0	-	-	-	-

producing three peak age periods in these persons in the groups 0-8 years, 13-17 years and age 36 and above (see Figure 2). Note, however, that in all samples this latter group constituted a relatively small percent (less than 20 percent) of their respective samples.

In Table 9, the percent of hemoglobin S in the AS individuals for the various age groups was tabulated. As indicated, the amount of S varied from 24.3 to 49.3 percent, with an overall average of 38.38 percent for all groups. Furthermore, the amount of S also varied slightly within each specific age grouping. When the mean percents of each age group were compared, the values obtained showed differences of less than four percent despite the small number of persons in some of the categories.

In an effort to compare the percent of sicklers with the mean percent of hemoglobin S for the age groups, Figure 3 was constructed from data in Tables 8 and 9. This was done for the random sample only. It can be seen that in spite of the small fluctuations in the mean amount of S for each group, the percent of sicklers in the age groups appears to vary inversely with the amount of S hemoglobin. This relationship holds for all age groups except in the category of age 66 up.

To facilitate comparisons of the random sample population to other geographical areas of the U.S., the places of birth by regions were tabulated for individuals eighteen

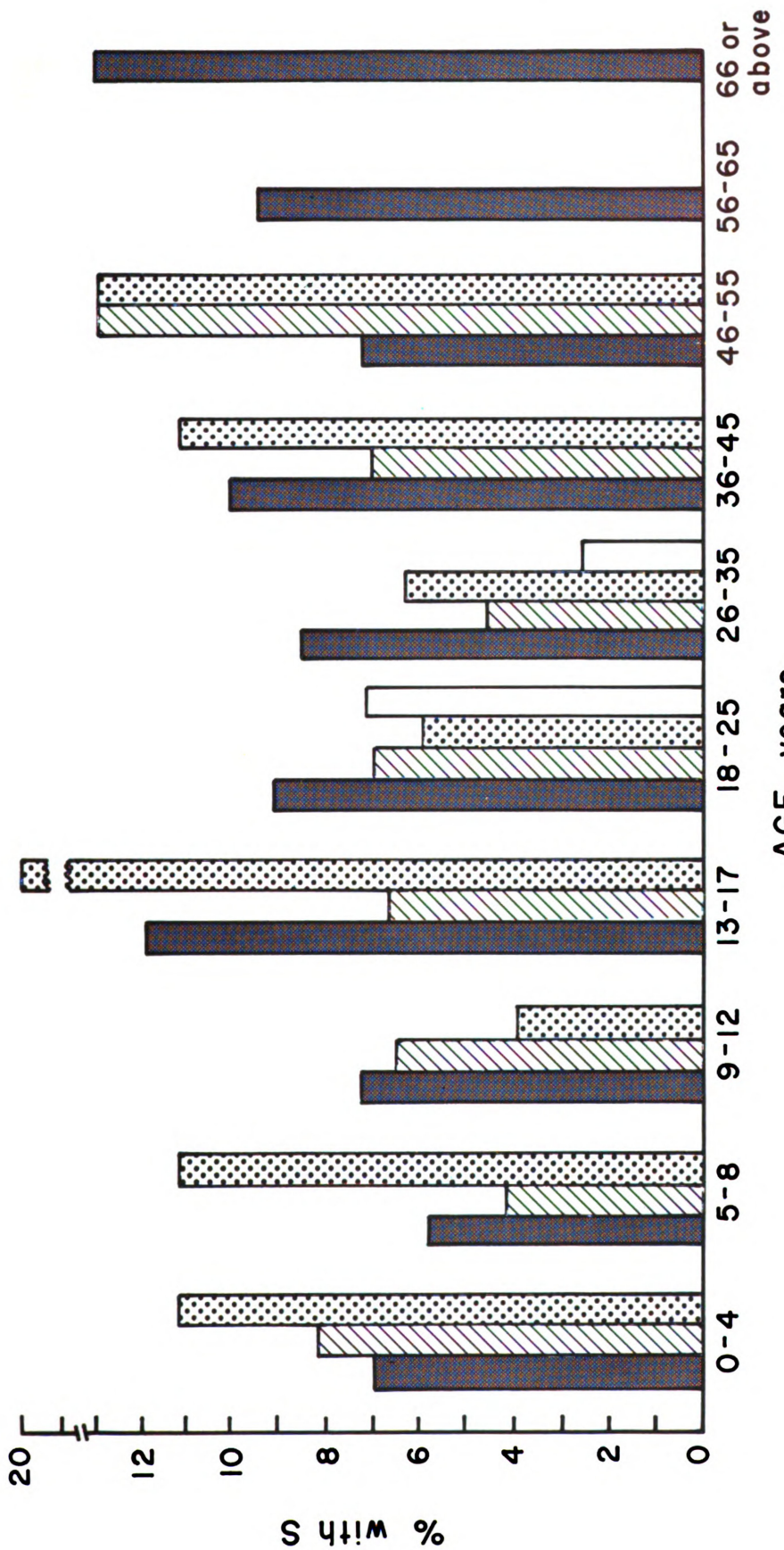

Figure 2. Percent of homoglobin S for age group distributions.

Table 9. Percent of hemoglobin S in AS individuals for age groups studied.

	Age Group	Hemoglobin S % - Range	Mean % of S	Std. Deviation
1.	0 - 4	32.8 - 42.1	37.56	2.72
2.	5 - 8	35.0 - 54.7	39.93	3.25
3.	9 - 12	30.8 - 44.7	38.1	3.24
4.	13 - 17	30.5 - 45.5	37.43	3.51
5.	18 - 25	31.5 - 45.2	39.30	3.6
6.	26 - 35	33.1 - 49.3	39.74	4.43
7.	36 - 45	26.9 - 43.4	36.59	4.58
8.	46 - 55	36.3 - 42.3	40.3	2.47
9.	56 - 65	24.3 - 41.7	36.78	5.9
10.	65 up	32.8 - 47.7	39.8	5.3

Mean % of S for all AS individuals = 38.38

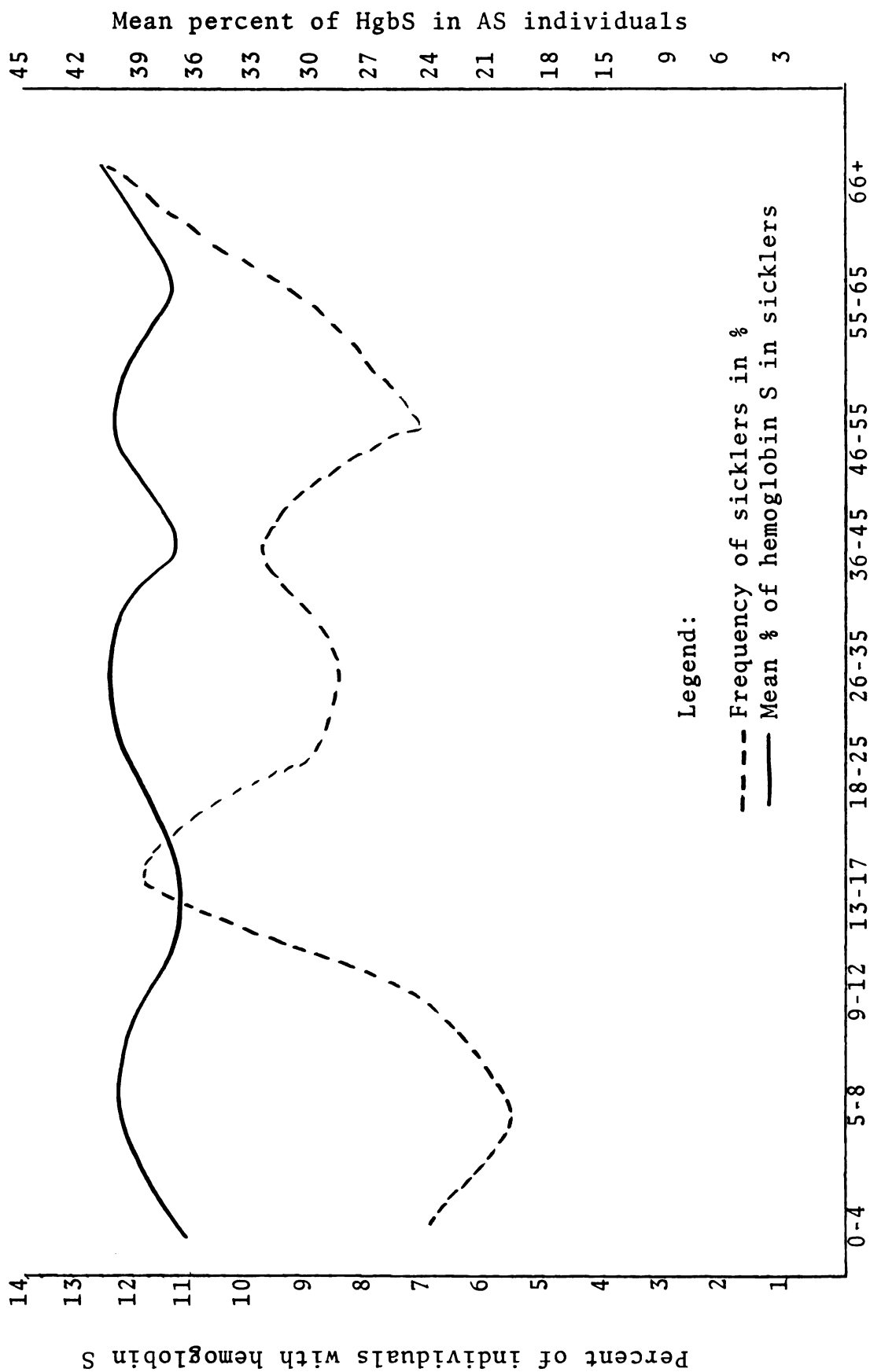

Figure 3. Comparison of the percent of sicklers and the mean percent of hemoglobin S in age groups studied.

or older in Table 10. It was found that the majority of these persons originated from the deep south central states of Alabama, Louisianna, Mississippi, Arkansas and Tennessee (51.22 percent) while 27.6 percent came from the immediate Midwestern states of Michigan, Illinois, Indiana and Ohio with all the other regions combined giving a total of 21.16 percent.

Table 10. Place of birth by state (Age 18 or above).

	A Immediate Midwest		B Deep South-Central		C South-Costal		D Mid-South		E North Atlantic		F Others		Over 18
Genotype	#	%	#	%	#	%	#	%	#	%	#	%	Totals
AA	213	24.7	385	44.7	85	9.9	62	7.2	3	0.4	8	0.92	756
AS	19	2.2	40	4.6	9	1.0	6	0.7	0	0	2	0.2	76
SS	0	0	1	0.12	0	0	0	0	0	0	0	0	1
AC	5	0.58	9	1.0	1	0.12	3	0.4	0	0	0	0	18
CC	0	0	0	0	0	0	0	0	0	0	0	0	0
AAF↑	1	0.12	7	0.8	1	0.12	2	0.2	0	0	0	0	11
TOTAL	238	27.6	442	51.2	96	11.2	73	8.5	3	0.4	10	1.12	862

Region A - Mich., Ill., Ind., Ohio.
Region B - Ala., La., Miss., Ark., Tenn.
Region C - Md., Va., NC, SC, Ga., Fla.
Region D - Mo., Tex., Ky., W. Va., Okla., Kans.
Region E - Me., Mass., Conn., NH, NJ, NY, Penn., RI, Vt.
Region F - All other states.

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AA	213	24.7	385	44.7	85	9.9	62	7.2	3	0.4	8	0.92	756
AS	19	2.2	40	4.6	9	1.0	6	0.7	0	0	2	0.2	76
SS	0	0	1	0.12	0	0	0	0	0	0	0	0	1
AC	5	0.58	9	1.0	1	0.12	3	0.4	0	0	0	0	18
CC	0	0	0	0	0	0	0	0	0	0	0	0	0
AAF↑	1	0.12	7	0.8	1	0.12	2	0.2	0	0	0	0	11
TOTAL	238	27.6	442	51.2	96	11.2	73	8.5	3	0.4	10	1.12	862

Region A - Mich., Ill., Ind., Ohio.
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Region E - Me., Mass., Conn., NH, NJ, NY, Penn., RI, Vt.
Region F - All other states.

DISCUSSION

The pathology and frequently fatal outcomes of individuals homozygous for hemoglobin S at early ages has long been recognized. The importance of the sickle cell trait in individuals heterozygous for this defect has been recently underscored as potentially fatal in special situations involving oxygen deprivation. Other hemoglobin S genotypes such as SC disease and S-β thalassemia have also been shown to lead to complications under certain conditions. At present, then, there is the need to identify individuals who are affected, for their own health reasons as well as for the potential risks to their future offspring.

Not insignificant also is the need to obtain extensive incidence data and age related frequencies to make intelligent assessments of the prevalence of this defect and to determine the fitness of these various S-genotypes in the absence of any obvious selective advantages. Rucknagel and Neel (1961) have stated that "although it may seem an anachronistic stand in these days of biochemical excitement, in the opinion of the authors, the outstanding gap in our knowledge of the population genetics of the Hb^{S}_{β} gene is this

question of the age-incidence trend under nontropical conditions." Since this statement by these authors, little has been done to illuminate this area. Those studies which have been undertaken have traditionally used hospital populations.

In the present study, both random and nonrandom populations were examined and resultant gene and genotype frequencies compared in an effort to detect and eliminate any biases which may have been included in earlier samplings. The data obtained from all of the five samples studied were consistent. When calculation of gene frequencies of the three hemoblobin alleles A, S, and C were made and the expected genotype frequencies were computed, the subsequent X^2 analysis for each sample revealed no significant differences between these and the observed genotype frequencies in any of the samples despite the differences in methods of sampling and ascertainment.

The individuals in sample #1 were randomly selected and located from census information. Contacts with them were made directly by letter and subsequent home visit when health surveys and blood samples were taken with their consent. While it may be argued that the lack of consent by some individuals so selected may in itself constitute a bias, it seems unlikely that any correlation would exist between an individual's hemoglobin type and his consent or refusal to be tested. Indeed a sampling of the reasons for

refusals indicate reasons such as age, preference of testing by family physicians, fear of needles and indifference to testing programs in general. A few declined due to previously being tested for this defect.

Strict comparisons of the frequencies obtained from sample #1 are difficult since there are no other random samplings from this geographical area reported in the literature. Only one other random study has been published. This was made by Boyle and coworkers (1968) in Charleston, S.C., an area of the U.S. which has consistently reported the highest hemoglobin S frequencies in North America. Moreover, in their study, no individuals under age 35 were included. Despite this limitation, these workers did report some age incidence data which will be discussed later.

Although there have been no other random samplings to use as comparisons, the average frequency of 8.2 percent for AS individuals from sample #1 compares favorably with the overall frequency for Michigan of 7.9 percent as reported in Table 2. This combined frequency for the state represents the results of four studies. Cooley and Lee (1926) and Neel (1951) from samples of Negro hospital patients obtained frequencies of 7.5 and 9.1 respectively. Nalbandian, et al. (1971), in a sample of black school children, found an incidence of 6.0 percent. In a 1972 study of black emergency room patients, Nalbandian et al. recorded a frequency of 13.7 percent for AS individuals.

This latter value as well as the overall incidence for all hemoglobin S genotypes of 27.6 percent found by these workers, differs greatly from other reported data for Michigan and is probably due to the method of sampling utilized. Upon consideration of this potential bias and noting the small size of the sample, it may be concluded that these frequencies are probably not significant. If one excludes this latter study from the frequency calculations for the state, the value obtained is reduced to an overall incidence of 6.6 percent. This figure is still not significantly different from the 8.26 percent found in the random sample of the present study.

Additional comparisons of the 8.26 percent value from this study with the data published for other areas of the Midwest are again favorable since the average frequencies reported for Illinois, Ohio and Indiana were 8.2 percent, 8.0 percent and 8.6 percent respectively. These are also in agreement with values reported for Louisiana of 8.5 percent and Tennessee of 8.4 percent, the states from which much of the Michigan black population has originated.

As has been noted, the pooling of family group data in sample #1 has little if any effect on overall frequency since the frequency values for unrelated males and females correlated closely with the combined data. In the males and females thus compared, however, a slightly higher

incidence was found in the males but this difference was not statistically significant.

As indicated earlier, sample #2 individuals were the results of a mobile screening program in which persons requesting sickle cell evaluations came to different sites for testing. Since most of these were located in school facilities, this sample contains a disproportionate number of school aged children, hence a lower mean age. This sample would, therefore, be comparable to the many current school screening programs across the country. While its overall results documented here are not significantly different from the random population, its frequency of 6.04 percent for the AS genotype is almost identical to the 6.0 percent found in a similar group in Grand Rapids, Michigan black school children by Nalbandian, et al. (1971).

The population in sample #3 was derived from a health services clinic and is, therefore, typical of many previous studies. The lower mean age of 16.1 years for this group is due to the larger number of young females attending the prenatal facilities. That these mothers subsequently brought their young children in for testing is reflected in the relatively large number of infants in this sample with an excess of elevated fetal hemoglobin. The frequencies reported here are also well within limits for all genotypes despite the slightly higher incidence of the AC persons of 4.08 percent.

In sample #4, a college population was sampled. Results reported here were calculated for American black students only although other groups were tested. Again, none of the frequencies derived deviated significantly from those predicted. Even the mean age more closely approximated that of the random sample than that of any of the other nonrandom groups. Additionally, the AS frequency of 6.57 percent is in close agreement with that of 7.0 percent reported by Rhatigan (1972) in Negro college students in Witchita, Kansas.

For sample #5, the smallest group, the black residents of the Lapeer State Home (institution for the mentally retarded) were tested. As with sample #1, there are no published studies of this type with which to make comparisons, however, as indicated, no significant differences were obtained from this data.

As previously suggested, there is a need to explore the question of age-incidence trends for the hemoglobin S gene, especially in relationship to the AS genotype under nontropical conditions. If there are certain disadvantages to this sickle cell trait as recent case reports have alluded, one would expect a significant decline in the numbers of these carriers in older age groups. In the present study, no such decline was noted. This finding is at variance with some of the earlier age incidence studies but is

in agreement with others. Table 11 summarizes results from these studies.

In the first four series (Diggs, et al., 1933; Switzer, 1950; Neel, 1951; Pollitzer, 1958), all based on hospital patients, agree in indicating less sickling among older patients. The results could be interpreted as compatible with less disease (and less cause for hospitalization) in older sicklers or a higher death rate on the part of sicklers. Neither of these alternatives seem plausible in view of the study by McCormick and Kashgarin (1965), also on hospital patients which found no decrease in the sickle cell trait percentages with increasing age. Moreover, these same workers, when comparing death rates by age of 305 individuals found to have sickle cell trait upon autopsy to those autopsied without the trait, failed to demonstrate a significant decrease in the number of sicklers with increasing age but did find random fluctuations in the percent of AS individuals in some age groups.

Other studies including the present one have concurred with these latter findings. Rucknagel (1961) for example, found an increase in the percentages of AS individuals in older age groups since this figure was 19.1 percent in groups from 0-50 years but was 26.1 percent in persons aged 50 or older. Petrakis, et al. (1970) found a slight decrease in the trait with age but it was not statistically significant. Boyle, et al. (1968) found no

Table 11. Relationship between age and the sickle cell trait in American Negroes.

Investigator	Age Group	Number Tested	Number Sickling	Percent Sickling
Diggs, et al., 1933	6-20	1,112	108	9.7
	21-50	1,102	96	8.7
	51-	403	28	7.0
Switzer, 1950	6-20	843	130	15.4
	21-50	1,233	165	13.4
	51-	302	42	13.9
Neel, 1951	12-20	147	15	10.2
	21-50	703	67	9.6
	51-	146	7	4.8
Pollitzer, 1958	14-29	232	42	18.1
	30-	241	26	10.8
Rucknagel and Neel, 1961	0-20	1,476	282	19.1
	21-50	530	101	19.1
	51-	176	46	26.1
McCormick and Kashgarin, 1965	11-20	126	10	8.0
	21-50	657	60	9.1
	51+	630	55	8.7
Boyle, et al., 1968	35-44	335	49	14.6
	45-54	183	24	13.1
	55-64	140	22	15.7
	65+	117	18	15.4
Petrakis, et al., 1970	5-20	417	43	10.3
	21-49	2,665	233	8.7
	51+	946	75	7.9
Present Study all samples combined	0-17	1,835	136	7.4
	18-35	1,652	120	7.3
	36-55	356	30	8.4
	56+	109	10	9.2

evidence of an age trend in adults nor a decrease in the prevalence of the trait in older persons although this sample excluded persons under age 35. In the present study a slight though not significant increase in the percent of AS individuals with age was observed. This may possibly be due to the small numbers of individuals included in these latter age groups. Clearly, the majority of evidence would tend to indicate that AS individuals are not at any great risk for early death due to sickle cell trait.

The additional finding in this study of similar fluctuations in the S frequency in all samples examined is interesting. While other workers have reported frequency changes for certain age groups, comparisons are difficult since the age categories of each were variable with most groupings encompassing a wide range of ages. When the present age data were grouped in this manner, no significant differences were observed. Furthermore, regression analysis of the small changes in the mean percent of S hemoglobin produced by the different age groups and subsequent t-values also indicated no significance. Thus in this study, the changes in the frequencies of hemoglobin S genotypes in certain age groups and the amount of S produced by them may show random variation from time to time but is not statistically significant.

CONCLUSIONS

The principle purposes of this study were to determine the incidence and age related frequencies of hemoglobin S in random and nonrandom populations. Specific screening of 4208 black individuals for hemoglobin S was conducted by use of cellulose acetate electrophoresis and by determination of hemoglobin solubility in a 2.3 molar phosphate buffer. Of the five sample groupings generated, one resulted from a random selection process. The random group was compared to the results of a nonrandom school-community screening program, and a clinic population from the same general area, a sample of college students and one sample from a State institution for the mentally retarded.

The frequency of the hemoglobin S allele in the random sample was found to be 0.043. No significant differences were found between this figure and the allele frequency in any of the nonrandomly selected groups although the corresponding values varied from 0.031 to 0.047. When the incidences for the AS genotypes were compared, the value for the random population was 8.3 percent, for the general screen it was 6.2 percent, the clinic population was 8.8 percent, the college group, 6.6 percent, and the institution was 7.1

percent with a mean AS frequency for all samples of 7.39 percent. While these values are slightly lower than the national average of 8.5 percent, they are in agreement with other figures reported for Michigan by other workers as well as with values for the Southern states from which the Michigan population has originated.

Examination of the age related frequencies revealed no significant differences in the number of individuals with hemoglobin S. This finding was consistent in all samples, despite the differences in the mean ages of the samples due to the methods of ascertainment. The mean percent of hemoglobin S fluctuates with age but those values were not statistically significant.

The results of this study have two major implications. Firstly, the incidence of hemoglobin S is no different whether the samples are randomly or nonrandomly selected. The frequencies obtained from both selection methods will be similar providing they are drawn from sympatric populations. The second and perhaps more significant implication is the finding of the lack of any decline in the frequency of AS individuals with age in a non-malarial environment. Within the limits of the population size sampled, this implies the lack of negative selection pressure against these individuals as some of the current literature has alluded. While a study of this nature does not explore the overall fitness of the AS genotype, it does suggest that

these individuals are not at any great risk for a shortened life span. In this regard, this study should prove reassuring to these persons as well as informative to the genetic counselor.

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APPENDIX

APPENDIX

Table 12. Incidence of hemoglobin S in the United States.

Reference	Year	Place	Population type and characteristics	Number tested	Methods used*
Greenberg	1970	Boston, Mass.	Negro children-Head start program	650	5
TOTALS		Massachusetts	NEGRO	650	
Barnes et al.	1972	New Haven, Conn.	Negroes-pediatric health center	1000	2
Barnes et al.	1972	New Haven, Conn.	Negro hospital patients	358	2
TOTALS		Connecticut	NEGROES	1358	

*Testing Methods Used

1. Sealed coverslip on slide - no sodium metabisulfite
2. Electrophoresis
3. Moist preparation
4. Sickle cell preparation - with sodium metabisulfite
5. Solubility test
6. Test tube method
7. Supravital staining
8. Cited in Livingstone (1967) - methods not given.
9. Not Given

Overall % sickle cell	Percent SS	Percent AS	Percent AC	Percent SC	Percent AD	Others
47(7.3%)						
47(7.3%)						
70(7.0%)	2(0.2%)	67(6.7%)	26(2.6%)	1(0.1%)		11(1.1%)
45(12.6%)	2(0.6%)	42(11.5%)	14(4.0%)	1(0.3%)		4(1.1%)
115(8.5%)						

Table 12. Continued.

Reference	Year	Place	Population type and characteristics	Number tested	Methods used
Wallstein and Kreidel	1928	New York, N.Y.	Negro-babies and Hospital patients	150	1
Dolgopol and Stitt	1929	New York, N.Y.	Negro-TB patients	77	1
Rosenblum et al.	1955	Brooklyn, N.Y.	Negro-TB patients	200	4 and 5
Trouillot	1971	Brooklyn, N.Y.	Negroes-Clinic	approx. 4500	5
Levy	1929	New Rochelle, N.Y.	Negro-Hospital patients	213	1
Watson	1948	Long Island, N.Y.	Negro-newborns and mothers	226	1
Kelly	1966	Albany, N.Y.	Negroes-Headstart	134	4
Rosner	1971	Queens, N.Y.	Negroes-Clinic patients	1328	2
TOTALS		New York	NEGROES	6828	
Beck and Hertz	1953	Philadelphia, Pa.	Negro maternity patients and children	100	1
Margolies	1951	Philadelphia, Pa.	Negro clinic and Hospital patients	1000	1
Myerson	1959	Philadelphia, Pa.	Negro-hospital patients	1000	2
Weiss and Strecher	1952	Philadelphia, Pa.	Negro TB patients	150	4
Weiss and Strecher	1952	Philadephia, Pa.	Negro-hospital patients	150	4

Overall % sickle cell	Percent SS	Percent AS	Percent AC	Percent SC	Percent AD	Others
13(8.66%)						
4(5.2%)						
16(8.0%)	1(0.5%)	15(7.5%)				
270(6.0%)						
12(5.6%)						
18(8.0%)						
12(8.96%)						
106(8.0%)		105(7.9%)	32(2.4%)	1(.08%)		12(0.9%)
451(6.6%)						
13(13%)						
72(7.2%)						
79(7.9%)	3(0.3%)	74(7.4%)	23(2.3%)	2(0.2%)		4(0.4%)
19(12.6%)						
8(5.3%)						

Table 12. Continued.

Reference	Year	Place	Population type and characteristics	Number tested	Methods used
Laros	1967	Philadelphia	Negro clinic patients	3701	2 and 4
TOTALS		Pennsylvania	NEGROES	6101	
Josephs	1928	Baltimore, Md.	Negro children and random hospital selection	250	9
Smith and Conley	1953	Baltimore, Md.	Negroes-hospital outpatient clinic	500	2
Weatherall	1963	Baltimore, Md.	Negroes-cord blood samples	900	2
Boyer et al.	1963	Maryland	Unrelated Negroes prisoners, hospital personnel, preg. women	681	2
Rucknagel	1964	Maryland-Charles and Prince George Counties	Wesorts Triracial Isolate	2578	2
Rucknagel	1964	Charles County Maryland	Negroes	191	2
TOTALS		Maryland	NEGROES	1772	
Ryan et al.	1960	Washington, D.C.	Negroes-Preg. females	3000	2 and 4
Jenkins and Clark	1962	D.C.	Negroes-prenatal clinic	828	2
Ryan et al.	1960	D.C.	Negro TB patients	330	2 and 4
McCurdy	1964	D.C.	Negroes-prenatal clinic	3333	2 and 4
McCurdy	1964	D.C.	Hospital employees	328	2 and 4

Overall % sickle cell	Percent SS	Percent AS	Percent AC	Percent SC	Percent AD	Others
333(9.0%)	3(0.008%)	320(8.7%)		7(0.02%)		3(0.008%) s-thal
524(8.6%)						
16(6.4%)						
42(8.4%)	5(1.0%)	36(7.2%)	9(1.8%)	1(0.2%)		
67(7.4%)		67(7.4%)	26(2.8%)			23(2.6%)
44(6.5%)		44(6.5%)	11(1.6%)			
520(20.2%)	20(0.78%)	496(20.2%)	5(0.19%)	4(0.16%)		
10(5.2%)		10(5.2%)	4(2.1%)			
121(6.8%)						
201(6.7%)						
37(4.5%)	1(0.12%)	36(4.5%)	18(2.2%)			
28(9.0%)		28(9.0%)	4(1.2%)			
212(6.4%)	3(0.09%)	206(6.4%)	43(1.3%)	3(0.09%)	14(0.4%)	
20(6.1%)	1(.3%)	19(5.8%)	12(3.3%)			

Table 12. Continued.

Reference	Year	Place	Population type and characteristics	Number tested	Methods used
Webb	1971	D.C.	Negroes-clinic ages 0-21	976	2 and 4
Standard	1972	D.C.	Negro-children	708	4 and 5
TOTALS		D.C.	NEGROES	9503	
Moran	1972	Danville Va.	Negro hospital patients	3822	2 and 5
TOTALS		Virginia	NEGROES	3822	
Tomlinson	1941	W. Virginia	Negro hospital patients	275	7
TOTALS		W. Virginia	NEGROES	275	
Mujamoto and Korb	1927	St. Louis, Mo.	Negro hospital patients	300	1
Chernoff	1956	St. Louis, Mo.	Negro hospital patients	1000	2 and 4
Goldstein	1964	St. Louis, Mo.	Negro hospital patients	500	2
Minnich et al.	1962	St. Louis, Mo.	Negro newborns-cord bloods	449	2 and 4
TOTALS		Missouri	NEGROES	2249	
Lawrence	1927	Nashville, Tenn.	Negro students and patients	100	1
Lawrence	1927	Nashville	White students	100	1
Diggs et al.	1933	Memphis, Tenn.	Negroes from hosp., school children and teachers	2539	1
Adams et al.	1953	Memphis, Tenn.	Negro maternity patients	2011	4

Overall % sickle cell	Percent SS	Percent AS	Percent AC	Percent SC	Percent AD	Others
84(8.6%)						
58(8.3%)						
640(6.7%)						
236(6.2%)	7(0.18%)	203(5.3%)		7(0.18%)		
236(6.2%)						
18(6.5%)						
18(6.5%)						
19(6.3%)						
94(9.4%)		94(9.4%)	26(2.6%)		4(0.4%)	
43(8.6%)		43(8.6%)	12(2.4%)			
47(10.5%)		47(10.5%)	9(2.0%)		2(0.4%)	
203(9.0%)						
5(5.0%)						
3(3.0%)						
211(8.3%)						
159(7.0%)						

Table 12. Continued.

Reference	Year	Place	Population type and characteristics	Number tested	Methods used
Adams et al.	1953	Memphis, Tenn.	Negro newborns	824	4
McCormick	1960	Memphis, and West Tenn.	Negroes-clinic, autopsy, TB and hosp. patients	2800	2 and 4
McCormick	1965	Memphis, Tenn.	Negro-Autopsies	3199	2 and 4
TOTALS		Tennessee	NEGROES	11473	
Pollitzer et al.	1959	Robson Co, N.C.	Lumbee Indians	1332	2
Pollitzer et al.	1966	Indian Sec., N.C.	Indian triracial isolate	232	2
Pollitzer et al.	1966	Northeast, N.C.	Non-Indian	145	2
Hansen-Preuss	1936	Durham, N.C.	Negro hospital patients	100	1
Chernoff and Weichselbaum	1958	Durham, N.C.	V.A. hosp. patients-non-Negroes	734	2
Chernoff and Weichselbaum	1958	Durham, N.C.	Negro hospital patients	390	2
TOTALS		N. Carolina	NEGROES	490	
Pollitzer et al.	1966	Walterboro, S.C.	Triracial Isolate	74	9
Johnson and Townsend	1937	S.C.	Negroes	719	8
Switzer and Fouche	1948	Charleston, S.C.	Negro OB patients, hosp. employees and patients	1000	1
Switzer	1950	Charleston, S.C.	Negro school children and hosp. patients	3066	1

Overall % sickle cell	Percent SS	Percent AS	Percent AC	Percent SC	Percent AD	Others
9(1.1%)						
277(9.9%)	19(0.7%)	254(9.1%)	60(2.1%)	4(0.14%)	1(0.04%)	4(0.14%)
305(9.5%)		305(9.5%)				
966(8.4%)						
23(1.7%)		23(1.7%)	23(1.7%)		1(<0.01%)	
4(1.7%)		4(1.7%)	13(5.6%)			
1(0.7%)		1(0.7%)	19(13.1%)			
7(7.0%)						
1(0.13%)		1(0.13%)			1(0.13%)	
34(8.7%)	1(0.25%)	33(8.5%)			1(0.25%)	3(0.8%)
41(8.4%)						
9(12.2%)		9(12.2%)				
57(7.9%)						
140(14%)						
412(13.4%)						

Table 12. Continued.

Reference	Year	Place	Population type and characteristics	Number tested	Methods used
Pollitzer et al.	1966	James Is., S.C.	Negroes	276	2
Pollitzer	1958	Costal S.C.	Negroes-clinic	483	2
Boyle, et al.	1968	Charleston, S.C.	Negroes-randomly selected from ages 35 or above	775	2
Ludvigsen and Smith	1972	Greenville, S.C.	Negroes-screening program	1878	2 and 5
TOTALS		S. Carolina	NEGROES	8197	
Sydenstricker	1924	Augusta, Ga.	Negroes	300	1
Sydenstricker	1924	Augusta, Ga.	Negroes	1800	1
Cooper et al.	1963	Southeast, Ga.	Negroes	247	2
TOTALS		Georgia	NEGROES	2347	
Diggs et al.	1933	Gainesville, Fla.	Negro school children and teachers	674	1
Cotter and Prystowsky	1963	Gainesville, Fla.	Negroes-prenatal clinic	944	2
Pollitzer et al.	1966	Hollywood, Fla.	Seminoles-Indians	374	9
TOTALS		Florida	NEGROES	1618	
Thompson et al.	1964	Mississippi	Whites	1045	2
Thompson et al.	1964	Mississippi	"Americans"	429	2
Thompson et al.	1964	Mississippi	Negroes	1310	2
TOTALS		Mississippi	NEGROES	1310	

Overall % sickle cell	Percent SS	Percent AS	Percent AC	Percent SC	Percent AD	Others
57(20.6%)		57(20.6%)	2(0.7%)			
75(15.5%)	2(0.4%)	73(15.1%)	14(3.1%)			
114(14.7%)	1(0.13%)	113(14.6%)	17(2.2%)			
138(7.4%)	1(0.05%)	130(6.9%)	8(0.43%)			
993(12.1%)						
13(4.3%)						
101(5.6%)						
21(8.5%)	2(0.8%) s-thal	19(7.7%)	4(1.6%)			
135(5.8%)						
65(9.6%)						
66(7.0%)	1(0.1%)	65(6.8%)	9(0.9%)			
36(9.6%)		36(9.6%)				
131(8.1%)						
1(0.1%)	1(0.1%)					
7(1.6%)		7(1.6%)	7(1.6%)			
155(11.8%)	37(2.8%)	114(8.7%)	38(2.9%)	4(0.3%)	14(1.1%)	
155(11.8%)						

Table 12. Continued.

Reference	Year	Place	Population type and characteristics	Number tested	Methods used
Smith	1928	New Orleans, La.	Negroes-random patients	100	1
Ogden	1943	Louisiana	White patients	910	8
Ogden	1943	Louisiana	Negro patients	692	8
Beacham and Beacham	1950	New Orleans La.	Negroes-Preg. females	1200	4
Moffitt and McDowell	1959	Southern Louisiana	Whites	140	8
Moffitt and McDowell	1959	Southern Louisiana	Negroes	564	8
Cherrie	1963	Louisiana	Negroes-college students	134	2 and 4
Coulter	1965	New Orleans, La.	Negro TB patients	220	8
TOTALS		Louisiana	NEGROES	2910	
Schneider	1954	Galveston, Texas	Negroes-Blood bank donors	505	2 and 4
Schneider	1956	Galveston, Texas	Negro hosp. patients	1550	2
Schneider	1956	Galveston, Texas	Negro clinic patients	2055	8
Bandau	1932	Houston, Texas	Negroes	150	9
Haynie et al.	1957	Houston, Texas	Negro hosp. patients	400	2
Killingsworth and Wallace	1936	Dallas, Texas	Whites	322	1
Killingsworth and Wallace	1956	Dallas, Texas	Mexicans	239	1

Overall % sickle cell	Percent SS	Percent AS	Percent AC	Percent SC	Percent AD	Others
5(5.0%)						
0						
45(6.5%)						
100(8.3%)						
1(0.7%)		1(0.7%)				
65(11.5%)	18(3.2%)	47(8.3%)	10(2.5%)	4(0.7%)		
15(11.1%)	7(5.2%)	5(3.7%)	3(2.2%)	3(2.2%)	3(2.2%)	
16(7.8%)	1(0.5%)	15(7.3%)	9(4.1%)			
246(8.5%)						
57(11.3%)		57(11.3%)	15(3.0%)			
146(9.4%)	6(0.4%)	139(9.0%)	35(2.3%)	1(<0.1%)		
203(9.9%)	6(0.3%)	196(9.9%)	50(2.4%)	1(0.05%)		
10(6.7%)						
42(10.5%)	5(1.3%)	36(9.0%)	6(1.5%)	1(0.25%)		1(0.25%)
0						
3(1.2%)						

Table 12. Continued.

Reference	Year	Place	Population type and characteristics	Number tested	Methods used
Killingsworth and Wallace	1956	Dallas, Texas	Negroes-school children and hospital patients	1205	1
Whalley et al.	1963	Dallas, Texas	Negro-preg. females	13835	2 and 4
Whalley et al.	1964	Dallas, Texas	Negro patients at OB clinic	3631	2 and 4
Long	1965	Dallas, Texas	Negroes	1165	8
TOTALS		Texas	NEGROES	24496	
Rucknagel	1957	Oklahoma	Indians-Cherokees, Caddoans, Muskhogeans	175	2
Cardoza	1957	Chicago, Ill.	White-Hosp. patients	307	1
Cardoza	1957	Chicago, Ill.	Negro-Hosp. patients	1263	1
Blanksma and Breen	1966	Chicago, Ill.	Negroes-Preg. Clinic	6360	2
TOTALS		Illinois	NEGROES	7930	
Cooley and Lee	1926	Detroit, Mi.	Negroes-Hospital patients	400	1
Neel	1951	Detroit and Ann Arbor, Mi.	Negro-Hosp. Patients	1000	1 and 6
Nalbandian, et al.	1972	Detroit, Mi.	Negro children Hosp. patients	380	2 and 5
Nalbandian, et al.	1971	Grand Rapids, Mi.	Negro-School children	4465	2 and 5
TOTALS		Michigan	NEGROES	6245	
Whalley	1963	Cleveland, Ohio	Negroes-Preg. females	988	8
TOTALS		Ohio	NEGROES	988	

Overall % sickle cell	Percent SS	Percent AS	Percent AC	Percent SC	Percent AD	Others
65(5.3%)						
1138(8.2%)		1138(8.2%)				
475(13.1%)		475(13.1%)				
85(7.3%)	7(0.6%)	78(6.7%)	40(3.1%)			
2221(9.1%)						
3(1.7%)						
1(0.3%)						
119(9.4%)						
528(8.3%)						
648(8.2%)						
30(7.5%)						
91(9.1%)						
105(27.6%)	7(1.8%)	52(16.1)		7(1.8%)		SSF 39(10.3%)
266(6.0%)	1(0.02%)	264(5.9%)		1(0.02%)		
492(7.9%)						
79(8.0%)						
79(8.0%)						

Table 12. Continued.

Reference	Year	Place	Population type and characteristics	Number tested	Methods used
Rhatigan	1972	Witchita, Kansas	Negro college students	400	2 and 5
Rhatigan	1972	Witchita, Kansas	Negroes-screening program	1049	2 and 5
TOTALS		Kansas	NEGROES	1449	
Loh	1971	Gary, Ind.	Negro hosp. patients	2355	4 and 5
Bemis	1972	Milwaukee, Wisc.	Negro hosp. patients and screening program	9881	2 and 5
Long	1967	Polk County, Iowa	Negro hosp. patients	approx. 3000	9
Petrakis et al.	1970	San Francisco, Calif.	Negro clinic patients and screening program	4028	2
Binder and Jones	1970	Ft. Bliss, Texas	Negro-military	1000	2
Nalbandian, et al.	1972	Ft. Knox, Ky.	Military-Negro and white	2939	2 and 5
Fielding, et al.	1972	U.S.	Negro Job Corpsmen	11182	2
Fielding, et al.	1972	U.S.	Puerto Rican-Job Corpsmen	469	2
Fielding, et al.	1972	U.S.	Mexican/Latin Am. Job Corpsmen	906	2
Fielding, et al.	1972	U.S.	White-Job Corpsmen	3426	2
Fielding, et al.	1972	U.S.	Caribbean(other than Puerto Rican Job Corpsmen	37	2

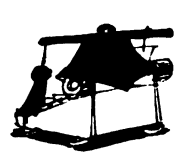
Overall % sickle cell	Percent SS	Percent AS	Percent AC	Percent SC	Percent AD	Others
28(7.0%)		28(7.0%)				
approx. 10%	approx. 2(0.2%)	approx. 10%				
132(9.1%)						
202(8.6%)						
931(9.4%)	29(0.3%)	880(8.8%)		22(0.2%)		
267(8.9%)	12(0.4%)	171(5.7%)	12(0.4%)	3(0.1%)		
351(8.7%)		351(8.7%)				
75(7.5%)	1(0.1%) s-thal	73(7.3%)		1(0.1%)		
65(2.2%)	1(0.03%)	63(2.1%)		1(0.03%)		
967(8.6%)	9(0.8%)	942(8.4%)	246(2.2%)	16(0.14%)		
18(3.8%)		18(3.8%)				
2(0.22%)		2(0.22%)				
5(0.15%)		5(0.15%)				
2(5.4%)		2(5.4%)				

Table 12. Continued.

Reference	Year	Place	Population type and characteristics	Number tested	Methods used
Fielding, et al.	1972	U.S.	American Indian, Oriental Job Corpsmen	398	2
TOTALS		U.S.	NEGROES	133,457 Tested	

Overall % sickle cell	Percent SS	Percent AS	Percent AC	Percent SC	Percent AD	Others
0						

11,387(8.5%)
with Hgb S



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