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# The effects of desferrioxamine iron chelation therapy in hypertransfused beta-thalassemia patients

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# The Effects of Desferrioxamine Iron Chelation Therapy in Hypertransfused Beta-Thalassemia Patients

A Thesis Presented to the Yale University
School of Medicine in Partial Fulfillment
of the Requirements for the Degree of
Doctor of Medicine

Gail Elizabeth Mizner
1986

## ABSTRACT

Twenty-one patients with beta-thalassemia treated with hypertransfusion and subcutaneous Desferrioxamine (DF) chelation received annual evaluations of serum ferritin level and hepatic, endocrine, and cardiac function. To assess the effectiveness of DF therapy, the patients were divided into compliant (N=11) and noncompliant (N=10) groups and the results of their annual evaluations were compared.

The compliant group showed a statistically significant drop in mean serum ferritin and SGOT levels not seen in the noncompliant population. However, the mean serum ferritin levels of the compliant group plateaued at 3,000 ng/ml, indicating that the DF dose was not sufficient to further reduce body iron stores. There was a significantly decreased incidence of impaired carbohydrate metabolism in the compliant group as compared with the noncompliant one. No difference in the incidence of growth curve abnormalities was found, but bone age and pubertal development both appeared to be normalized by compliance with DF if the drug was begun at an early age. Echocardiograms suggested that left ventricular hypertrophy might be prevented or delayed by compliance with a 5 night/week regime, but this regime's overall effectiveness in delaying or preventing arrhythmias and myocardial dysfunction was less apparent.

It is concluded that subcutaneous DF chelation is helping to at least delay some of the complications of iron overload but that earlier, more intensive chelation is needed.

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## DEDICATION

This thesis is dedicated to the children and young adults with thalassemia who made it possible and whom it is intended to help.

CONTENTS

# Chapter I

# LITERATURE REVIEW

## 1.1 INTRODUCTION

In 1925, Thomas Cooley and Pearl Lee first described the clinical syndrome of "anemia, splenomegaly, and some enlargement of the liver,...[as well as]... a peculiar mongoloid appearance, caused by enlargement of the cranial and facial bones, combined with the skin discoloration." (Cooley 1925) This syndrome later came to be known (after the senior author) as Cooley's anemia. Since then, the medical profession has learned a great deal about the genetics, clinical manifestations, and treatment of this disease, which is now commonly referred to as homozygous beta thalassemia, the eponym "Cooley's anemia" having fallen into slight disfavor because of its unintended racial connotations. The term "thalassemia" is derived from the Greek words for "sea" and "blood", indicating that the population most afflicted by this disorder is from the countries bordering the Mediterranean Sea, particularly Greece and Italy. (Lin-Fu 1981)

Thalassemia is an autosomal recessively inherited genetic defect in which there is diminished or absent synthesis of the beta-globin chain of normal hemoglobin A. Recombinant DNA technology has revealed that the molecular basis for thalassemia is quite heterogeneous. More than 25 distinctive thalassemia mutations have been identified. They vary

from point mutations that change "sense" codons to give incorrect signals to unequal crossover and recombination of closely linked genes to full or partial deletion of the gene. All of the mutations in some way prevent the beta-globin gene from producing mRNA template in sufficient amounts; most commonly, by causing "premature termination of translation or abnormal processing of mRNA precursor." (Pearson and Benz)

The anemia of the homozygous state of beta-thalassemia, known as thalassemia major, is due not only to the decrease or lack of production of Hb A, but also to the increased hemolysis thought to be brought about by an excess of unpaired alpha chains that precipitate and form inclusion bodies called Fessas bodies. Beta thalassemia major is characterized by an untransfused hemoglobin level of 5 g/dl or less. Microcytes, bizarre poikilocytes, target cells, and increased numbers of white blood cells and nucleated red blood cells are typically seen on peripheral blood smear. Hemoglobin electrophoresis reveals reduced amounts or a complete absence of Hb A, with relatively increased Hb $A_2$ and Hb F. (Pearson and Benz)

The heterozygous state, referred to as thalassemia trait, produces only a mild anemia (hemoglobin 1-2 g/dl below normal) that is usually asymptomatic and may go undiagnosed. Peripheral smear shows many of the same abnormalities seen in thalassemia major but to a lesser degree. Definitive diagnosis is usually made by demonstration of increased amounts of Hb $A_2$ relative to Hb A, but for mass screening purposes, simpler tests such as determination of MCV or osmotic fragility are generally employed. (Pearson and Benz)

Thalassemia intermedia is a clinical classification for thalassemics who are able to maintain their hemoglobin levels between 7 and 9 g/dl without regular transfusions. The genetic disorder behind this clinical presentation can be either mild homozygous beta thalassemia or severe heterozygous beta thalassemia, more commonly the former. (Lin-Fu 1981) These patients tend to do better clinically, but may still suffer many of the complications of anemia and iron overload seen in thalassemia major and described below. (Pearson and Benz) The treatment of thalassemia intermedia is controversial. The criteria used by the Yale New Haven Hospital Pediatrics Department in deciding to put a patient with thalassemia intermedia on hypertransfusion therapy include nonspecific symptoms such as weakness and fatigue, progressive disfiguring bone changes, and evidence of cardiac dysfunction secondary to anemia such as cardiomegaly or tachycardia.

## 1.2 CLASSICAL PRESENTATION OF THALASSEMIA MAJOR

Before the introduction of hypertransfusion and iron chelation therapies, the classical presentation of thalassemia major was quite distinctive. Because the production of fetal hemoglobin, the predominant hemoglobin during intrauterine life, is normal, the child appears normal at birth. But within three to twelve months some of the first symptoms, including pallor, fever, recurrent infections, jaundice, epistaxis, constipation, diarrhea, splenomegaly, and general weakness, begin to appear. (Lin-Fu 1981) With increasing age, the child develops symptoms related to the body's attempt to cope with the severe anemia. Ineffective hematopoiesis causes expansion of the bone marrow and

resulting skeletal changes in the ribs, skull, long bones of the extremities, and short bones of the hands and feet. Overgrowth of the maxilla produces the typical facies of thalassemia with short nose, prominent eyes, high, protruding cheek bones, overbite and malocclusion. (Lin-Fu 1981) Osteoporosis and pathological fractures are fairly common. Cardiomegaly secondary to anemia and abdominal enlargement due to hepatosplenomegaly are inevitable. Because intestinal iron absorption is increased, even completely untransfused children develop iron overload and hemosiderosis. Growth retardation is usually severe, (Logethetis 1972), and secondary sex characteristics rarely develop. (Zaino 1969, Landau 1984) Children who are not given transfusional therapy at all survive an average of less than four years. Those who are minimally transfused to maintain a hemoglobin level between 5 and 6 g/dl are symptomatic much of the time and generally die between the ages of fifteen and twenty years. (Pearson and Benz)

## 1.3 HYPERTRANSFUSION THERAPY

Until about twenty years ago, because of the fear of accelerating hemosiderosis and its fatal consequences, such minimal transfusions were the standard therapy for thalassemics. But in 1963, Irving J. Wolman published his seminal study "Transfusion therapy in Cooley's anemia: growth and health as related to long-range hemoglobin levels. A progress report." Wolman reported that a study of three groups of children maintained at different pretransfusional hemoglobin levels showed that the group with the highest levels had the fewest and least severe health problems. Those problems relating to anemia such as

stunted growth, bone abnormalities, and cardiomegaly were particularly improved. (Wolman 1964)

With this study, Wolman introduced the concept of hypertransfusion therapy in which transfusions are given regularly enough to maintain a hemoglobin level of 9 to 10 g/dl. Since then, hypertransfusion has become standard practice, and the classical features of beta thalassemia major described above have virtually disappeared. Thalassemic children on hypertransfusion therapy are able to lead almost normal lives. They do not suffer the fatigue, irritability, and exercise intolerance associated with severe anemia. Cardiomegaly, hepatosplenomegaly, and hypersplenism are all reduced. Because erythropoiesis is suppressed, facial changes and osteoporosis of the long bones do not develop. Any such changes that are already present when hypertransfusion therapy is begun tend to regress. Growth, at least during the first decade of life, is much more normal. (Beard 1969, Piomelli 1969, Necheles 1974) The acceleration of hemosiderosis and consequent earlier deaths that were feared have not proved to be the case. This may be because Wolman was correct when he suggested that it was "not unlikely that the maintaining of normal hemoglobin levels by more frequent transfusions prevents undue iron absorption from the intestine." (Wolman 1964) The decrease in intestinal iron absorption may offset the increase in the amount of iron received through transfusion.

## 1.4 SPLENECTOMY

An additional advancement in the care of thalassemic patients has been the use of splenectomy when there is evidence of hypersplenism. Hypersplenism usually manifests itself as an increased transfusional requirement because of decreased survival time of transfused red blood cells, but neutropenia and thrombocytoenia may also be present. A transfusion requirement of greater than 200 ml/kg/yr (to maintain hemoglobin levels greater than 9.5 g/dl) is generally felt to be an indication for splenectomy. Because of the increased risk of infection in splenectomized patients, splenectomy is deferred as long as possible, at least until after age five or six, to allow broader humeral immunity to develop. Polyvalent pneumococcal polysaccharide vaccine is administered before splenectomy, and prophylactic oral penicillin may be used afterwards. (Pearson and Benz) By decreasing the transfusional requirement in the presence of hypersplenism, splenectomy is believed to reduce the rate of iron loading.

## 1.5 COMPLICATIONS OF IRON OVERLOAD

Despite these advances in treatment, thalassemics suffer considerable morbidity and mortality beginning in their second decade due to the complications of iron overload. The finding of hepatic fibrosis associated with siderosis in thalassemics was first described in 1954 in post-mortem histological studies by Ellis et al. The patients in this study were on low transfusion regimens but presumably had additional iron load from increased gastrointestinal absorption on top of their

transfusional load. More recent studies of liver biopsy specimens have confirmed this finding in hypertransfused children. (Barry 1974) A large study by Jean et al revealed that fibrosis was limited to the portal spaces in children aged three to five but that by age fifteen to sixteen, most showed features of cirrhosis. (Jean 1984) Elevated transaminase levels as well as more clinically important abnormalities of liver function, such as hypergammaglobulinemia, hypoalbuminemia, and moderately decreased production of coagulation factors, are found and generally become worse with increasing age. (Hilgartner 1964, Pearson and Benz)

The pancreas, too, undergoes siderosis and fibrosis (Ellis 1954), and, as might be expected, impaired carbohydrate metabolism, and even overt diabetes mellitus, is often seem in the second decade. (Lassman 1974, Necheles 1974, Flynn 1976, McIntosh 1976, Costin 1977) It is unclear, however, to what degree this is due to decreased insulin secretion from iron damaged beta cells and to what degree it is due to insulin resistance and hyperglucagonemia, probably related to cirrhosis. (Costin 1977, Zuppinger 1979) There is also some evidence that a familial history of diabetes increases the likelihood that a thalassemic patient will develop this complication. (Wolman and Ortolani 1969, Costin 1977, Zuppinger 1979, Modell and Berdoukas 1984)

Growth retardation remains a confusing issue with thalassemic children. Although growth during the first decade of life seems to be improved by hypertransfusion therapy, the adolescent growth spurt and the onset of puberty are delayed or absent under any transfusional

program. (Canale 1974, Flynn 1976, Landau 1984) In general, it appears that it is largely anemia that retards growth during the first decade and hemosiderosis that retards it during the second decade. (Lin-Fu 1981) Bone maturation is impaired. Girls frequently are oligo- or amenorrheic and have poor breast development. Boys often have sparse facial and body hair, decreased phallic development, and varying degrees of spermatogenesis. In general, males are somewhat more likely to experience difficulties with pubertal development than are females. (Landau 1984, Flynn 1976) Iron damage to the pituitary gland appears to play a larger role in the lack of pubertal development than does hypothalamic or end organ damage. (Kletsky 1979)

Cardiac complications due to hemosiderosis are usually the cause of death in thalassemic patients. Analogous to the situation with growth, the cardiomegaly due to anemia during the first decade of life is greatly reduced or eliminated by hypertransfusion therapy, but during the second decade hemosiderosis produces cardiomegaly and left ventricular hypertrophy which progress to chronic refractory congestive heart failure. Pericarditis, heart block and atrial and ventricular arrhythmias are also common. (Engle 1964, Ehlers 1930) Most patients succumb to these complications between sixteen and twenty-four years of age. (Modell 1976)

## 1.6 DESFERRIOXAMINE

With the introduction of hypertransfusion therapy the interest in finding a good iron chelator to combat transfusional iron overload was increased. A number of chelating agents, including penicillamine, EDTA, tricalcium diethylene triamine pentaacetate, DPTA, and desferrioxamine B were studied. All but desferrioxamine B were found to either lack specifity or carry significant toxicity. (Pearson and Benz)

Ferrioxamine B is a sideramine obtained from the bacterium Streptomyces pilosus. The iron is removed by chemical means to produce desferrioxamine B. As seen in Figure 1, desferrioxamine (DF) is made up of one molecule of acetic acid, two molecules of succinic acid, and three molecules of 1-amino-5-hydroxylaminopentane. When DF comes in contact with a ferric ion, it wraps around it forming three hydroxamic acid bonds, which makes for a very stable iron complex, as shown in Figure 2. (DF does not bind ferrous ions.) This chelating agent is not only potent, it is also very specific, showing a much higher affinity for iron than for any other metal. It removes iron from ferritin and hemosiderin but not from transferrin or from the porphyrins of hemoglobin, myoglobin, and the respiratory chain enzymes where the iron plays an essential role. (Keberle 1964, Graziano 1978)

Unfortunately, DF is not absorbed well through the gastrointestinal tract, and so must be administered parenterally. Numerous studies have shown that continous subcutaneous or intravenous administration are much more effective in producing high levels of urinary iron excretion than are painful, intermittent intramuscular injections. (Hussain 1976,

Weiner 1978) Because continuous subcutaneous infusion with a portable pump is much more convenient and is 80-90% as effective as the intravenous route, (Propper 1977, Graziano 1978), subcutaneous infusion has become the preferred method of DF administration. With this method, as much as 100 mg of daily urinary excretion can be achieved in iron overloaded patients, (Pearson and Benz), although this amount decreases with decreasing iron load. (Piomelli 1985) In addition, some recent studies have shown that fecal iron excretion is also increased by use of DF and can represent approximately 25-50% of the total iron excretion. (Cohen 1985, Bianco 1984)

Although DF frequently produces localized irritations and swellings around the site of injection, until very recently, only extremely rare instances of more serious side effects such as cataract formation, allergic reaction secondary to histamine release, and visual and auditory neurotoxicity had been reported. (Bloomfield 1978, Romeo 1984, Orton 1985) However, Olivieri's group has just published a study of 89 transfusion-dependent patients on subcutaneous DF which found symptomatic auditory or visual neurotoxicity in 13 patients and asymptomatic toxicity in 27 more. These side effects usually occurred in young patients on very high doses of DF per kilogram of body weight. In some cases, the damage was partially or fully reversible with discontinuation of the DF. (Olivieri 1986)

## 1.7 THE EFFECTIVENESS OF DESFERRIOXAMINE

Several studies have found that serum ferritin levels, which have been shown to correlate with total body iron stores (Saarinen 1979, Letsky 1974, Lipschitz 1974), are markedly reduced by use of DF. (Hyman 1985, Wolfe 1985) In most cases these reduced levels are still in the thousands, far above the normal serum ferritin range of 12-300 ng/ml, but in one small study, Cohen et al were able to decrease serum ferritin levels to within normal limits through a combination of intensive subcutaneous and intermittent intravenous DF therapy. (Cohen 1981 and 1985)

Hence, the decrease in total body iron stores produced by DF therapy has been well established, but the question of its efficacy in delaying or preventing the clinical complications of iron overload remains to be answered. Some preliminary evidence is available. Barry et al found decreased amounts of hepatic fibrosis on liver biopsy in chelated as compared to unchelated thalassemics, (Barry 1974), and Cohen, with his small intensively chelated group of patients, was able to reduce liver iron stores to normal or near normal levels and halt the progression of fibrosis. (Cohen 1984) Decreased serum aminotransferase levels following DF chelation have also been reported. (Hoffbrand 1979, Wolfe 1985)

Barry et al also found that only one out of four children on intramuscular DF had delayed puberty as compared to four out of five controls who were delayed. However, in a large study done in Italy, Borgna-Pignatti et al found that growth and sexual maturation were not

improved by DF therapy. Out of 250 hypertransfused adolescents on intramuscular DF for 7-10 years and subcutaneous DF for 3 years, 37% were found to be two standard deviations below the mean for normal height. Sixty-seven percent of the males and 38% of the females aged 12-18 had a complete lack of pubescent changes, and only five out of 121 females went through menarche at a normal age and continued to have normal menstrual cycles thereafter. In addition, a separate analysis of younger children (<14 years) showed that the age at which DF treatment was started had no influence on sexual maturity. (Borgna-Pignatti 1985)

The prevention of cardiac dysfunction has been given particular attention because of the high frequency of death from this complication. Kaye and Owen (1978) found fewer cardiac arrhythmias in "well-chelated" children on intramuscular DF, and Giardina et al (1985) showed a reduction in the incidence of arrhythmias in younger patients started on subcutaneous DF before age 13. A study of left ventricular function by Freeman et al (1983) suggested that in some cases cardiac function can be improved by intensive subcutaneous DF, and a preliminary study by Wolfe et al (1985) shows that DF may at least delay the onset of cardiac disease. In contrast, Borow et al (1982) found abnormal cardiac function in older patients despite what was believed to be "adequate" chelation therapy, and Hyman et al (1985) found asymptomatic cardiac abnormalities in three out of six young compliant patients on subcutaneous and intravenous DF. Overall survival rates were shown to be improved by DF use in a study of 92 British patients by Modell et al (1982), though these findings must be seen as preliminary since the average age of their well-chelated population was only twenty.

## 1.8 CURRENT STUDY

As part of this ongoing effort to assess the clinical effectiveness of DF chelation therapy, this study reviews the annual evaluations of cardiac, hepatic, and endocrine function done on a group of thalassemic patients treated with hypertransfusion and subcutaneous DF at Yale-New Haven Hospital. A number of these patients have been on DF since pre-adolescent years and are now in the age range when complications might be expected. The patients are divided into groups according to compliance, and these groups are compared looking for statistically significant differences in the incidence of complications with attention paid to current age and the age at which DF was begun. Some particularly interesting individual cases are examined as well.

# Chapter II

# METHODS AND MATERIALS

## 2.1 POPULATION

The population for this study consisted of a group of twenty-five patients with beta-thalassemia major or thalassemia intermedia treated with hypertransfusion and subcutaneous DF therapy since 1977 at Yale-New Haven Hospital (YNHH). Four of these patients were eliminated from the study because they were followed on DF for less than three years and, therefore, evaluation of long-term effects could not be made. Two of the four were quite old (>21 years) when they started DF and died (one in an automobile accident and the other from cardiac failure) within three years of beginning the drug. The other two started DF at a young age but moved out of the area shortly thereafter and were lost to follow-up.

As shown in Tables 1A and B, of the twenty-one patients used in the study, three (all sisters) have thalassemia intermedia treated with hypertransfusion therapy since age 6 or 7, and eighteen have beta-thalassemia major. There are 12 females and 9 males, and 12 italians and 9 greeks. The patients range in age from 9 1/2 to 22 years. All but four of them have undergone splenectomy. The age at which they began DF ranges from 4 to 14 1/2 years. Transfusional iron load, which was calculated by estimating a load of 200 mg/kg/yr since

the beginning of hypertransfusion therapy, was >30 gm in all patients, with a range of 31 gm to 155 gm. Three of the older, noncompliant patients have died within the past year. There were no other deaths (aside from the ones in patients excluded from the study) up to this time.

## 2.2 COMPLIANCE

Compliance with chelation therapy was variable. Four of the older patients were put on a dose of 2 gm DF per night, and the other seventeen were put on 1.5 gm per night, giving a dose range of 23.5 to 54.5 mg/kg/night based on weights in 1985. The patients were instructed to use their subcutaneous DF infusion pump for a minimum of eight hours per night for five consecutive nights per week. Compliance with this regimen was judged in two ways. First, the thalassemia nurse specialist who works closely with all of the patients was asked to evaluate their compliance based on patient and parent reports to her and on the frequency with which they requested new DF prescriptions. (Follow-up at the pharmacies was not possible because of the wide number of pharmacies used by our population.) The nurse gave each of the patients a rating of compliant, borderline, or noncompliant. Then, a confidential questionnaire was sent to each of the patients still living with a cover letter assuring them that their answers would be seen only by this investigator. They were asked to state whether they used their DF >5 times/week, 5 times/week, 4-5 times/week, 4 times/week, 3-4 times/week, etc. Patients who used their DF 4-5 times/week or more were judged compliant; those who used it 3 or 4 times/week were called borderline

compliant, and those who used it less were called noncompliant. Patients were also asked if they used their DF any more or less in 1985 than in previous years and whether they had periods when they did not use it at all in order to judge the consistency of their reports. All but one of the eighteen living patients responded, and all but two of these responses were consistent with the judgement of the nurse. The compliance ratings of the deceased patients were confirmed by conversations between their physician and their parents following their deaths.

Eleven patients were clearly compliant with their DF regimen, and ten patients were judged to be borderline or noncompliant. One of these ten, patient R, was very compliant (6x/week) for the first four years of DF use, then became noncompliant for two years and has been borderline compliant for the past two years. She was given an overall rating of borderline, but her initial compliance is taken into account in several instances. For most of the data analysis and discussion in this study, the noncompliant and borderline patients are grouped together and called "noncompliant". The mean age of the compliant population in 1985 was 13.9, significantly lower (by 2-tailed t-test, $p < .05$) than the mean age of 17.2 of the noncompliant population. The mean ages at which the two groups began DF is also significantly different at 8.6 yrs for the compliant group and 10.8 years for the noncompliant group, ($p < .05$). These differences in age are taken into account in parts of the data analysis in which age could play an important role.

## 2.3 ANNUAL EVALUATIONS

In order to assess the effectiveness of DF therapy, evaluations of iron load and liver, endocrine and cardiac function were done on each patient before beginning DF and every year thereafter. These annual evaluations consisted of a physical exam, including height, weight, and Tanner staging of sexual maturity, a serum ferritin level, liver function tests, an oral glucose tolerance test, an electrocardiogram or 24-hour Holter monitor, and an echocardiogram.

## 2.4 SERUM FERRITIN

Serum ferritin levels were measured by radioimmunoassay in the YNHH immunology laboratory. The laboratory's range of normal for serum ferritin is 9-200 ng/ml. The range found in the thalassemic patients was approximately 800 to 8,500 ng/ml. Extremely high values (9,000 to 11,000) associated with temporarily elevated liver function tests were considered to be secondary to non-A non-B hepatitis and were not included in the analysis.

## 2.5 LIVER FUNCTION TESTS

Measurements of SGOT, alkaline phosphatase, and direct and indirect bilirubin were done in the usual fashion in the chemistry laboratory of YNHH. The laboratory's ranges of normal are as follows: SGOT 15-30 units, alkaline phosphatase 10-70 units, direct bilirubin <.30 mg/dl, and total bilirubin <1.50 mg/dl. Statistical analysis was carried out only on the SGOTs in part because of a large number of missing values

for the other tests. In addition, bilirubin levels were found to be within normal limits with only a few sporadic exceptions and, therefore, were not felt to be of much value in assessing liver function in this population. The alkaline phosphatase levels also posed problems because of their variability in growing children.

## 2.6 ORAL GLUCOSE TOLERANCE TESTS

Oral glucose tolerance tests (OGTTs) were done with a glucose load of 1.75 gm/kg up to 100 gms. Samples of venous blood were drawn in the fasting state and at 15, 30, 60, 90, and 120 minutes after the glucose load. The tests were rated normal, impaired, or diabetic based on the 1979 classifications of diabetes mellitus in children by the National Diabetes Group. (See Table 2.) Two patients who had OGTTs consistent with borderline impairment for one year whose tests the next year were well within normal limits were called normal. Family histories of diabetes were obtained through telephone conversations with the patients' parents, most of whom were felt to be reliable historians.

## 2.7 GROWTH AND PUBERTAL DEVELOPMENT

Three criteria were used to evaluate the normalcy of growth and development in the thalassemic children: growth curves, bone age, and Tanner staging numbers. The patients' heights and weights were measured on a regularly calibrated medical step-up scale located in the clinic where they receive their monthly transfusions. Growth curves for height were plotted on standard growth charts based on these measurements. The

curves were judged to be normal or abnormal by three pediatricians who were blinded to the compliance ratings. Curves that the clinicians found difficult to categorize or over which there was disagreement were rated borderline. Both parental heights and the use of exogenous reproductive hormones to promote pubertal development were taken into account in assigning a final rating to each curve.

Bone ages were determined from radiographs of the left hand and wrist and were rated normal or delayed in comparison with chronological age based on the standards of Greulich and Pyle. There were no patients with significantly advanced bone age.

Tanner stage was judged each year by one of two pediatric attending physicians or, occasionally, by members of the pediatric housestaff. The standard scale of 1 to 5 established by Tanner, with 1 being prepubertal and 5 being full adult development, was used. (Tanner 1978) A single number was assigned for each child for each year, representing the average of the ratings for breasts and pubic hair in girls and phallus and pubic hair in boys. Patients who required testosterone or estrogen supplementation to begin or complete puberty were automatically considered abnormal. Those who were at a Tanner stage within normal limits for their age without the help of exogenous hormones were considered to be normal.

## 2.8 CARDIAC FUNCTION

Echocardiograms, ekgs, and, in 1985, 24-hour Holter monitors were used to evaluate cardiac function. Standard 12 lead ekgs were done each year looking for arrhythmias, T wave changes, and evidence of LVH. However, the ekgs were found to be inaccurate in assessing LVH (as compared to echocardiographic measurements of left ventricular wall thickness) and inadequate for picking up minor arrhythmias. For this reason, 24-hour Holter monitors were done on each child during the 1985 evaluations. Patients with only extremely rare premature atrial contractions (PACs) were called normal. Those with more frequent PACs, rare atrial couplets, junctional ectopic beats, or rare premature ventricular contractons (PVCs) were called borderline, and those with more frequent PVCs or supraventricular tachycardias such as atrial flutter or fibrillation or ectopic atrial tachycardia were called abnormal.

Ejection fractions were calculated using the Teicholz method from standard M-mode echocardiograms by two pediatric cardiologists who double checked each other's work. The normal range for ejection fractions calculated by echocardiogram at YNHH is considered to be between 50-70%. During 1983 (and, in some cases, 1984), the left ventricular posterior wall thickness during end-diastole (LVPW) was also recorded. Normal values for LVPW based on body surface area were taken from the work of Roge et al. (Roge 1978) Measurements of left ventricular end-diastolic chamber dimension were taken into account in rating the LVPWs normal, borderline, or abnormal in order to assure that

wall thickness did not appear to be within normal limits simply because the wall was stretched due to ventricular dilatation.

## 2.9 ANALYSIS

Analysis of variance for repeated measures was used to determine the difference in trends between compliant and noncompliant groups for both serum ferritin levels and SGOTs. This was followed up by t-tests to evaluate the difference in the change in the means for each year out from DF. For more qualitative tests, chi-square was used to evaluate the differences between compliant and noncompliant groups. Because in most instances there was a number less than five in at least one of the cells for chi-square, a Fischer exact test was used to confirm the findings. The analyses of variance and graphs of serum ferritin and SGOT levels were done on Clinfo Computer Systems sponsored by the NID, DRR, and the Yale General Clinical Research Center.

# Chapter III

# RESULTS

## 3.1 SERUM FERRITIN

Analysis of variance for repeated measures was done to look at the difference in the change in serum ferritin levels over time between the compliant and noncompliant groups. Two noncompliant patients and one compliant patient were eliminated from the analysis because their pre-treatment serum ferritin levels were either missing or believed to be falsely elevated due to hepatitis. Patient R, whose overall compliance rating is borderline but who was compliant with the first four years of DF, was grouped with the compliant patients for this analysis. The analysis was performed for all patients who had no missing values every year up to three years, four years, and five years out on DF. A significant difference in the trends between the two groups was found for all three time spans. The analysis was not done beyond five years because less than half of the patients have been on DF more than five years, and the numbers became too small.

Two-tailed t-tests for repeated measures done for the noncompliant group for each year after beginning DF up to five years out showed that there was no significant change in serum ferritin level from pre-DF levels for any of the years. In the compliant group, however, there was a significant change in ferritin level from pre-DF levels for every year on DF up to five years out.

The direction of this change is shown in Figure 3, which gives the means of the serum ferritin levels of the compliant and noncompliant groups prior to starting DF (year 0) and every year afterward. Patient R's levels were averaged with the compliant group for the first four years and with the noncompliant group for the last four years. The standard errors are plotted on either side of the means to give a precision band. This band clearly widens after the fifth year as might be expected from the fall in the number of values used to determine the means. Years seven and eight are not shown for the compliant group because there were less than three measurements in those years.

As seen in Figure 3, the mean ferritin levels for the two groups are almost identical at about 4,300 ng/ml prior to beginning DF. The mean of the compliant group falls precipitously with the start of DF to 2,785 and remains at a level of 3,000 or less. The noncompliant group, on the other hand, shows no fall in serum ferritin and remains at a level >4,000 ng/ml for all nine years. Thus, compliance with DF produced a significant and sustained decrease in serum ferritin levels that was not seen in the noncompliant population.

## 3.2 LIVER FUNCTION TESTS

None of the twenty-one patients had SGOTs within the normal range of 15-30 units prior to beginning DF therapy. Four of the compliant patients had their SGOTs fall within normal range after starting DF as shown in Table 3. In some others, levels fell but were still above normal limits. None of the noncompliant patients achieved a normal SGOT.

Analysis of variance for repeated measures revealed that there was a significant difference ($p < .05$) in the change in SGOT over time between the compliant and noncompliant groups up to three years after starting DF. However, when the analysis was carried out to five years, the difference in the trends did not reach significance.

The changes in the mean SGOT levels in each group over time are illustrated in Figure 4. The mean SGOTs of the two groups begin within ten units of each other at time 0 prior to DF but then diverge as the mean for the compliant group falls during the first three years on DF. However, in the forth and fifth years the mean SGOT of the compliant group rises and the widening standard errors of the two groups overlap. Thus, DF appears to have been effective in decreasing ongoing liver damage as measured by SGOT during the first few years of use but not over a longer period.

## 3.3 ORAL GLUCOSE TOLERANCE TESTS

As seen in Table 4, eight out of ten noncompliant patients had abnormal OGTTs. Five of these were rated impaired and three were fully diabetic. Of the eleven compliant patients, only one, pt O who became impaired just this year at age 18, had an abnormal OGTT. Of note is that patient R, who has an overall compliance rating of borderline but who was extremely compliant for the first four years of DF treatment, developed impairment relatively late, at age 17, two years after she became noncompliant with therapy. The average age of onset of impairment in the noncompliant population is 14.1 years with a range of

9-19 years. The average age of the compliant population is now 13.9 years (with a range of 9 1/2 to 19 years), closely approaching the average age of onset of impairment in the noncompliant group. Five of the compliant patients are now 15 years or older, well within the age range for developing diabetes, and only one of them has developed any abnormality. This suggests that DF is at least delaying the onset of impaired carbohydrate metabolism in compliant patients.

The calculated risk ratio for the entire age range of patients is 8.9, indicating that the risk of noncompliant patients developing diabetes was about nine times greater than the risk among compliant patients. (Chi-square is significant at $p < .05$.) Because abnormal glucose tolerance is much less commonly seen in thalassemics on any treatment regimen before age 11 (Zuppinger 1979, Flynn 1976, Necheles 1974), the risk ratio was also calculated using only patients eleven years or older to eliminate the possibility that the younger patients were biasing the sample in favor of the compliant group. The risk of developing diabetes was 5.4 times greater in the noncompliant patients using this subgroup, (chi-square significant at $p < .05$), indicating that the age difference between the two groups did not bias the results.

Previous studies have shown that there is a positive correlation between a family history of diabetes and the development of diabetes as a complication of iron overload in thalassemia (Costin 1977, Flynn 1976, Wolman 1969). In this study, three compliant patients and four noncompliant patients had a family history of adult onset diabetes mellitus (AODM). (Table 4) The one compliant patient who is impaired

did have a maternal great grandmother with AODM. Only three of the eight noncompliant patients with impairment had a known family history of diabetes, and none of the noncompliant children whose impairment is known to have begun at age 13 or less had a positive family history. This finding indicates that the early onset of impairment may have had more to do with noncompliance with DF than with genetic factors.

## 3.4 GROWTH AND PUBERTAL DEVELOPMENT

As seen in Table 5, there was essentially no difference between the compliant and noncompliant groups in the proportion of children with normal, borderline, or abnormal growth curves. Thus, growth did not correlate with DF use in a general sense, but there were some more subtle findings that should be noted. Despite the reported highly beneficial effects of hypertransfusion therapy on prepubertal growth, only one of the five prepubertal children had a completely normal growth curve. The other three compliant prepubertal children had borderline curves, and the one noncompliant child had an abnormal curve. The child with the normal curve was also the one who began DF at the earliest age (4 yrs). These findings suggest that early DF use may be important for preadolescent growth.

A difference in propensity for growth disturbance between males and females was also noted. Only one of the eight males, (the 10 yr old boy who began DF at age 4), had a completely normal growth curve, whereas six of the twelve females had normal curves. Of the compliant patients,

80% of the males and 66.7% of the females had borderline or abnormal curves. In the noncompliant group, 100% of the males and only 33.3% of the females had borderline or abnormal curves. These figures indicate that noncompliant males were at greater risk of having stunted growth than were females but that compliant males were at no significantly increased risk.

Contrary to the finding with growth curves, there was a marked difference in the incidence of delayed bone age between compliant and noncompliant groups. (Table 5) Only one compliant patient, the one who began DF the latest, had a delay in bone age, whereas all but two of the noncompliant patients had delayed bone ages. Delay in bone age also correlated very closely with abnormal pubertal development. The only two noncompliant patients who had normal bone ages were also the only two who had normal Tanner staging for their ages.

Because pubertal development would not be expected before age 11, only patients aged 11 or more were included in the analysis of Tanner staging. Nine of these patients had abnormal pubertal development. (Table 7) All nine of them eventually received hormone replacement therapy. Seven of the nine abnormal patients were noncompliant with DF. Seven patients age 11 or more have gone through normal puberty or have normal Tanner ratings for their ages. (Table 6) Only two of the seven are noncompliant with DF. Though the more successful outcome for the compliant patients is obvious, chi-square did not reach significance.

The mean age at pubarche in the normal group was 12.1 yrs with a range of 11.5 to 13 yrs. This differs sharply from the delayed mean age

at pubarche (for those who experienced it) in the abnormal group of approximately 14 with a range of 13-16. The two noncompliant patients with normal Tanner ratings for their ages did not experience pubarche until age 13, the late end of the normal range.

Although the normal group is younger than the abnormal group, the average Tanner number at the time hormones were begun in the abnormal group was 2.3, and the four compliant patients aged 13 or more have now far surpassed that rating. On the other hand, the two noncompliant 13 yr old boys with a Tanner rating of 2, within normal limits for their age, have not yet surpassed it, suggesting that their future normal development may be less certain.

The mean age at which DF was begun in the normal group is 8.8 years, significantly lower than the mean age of 11.9 years for beginning DF therapy in the abnormal group. (T-test significant at $p < .05$.) All the normally developed patients, except patient K, began DF at less than ten years of age, whereas all of the patients with abnormal pubertal development began DF at 10 years of age or more.

The importance of the age at which DF therapy is started is exemplified by the case of the three sisters with thalassemia intermedia. All three sisters have been compliant with DF, but their growth and pubertal development have been very different. The eldest sister, who began DF at age 13, has an abnormal growth curve, delayed bone age, and abnormal pubertal development. Her two younger sisters, on the other hand, who began DF at ages 10 and 12 have had completely normal adolescent growth spurts and bone ages. They each underwent

menarche two years after beginning DF and have continued to have normal menstrual cycles with Tanner stages almost at the adult level. It could be argued that these two girls, (who are the only ones who have experienced spontaneous menarche without secondary amenorrhea), had an advantage because they have thalassemia intermedia and did not start hypertransfusion therapy until after age 5. However, in light of what happened to their sister and the fact that iron absorption from the gastrointestinal tract can be assumed to have been very high prior to the beginning of regular transfusions, much of their successful development can probably be attributed to their relatively early use of DF.

## 3.5 CARDIAC STATUS

Cardiac status was evaluated by means of LVPW thickness during end-diastole to look for evidence of left ventricular hypertrophy (LVH), 24-hour Holter monitors to look for arrhythmias, and ejection fractions calculated from echocardiograms to look for changes in myocardial function.

The LVPW thicknesses taken from 1983, before any of the patients developed serious heart failure, show a marked difference between the compliant and noncompliant populations. (Table 8) All ten of the compliant patients measured had normal ventricular wall thickness based on body surface area, whereas four out of the eight noncompliant patients measured had borderline or abnormally thick left ventricular walls. (Significant at $p<.05$ level by Fischer's exact test.) These

findings provide preliminary evidence that LVH as measured by LVPW on echocardiogram may be prevented or delayed by DF.

Four out of eleven compliant patients had borderline or abnormal Holter monitors. However, one of the four is a 12 yr old girl (pt F) with frequent PVCs that she has had since early childhood and that disappear with exercise. Her arrhythmia is not believed to be related to her thalassemia, and her inclusion amongst the abnormal patients is questionable. Five out of the nine noncompliant patients who got Holter monitors in 1985 had abnormalities. Details of these abnormalities are shown in Table 9. Though by far the most serious arrhythmias were seen in the three noncompliant patients who died, there is not a statistically significant difference in the incidence of arrhythmia between the compliant and noncompliant groups when minor abnormalities are counted. The importance of these minor arrhythmias in predicting future, more serious irregularities is not clear, however.

As can be seen in the samples from 1985 shown in Table 9, most of the calculated ejection fractions for both compliant and noncompliant patients fall well within the normal range of 50-70%. A few are greater than 70, representing a hyperdynamic state, possibly related to mild anemia at the time of testing. Through out the years of testing, most of the patients' ejection fractions have fluctuated randomly but have remained within normal limits. However, two of the patients have shown fairly consistent trends that are of interest. (Table 10) Patient S, who is noncompliant, has a borderline Holter, and has already had an episode of pericarditis, has shown a slow steady fall in her ejection

fraction from 63 in 1978 to 52, (the lower limits of normal), in 1985. Patient O, on the other hand, who is one of the most compliant patients, began DF therapy with an ejection fraction of only 46. Her ejection fraction continued to fall slightly for the first three years of DF, but over the past five years it has risen to 66, a value well within normal limits.

Calculation of the mean of the ejection fractions before DF and each year thereafter showed that there was no difference between the compliant and noncompliant groups until six to eight years out when there were very few data points and the falling values of the three patients who died pulled the mean of the noncompliant group down. In two out of the three patients who died, the ejection fraction was well within the normal range until one to two years prior to death. The third patient, (pt U), had an ejection fraction consistently below normal for the six years preceding her death. However, her fall in ejection fraction corresponded to the start of disopyramide for atrial fibrillation, so the decrease in myocardial contractility may, at least in part, have been a drug effect. Hence, ejection fractions calculated by echocardiogram do not seem to have been a very sensitive measure of cardiac status since abnormalities appear only very late in the course.

# Chapter IV

# DISCUSSION

## 4.1 INTRODUCTION

By dividing the patients in this study into compliant and noncompliant groups, we were able to compare a relatively well-chelated population with a minimally chelated one. This comparison revealed that steady use of subcutaneous DF produces a significant drop in serum ferritin and SGOT levels not seen with sporadic or infrequent use. Impairment of glucose tolerance was at least delayed if not prevented in the compliant group as compared to the noncompliant one, and bone age and pubertal development were markedly improved when DF was begun at an early age. On the other hand, there was essentially no difference in the incidence of growth curve abnormalities between the "well-chelated" and minimally chelated populations. Although some preliminary evidence revealed that left ventricular hypertrophy may be prevented or delayed by a five night/week subcutaneous DF regime, this regime's overall effectiveness in delaying or preventing arrhythmias and myocardial dysfunction was less apparent. In examining the specifics of these findings, a number of recommendations for future evaluation and treatment of thalassemic patients can be made.

## 4.2 COMPLIANCE

Though they made a good, if imperfect, control group, the fact that ten out of twenty-one patients were borderline or noncompliant with their DF treatment is distressing. The significantly older age of the noncompliant group is at least in part a reflection of the tendancy of adolescents to rebel against medical intervention. This tendancy towards noncompliance with DF in the teenage years has been widely noted (Cohen 1985, Giardina 1985, Piomelli 1985), and some authors have argued that starting DF at a very early age would make use of the subcutaneous infusion pump a routine, accepted habit so that noncompliance might be less of a problem in later years. (Modell and Berdoukas) The fact that the mean age at which DF was begun is significantly lower in the compliant population than in the noncompliant one supports this reasoning, and is just one of numerous findings in this study supporting the idea that the early initiation of DF chelation therapy is crucial. Other suggestions that have been made for reinforcing compliance include asking patients to keep a daily record of DF use (Modell and Berdoukas, Lancet 2/28/84) and asking them to use their DF more often than is actually felt to be necessary. (HA Pearson, personal communication) Sherman et al found a positive correlation between poor psychiatric adjustment and poor compliance in thalassemic patients and recommended psychiatric intervention in such cases as a means of improving compliance. (Sherman 1985) Persistance in the area of compliance is a necessity. In the words of Modell and Berdoukas, "all patients need unflagging encouragement to persevere with their chelation therapy." (Modell and Berdoukas 1984)

## 4.3 SERUM FERRITIN

Since the ability of DF to lower serum ferritin levels is fairly well established, (Wolfe 1985, Hyman 1985), the fact that there was a significant difference in mean ferritin levels between the compliant and noncompliant groups acts as a confirmation of their compliance ratings. The lack of any fall in serum ferritin levels in the noncompliant group, many of whom were actually rated borderline compliant, suggests that use of DF less than four nights/week is basically ineffective. It is encouraging that the use of DF five nights/week kept serum ferritin levels lower on a long-term basis. However, on this regimen, the ferritin levels fell to a mean of approximately 3,000 ng/ml and remained there rather than continuing to fall. Only one child ever reached a level below 1,000 ng/ml (which is still five times the normal level). In other words, these children are clearly still iron overloaded. Cohen et al have shown that with a combination of intensive subcutaneous and intermittent intravenous DF it is possible to lower the serum ferritin levels of thalassemic children to within normal range. (Cohen 1981) Although no other group has yet been able to completely reproduce Cohen's results, perhaps this is the goal we should be striving for.

## 4.4 LIVER FUNCTION TESTS

The fall in SGOT levels seen in the compliant group over the first three years on DF is as might be expected with a decrease in iron load and presumably, therefore, decreased hepatocellular damage due to iron toxicity. The subsequent rise in SGOT in this group after three years

may in part have been due to chronic underlying non-A, non-B hepatitis secondary to transfusion. (Moroni 1984) However, the possibility of ongoing liver damage secondary to iron overload must be entertained. Cohen et al were able to demonstrate a drop in stainable liver iron in liver biopsy specimens from Grade IV to Grades 0-I in his group of heavily chelated thalassemics whose serum ferritin levels concurrently fell to within normal range. We cannot assume that with serum ferritin levels in the 800-4,000 range our compliant patients' livers are not still iron overloaded or that hepatocellular damage is not still occurring.

Even though SGOT levels seem more sensitive and reliable than other routine liver function tests in assessing the presence of ongoing hepatocellular damage in our thalassemic population, they are in no way specific to damage from iron overload and, so, are not ideal as a measure of the effectiveness of DF in reducing hepatic siderosis and fibrosis. Yet they remain our most reasonable, if imperfect, measure of liver status since other methods, such as nuclear magnetic resonance (Brasch 1984) or liver biopsy, are either too expensive and poorly worked out or too invasive to be used for routine evaluation.

## 4.5 ORAL GLUCOSE TOLERANCE TESTS

The use of subcutaneous DF clearly delayed the onset of impaired glucose tolerance in our compliant population as compared to our noncompliant one. This represents a new and exciting finding in that none of the other studies of the effectiveness of subcutaneous DF have

specifically addressed the problem of impaired carbohydrate metabolism. Unfortunately, it cannot yet be stated that the current level of DF use will completely prevent impaired glucose tolerance and diabetes, especially in light of the fact that one of our most compliant teenagers has developed impairment at age 18.

Oral glucose tolerance tests seem to be a relatively sensitive and uncomplicated way of detecting early impairment of carbohydrate metabolism and can be recommended for use in yearly evaluations of thalassemic patients. Simultaneous measurement of insulin levels would give further information as to whether abnormalities were due more to decreased insulin secretion from the pancreas or to increased peripheral resistance.

## 4.6 GROWTH AND DEVELOPMENT

Our finding that the incidence of growth curve abnormalities did not differ significantly between compliant and noncompliant populations is in agreement with the findings of the large study of Italian children done by Borgna-Pignatti's group. Given the positive effect of DF seen in other areas, these findings are somewhat surprising. Their explanation may lie in our observation that the only prepubescent child with a completely normal growth curve was the one who began DF the earliest, at age 4, (earlier than most, if not all, of Borgna-Pignatti's population). Growth may be such a sensitive parameter that it can be effected by even moderate iron overload. Although prepubescent growth abnormalities were thought to have been corrected by hypertransfusion

therapy, the finding of more subtle, "borderline" abnormalities in the three compliant pre-adolescent children who started DF later suggests that iron overload may already be effecting their growth and, again, argues for the earlier initiation of DF.

Our data show that DF is preventing delayed bone age in compliant patients. The fact that DF seems to be helping bone age but not growth in the compliant patients and that normal growth was seen in noncompliant patients with delayed bone ages is curious. (Table 5) A more careful examination of the growth curves of the four noncompliant females with delayed bone age and abnormal pubertal development but "normal" growth curves may provide an explanation. Two of these patients had no recorded heights prior to the end of the adolescent growth spurt so that only the "tail-end" of their growth curves could be seen. Three of them showed an upward rise in their growth curves (two from the 5th to the 25th percentiles and one from the 10th to the 50th percentile) after age 13 1/2, that is, after the normal adolescent growth spurt should have been over. These observations suggest that the delay in bone age may actually have worked in favor of these patients allowing them to eventually, though belatedly, reach a normal height before their epiphyses fused. The observations also call into question the normal ratings originally given to these curves. Further study of the bone age-growth curve relationship in thalassemic patients would be of interest.

Pubertal development in our population seemed to be improved by subcutaneous DF if it was begun at an early enough age. This finding is

not in agreement with the results of the Borgna-Pignatti study. While it is entirely possible that our current data, which was suggestive but not statistically significant, will not hold out as our compliant group becomes older, examination of the data from Borgna-Pignatti's work reveals one possible explanation for the disagreement. The median age of their population was 13 yrs 10 mos (with 25th and 75th percentiles of 12 and 15 1/2 years), and the median amount of time on subcutaneous DF was 3 yrs 4 mos (with 25th and 75th percentiles of 3 yrs 0 mos and 3 yrs 9 mos). By these numbers, it appears that only a small percent of their population within the age range of expected puberty began subcutaneous DF before age 10 and an even smaller number began it before age 9. In contrast to this, the mean age of beginning DF in our normally developed population was 8.9 yrs with the majority of the patients being in the 7 to 8 year range. In addition, the ability of Borgna-Pignatti's group to judge compliance in such a large, scattered population of patients might be questioned. The major conclusion to be drawn from this data is that the final word is not yet in. As Borgna-Pignatti herself states:

> It is possible that endocrine glands are extremely sensitive to iron toxicity and that even small amounts of it, accumulated in the first years of life, produce damage that cannot be reversed. Perhaps both hypertransfusion and aggressive chelation therapy must be initiated very early in life, around age 3 years...or possibly even earlier, to ensure a favorable prognosis for growth and puberty.

## 4.7 CARDIAC FUNCTION

Our finding that ejection fractions in thalassemics did not deteriorate until approximately one year before death confirms the findings of numerous other authors. (Giardina 1985, Nienhuis 1980, Henry 1978, Weiner 1978) Because most thalassemics patients die of cardiac failure, myocardial function is one of the most important parameters to be measured in following their course and evaluating the effectiveness of DF. Ejection fractions calculated from echocardiograms are clearly inadequate for this task, and more sensitive techniques need to be employed. Several such techniques have already been developed. In a study of twenty-four thalassemic patients, Leon et al found that using radionuclide cineangiography to determine ejection fractions during excercise often reveals myocardial dysfunction not yet apparent when ejection fractions are determined at rest. (Leon 1979) These findings were confirmed in a study by Nienhuis et al in 1980. The measurement of left ventricular end-systolic pressure-dimension relation developed by Borow's group may be even more helpful in revealing early cardiac abnormalities in thalassemic patients because it evaluates intrinsic contractile state independent of the changes in sympathetic tone and preload and afterload that occur with exercise, and it requires minimal patient cooperation. (Borow 1982)

While echocardiograms are not very useful in detecting early myocardial dysfunction, they can be used to look for left ventricular hypertrophy. Several authors have noted increased left ventricular wall thickness in asymptomatic thalassemics with normal ejection fractions.

(Weiner 1978, Henry 1978) Our measurements of LVPW for 1983 showed a significant difference between the compliant patients, who all had normal wall thickness based on body surface area, and the noncompliant patients, four of whom showed borderline or abnormally increased wall thickness. This finding gives a preliminary indication, first, that DF may be delaying or preventing left ventricular hypertrophy and, second, that measurement of LVPW by echocardiogram might be one reasonable method of detecting asymptomatic cardiac abnormalities in thalassemics. Yearly measurement of LVPW and correlation with both compliance and other measures of cardiac status will be necessary to verify these two hypotheses.

The presence of mild arrhythmias in three of our patients less than 14 years of age (two of whom were compliant) is in accord with the findings of mild cardiac abnormalities in young, compliant patients in two other recent studies. (Giardina 1985, Hyman 1985) Although the significance of these minor arrhythmias is not completely clear, their presence is somewhat alarming. Hyman et al argue that

> the presence of cardiac abnormalities in...[young, compliant patients]...suggests that to prevent heart damage in thalassemia, it may be necessary to start the hypertransfusion and effective chelation at a very young age, realizing that the amount of iron removed will be small. (Hyman 1985)

Giardina was able to demonstrate a decreased incidence but not a complete disappearance of these arrhythmias with prolonged DF use. (Giardina 1985) The presence of arrhythmias in older, compliant thalassemics, though less surprising, also suggests the need for earlier, more intensive chelation.

Prolongation of life is both the ultimate measure of cardiac function in thalassemia and the most important goal of DF therapy. In this respect, the compliant group, with no deaths and no symptomatic cardiac dysfunction, is faring much better than the noncompliant group, indicating some positive effect of chelation therapy. However, the mean age of death in nonchelated patients as calculated by Modell and Berdoukas is 18 years with a range of 11-24 years, and only two of the compliant patients have even reached 18 much less lived beyond age 24. The large study of British patients done by Modell gave preliminary evidence that DF is prolonging life, but the definitive answer to this question will not be available until patients started on subcutaneous DF at a relatively early age have had the chance to live well into their 20's.

## 4.8 CONCLUSION

The results of this study indicate that many of the complications of iron overload seen in thalassemic patients are being delayed or decreased in severity by subcutaneous DF therapy. However, the results also indicate that earlier more intensive chelation is necessary in order to have any hope of actually preventing complications and allowing these children to live relatively normal lives.

The argument for not starting DF chelation before age 5 or 6 has been that net negative iron balance could not be achieved before that age because the iron load would not be high enough. However, Fargion et al, in a study of twenty-eight thalassemics from 11 months to 4 years of

age, showed that with a subcutaneous DF dose of 40 mg/kg, high rates of iron excretion could be achieved in even the youngest patients. They recommended that subcutaneous chelation be started as early as the third transfusion. (Fargion 1982) In addition, several authors have argued that even if net negative iron balance cannot be achieved in the very early years, chelation should be started to slow the rate of iron accumulation. An iron load high enough to easily achieve net negative iron balance with current doses of DF is already much too high. (Hyman 1985, Piomelli 1985) In a study of labelled iron storage and excretion, Bianco et al found evidence that newly released iron "concentrates initially in a reticuloendothelial pool which is readily chelatable, and then 14-16 days]...moves to a larger and hardly chelatable storage pool." They, too, advise that DF chelation begin early in life before large amounts of iron have become part of the permanent storage pool. (Bianco 1984)

The need for not only earlier but also more intensive chelation is suggested by serum ferritin levels of 1500 or more (1480-2800) even in the four patients who began DF the earliest (ages 4-6 1/2) and by levels up to 4000 in some of the older compliant patients. Conceivably, this more intensive chelation might be achieved by increasing use of the subcutaneous pump to 7 nights per week. But because of the difficulties with compliance with the pump, two investigators have found it beneficial to also give high dose intravenous DF with each transfusion. Using subcutaneous DF at 20-60 mg/kg/night for 5-6 nights per week plus 48 hour intravenous DF (at 0.5 gm/hr) with each transfusion, Hyman has achieved a median serum ferritin level of 945 (with a range of 555-2300)

in her youngest group of patients (ages 4-9 1/2). (Hyman 1985) Cohen's data is less clearly presented, but he appears to have ten patients with serum ferritin levels less than 1000 on 2 gm/night of subcutaneous DF 6-7 nights per week plus 24 hour intravenous infusion of 9-16 grams of DF every three weeks in six of the ten patients. (Cohen 1985) Cohen's patients begin the intravenous DF in the hospital setting with their transfusions and then finish it at home. Hyman has her patients hospitalized in socially compatible groups to receive their transfusions and intravenous DF, thus allowing her to incorporate an intensive psychosocial support program into the treatment.

The recent finding of visual and auditory neurotoxicity in young thalassemics on high doses of subcutaneous DF (Olivieri 1986) suggests that there are limits to the dose of subcutaneous DF that can safely be used. However, it does not suggest that the dose of DF used in our population cannot be increased or that earlier chelation is unsafe if dosage is controlled for the weight of the child. The higher incidence of neurotoxicity in younger children found in the study appeared to be due mainly to the fact that these children were smaller and therefore their dose of DF per kilogram of body weight was higher. The DF dose in the Olivieri study ranged as high as 150 mg/kg/night whereas the dose in our population ranged from 23.5 to 54.5 mg/kg/night. Only three children in our study were on a dose as high as the 50 mg/kg/night recommended by the Olivieri group as being safe for younger patients. The most cautious approach would be a yearly analysis of DF-induced urinary iron excretion to find the minimum DF dose at which maximum iron excretion can be achieved in each patient, keeping in mind the 50

mg/kg/night recommendation. Complete eye examinations, audiologic testing, and studies of visual evoked potentials should also be done at least every six months, if not every three months as recommended by Olivieri.

Desferrioxamine chelation is a burdensome, difficult, and expensive therapy. It demands an enormous expenditure of time and energy on the part of both the patients and their physicians, nurses, and social workers, who must coordinate complicated treatment plans and continually reinforce and encourage compliance. Successful implementation of DF chelation is even more difficult in many of the poorer, less medically well-developed Mediterranean countries in which thalassemia is most prevalent. However, despite these drawbacks, hypertransfusion and intensive DF chelation are the most promising treatment for thalassemia currently available. No successful oral chelating agent has yet been found, and hopes for a cure through bone marrow transplantation or genetic manipulation of the fetal hemoglobin gene are distant. (Lancet 8/24/85, Lancet 1/8/83) If used intensively, desferrioxamine chelation offers thalassemic children the best possibility of normal growth and development and an extended life span, and its use should be vigorously continued until other solutions for the treatment of this complicated disease can be found.

Table 1A PATIENT POPULATION (part 1)

| Pt | Age (yr:mo) | Sex | Ethnic back-ground | Diagnosis | Age of Splenectomy (yr:mo) |
|---|---|---|---|---|---|
| A | 9: 6 | F | italian | thal maj | 8: 0 |
| B | 9: 6 | M | greek | thal maj | 6: 8 |
| C | 10: 0 | F | italian | thal maj | 5:10 |
| D | 10: 6 | M | greek | thal maj | 9: 8 |
| E | 10: 6 | M | italian | thal maj | 7: 8 |
| F | 12: 6 | F | italian | thal maj | 7: 7 |
| G | 13: 0 | M | italian | thal maj | 5: 8 |
| H | 13: 6 | M | greek | thal maj | 10: 7 |
| I | 13: 6 | F | greek | thal int | not done |
| J | 15: 0 | M | italian | thal maj | not done |
| K | 17: 0 | F | greek | thal int | not done |
| L | 17: 0 | M | greek | thal maj | not done |
| M | 17: 0 | M | italian | thal maj | 5: 9 |
| N | 17: 0* | F | italian | thal maj | 5: 0 |
| O | 18: 0 | F | italian | thal maj | 5: 0 |
| P | 19: 0 | F | greek | thal int | 8: 1 |
| Q | 19: 0 | F | italian | thal maj | 12: 4 |
| R | 19: 0 | F | italian | thal maj | 3: 0 |
| S | 20: 6 | F | greek | thal maj | 12: 8 |
| T | 21: 6* | M | greek | thal maj | 12: 1 |
| U | 22: 0* | F | italian | thal maj | 10: 5 |

*deceased

Table 1B PATIENT POPULATION (part 2)

| Pt | Age Hypertx Begun (yr:mo) | Units of Blood Tx'd (250cc/unit) | Estim Fe Load (gms) | Age DF Begun (mo:yr) | Dose of DF (gms) | Compliance |
|---|---|---|---|---|---|---|
| A | 0: 7 | 175 | 35 | 6: 0 | 1.5 | borderln |
| B | 0: 4 | 182 | 36 | 6: 7 | 1.5 | comp |
| C | 3: 0 | 155 | 31 | 5:10 | 1.5 | comp |
| D | 3: 0 | 198 | 40 | 4: 0 | 1.5 | comp |
| E | 0: 9 | 229 | 46 | 7: 8 | 1.5 | comp |
| F | 2: 0 | 272 | 55 | 7: 7 | 1.5 | comp |
| G | 0: 9 | 343 | 69 | 8:10 | 1.5 | borderln |
| H | 0: 7 | 341 | 68 | 8: 8 | 1.5 | borderln |
| I | 6: 0 | 263 | 53 | 9:11 | 1.5 | comp |
| J | 1: 0 | 450 | 90 | 7: 5 | 1.5 | comp |
| K | 7: 0 | 400 | 80 | 12: 2 | 1.5 | comp |
| L | 0: 6 | 510 | 102 | 9: 6 | 1.5 | comp |
| M | 0: 6 | 495 | 99 | 10: 1 | 1.5 | noncomp |
| N | 2: 8 | 461 | 92 | 11: 2 | 1.5 | noncomp |
| O | 2: 0 | 585 | 117 | 10: 3 | 2.0 | comp |
| P | 9: 0 | 360 | 72 | 13: 2 | 1.5 | comp |
| Q | 0: 5 | 459 | 92 | 11: 8 | 1.5 | borderln |
| R | 5: 0 | 545 | 109 | 11: 2 | 2.0 | borderln |
| S | 0: 6 | 666 | 133 | 12:11 | 1.5 | borderln |
| T | 5: 0 | 775 | 155 | 13: 4 | 2.0 | borderln |
| U | 2:10 | 610 | 122 | 14: 5 | 2.0 | borderln |

Figure 1 Desferrioxamine B

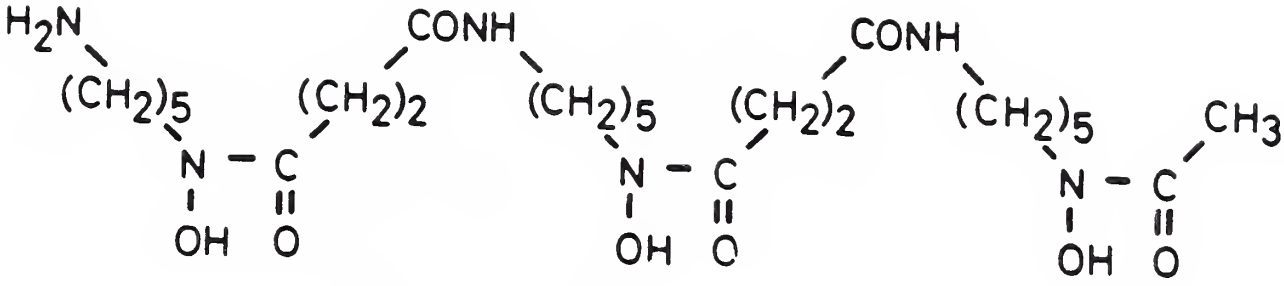

Figure 2 Desferrioxamine B binding a ferric ion to form Ferrioxamine B

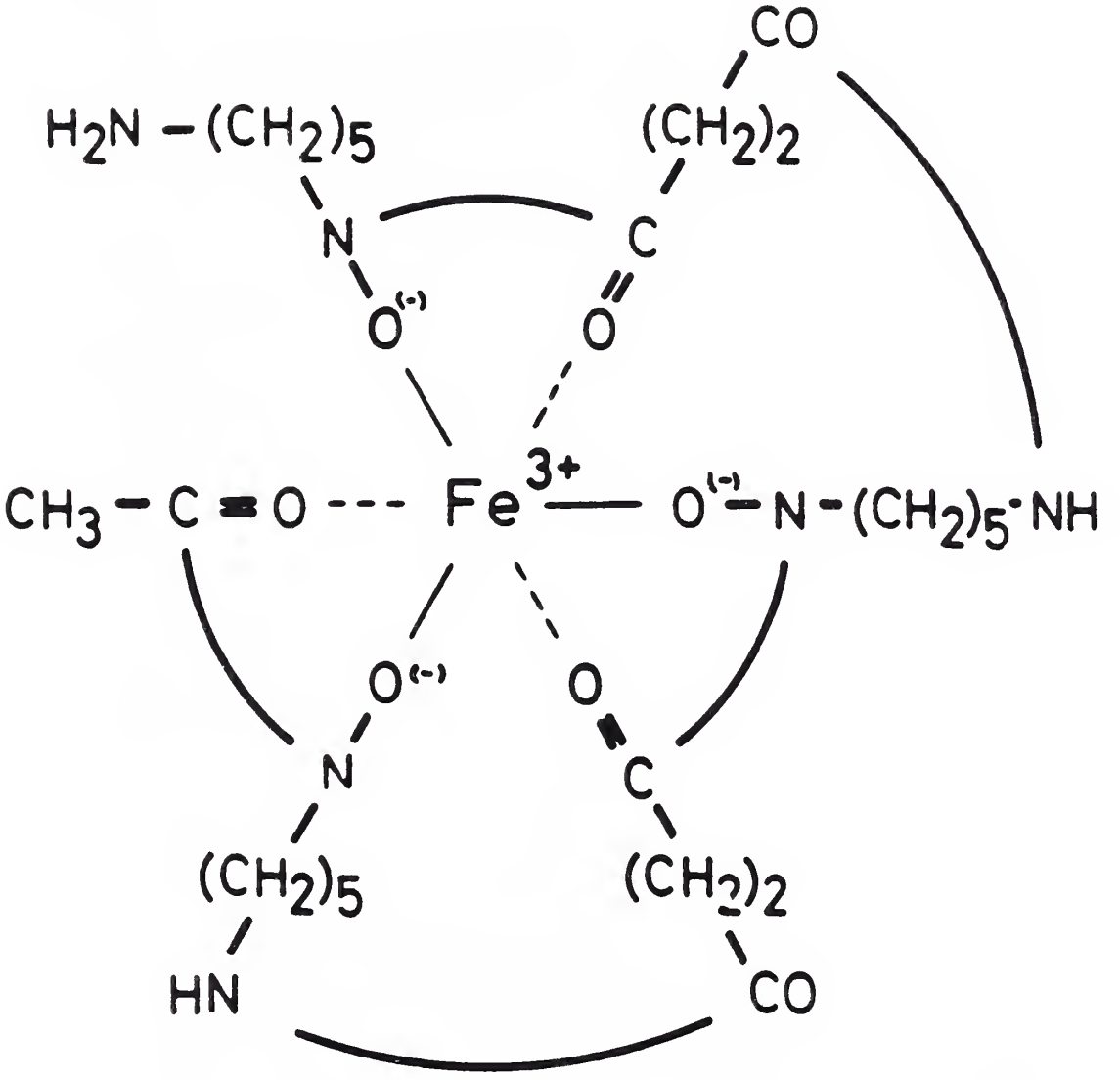

Table 2 CLASSIFICATIONS OF DIABETES IN CHILDREN BASED ON OGTTS
(National Diabetes Group, 1979)

Normal = fasting venous plasma glucose <130 mg/dl
2 hour OGTT venous plasma glucose <140 mg/dl

Impaired = fasting venous plasma glucose <140 mg/dl
2 hour OGTT venous plasma glucose >140 mg/dl

Diabetic = fasting venous plasma glucose >140 mg/dl
2 hour OGTT venous plasma glucose >200 mg/dl

Figure 3

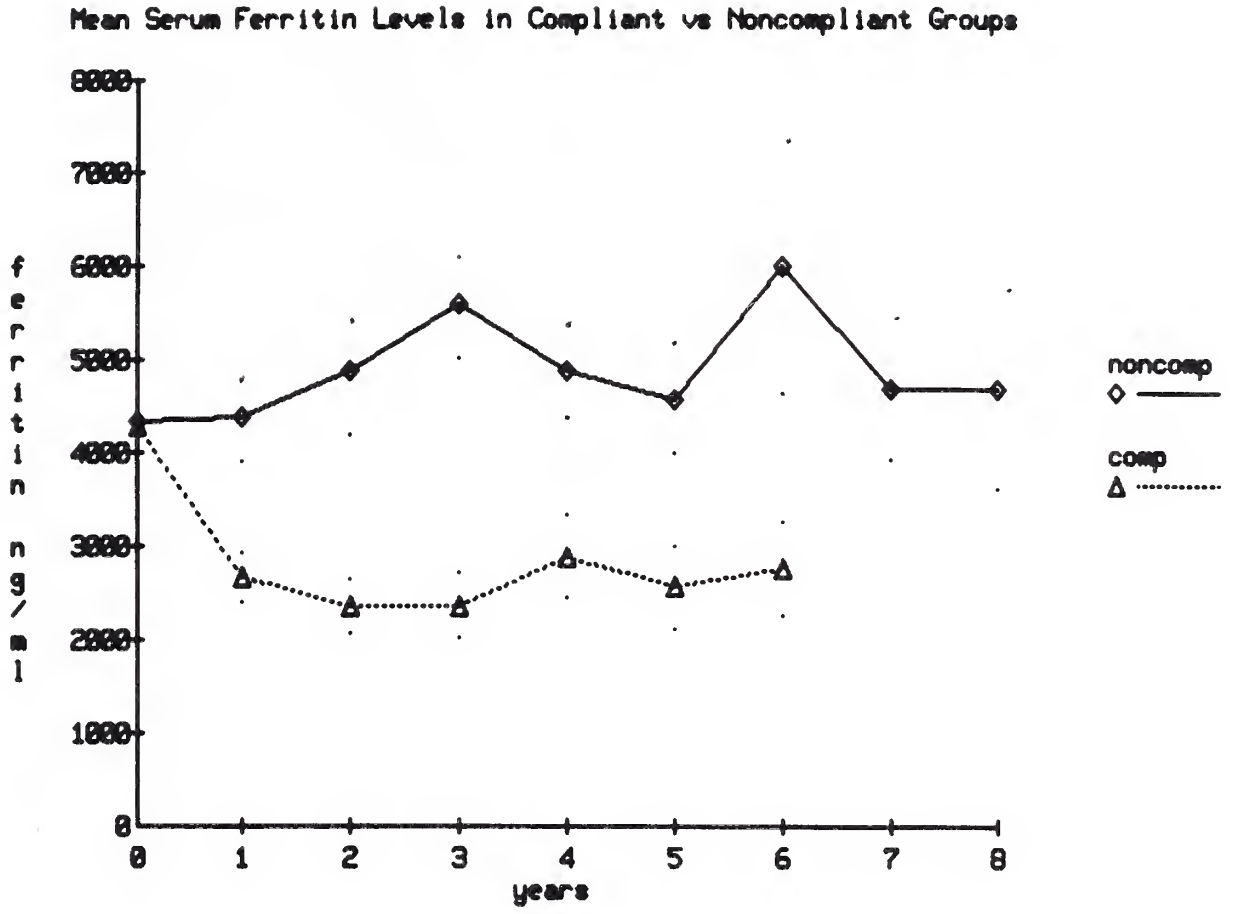
Mean Serum Ferritin Levels in Compliant vs Noncompliant Groups
ferritin ng/ml
8000
7000
6000
5000
4000
3000
2000
1000
0
0
1
2
3
4
5
6
7
8
years
noncomp
comp

Table 3

FOUR COMPLIANT PATIENTS WITH SGOTS THAT FELL INTO THE NORMAL RANGE FOLLOWING DESFERRIOXAMINE THERAPY

| Year | Pt E | Pt F | Pt O | Pt R |
|---|---|---|---|---|
| 0 | 101 | 105 | 59 | 86 |
| 1 | 58 | 41 | 26 | 40 |
| 2 | 29 | 38 | 26 | 24 |
| 3 | 26 | 29 | 22 | 69 |
| 4 | | 29 | 29 | 40 |
| 5 | | 26 | 29 | 21 |

Figure 4

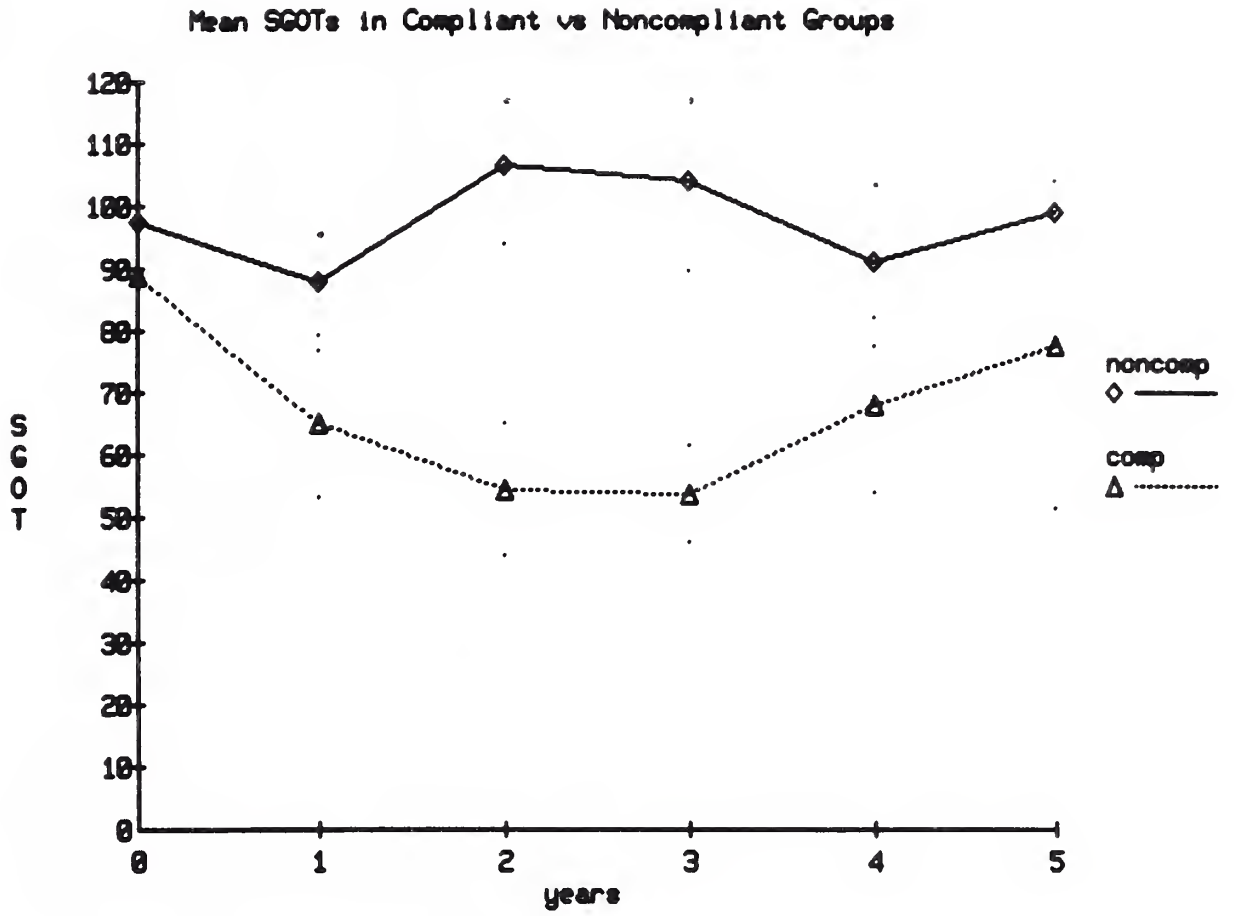
Mean SGOTs in Compliant vs Noncompliant Groups
120
110
100
90
80
70
60
50
40
30
20
10
0
S
G
O
T
0
1
2
3
4
5
years
noncomp
comp

Table 4 ORAL GLUCOSE TOLERANCE TESTS

| Pt | Age | Age DF begun | Compliance | OGTT | Age of onset of impairment | Family hx of AODM |
|---|---|---|---|---|---|---|
| B | 9: 6 | 6: 7 | comp | nl | | none |
| C | 10: 0 | 5:10 | comp | nl | | pat great uncles pat 2nd cousin |
| D | 10: 6 | 4: 0 | comp | nl | | none |
| E | 10: 6 | 7: 8 | comp | nl | | none |
| F | 12: 6 | 7: 7 | comp | nl | | none |
| I | 13: 6 | 9:11 | comp | nl | | none |
| J | 15: 0 | 7: 5 | comp | nl | | mat great aunt |
| K | 17: 0 | 12: 2 | comp | nl | | none |
| L | 17: 0 | 9: 6 | comp | nl | | none |
| P | 19: 0 | 13: 2 | comp | nl | | none |
| O | 18: 0 | 10: 3 | comp | impaired | 18: 0 | mat great gm |
| M | 17: 0 | 10: 1 | noncmp | nl | | none |
| Q | 19: 0 | 11: 8 | brdrln | nl | | father mat uncle & gm |
| A | 9: 6 | 6: 0 | brdrln | impaired | 9: 0 | none |
| G | 13: 0 | 8:10 | brdrln | impaired | 13: 0 | none |
| H | 13: 6 | 8: 8 | brdrln | impaired | 13: 0 | none |
| N | 17: 0 | 11: 2 | noncmp | diabetic | 11: 0 | info not avail |
| R | 19: 0 | 11: 2 | brdrln | impaired | 17: 0 | pat gm |
| S | 20: 6 | 12:11 | brdrln | diabetic | 16: 0 | none |
| T | 21: 6 | 13: 4 | brdrln | impaired | 19: 0 | pat gf |
| U | 22: 0 | 14: 5 | brdrln | diabetic | <16: 0 | pat uncle |

Table 5 GROWTH AND DEVELOPMENT

| Pt | Age | Age DF Begun | Compliance | Sex | Growth Curve Rating | Bone Age | Pubertal Development |
|---|---|---|---|---|---|---|---|
| D | 10: 6 | 4: 0 | comp | M | normal | normal | not applic |
| I | 13: 6 | 9:11 | comp | F | normal | normal | normal |
| K | 17: 0 | 12: 2 | comp | F | normal | normal | normal |
| B | 9: 6 | 6: 7 | comp | M | brderln | normal | not applic |
| C | 10: 0 | 5:10 | comp | F | brderln | normal | not applic |
| E | 10: 6 | 7: 8 | comp | M | brderln | normal | not applic |
| J | 15: 0 | 7: 5 | comp | M | brderln | normal | normal |
| F | 12: 6 | 7: 7 | comp | F | abnormal | normal | normal |
| L | 17: 0 | 9: 6 | comp | M | abnormal | normal | normal |
| O | 18: 0 | 10: 3 | comp | F | abnormal | normal | abnormal |
| P | 19: 0 | 13: 3 | comp | F | abnormal | delayed | abnormal |
| N | 17: 0 | 11: 2 | noncmp | F | normal | delayed | abnormal |
| R | 19: 0 | 11: 2 | brderln | F | normal | delayed | abnormal |
| S | 20: 6 | 12:11 | brderln | F | normal | delayed | abnormal |
| U | 22: 0 | 14: 5 | brderln | F | normal | delayed | abnormal |
| A | 9: 6 | 6: 0 | brderln | F | brderln | delayed | not applic |
| G | 13: 0 | 8:10 | brderln | M | brderln | normal | normal |
| H | 13: 6 | 8: 8 | brderln | M | abnormal | normal | normal |
| M | 17: 0 | 10: 1 | noncmp | M | abnormal | delayed | abnormal |
| Q | 19: 0 | 11: 8 | brderln | F | abnormal | delayed | abnormal |
| T | 21: 6 | 13: 4 | brderln | M | abnormal | delayed | abnormal |

Table 6 THALASSEMICS >AGE 11 WITH NORMAL PUBERTAL DEVELOPMENT

| Pt | Age | Age DF Begun | Sex | Age at Pubarche | Tanner Rating | Compliance |
|---|---|---|---|---|---|---|
| G | 13: 0 | 8:10 | M | 13: 0 | 2 | borderln |
| H | 13: 6 | 8: 8 | M | 13: 0 | 2 | borderln |
| F | 12: 6 | 7: 7 | F | 11: 6 | 2 | comp |
| I | 13: 6 | 9:11 | F | 11: 6 | *4 | comp |
| J | 15: 0 | 7: 5 | M | 11: 0 | 3.5 | comp |
| L | 17: 0 | 7: 5 | M | <14: 0 | 4 | comp |
| K | 17: 0 | 12: 2 | F | 12: 0 | **4.5 | comp |

*Underwent spontaneous menarche at age 12 and continues to have normal menstrual cycles.

**Underwent spontaneous menarche at age 14 and continues to have normal menstrual cycles.

Table 7 THALASSEMICS >AGE 11 WITH ABNORMAL PUBERTAL DEVELOPMENT

| Pt | Age | Age DF Begun | Sex | Age at Pubarche | Age Hormone Rx Begun | Tanner When Hormones Begun* | Compliance |
|---|---|---|---|---|---|---|---|
| O | 18: 0 | 10: 3 | F | 14? | 15 | 2 | comp |
| P | 19: 0 | 13: 3 | F | 16 | 16 | 1.5 | comp |
| M | 17: 0 | 10: 1 | M | none | 14 | 1 | noncmp |
| N | 17: 0 | 11: 2 | F | 13-14? | 15.5 | 3 | noncmp |
| Q | 19: 0 | 11: 8 | F | 14 | 16 | 2.5 | borderln |
| R | 19: 0 | 11: 2 | F | 14? | 15 | 2.5 | borderln |
| S | 20: 6 | 12:11 | F | 13-14? | 18 | **3 | borderln |
| T | 21: 6 | 13: 4 | M | <16? | 16.5 | 2 | borderln |
| U | 22: 0 | 14: 5 | F | 13 | 19 | ***3.5 | borderln |

*Tanner ratings all rose to 4-5 following hormone therapy.

**Underwent spontaneous menarche at age 14 but developed secondary amenorrhea after 6 months.

***Underwent spontaneous menarche at age 13 but developed secondary amenorrhea after 1 year.

Table 8 CARDIAC STATUS

| Pt | age | Compliance | Holter | EF by Echo('85) | 1983 LVPW | Clinical Complications |
|---|---|---|---|---|---|---|
| B | 9: 6 | comp | nl | nl (66) | nl | none |
| C | 10: 0 | comp | nl | nl (58) | nl | none |
| D | 10: 6 | comp | nl | nl (64) | n.d. | none |
| E | 10: 6 | comp | brdrln | nl (59) | nl | none |
| F | 12: 6 | comp | *abnormal | nl (60) | nl | none |
| I | 13: 6 | comp | nl | nl (58) | nl | none |
| J | 15: 0 | comp | nl | nl (68) | nl | none |
| K | 17:0 | comp | nl | nl (73) | nl | none |
| L | 17: 0 | comp | nl | nl (72) | nl | none |
| O | 18: 0 | comp | brdrln | nl (66) | nl | none |
| P | 19: 0 | comp | abnormal | nl (54) | nl | none |
| A | 9: 6 | brdrln | nl | nl (73) | nl | none |
| G | 13: 0 | brdrln | brdrln | nl (70) | nl | none |
| H | 13: 6 | brdrln | n.d. | nl (63) | brdrln | none |
| M | 17: 0 | noncmp | nl | nl (66) | n.d. | none |
| N | 17: 0 | noncmp | abnormal | abnl (44) | abnormal | Pericard.(12)<br>CHF,Death (17) |
| Q | 19: 0 | brdrln | nl | nl (66) | brdrln | none |
| R | 19: 0 | brdrln | nl | nl (57) | n.d. | none |
| S | 20: 6 | brdrln | brdrln | brdrln(52) | abnormal | Pericard.(19) |
| T | 21: 6 | brdrln | abnormal | abnl (26) | nl | Afib (20)<br>CHF,Death (21) |
| U | 22: 0 | brdrln | abnormal | abnl (33) | nl | Afib (16)<br>CHF (20)<br>Death (22) |

Table 9 HOLTER MONITOR ABNORMALITIES

| Pt | Age | Compliance | Holter rating | Specific Abnormalities |
|---|---|---|---|---|
| E | 10: 6 | comp | brdrln | ext. rare JEBs, 1 PVC |
| F | 12: 6 | comp | abnormal | **frequent PVCs |
| O | 18: 0 | comp | brdrln | 20 PACs in 24 hrs & 1 atrial couplet |
| P | 19: 0 | comp | abnormal | ext. rare PACs with 1 episode EAT |
| G | 13: 0 | brdrln | brdrln | 10-30 PACs/hr, 2 conducted PACs & 4 atrial couplets |
| N | 17: 0 | noncmp | abnormal | Ectopic atrial tach(EAT) beginning at age 16 freq. PVCs at age 17 |
| S | 20: 6 | brdrln | brdrln | 15 PACs & 1 atrial couplet 15-30 PVCs/16 hrs |
| T | 21: 6 | brdrln | abnormal | Afib beginning at age 20 freq PVCs close to death |
| U | 22: 0 | brdrln | abnormal | Begun on disopyramide for afib at age 16 |

**PVCs have been present since early childhood and disappear with exercise. They are probably not related to thalassemia.

Table 10

TWO PATIENTS WITH SIGNIFICANT TRENDS IN THEIR EJECTION FRACTIONS

| Year | Patient S (noncmp) | Patient O (comp) |
|---|---|---|
| 0 | 62 | 46 |
| 1 | 63 | 53 |
| 2 | 62 | 45 |
| 3 | 59 | 41 |
| 4 | 59 | 54 |
| 5 | 56 | 58 |
| 6 | 57 | 58 |
| 7 | 56 | 50 |
| 8 | 52 | 66 |

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