PART I: OVERVIEW OF STUDY

INTRODUCTION

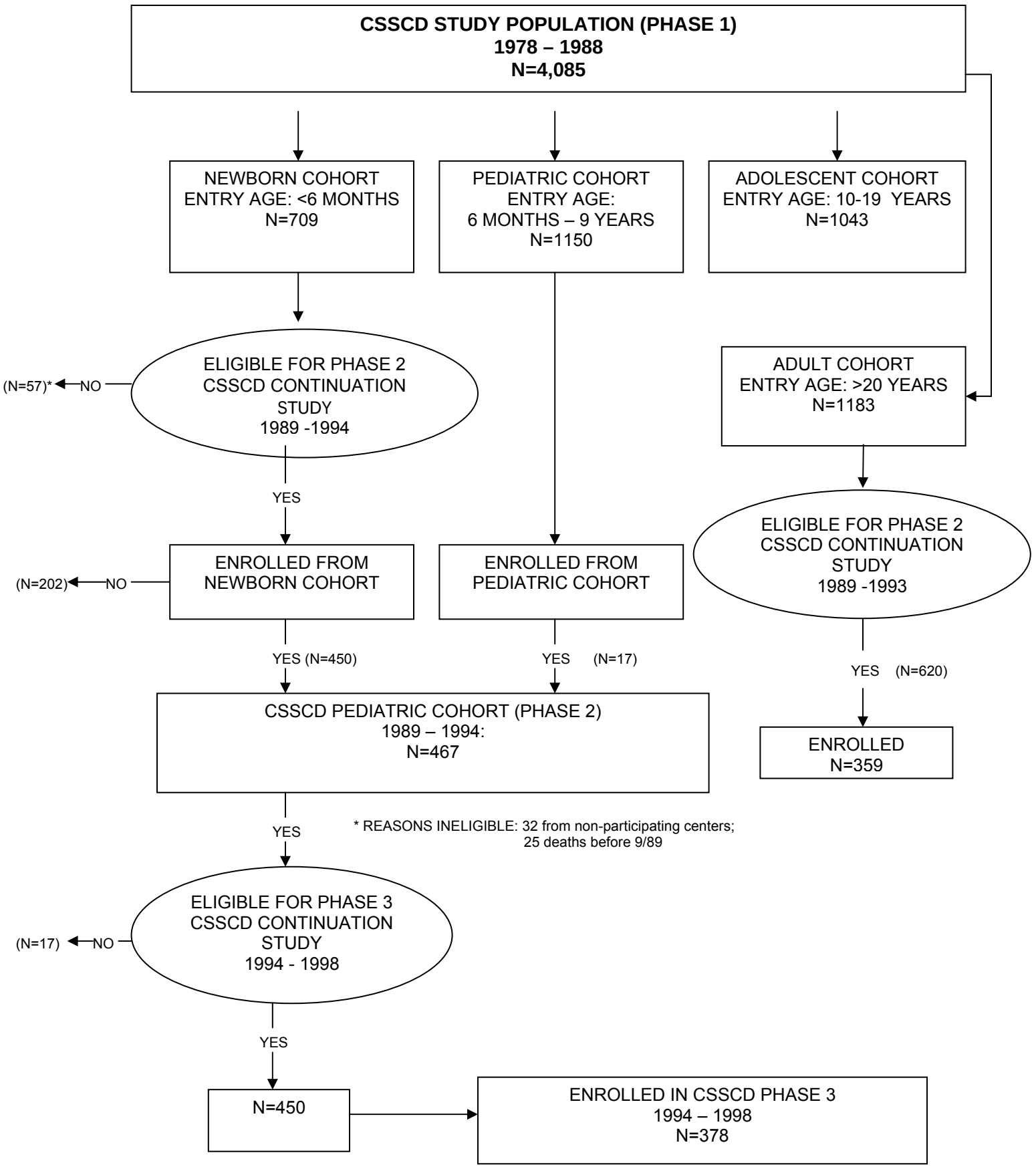
This manual provides documentation for the SAS datasets which contain data collected during Phase 1 of the Cooperative Study of Sickle Cell Disease (CSSCD). The original CSSCD study was conducted between March 1979 and September 1988 at 23 clinical centers (see Appendix A) and a Statistical Coordinating Center located at the University of Illinois School of Public Health, Chicago, Illinois. The study was funded by the National Heart, Lung and Blood Institute (NHLBI).

BACKGROUND

A cooperative group for the study of sickle cell disease was formed in *1977* at the National Institutes of Health (NIH) to organize a multicenter, prospective study of the clinical course of sickle cell disease in patients from the newborn period through adulthood. The primary motivation for the study was the lack of systematically obtained knowledge about this prevalent and costly hereditary disease. Although the molecular and genetic features of sickle cell disease have been known for years and many of its manifestations have been recognized, the clinical course from birth to death is not well understood. This is largely due to the variable degree of severity, the variability of the many manifestations, and the complexity of the interaction of the disease process with other health-related events. Therefore, information obtained was largely anecdotal, retrospective in nature, and lacking statistical validity. There were many unanswered questions about this disease.

In 1978, the Division of Blood Diseases and Resources established a multi-institutional study, the Cooperative Study of Sickle Cell Disease, to answer these questions. During the first 10 years of the study (October 1978 through September 1988), 4,085 patients from 23 clinical centers within the United States were enrolled in the study (See Figure 1). Patient entry was completed in 1981 for all except patients less than 6 months of age who continued to enter the study through September *1988.* Protocol requirements for patients during the original study varied depending on age, phenotype, and time period.

FIGURE 1


CSSCD STUDY POPULATION (PHASE 1)
1978 – 1988
N=4,085
NEWBORN COHORT
ENTRY AGE: <6 MONTHS
N=709
PEDIATRIC COHORT
ENTRY AGE:
6 MONTHS – 9 YEARS
N=1150
ADOLESCENT COHORT
ENTRY AGE: 10-19 YEARS
N=1043
ELIGIBLE FOR PHASE 2
CSSCD CONTINUATION
STUDY
1989 -1994
(N=57)*
NO
YES
ADULT COHORT
ENTRY AGE: >20 YEARS
N=1183
ELIGIBLE FOR PHASE 2
CSSCD CONTINUATION
STUDY
1989 -1993
ENROLLED FROM
NEWBORN COHORT
(N=202)
NO
ENROLLED FROM
PEDIATRIC COHORT
YES (N=450)
YES (N=17)
YES (N=620)
CSSCD PEDIATRIC COHORT (PHASE 2)
1989 – 1994:
N=467
ENROLLED
N=359
YES
* REASONS INELIGIBLE: 32 from non-participating centers;
25 deaths before 9/89
ELIGIBLE FOR PHASE 3
CSSCD CONTINUATION
STUDY
1994 - 1998
(N=17)
NO
YES
N=450
ENROLLED IN CSSCD PHASE 3
1994 – 1998
N=378

In 1989, a 5-year study (Phase 2) to continue follow-up of two patient groups (the infant cohort (patients entered at < 6 months of age in Phase 1) and adults 35 years of age and older) was initiated. During Phase 2, the objectives for the original infant cohort were 1) to identify factors related to overall disease severity, 2) to determine the onset of early organ damage, and 3) to examine risk factors contributing to certain complications and outcomes. The primary areas of interest for this cohort were the brain and lungs. A 5-year follow-up study (Phase 3) was initiated in 1994 to continue collecting these data to allow for longer longitudinal evaluation of factors affecting overall disease severity, brain abnormalities, neurocognitive and psychosocial functioning, and pulmonary dysfunction.

A list of center letter codes and number of patients enrolled at each center during Phases 1, 2, and 3 are presented in Table 1.

TABLE 1

DISTRIBUTION OF PATIENTS BY CLINICAL CENTER AND PARTICIPATION PHASE

Center Letter Code	# enrolled in		
	Phase 1	Phase 2	Phase 3
A	128	28	27
B	84	0	
C	259	70	
D	117	15	10
E	185	20	15
F	175	53	46
G	136	36	11
H	113	27	23
I	142	0	
J	173	40	38
K	78	0	
L	23	10	
M	282	80	40
N	132	27	
O	246	49	29
P	68	10	7
Q	227	28	26
R	117	6	
S	155	21	
T	83	0	
U	94	7	
V	146	37	
W	119	42	39
X	125	40	
Y	164	36	
Z	315	104	67
AA	69	0	
BB	130	40	
TOTAL	4085	826	378

Cooperative Study of Sickle Cell Disease
Phase 1 Original Protocol
1979-1983

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I. Introductory Summary

This protocol describes the essential aspects of the Cooperative Study of Sickle Cell Disease (CSSCD), a collaborative study of the "natural history" of sickle cell disease from birth through adulthood. This study, which documents the clinical course of sickle cell disease prospectively, will involve the participation of 15 Clinical Centers (23 hospitals) following approximately 3500-4000 patients over a period of four and one half years. Approximately 800 controls will be followed with the newborn cohort. Therapeutic interventions have not been incorporated into this study but will be recorded for specific complications.

The Manual of Operations (MOO) will specify in detail the operations and procedures needed for the conduct of this study.

II. Background

Sickle cell anemia was described by Herrick in 1910 and although there is a large collection of clinical and scientific data concerning the disease in the literature, there is a paucity of prospective studies and as a result the "natural history" of sickle cell anemia remains largely undefined. The variable degree of severity and clinical manifestations and complexity of the interaction of the disease process with other health-related events further contribute to our lack of understanding about the "natural history" of the disease from early childhood to death.

The clinical manifestations of sickle cell disease are legion, but all can be related to the single amino acid substitution of valine for glutamic acid in the 6^{th} position of the β chain of hemoglobin yielding the mutant hemoglobin S. When a critical concentration of deoxyhemoglobin S is achieved within the red blood cell, it polymerizes and distorts the red cell membrane producing the typical sickled erythrocyte. Such cells are prematurely removed from the circulation resulting in a chronic hemolytic anemia which under certain circumstances is compensated by increased erythropoiesis. The medullary and extramedullary compensatory response to chronic anemia has been amply documented, as have the results of increased hemoglobin catabolism. The chronic effects of this dynamic process have not been clearly identified.

The clinical course of sickle cell disease is punctuated by acute painful episodes traditionally referred to as “crises” which are the hallmark of the disease. The clinical consequences of sickle cell “crises” are diverse and have been the subject of numerous varied reports in medical literature. However, their toll in terms of morbidity and mortality is not known due to the lack of careful prospective studies that record their frequency, cause, association, duration, and effects both acutely and chronically over time.

The clinical image of sickle cell disease is greatly influenced by the perspective and discipline of the observer. This is perhaps best illustrated by the somewhat divergent perceptions of the disease on the part of pediatricians and internists. The pediatrician views it as a recurrent acute process with infection as the major threat to life, and painful vaso-occlusive episodes as the major factor influencing morbidity. The internist views the disease as a chronic process that has affected the function of different organ systems to various degrees and is aggravated by repeated acute episodes. A cohesive and comprehensive understanding of the clinical course of the disease is lacking and is needed to appreciate its “natural history”. An understanding of the clinical course of the disease would facilitate the identification of those areas where medical intervention would be expected to favorably influence its morbidity and mortality.

III. Objectives

Although sickle cell has been known for many years and many of its manifestations recognized, the “natural history” or clinical course of the disease from early childhood to death is not well understood. It is for this reason, a project has been initiated which seeks to delineate the course of sickle cell disease from birth through adulthood. The specific objectives of the study are:

a. to study the effect of sickle cell disease on growth and development from birth through adolescence.
b. To study the “painful crises”
    - Conditions or events which may be related to onset
    - Nature of the effects
    - Record therapeutic interventions
c. To obtain data on the nature, duration, and outcome of selected major complications of sickle cell disease.

d. To determine the nature, prevalence, and age-related incidence of selected organ damage due to sickle cell disease
e. To study the role of sickle cell disease and its interaction with selected health events
f. To examine the economic, educational, and vocational levels in patients with sickle cell disease
g. To develop a data base which will be used:
    - To evaluate treatment modalities currently used in the care of patients with sickle cell disease
    - To describe the spectrum of disease severity in patients with sickle cell disease;
    - To establish criteria for potential therapeutic interventions based upon the knowledge gained in the study

A. Rationale and Methodology for the Objectives

(1) Objective: To study the effect of sickle cell disease on growth and development from birth through adolescence.

Rationale: It has been reported that patients with sickle cell disease have retardation in growth and development during the two phases of life when this is most critical, i.e., the years 1 though 5 and the years of puberty. It is important to determine whether the rate of growth and development is influenced by the presence of the disease.

Methodology: Patients admitted to the Newborn, Pediatric, and Adolescent protocols will be carefully measured for certain physical and developmental milestones. These measures will be standardized to test commonly in use so that comparisons can be made with patients who do not have sickle cell disease. In adolescent children careful measurements of sexual development will be made. If there appears to be abnormalities of growth and development, special studies, especially endocrinological, will be instituted in an attempt to determine the etiology.

(2) Objective: To study the painful episodes or “crises” including conditions or events which may be related to onset, and the nature of the effects of the episodes, and to record therapeutic interventions.

Rationale: The painful episodes are certainly the best known of all complications of sickle cell disease. Although they are well recognized, relatively little is known concerning the conditions which surround their onset. The exact descriptives have not been obtained on a large enough number of patients to determine the spectrum of the painful episodes and there are no objective parameters to assess these events. In addition, no optimum treatment has evolved.

Methodology: Careful documentations of the occurrence of painful episodes, including the surrounding events, descriptives of the episodes, and therapeutic interventions which are undertaken will be made. Although no prescribed or comparative therapeutic trials will be attempted, the data concerning therapy will be recorded in such a way that it may be collated and reviewed.

(3) Objective: To obtain data on the nature, duration and outcome of selected major complications of sickle cell disease.

Rationale: In addition to the painful episode there are many acute and chronic complications which usually cause the patient to seek medical attention and which may lead to organ damage. It is important to know the nature and prevalence of the selected major complications of sickle cell disease in order to understand the health problems and hopefully design therapeutic interventions for their prevention in the future.

Methodology: Information concerning the acute and chronic complications of sickle cell disease will be obtained in two ways:

- Complications in the past history of the patient will be gathered by a careful review of the patient's autobiographical data, hospital records, and information from patients, family members, and health care workers. This information is clearly retrospective and will be treated as such.
- During the period of study, selected complications related to sickle cell disease will be careful recorded, including historical data, measures of organ change, and progression or regression of the complications of sickle cell disease.

(4) Objective: To determine the nature, prevalence, and age-related incidence of selected organ damage due to sickle cell disease.

Rationale: One of the most serious consequences of sickle cell disease is the impairment of organ function. Many of these impairments have been anecdotally reported e.g., renal failure, cardiac failure, and pulmonary disease. However, in

most instances, patients are studied at a time when organ damage is already severe and accurate assessment of time of onset and rate of progression of organ damage cannot be made from such investigations. This information is of vital importance for designing a realistic approach to medical management including both treatment and prevention of organ damage.

Methodology: Evidences of organ damage will be sought by assessing the structure and function of specific organs. Evaluation will be done upon entry of all patients other than newborns into the study. Since the age span of patients to be studied will be great, the prevalence and age-related incidence of abnormalities or organ structure in a large series of patients may be determined. These baseline studies will be repeated to determine the occurrence and progression of organ damage.

(5) Objective: To study the role of sickle cell disease and its interaction with selected health events.

Rationale: Patients with sickle cell disease are subject to increased risk of complications from other health problems.

Methodology: The information to be obtained will be detailed on a series of forms similar to those used to study the specific complications of sickle cell disease. These forms will reflect the nature of the underlying disorder and its progression. The conditions which will be studied in this way include:

i. pregnancy
ii. surgery with anesthesia
iii. transfusion complications
iv. acute febrile events
v. septicemia

(6) Objective: To examine the economic, educational and vocational levels in patients with sickle cell disease.

Rationale: One of the major effects of sickle cell disease is its influence on the economic situation of the patient and family. The expenses of medical care are great, but perhaps even greater are the ability of the patient to achieve educational and vocational goals. Although these influences are recognized, they have not been carefully documented in a large group of patients.

Methodology: We propose to collect information on the patients with sickle cell disease which will delineate the economic status. This information will be

carefully chosen so as not to intrude upon privacy and will be kept strictly confidential. Demographic data obtained on sickle cell patients and their families will then be compared to data available for the general population.

(7) Objective: To develop a data base which will be used to evaluate treatment modalities currently used in the care of patients with sickle cell disease; to describe the spectrum of disease severity in patients with sickle cell disease; and to establish criteria for potential therapeutic interventions based upon the knowledge gained in the study.

Rationale: A better understanding of the clinical course will augment our understanding of the mild and moderately ill patient, allow an examination of possible "risk" factors and provide data for a statistical determination of an index of severity. This will aid in the establishment of specific criteria for future therapies and should improve the quality of life of patients with sickle cell disease.

Methodology: This prospective study will document parameters of organ function and occurrences of specific events. Patient selection for the study will entail not only inclusion of patients frequently seen because of problems with their illness, but every effort will be made to include patients with few to no problems and presently seen infrequently. This should further delineate the spectrum of illness and permit development of a classification of illness severity based on observed and clinical parameters.

IV. Project Design:

A. General: The study of the clinical course of sickle cell disease has been arbitrarily divided into three age groups. Although many of the problems are the same through out life, often the problems are age-related and therefore three protocols have been formulated:

- Newborn and the first decade: This protocol outlines the procedures to analyze the effects of sickle cell disease on growth and development of the early years. It will look at the early development of organ dysfunction, especially the spleen and its effect on the health of the patient, the incidence of the complications of childhood, specifically related and unrelated to sickle cell disease, and the morbidity and mortality due to acute infections.
- Adolescence (Age 10-19): This protocol outlines the procedures to examine the effects of sickle cell disease on growth and maturation in puberty and on

the adjustment to the disease made by young patients. It will specifically look at complications, the onset of organ failure, and the different manifestations of sickle cell disease during this period.

- Adulthood (Ages 20 and Older): this protocol outlines the procedures to determine the prevalence of chronic organ damage, and acute events and other health related events, and their effects in patients with sickle cell disease.

Each protocol is designed so that similar data are collected at similar times. Thus, it facilitates patient movement from one protocol to another. Where complications are identical in all age groups, identical data will be collected. By the close intermeshing of the protocols, comparable data concerning patients with sickle cell disease, from the very young to the very old, will be possible.

B. Recruitment: Recruitment of participants for this study will be primarily from:

- identified existing populations of sickle cell disease patients at the clinical centers;
- local physicians in the community; and
- those identified through screening programs.

Special emphasis is given to recruiting “mild patients” so that the clinical course reflects the full spectrum of the disease.

At clinical centers recruiting a significantly smaller group of patients than the number of patients available for the study, entry will be performed according to a randomized scheme developed by the Statistical Coordinating Center.

Patients may enter the study by:

- Direct entry from the population of patients presently under care in the participating clinical centers. These patients will be entered on a pre-arranged schedule from randomized lists prepared by the Statistical Coordinating Center.
- Referral from another health provider, within the same hospital system or outside. This may include other clinics, hospitals or private physician referrals.
- Referral by self, relative, friend, etc.

Screening of newborns “at-risk” will identify newborns with sickle cell disease who can be recruited into the study. For the newborn study, two AA controls will be followed for each newborn. Controls will be matched for race, birthdate, sex, gestational age, birth weight, and five minute APGAR. Upon enrollment, controls will be followed in the same way as the newborn with sickle cell disease with the exception of radiologic and isotopic studies and many of the blood studies.

C. Enrollment: Patients with major sickle cell hemoglobinopathies (SS, SC, S β-thal) are eligible for enrollment in this study. This includes all age groups, beginning with the newborn. Pediatric, adolescent and adult patients will be entered into the study through February 1981. Newborns will be entered through September 1982. Patient entry will begin March 1, 1979.

D. Exclusion Criteria:

- Patients (or parents) unable or unwilling to give informed consent.
- Patient involvement in other research projects requiring a large commitment.
- Unreasonable distance for patients to travel to clinical centers as expressed by their unwillingness to return for follow-up on a regular basis.

E. Data Collection:

Pre-Consent Period:

- List of potential participants will be prepared by each Clinical Center and submitted to the Statistical Coordinating Center for the purpose of randomization and scheduling of entry.
- Potential participants will be informed of the purpose, methods, and demands of the study.
- Informed consent, previously approved by the local hospital’s or University’s Human Investigation Committee, will be signed to verify the patient’s willingness to be included in the study.
- Blood will be submitted to the Hematology Section of the Center for Disease Control (CDC, Atlanta, Georgia) for laboratory verification of each patient’s diagnosis.

F. Scheduled Visits: A thorough evaluation of each patient will be made upon entry and exit from the study: Infants with sickle cell disease and control infants will be followed every two months until the age of six months and every three months from six months to two years of age. Pediatric Patients (2 yrs. – 9 yrs.), Adolescent Patients (10 yrs. – 19 yrs.) and Adult Patients (20 yrs. and older) will be followed every six months for regular visits. Laboratory tests, clinical evaluation, diagnostic studies, and consultations with specialists will be carried out according to the specific schedule outlined on the Composite Flow Sheet.

In addition, emergency services and other health care will be provided and data obtained according to the specific protocol designed for each "special event".

G. Timetable of Study:

Planning and organization of Study…….October 197 through February 1979
Recruitment and Patient Entry……..March 1979 through February 1981
Newborns……...October 1978 through September 1982
Study Period…….March 1979 through September 1983
Phase-Out of Study……...October 1983 through March 1984
Data Analysis……October 1983 through February 1985

H. Newborn and Pediatric Protocol

1. Definition: Individuals from birth to the 10th birthday.
2. Questions:
   a. Are there clinical effects of sickle cell disease in utero in the first month of life?
      The fetus in utero produces large amounts of hemoglobin F, and therefore, the proportion of Hgb S in the fetus and newborn is quite low in infants with homozygous Hgb S disease. The proportion of Hgb S in these infants is 5-15%. Because of these low proportions, conventional screening tests such as sickle cell preparation and solubility techniques are unreliable. Special procedures such as acid agar gel electrophoresis and microcolumn chromatography are required for accurate definition of hemoglobin genotype. The high levels of Hgb F are also believed to be "protective" so that hematologic changes and clinical symptoms attributable to in vivo sickling are considered rare before 3-4 months of age. Despite this prevailing opinion, isolated cases of severe neonatal morbidity and mortality have been described as a consequence of sickle cell disease.
      This study will examine the intrauterine growth, development and neonatal course of a group of infants with homozygous Hgb S disease diagnosed at birth. The birth weight, length, head circumference and incidence of congenital anomalies in this group will be compared to control infants of similar characteristics with normal hemoglobin genotypes who are born in the same institution.
      Examination of this data will determine whether sickle cell disease is associated with intrauterine growth retardation or other fetal pathology. The neonatal course of infants with sickle cell disease will be examined to see if the rate of neonatal complications such as respiratory distress, hyperbilirubinemia, sepsis, hypoglycemia and others differ significantly from control infants.

   b. What is the incidence of hemoglobinopathy in the newborn population at highest risk? Are there differences in the frequencies and sex ratios predicted by the known gene frequencies and presumed genetic transmission?

The frequency of sickle cell trait in the American black population has been reported to be 5.0-10.0%. Using these estimates, the frequency of mating involving two heterozygotes for Hgb S would be 1/100 – 1/400 and the rate of Hbg SS at conception would be 1/400 to 1/1600. An unexpected large number of Hgb SS babies has been reported in one study and a preponderance of females over males with sickle cell trait and Hgb SS disease have been described in several reports. These observations may represent statistical variations; however, should they be confirmed, they may indicate that the genetic mechanism involved in the inheritance of sickle hemoglobin needs further examination and definition. In addition, in order to determine the "natural history" of sickle cell disease, it will be necessary to determine the incidence of sickle cell disease at birth in the population at highest risk.

The newborn screening programs at the participating centers will provide precise information concerning this incidence. The hemoglobin genotypes of newborn infants at the Clinical Centers will be determined. The frequencies of Hgb AS, SS, etc., can be determined and compared statistically with expected frequencies. Sex ratios of sickle cell trait and disease will be determined on large numbers of newborn infants.

The newborn electrophoretic studies will also document the frequency of other genetically determined hemoglobinopathies such as HbC, D, and alpha thalassemia. Carefully studies will be don to differentiate infants with Hgb FS patterns at birth. Hgb SD, S-HPFH, S-β thalassemia will be diagnosed by appropriate biochemical and family studies.

c. <u>What are the hematologic and clinical manifestations of sickle cell disease in the first ten years of life and what are their correlations?</u>

Relatively few systematic prospective data have been assembled which document changes in hematologic values in infants with sickle cell disease. The few data available suggest that these children are born with normal hemoglobin levels but develop hemolytic anemia after three of four months of age as the proportion of hemoglobin S increases in the circulating red cells. There is considerable individual increases in the circulating red cells. There is considerable individual variability. Irreversibly sickled cells appear in the

circulation much later. Vaso-occlusive phenomena often manifested as the hand foot syndrome (sickle cell dactylitis) may be seen as early as 4-6 months, but often do not occur until the second year of life or even later. This study will prospectively assess the degree of variability of these hematologic and clinical findings and attempt to relate this variability to other factors.

d. <u>What are the effects of sickle cell disease on growth and development during the first decade of life?</u>

It has been traditionally accepted that the patient with sickle cell anemia has a retarded growth rate during infancy and childhood and tends to be shorter than his peers with normal Hgb genotypes. Prospective studies are necessary to determine the time of onset and pattern of this growth retardation. Prospective data will be assembled for correlations of growth and development patterns and hematologic parameters.

This study will be possible because of the selection of two control infants with Hgb AA genotype for each SS patient enrolled in the study. Periodic examinations will be performed. By comparing the patients with sickle cell disease and controls, perturbations of the pattern of growth in sickle cell disease in the first decade of life can be determined.

e. <u>What are the types of severe infections and their frequency in children with sickle cell disease?</u>

Retrospective studies indicate a high frequency of at least two types of serious infections in children with sickle cell disease during the first decade of life. These include first a particular propensity to septicemia and meningitis caused by encapsulated organisms such as *D. pneumoniae* and *H. influenzae* type B. The incidence of these kinds of life threatening infections may be as high as 30% during the first 5 years of life resulting in mortality estimated to be as high as 15-30%. Children with sickle hemoglobinopathies also have increased susceptibility to osteomyelitis. Bone infections caused by salmonellae are frequently associated with sickle hemoglobinopathies.

Unusually severe pulmonary infections by mycoplasma pneumoniae have also been described in sickle cell disease patients. These types of infections are relatively infrequent in the general childhood population. This prospective

study is designed to establish more precisely the incidence rates of these various infectious complications in a large population of infants and children.

f. <u>What is the natural course of splenic function and dysfunction during the first years of life? How does this correlate with morbidity and mortality? Are there factors which serve to preserve splenic function?</u>

A state of splenic dysfunction in children with sickle cell disease is suggested by several clinical and hematologic observations. First, young children with sickle cell disease develop the typical acute and overwhelming bacterial septicemia and meningitis which occurs in the young child splenectomized for hematologic indications. The peripheral blood smears of young children with sickle cell disease also manifest hematologic changes such as the presence of Howell Jolly bodies and other "intraerythrocytic rubbish" as well as a high proportion of red cells with membrane "pocks". All of these are consistent with decreased splenic function even though a majority of children with sickle cell have enlarged spleens on palpitation.

The concept of a state of "functional" hyposplenia in sickle cell disease was advanced on the basis of the inability of the enlarged spleens of young children with sickle cell anemia to clear and concentrate radiocolloidal material (99mTc gelatin sulfur colloid). Functional hyposplenia was subsequently shown to be temporarily reversible by transfusions of normal red blood cells. Further, it is not congenital but rather a defect acquired when the proportion of hemoglobin F decreases post natally.

The study will investigate splenic function serially in a cohort of infants with sickle cell and other major Hgb S syndromes followed form birth. Splenic function will be assessed by quantification of the percentage of "pocked" or pitted RBC as determined by interference phase contrast microscopy (Normarsky optics) and 99mTc spleen scans will be done at specific times to compare the validity of the two indirect methods to identify splenic dysfunctions. Serial measurements of Hgb F will be correlated with the percent of "pocked" RBC, presence of Howell Jolly bodies and the 99mTc scans to see if there is a critical level of fetal hemoglobin at which splenic dysfunction occurs.

Scans will be done at 9 months of age or earlier, if the level of Hb F falls consistently below 20% or if Howell Jolly bodies are noted or if a severe bacterial infection occurs or if the % of "pocked" RBC exceeds 3.5. If the spleen does not take up sulfur colloid, a diagnosis of functional hyposplenia is established. If the spleen is still functional, a repeat spleen scan should be performed when the following occur: (1) severe bacterial infection; (2) Hb F persistently less than 20%; (3) presence of Howell Jolly bodies on smear; (4) % of "pocked" RBC greater than 3.5. Spleen scans will not be routinely performed on other patients.

g. <u>When do specific organ degenerations found in the adult patient begin?</u>
Progressive vaso-occlusion may involve many organs and tissues of the patient with sickle cell disease. The ultimate result of inexorable tissue destruction is a panoply of degenerative phenomena such as aseptic necrosis of the hip, renal failure, decreased visual acuity, hepatic fibrosis, congestive heart failure and many others. These "adult" manifestations of sickle cell disease must have their onset in childhood and teenage years. Determination of the developmental aspects of these degenerative diseases is necessary not only to permit strategies for prevention but also to guide clinical management. For example, if changes in the hips do not occur before teenage years, optimal management does not require regular skeletal x-rays before that time. Similar observations and recommendations could be made for other organ systems.
The study will address these issues in two ways. First, a cross-sectional survey of <u>all</u> patients will be available by data gathered on enrollment. Second, prospective data will be collected by follow-up clinical evaluations of all patients at least twice, once on admission and again four-five years later on exit from the study.

h. <u>What are the incidence, frequency of occurrence, and pattern of acute episodes ("crises")? How are these associated with environmental changes or other precipitating factors?</u>
The clinical course of patients with sickle cell disease is punctuated by acute episodes which have traditionally been designated as "crises". Actually, there

are several types of “crises” which have different pathophysiological mechanisms, clinical manifestations and laboratory findings.
Specific protocols have been formed to examine the clinical and hematological features of these various episodes. Possible inciting clinical events will be correlated with these episodes. Possible seasonal and geographical variability will be sought and quantitated.

I. Adolescent Protocol (Second Decade)

1. Definition: Individuals from the 10th to the 20th birthday.

   This protocol was selected to include the second period of rapid somatic growth and time of sexual maturation. Thus, if these major events have a significant effect on the acute manifestations and complications of the disease or on the progression or regression of organ damage, the analysis of data collected should document these relationships. The turbulence of the adolescent period with its sensitiveness and uncertainties, its acute awareness of self image and intense response to peer pressures, can make these patients the most difficult of recruits. Special attention must be given to this age group to encourage participation and retention by means which will clearly differ from those needed for either the child or the adult.

2. Questions:

   Because of the frequent difficulty in attracting and retaining the adolescent in ongoing systems of health care, relatively little is known about the disease in this age group. The questions to be answered focus on determining whether this is really the quiescent period, it seems, and on analyzing the step by step progression of events in the transition to adulthood.

   a. What are the clinical and hematologic measurements of sickle cell disease in the second decade and what are their correlations?

   During adolescence the threat of infection so prevalent in the first decade of life subsides, and chronic organ damage is not well established. Some patients show amelioration of the frequency and severity of painful episodes and others show the recurrence of previously established patterns at the same or increased rates. The documentation of the range of these changes is critical, as well as their correlation with other events in the medical and psychological life of the adolescent, e.g., menarche, life stress, as well as the hematologic changes in the individual patient. These events can be assessed through recording of data at varying intervals and at the time of the actual event.

   The sudden increase of muscle mass in the male adolescent, the onset of menarche in the female, and the increased nutritional demands of the growing body, will cause changes in iron requirements and iron stores which in turn may effect the basic laboratory parameters of the disease, as well as the symptomatology. Data assessed

through interval laboratory tests, and clinical evaluation will assist in evaluating these effects.

b. What are the acute and chronic complications of sickle cell disease in the second decade of life? Can the incidence, age at onset and frequency of recurrence be predicted?

The study will attempt to differentiate the type and frequency of complications in the adolescent from those seen in the first decade of life and adulthood. Periodic thorough assessment of each adolescent as shown on the flow sheet, as well as data collected on acute events will serve to answer these questions.

c. What is the prevalence, incidence and age of onset of selected chronic organ damage in the adolescent?

The patient in the first decade shows little evidence of chronic organ damage. The patient who presents as an adult frequently seeks medical care after evidence of chronic organ damage is well established. This study is designed to describe interim events.

Gallbladder:

A cross-section of patients will be evaluated initially at entry in the age group of 10-14 years. This will be done by a cholecystogram on adolescents who do not show stones on abdominal roentgen examinations. Since the age of onset is unknown, it is not considered feasible to assess all adolescents, but to review the data in the above stated age groups and later determine if the age range should be revised in either direction after the first year of the study. Patients who do not show gall stones will be reassessed upon exit from the study or graduation into the adult protocol.

Renal:

A routine urinalysis, urine culture, serum creatinine and uric acid will be performed on each patient yearly. A creatinine clearance will be performed if the serum creatinine is elevated. When abnormalities are detected, specific studies will be performed (outlined under renal complications) to evaluate the degree of abnormality and to follow its progression during the course of the study.

Lung:

Pulmonary function tests will be done at age 12 and data will be assessed at six months to one year into the study. These findings and analysis of results will permit early decisions regarding the most appropriate ages for carrying out these studies in a large number of patients.

Bone:

Aseptic necrosis of the femur and humerus is frequently first manifested in the second decade. The periodic examination of the youth plus evaluation of acute skeletal events will allow a more accurate assessment of this complication and give evidence of antecedent factors, symptomatology, and effects of various therapeutic regimens.

Eye:

The three scheduled eye examinations at entry, second year of the study and exit, performed under standardized conditions, should afford valuable data on the age of onset and extent of eye lesions in each of the hemoglobinopathies.

d. What is the effect of sickle cell disease on growth and sexual maturation? Which youths are most likely to show the extreme of these changes? How do the changes differ among the different hemoglobinopathies?

   Growth will be assessed through semi-annual measurements of weight and height. Tanner staging will be done yearly on both girls and boys in order to evaluate sexual development. The age of menarche will be recorded for the females.

e. What is the effect of hospitalization on the educational outlook on the youth with sickle cell disease using his peers and his own performance for comparison? What medical and social factors can be correlated within these variations?

   From the second year of the study, school reports will be requested at regular intervals. The youth's individual progress can be assessed and correlated with such factors as frequency of hospitalization, occurrence of complications and other factors.

f. How many patients in the second decade of life still have residual splenic function? Is a palpable spleen in youths with SC and Sβ Thal an indication of a

critical amount of residual function? How does splenic function correlate with other medical events in the life of the patient?

Patients in the adolescent group will have RBC's examined for pitting on entry. Comparison of finding in the adolescent cohort should go far towards answering these above questions. Perhaps other studies will be indicated.

J. Adult Protocol

1. Definition:

   Individuals having attained their 20th birthday.

2. Questions:

   a. What is the prevalence, incidence, rate of progression, and outcome of chronic organ damage occurring in adult patients with sickle cell disease?

   - Renal:

   A routine urinalysis, urine culture, serum creatinine and uric acid will be performed on each patient yearly. When abnormalities are detected, specific further studies will be performed (outlined under renal complications) to evaluate the degree of abnormality and to follow its progression during the course of the study.

   - Eye

   Upon entry, in the second year, and upon exit each adult patient will be evaluated by an opthalmologist who will examine the fundi for the presence of vascular disease, measure visual acuity, map visual fields, and photograph fundal abnormalities. If Stage III or IV retinopathy (Goldberg classification) is present, flourescein angiography will be performed.

   - Cardiopulmonary

   Chest x-rays will be performed on entry, at the second annual visit, and upon exit for evaluation of cardiomegaly and pulmonary abnormalities. Pulmonary function studies including spirometry, total lung capacity, diffusing capacity and blood gases will be performed upon entry and exit in patients between the ages of 20-22 and 30-32 years.

   Depending upon the initial findings these studies may be extended to other age groups in year two and upon exit. Cardiac function will be assessed through special studies in selected Clinical Centers.

   - Bone

   Upon entry and exit, x-rays of the hips, pelvis and shoulders will be obtained. The primary concern is to answer the above questions pertaining to aseptic necrosis of the heads of the femur and humerus. In other portions of the protocol, the short and long term effectiveness of surgical intervention (complete hip prosthesis) will be determined.

b. What is the incidence and frequency of recurrence of painful episodes, and what factors predispose patients to these episodes? What methods are used to treat these episodes and what is the outcome? Is there a relationship between painful episodes and environmental factors such as temperature and humidity, alcohol consumption, infection, pregnancy, etc. Is there a relationship between the occurrence of painful episodes and the patient's genotype, the level of hemoglobin, the ISC count, and the percentage of hemoglobin F?

- Painful episodes in all patients will be documented and details of antecedent events, symptomatology, physical findings, laboratory studies, therapy and clinical course will be recorded on the appropriate data forms. When direct observation is not possible, the data are to obtained from emergency room records, hospital charts, conversation with the private physician, etc.

c. What is the incidence and frequency of recurrence and outcome of the various complications and associated health events observed in persons with sickle cell disease? Are there means of predicting which patients are likely to develop certain complications? What modalities of therapy are used for the different complications and is there statistical evidence to suggest that one form of therapy is superior to another?

- Acute complications to be studied, in addition to painful episodes, include the acute chest syndrome; right upper quadrant syndrome; skeletal events; neurological events; anemic episodes and priapism.
- Chronic complications include renal, eye, aseptic necrosis, and leg ulcers. There are specific questions to be answered and the required data appears on the appropriate data reporting forms.
- Associated other health events include delayed growth, delayed sexual maturation, pregnancy, surgery with anesthesia, septicemia, acute febrile events, and transfusions.

K. Clinical Evaluation

COMPOSITE FLOW SHEET

	NEWBORN & PEDIATRIC	ADOLESCENT	ADULT
* Complete P.E.	Yearly	Yearly (including Tanner)	Yearly
* Interval History and P.E.	q 2 months until 6 months of age q 3 months until age 2 years then q 6 months	q 6 months	q 6 months
* Denver Development	Each visit until age 2 then yearly to age 5		
‡ School Report	yearly	q semester	
Hematology *CBC, *Retic, ISC, H.J., Differential	q 2 months until 6 months of age q 3 months until age 2 years then q six months (controls entry, 12 mos of age, then yearly)	q 6 months	q 6 months
Platelet Count	yearly	yearly	yearly
Hgb F	q 2 months until 6 months q 3 months until age 2 yrs then q 6 months	yearly	yearly
† Allo Antibodies Blood Type	Entry only on patients previously transfused or expecting transfusion.		
† Hgb Evaluation	Entry Confirm diagnosis at age 2 yrs on SCD infants (confirm diagnosis on controls at 12 mos at the local lab)	Entry	Entry

COMPOSITE FLOW SHEET (continued)

	NEWBORN & PEDIATRIC	ADOLESCENT	ADULT
G-6PD (males & females)	Entry (or age 9-12 months)	Entry	Entry
Liver Liver Function Tests	Entry (or age 1 at earliest), 2nd annual visit Exit	Entry 2nd annual visit Exit	Entry 2nd annual visit Exit
Spleen Scan	(See Protocol)		
*†Pitting of rbc	Each visit up to 3 yrs of age on SCD infants (Controls – entry and 12 mos of age, then yearly)	Entry	Entry

COMPOSITE FLOW SHEET (continued)

	NEWBORN & PEDIATRIC	ADOLESCENT	ADULT
Gallbladder Plain film of abdomen If no stones perform cholecystogram		Entry (only 10-14 yrs) Exit (on negatives)	Entry (only 25-30 yrs) Exit (on negatives)
Heart Chest X-ray	Entry (or age 1 at earliest) 2nd annual P.E. Exit	Entry 2nd annual P.E. Exit	Entry, 2nd annual P.E. Exit
Renal * Routine Early A.M. Culture (colony count) Creatinine Uric Acid	Yearly (start age 3)	Yearly	Yearly
Ophthalmology	Entry (age 5 at earliest) 2nd annual visit Exit	Entry 2nd annual visit Exit	Entry 2nd annual visit Exit
Bone Hip & Pelvis Shoulders	Entry (or age 5 at earliest) Exit	Entry & Exit	Entry & Exit
Pulmonary Function Spirometry Total Lung Capacity Diffusing Capacity Blood Gases		Entry (only 12 yrs of age) Exit	Entry (20-22 yrs, 30-32 yrs) Exit

* these data will also be collected on newborn controls during routine well baby visits

† Will be performed at a central laboratory

‡ Not initiated until year 02

I. Complications of Sickle Cell Disease

Definition: The specific complications of sickle cell disease include evaluation of chronic organ damage and acute complications which occur as a direct result of sickle cell disease or as it interacts with other health events.

General Questions for all Complications

1. What is the incidence and frequency of recurrence of each event? (age-related, diagnosis related).
2. What are the preceding events? Can precipitating events be identified?
3. What are the possible predictors of risk?
4. What is the course of the event?
5. Is the outcome related to:
    a. type of therapy
    b. descriptives of the events
    c. possible risk factors

ACUTE COMPLICATIONS

Painful Episode

Acute Chest Syndrome

Right Upper Quadrant Syndrome
(Hepatobiliary Syndrome)

Skeletal Events

- Hand-Foot Syndrome
- Bone Infarction
- Osteomyelitis
- Joint Swelling with Effusion

Neurologic Events

- Seizures
- Meningitis
- C.V.A. (stroke)

Anemic Episodes

Acute Priapism

Fat Embolization Syndrome

CHRONIC COMPLICATIONS

Leg Ulcers

Renal

- Hematuria
- Urinary Tract Infection
- Persistent Proteinuria
- Renal Insufficiency

Aseptic Necrosis

Ocular Complications

Chronic Priapism

INTERACTION WITH OTHER HEALTH EVENTS

Delayed Growth and Development

Delayed Sexual Maturation

Pregnancy

Surgery and Inhalation Anesthesia

Septicemia

Acute Febrile Event

Transfusion

V. Laboratory Standards

Laboratory Standards for this study, were established in consideration of the: 1) desirability of having specific laboratory tests immediately available for the ongoing care of the patient, 2) expense of testing and 3) comparability of data and quality control. Therefore, laboratory procedures will be performed at both the individual clinical centers with centrally operated proficiency testing programs and at designated central laboratories for specific tests.

The methods to be employed for all testing were decided upon by the laboratory subcommittee, with appropriate consultants when required and are detailed in the Manual of Operations. The frequency and desirability of doing tests was a function of the protocol committee. Recommendations for proficiency testing procedures were made by the laboratory committee and consultants. The tests best done in central laboratories were designated by the laboratory committee and the assignment of central laboratories made by the Policy Board.

Complete blood counts will be done using the Coulter electronic cell counter method with proficiency testing coordinated by the Center for Disease Control (CDC). Red cell morphology, definition and counting of the ISC, Howell-Jolly bodies and reticulocytes will be done by standard methods with central training of all technicians and proficiency testing done by the central training laboratory at the Medical College of Georgia. Evaluation of red cell pitting will be done in a central laboratory designated at Yale University.

Blood chemistries and liver function tests will be done locally and controlled by the CDC proficiency testing program.

Alloantibodies will be performed in a central laboratory using standard blood banking procedures at Duke University. G-6-PD electrophoresis and assays of G-6-PD and hexokinase activity will be done centrally at the University of Mississippi.

Hemoglobin evaluation will be done at each center and confirmed centrally by the Center for Disease Control. Each individual will have electrophoresis at alkaline and acid pH, solubility testing and quantification of HbA_2 and F, by methods specified in the operations manual. The results of these tests will dictate the need for globin synthesis studies to be completed at a central laboratory. The following definitions have been adopted:

Sickle Cell Anemia:

1. Hbs, the major Hb band upon electorphoresis at both alkaline and acid pH and a positive solubility test for HbS.
2. Both parents with a HbS gene (desirable, but not required).
3. HbF levels less than 15% of the total with an uneven distribution of HbF upon acid elution stains of thin blood films.
4. HbA_2 less than 4%.

In children, the results of electrophoresis obtained during the first year of life or at birth, will be repeated again at age 2.

HbS-β^+-Thalassemia:

1. In the absence of blood transfusion for 4 months, any HbA present on both alkaline and acid pH electrophoresis, with HbS the major Hb type present and a positive solubility test for HbS.
2. HbA_2 greater than 4%.

HbS-β^0-Thalassemia:

1. Presence of β-thalassemia in a parent as well as confirmatory familial evidence in siblings or other family members.
2. HbS the major Hb band upon electrophoresis at both alkaline and acid pH and a positive solubility test for HbS.
3. Coulter MCV of less than 70 fl. under 2 years of age and less than 76 over 6 years.
4. HbA_2 greater than 4.0%.
5. α/β chain biosynthesis ratios of greater than 1.5.

HbSC Disease:

1. HbS and C present in approximately equal proportions upon electrophoresis at both alkaline and acid pH, with a positive solubility test for HbS.

Radiographic and scanning procedures will all be done locally. The specifications for testing have been formulated by radiologic consultants who will also monitor quality control.

Pulmonary function testing has been standardized and will be monitored by the Pulmonary Disease consultants to the study. The ophthalmologic examination has been

similarly standardized by the ophthalmology consultant by site visits to each clinical center and the center ophthalmologist.