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THE SPLEEN IN SICKLE CELL ANAEMIA

DURING EARLY CHILDHOOD

Dissertation for the degree of Doctor of Medicine
David Watson Rogers, Merton College

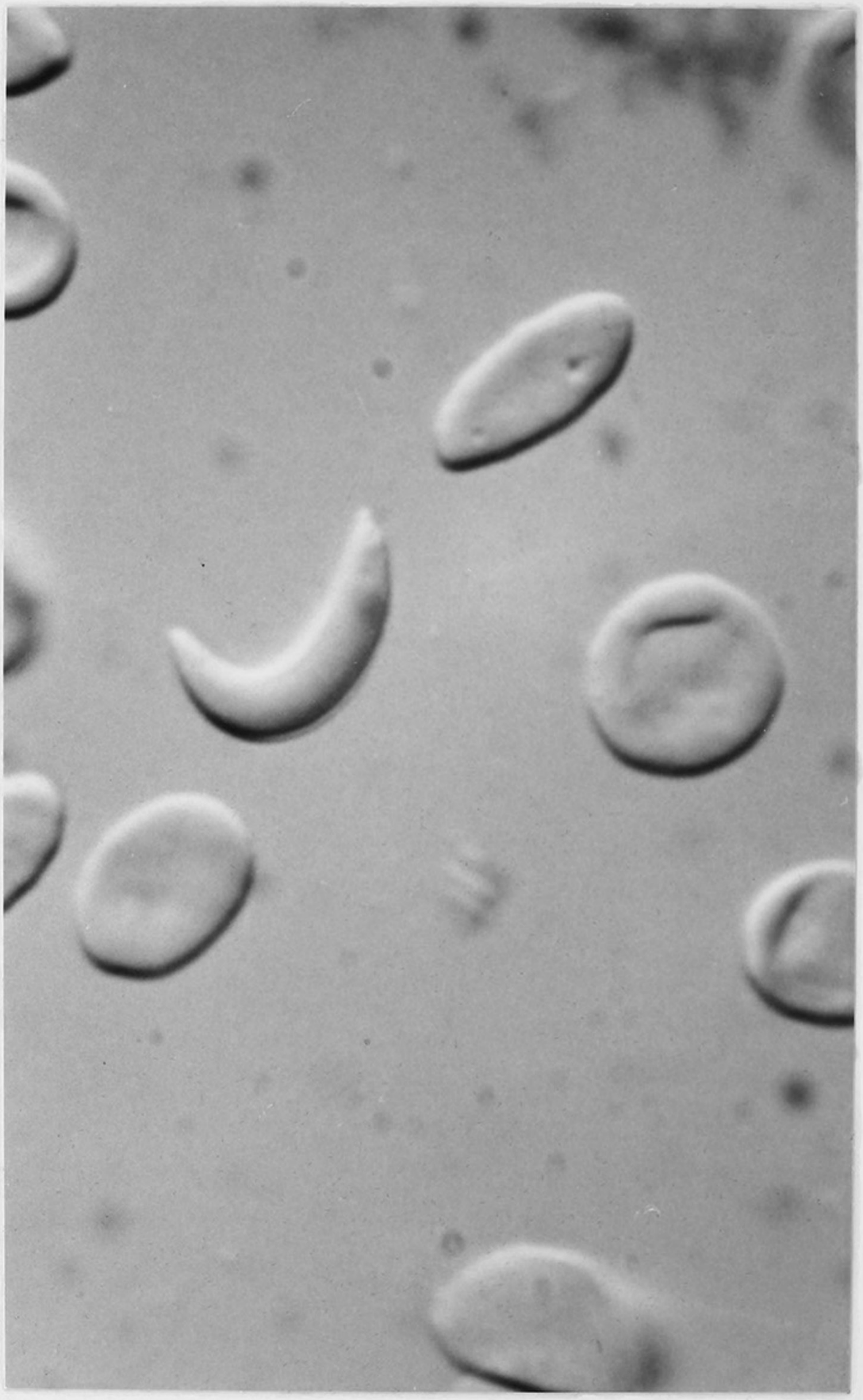

Plate A. Pitted red cells in sickle cell disease

THE SPLEEN IN SICKLE CELL ANAEMIA DURING EARLY CHILDHOOD

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This thesis is dedicated to the mothers and their children in recognition of their courage and cheerfulness in the face of great difficulties.

PREVIOUS PUBLICATIONS

Some of the findings reported in this thesis have been published in:

ROGERS D W, CLARKE J M, CUPIDORE L, RAMLAL A M, SPARKE B R, and SERJEANT G R (1978)
British Medical Journal, 1, 1515-1516.
Early deaths in Jamaican children with sickle cell disease.

ROGERS D W, VAIDYA S, and SERJEANT G R (1978)
Lancet, 2, 963-965.
Early splenomegaly in homozygous sickle cell disease: an indicator of susceptibility to infection.

ROGERS D W, SERJEANT B E, and SERJEANT G R (1982)
Archives of Disease in Childhood, 57, 338-342.
Early rise in 'pitted' red cell count as a guide to susceptibility to infection in childhood sickle cell anaemia.

THE SPLEEN IN SICKLE CELL ANAEMIA

DURING EARLY CHILDHOOD

SUMMARY

In a study of the natural history of homozygous sickle cell (SS) disease 154 of 160 Jamaican children in whom SS genotype had been diagnosed by cord blood haemoglobin electrophoresis were followed to age 1 to 5 years. Seventeen girls and five boys died. Severe bacterial infections and acute splenic sequestration (ASS) were the principal causes of death. The incidence of each of these complications of SS disease was studied in relation to the natural history of splenomegaly and of splenic reticuloendothelial function.

Splenomegaly was assessed by physical examination at each clinic visit. 37% SS children had had palpable spleens by age 6 months, 66% by age 12 months, 79% by age 24 months and 85% by age 36 months.

Splenic reticuloendothelial function was assessed by estimating the percentage of pitted red blood cells (pit count) using differential interference contrast microscopy. Pit counts in 139 control children with normal haemoglobin genotype AA were always below 9%. Pit counts performed serially in 130 of the SS children (in 46 from birth) rose with age, and were greater than 9% in 23% SS children at age 1 year, 42% at age 2 years and 52% at age 3 years. Radioactive colloid spleen scans showed no splenic uptake of colloid in 11 SS children with palpable spleens and pit counts greater than 9%, confirming impairment of their splenic reticuloendothelial activity.

Nineteen severe bacterial infections with Streptococcus pneumoniae, Haemophilus influenzae and Salmonella spp were diagnosed in 14 of the SS children at ages from 4 to 57 months, and carried a 32% mortality. These infections were seen only in the 66% SS children in whom the spleen was first felt at or before age 12 months, and occurred most commonly in the 37% SS children in whom the spleen was first felt at or before age 6 months. Only children with raised pit counts experienced severe infections. Among those with pit counts followed serially from birth severe infections were confined to those whose pit counts were first greater than 9% at or before age 12 months.

Twenty-four episodes of ASS occurred in 18 SS children at ages from 3 months to 53 months, and carried a 40% mortality. Successful treatment depended on prompt diagnosis and immediate red cell transfusion. Streptococcus pneumoniae was isolated from four of the nine fatal episodes; treatment should therefore include parenteral antibiotic therapy. In 14 of the 18 children the spleen was first felt before their first episode of ASS, but the age of onset of palpable splenomegaly showed no relation to the subsequent risk of ASS. Pit counts were usually less than 9% at the time of the first episode of ASS and rose to levels greater than 9% subsequently.

Analyses of the variations in haematological development in the SS children showed that earlier splenomegaly and earlier rise in pit count were associated with earlier and greater falls in total haemoglobin and red cell counts with earlier and greater rises in reticulocyte count. Earlier splenomegaly was associated with a lower haemoglobin F percentage and a higher reticulocyte count at age 6 months, and the risk of severe infection was related to a higher reticulocyte count at age 6 months independently of its relation to age of appearance of splenomegaly.

Analyses of two other significant clinical events in SS disease in early childhood, dactylitis and pneumonic episodes, showed that in the first two years of life significantly more of the children with early splenomegaly were affected by these complications.

In SS children diagnosed at birth and carefully followed, earlier onset of splenomegaly, associated with earlier falls in total haemoglobin concentration, haemoglobin F percentage and red cell count, and earlier rise in reticulocyte count, identifies children at greater risk of life-threatening bacterial infections, who may also be identified more sensitively by an early rise in pit count, indicating early failure of splenic reticulo-endothelial function. The onset of splenomegaly may be initiated or followed by episodes of ASS. Children who survive such episodes subsequently experience severe infections, develop raised pit counts and fail to take up radioactive colloid in their spleens; these findings suggest that splenectomy is unlikely to increase their susceptibility to infection and is therefore an acceptable prophylaxis against recurrence of ASS.

Early onset of the splenic complications of SS disease is thus associated with increased severity of disease. Neonatal diagnosis makes possible the subsequent identification of such SS children at high risk whose prognosis might be improved by close medical supervision.

INTRODUCTION

Splenomegaly is a common clinical finding in young children with homozygous sickle cell (SS) disease. Although the spleen usually involutes and becomes impalpable with age, splenomegaly in early childhood is significant because of its association with two life-threatening complications of SS disease, acute splenic sequestration (ASS), and susceptibility to fulminant and recurrent bacterial infections, especially pneumococcal septicaemia and meningitis.

Follow up of SS children diagnosed in a cord blood screening programme provided the opportunity for a study of the natural history of splenomegaly in early childhood and its relationship to these two complications. Serial pitted red cell counts were performed to assess their value as indicators of failure of splenic function and susceptibility to severe infection. The aims of the study were to identify the children at particular risk for severe infection or ASS and to make recommendations for prophylaxis and management based on an understanding of the natural history of these complications.

REVIEW OF THE LITERATURE

1. Haemoglobin S and the sickling process

The precision with which the molecular defect of sickle cell anaemia has been defined contrasts with our imprecise understanding of the pathophysiology of the disease's varied clinical manifestations and our corresponding failure to provide adequate therapy. The clinical observations on the disease reported in this thesis, which it is hoped may indicate appropriate clinical management for one aspect of the disease in children, will where possible be interpreted in the light of the current molecular and biochemical understanding of the disease, which is now reviewed.

Herrick's original description (1910) from Chicago of sickled-shaped red cells in a 20 year old Grenadan student with a history of leg ulcers, presentation with a pneumonic episode and subsequent painful crisis, included the suggestion that as normal red cells could not be induced to assume the sickle shape by varying the fluid in which they were suspended, the unusual sickle appearance might be due to some abnormality in the composition of the red cell itself.

Hahn and Gillespie (1927) examining red cells from a patient with sickle cells and massive splenomegaly, who showed a good response to splenectomy, defined the conditions under which sickling occurred *in vitro*. They demonstrated that provided the pH of the suspension was below 7.4 sickling would occur when the oxygen tension fell below 45mmHg, and could be reversed by increasing the oxygen or carbon monoxide tensions. They concluded that the sickle form was stable when haemoglobin was deoxygenated and that the influence of pH was probably related to its influence on oxygen dissociation, and suggested that sickle cell formation *in vivo* might be related to anoxia.

Neel (1949) undertook family studies for 29 patients with sickle cell anaemia and demonstrated sickling in all the parents tested. He concluded that the possession of red cells which would sickle in the absence of symptoms (then known as sicklemia) represented the heterozygote state, while the patient with symptomatic sickle cell anaemia was a homozygote for the sickle gene.

Pauling et al (1949) compared the results of electrophoresis of haemoglobin extracted from cells of patients with sickle cell anaemia (homozygotes), individuals with sickle cell trait (heterozygotes), and normal individuals. They demonstrated a difference in electrophoretic mobility between the haemoglobins of sickle cell anaemia patients and of normal individuals and showed that both haemoglobins were present in individuals with sickle cell trait. They thus demonstrated that sickle cell anaemia was likely to be the consequence of a specific molecular abnormality in sickle haemoglobin.

Ingram (1957) defined the nature of the abnormality by performing paper chromatography on tryptic digests of normal haemoglobin (HbA) and sickle haemoglobin (HbS). Analysis of the single peptide occupying a different position in the chromatograms revealed that a glutamic acid residue of HbA had been replaced in HbS by a valine residue.

Sickling of the red cells in the sickling disorders appears to result from aggregation of HbS molecules into long filaments which by forming fibres deform the cell (Murayama, 1966). Perutz and Lehmann (1968) explained the polymerisation of the HbS molecule by suggesting that the valine residue which substitutes in the globin beta chain of HbS for the glutamine of HbA might bind to a complementary site accessible only in deoxyhaemoglobin. Polymerisation would therefore depend on the presence of a certain minimum concentration of deoxyhaemoglobin S, and would be prevented by the presence of other haemoglobins (such as HbA or F)

lacking the valine residue, or by an increase in the proportion of haemoglobin in the oxy form lacking the specific complementary site.

Perutz (1970) described the changes in the structure of the haemoglobin molecule associated with oxygenation, showing the cooperative allosteric effect of increasing oxygen affinity with increasing oxygenation of each molecule. He also showed that globin chain combination with ligands, which physiologically might be hydrogen ions or 2-3 diphosphoglycerate, resulted in the breakage of the ionic bridges stabilising oxyhaemoglobin. These findings provided a molecular explanation for the observed shape of the oxygen dissociation curve and for the influence of pH and 2-3 diphophoglycerate concentration on oxygen dissociation. The findings have important implications for an understanding of the sickling process, in that as HbS polymerizes only in the deoxy form, conditions favouring deoxygenation of HbS will also favour polymerisation and therefore sickling.

Wishner et al (1975) studying the deoxy HbS crystal demonstrated that it was composed of double strands of molecules. They showed that the strands were linked by intermolecular contacts between residue 6 on a beta-chain of one strand and residue 73 on a beta-chain of the other strand. Dykes et al (1979) studying HbS fibres formed by stirring HbS solutions during warming demonstrated that the fibres were usually formed of 14 strands packed together in a hexagonal arrangement and suggested that within this arrangement the strands might be linked together in pairs. They argued from the similar appearances seen in crystals of both deoxy HbS and deoxy HbA that the fibre structure might already be marginally stable in both deoxy-haemoglobins, but was firmly stabilised by the cross-linkages in HbS.

The mechanism by which HbS polymerisation results in sickle cell formation and hence in vaso-occlusion has been studied by Eaton *et al* (1976). Both in solution and within the cell polymerisation of HbS resulted in the formation of a gel. Following an initial stimulus to polymerisation, for example warming the solution, the time-course of gel formation was found to show a significant delay before the gel formed. They suggested by analogy with crystallization that this delay time represented the time taken to form a nucleus (or molecular aggregate) for polymerisation. Noting that under physiological conditions this delay time was of the same order as capillary transit time (about 1 second) they argued that sickling within the capillary, and hence vaso-occlusion, would only take place under conditions reducing delay time or increasing capillary transit time. Factors reducing delay time *in vitro* included lower pO_2, lower pH, increasing HbS concentration and increasing temperature. Hypoxia, acidosis; dehydration and fever might therefore reduce delay time and so encourage vaso-occlusive sickling, while such sickling would be more likely to occur in organs with prolonged capillary transit times.

2. Splemomegaly and splenic pathology in the sickling disorders

In the first report of sickle cell anaemia diagnosed in childhood (Sydenstricker et al, 1923) necropsy of a 5 year old child showed a small spleen with the histological appearances of congestion, haemorrhages, scarring and atrophy. A 6 year old child had a hard spleen on clinical examination, two fingers' breadth below the costal margin. Because haemoglobin electrophoresis was not then available the haemoglobin genotypes of these children are unknown.

In Uganda (Trowell et al, 1957) 30 of 37 (81%) SS children developed splenomegaly under age 2 years. In the USA 15 of 26 (58%) SS children examined under age 2 years had splenomegaly (Haggard and Schneider, 1961), and 32 of 64 (50%) diagnosed under age 1 year had splenomegaly at diagnosis (Porter and Thurman, 1963). These populations of SS children were of course selected by symptomatic presentation prior to diagnosis, and in the Ugandan population malaria may have influenced the proportion with splenomegaly.

Cross-sectional analysis in Jamaica (Serjeant, 1970; Gray, 1971) showed that 70% of SS children age 6 months to 2 years had palpable spleens, but only 60% in the age group 3 to 4 years, 50% in the age group 5 to 9 years, 40% in the age group 10 to 14 years, and 25% in the age group 15 to 19 years. After 20 years, less than 10% SS patients had palpable spleens, and in patients followed prospectively spontaneous disappearance of the spleen might still occur after that age. In SS disease the pattern of splenomegaly is therefore of early appearance with subsequent disappearance usually before adulthood.

In contrast persistence of splenomegaly into adult life is common in sickle cell haemoglobin C disease. Bannerman et al (1979) found a palpable spleen in 16 of 31 (53%) Jamaican SC patients aged 17 to 59 years.

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Splenomegaly also persists in patients with sickle cell-beta thalassaemia (S beta thal). Serjeant et al (1973) studying 56 Jamaican S beta thal patients aged 3 to 60 years, including patients with and without haemoglobin A, found a palpable spleen in more than 50% of patients, irrespective of HbA concentration or age group. In a comparison of 41 patients with sickle cell beta^{o}thalassaemia (S beta^{o} thal) aged 5 to 60 years and 123 age and sex-matched SS patients Serjeant et al (1979) demonstrated a significantly increased prevalence of splenomegaly (24% against 6%) and of splenectomy (20% against 6%), together with a lower irreversibly sickled cell (ISC) count in the S beta^{o} thal group. Theyascribed these differences to a decreased tendency to intravascular sickling in the sickle cell beta^{o} thal group, and suggested that the smaller cells in S beta^{o} thal might negotiate capillary beds more easily.

Diggs (1935), reviewing the pathology of spleens from patients with sickle cell disease at a time when electrophoresis was not available to identify the genotypes, described four histological stages in the process of splenic enlargement and involution. In the first splenic cords are stuffed with erythrocytes while the sinuses are compressed and empty. Haemorrhages and infarcts are already evident. In the second the haemorrhages become organised, and there is a relative decrease in the number of erythrocytes as the pulp is replaced by fibrous tissue. In the third stage, characterised macroscopically by a small hard spleen, the anatomical pattern is confused with replacement of the pulp by connective tissue, obliterative vascular changes and absence of typical splenic corpuscles. In the fourth stage the spleen has shrunk to a wrinkled mass which histologically shows thick walled calcified blood vessels in a mass of disarranged degenerated connective tissue surrounded by a thickened and hyalinized capsule. Commenting specifically on the spleen in SS infants Diggs (1973) noted congestion of the pulp with sickled erythrocytes and stasis within or at the margins of the splenic corpuscles.

Tomlinson (1945), reporting from Panama on the splenic histology in necropsies in which sickle cells had been seen, noted pooling of red cells, compressed and packed to give the impression of rigidity, in dilated pulp spaces with empty venous sinuses, especially in the marginal zone of the spleen. In experiments in which he perfused spleens from patients with sickle cell disease by both the arterial and the venous route he found no evidence of a congenital abnormality of the splenic sinuses which Rich (1928) had suggested as an explanation for the red cell pools. Instead Tomlinson's experiments suggested that the accumulation of red cells in the pulp cords resulted from failure of the rigid sickle cells to pass into the venous sinuses. He postulated that in the individual with sickle cell anaemia, with sickle cells constantly present in the peripheral blood, pooling and packing of these cells in the spleen would lead to thrombosis, haemorrhage and scarring and so eventually to a small fibrotic spleen.

Edington (1955), reviewing necropsies in sickle cell disease patients in the Gold Coast (now Ghana), made an important distinction between patients dying with a fibrotic spleen and those in whom the spleen was grossly enlarged and congested with the remaining organs exhibiting either gross pallor or various degrees of congestion. He described the appearances in the enlarged congested spleen, with compression of the splenic sinuses to 'tiny chinks in a sea of red blood cells'. The age and sex distributions of the two splenic appearances at necropsy are of interest, the enlarged congested spleen being commonest among children aged under 5 years (24 cases in 306 total necropsies of all children in this age group) and females of childbearing age (32 cases in 235 total autopsies of all females age 16 to 25). No cases of fibrotic spleen were seen under age 5 years. He comments that in the deaths with congested spleens and pallor of the other organs associated with pregnancy many of the patients were likely to be heterozygous for haemoglobins, probably having haemoglobin SC disease.

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Among the necropsies listed are several children under age 5 with a history of sudden dyspnoea and massively enlarged spleens (for example spleen weight 192 gm at age 18 months, normal spleen weight at this age being 30 gm) and it is interesting to speculate that these children died with the then unrecognised acute splenic sequestration syndrome.

3. Pathophysiology of splenomegaly in the sickling disorders

Weisman et al (1954) transfused blood from a patient with sickle cell anaemia into a patient with normal haemoglobin who was about to undergo splenectomy, and demonstrated a higher concentration of sickle cells in the splenic pulp, blood from the splenic pulp containing 8% sickle cells while peripheral blood contained 3% sickle cells. In contrast a patient with sickle cell anaemia transfused with normal blood prior to splenectomy had 43% sickle cells with decreased osmotic fragility in the peripheral blood but 60% sickle cells with an extreme increase in osmotic fragility in splenic pulp samples. They concluded that sickle cell anaemia erythrocytes were sequestered in the spleen, probably because of their physical characteristics, and that the increased susceptibility of the sequestered cells to osmotic haemolysis presumably resulted from stasis within the spleen.

Watson et al (1956) reviewed twenty-one patients age 1 to 36 years with homozygous sickle cell disease. They demonstrated disappearance of splenomegaly with age in 7 of these patients. They showed an increase in survival in normal (HbA) blood in an SS patient after splenectomy, suggesting that extracorpuscular factors were contributing to the patient's anaemia. Splenic aspirations from eight patients showed that the irreversibly sickled cell (ISC) count in the splenic blood was always greater than that in peripheral blood by a factor ranging from 2 to 14. They concluded that ISC's were formed and sequestered within the spleen resulting in enlargement of the spleen and increasing sequestration and haemolysis.

Serjeant (1970) demonstrated a highly significant difference between the mean ISC count in SS patients over age 12 years with and without splenomegaly, patients without splenomegaly having a higher ISC count.

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He found that while the incidence of splenomegaly fell with age there was no corresponding increase in ISC count with age, nor was there any increase in ISC counts associated with splenectomy. However high ISC counts were associated with low HbF levels in the non-splenomegaly group, and high HbF levels were found to be associated with a high percentage of patients with persistent splenomegaly. He suggested that lower HbF levels might result in more ISC's entering the spleen or being formed within it and causing splenic atrophy, while higher HbF levels with fewer ISC's might allow persistence of splenomegaly.

These findings suggest that initial red cell pooling within the spleen might be due to sequestration of ISC's from the general circulation unable to pass through the splenic circulation. Once pooling is established further sickling and formation of ISC's might take place as a consequence of the impaired splenic circulation. The mechanism by which the splenic circulation might become obstructed is suggested by the work of Chen and Weiss (1973) who undertook electro-microscope studies of spleens of rats in whom an acute Heinz body anaemia had been induced by phenylhydrazine. They showed that red cells containing Heinz bodies became trapped when attempting to pass through slits between the endothelial cells forming the walls of the splenic sinuses. They suggested that these slits formed the common path for the exit of all red cells from the spleen, and that if these slits become blocked by damaged or rigid cells passage of red cells out of the spleen might be impeded. The resulting accumulation of red cells in the spleen would then provide a possible cause for splenomegaly.

4. Early mortality in SS disease

The rarity of SS disease in adults in some African populations has suggested a high childhood mortality (Lehmann and Raper 1956, Jacob 1957). 120 of 300 (40%) SS children diagnosed at a mean age of 1 year 5 months in the Belgian Congo (now Zaire) died before age 2 years and 144 (48%) before age 5 years (Lambotte-Legrands, 1955). Mortality was higher in girls than in boys at all ages, 81 of 155 (52%) girls and 63 of 145 (43%) boys dying before age 5. The mortality rate declined with increasing age at diagnosis, which was interpreted as indicating a slower evolution of the disease in less severely affected infants.

Survival of SS homozygotes to adult life is more common in West Indian and North American populations, but early mortality remains high. Reviewing 64 SS infants diagnosed before age 1 year, Porter and Thurman (1963) found a 16% mortality under age 1 year, and a 50% mortality under age 1 year in those presenting before age 4 months. The sex of infants who died was not reported. Nine of 10 children who died had splenomegaly. Three children with splenomegaly died in respiratory difficulty with haemoglobin concentration less than 3.5 g/dl, suggesting that they may have had acute splenic sequestration (ASS), a syndrome which had not then been described. Four children with splenomegaly died with bacterial infections caused by Haemophilus influenzae, Salmonella, Staphylococcus or Pseudomonas.

Seeler (1972) reviewed 19 deaths in SS children aged up to 13 years in Chicago. Six were males and 13 females. Four died with ASS at age 1, 2, 2, and 4 years respectively. Three died with pneumonoccal septicaemia, two aged 1 year and one aged 4 years, and a further child aged 1 year had purulent meningitis from which no organism was isolated. Other causes of death included Salmonella enteritidis septicaemia at age 13 years, cerebrovascular occlusion (3 cases), congestive heart failure (3 cases), fulminant

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hepatitis following transfusion, and epistaxis accompanying intracranial haemorrhage. Two further patients were moribund on admission to hospital. Mortality appeared to be higher in the younger age groups.

A similar review of deaths in 23 children in Los Angeles (Powars, 1975) showed six due to pneumococcal septicaemia or meningitis, two due to H influenzae septicaemia, one due to haemolytic streptococcal septicaemia, and one to staphylococcal pneumonia. Three were due to ASS in the age range 2-3 years. The sex of children who died was not reported.

These last two series (Seeler, 1972; Powars, 1975) include only one and two deaths respectively under age 1 year. Powars stresses that the diagnosis of SS disease as a cause of death in infancy may be missed unless haemoglobin electrophoresis is performed at autopsy. Following the introduction of routine post mortem haemoglobin electrophoresis and blood culture in the investigation of the sudden infant death syndrome, in 10 years seven children were found to have died from previously unsuspected SS disease and pneumococcal septicaemia.

5. Acute splenic sequestration

ASS has only recently been recognised as a significant cause of death in SS children, although review of the literature reveals several early case histories. The first is probably that of Josephs and Winocur (1938) who describe a boy aged 3 years who suddenly became severely anaemic with splenic enlargement while in hospital, with haemoglobin concentration falling from 8.2 to 2.6 gm/dl in 8 hours but no haemoglobinaemia or haemoglobinuria, and died in a few hours. At autopsy it was thought that he had bled to death into his spleen.

Jenkins, Scott and Baird (1960) described sudden death in a 16 month old SS girl who presented with a convulsion and extreme pallor. Her haemoglobin concentration was less than 1 gm/dl. At necropsy all organs were pale except the spleen which was grossly enlarged (weight 274 gm - normal for age 30 gm) and was found on histological examination to be packed with erythrocytes. The liver sinusoids were dilated and filled with sickled red cells. Deaths due to acute anaemia and collapse in nine other SS children, five under 20 months, were reviewed. It was suggested that the spleen, together with liver sinusoids and splanchnic vessels, might rapidly remove red blood cells from the circulation. Trapping of much of the total red blood cell volume in the engorged spleen might cause death from peripheral vascular collapse and cerebral anoxia.

Seeler and Shwiaki (1972) used the term 'acute splenic sequestration' to describe these episodes. They reviewed their experience of 20 such episodes in 14 children age 6 to 55 months at the time of the episode.

A sudden increase in pallor with adbominal distension and polydipsia was followed by weakness, marked dyspnoea, left-sided abdominal pain and vomiting. On examination the spleen was acutely enlarged, firm and tender, and there

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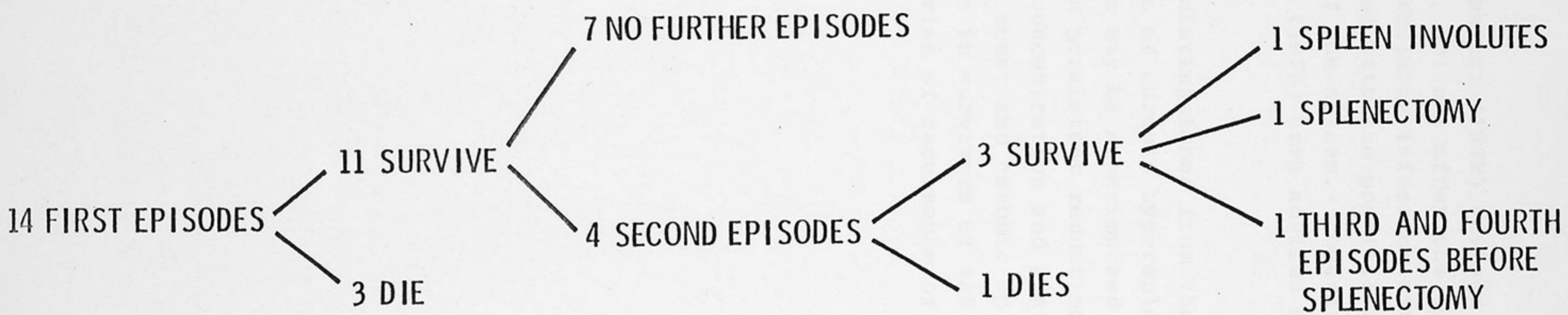
were signs of acute circulatory failure (tachypnoea, tachycardia, cardiomegaly, and hepatomegaly). In four episodes evidence of concurrent infection included pneumonia, diarrhoea, upper respiratory tract infection, and pyrexia of unknown origin. Haematocrits at the time of the episode ranged from 6-20 per cent and were always significantly lower than the child's usual value. Reticulocyte counts were from 12-50 per cent. Three of the 14 children died in their first episode, and four of the 11 survivors had recurrences, in which one died. Two of the three survivors of the second episode subsequently underwent splenectomy and the spleen of the third involuted spontaneously (Figure 1). Autopsies in three of the children who died showed massive splenomegaly with spleens weighing 200 gm, 300 gm, and 575 gm.

The extreme rapidity with which ASS may develop has been illustrated by Pearson (1973) who reported an episode in which the haemoglobin concentration fell from 7 gm% to less than 2 gm% within 3 hours, a moderately enlarged spleen expanding into the pelvis. This rapid progression provides some explanation for the high mortality seen even in patients who reach hospital before death.

Jenkins et al (1960) advised immediate packed red blood cell transfusion for treatment of the acute episode. Seeler and Shwiaki (1972) agreed that packed erythrocytes were preferable to whole blood, but felt that in the acute episode whole blood might be more rapidly available, and that the red cells could then be allowed to settle during transfusion. They reported that saline infusions to 'keep open' the catheter, presumably while awaiting the arrival of blood, produced pulmonary oedema in two patients. Following transfusion the spleen has been found to decrease in size and the haemoglobin concentration to rise to levels several gm/dl higher than expected for the amount of blood given (Robinson, 1975), findings compatible with release of sequestrated cells from the spleen.

FIGURE 1

OUTCOME OF ASS (Seeler and Shwiaki, 1972)



Seeler and Shwiaki (1972) advised splenectomy following two episodes, but not after one episode because of the risk of overwhelming infection after splenectomy in young children with the possibility of spontaneous involution of the spleen. However both Pearson (1973) and Robinson (1975) have advised splenectomy after one episode only.

ASS must be distinguished from the chronic red cell sequestration of chronic hypersplenism on which acute sequestration may be superimposed. Topley et al (1981) showed that a persistent reduction in steady state haemoglobin concentration and platelet count with raised reticulocyte count and chronic splenic enlargement was commonly seen in survivors of ASS. These children remained at risk of recurrence of ASS.

6. SS disease and bacterial infection

Robinson and Watson (1966) reported an increased incidence of pneumococcal meningitis in SS patients, 6% of 252 SS patients experiencing one or more episodes during a ten year period, in contrast to only 0.2% of the general population. Eleven patients age 5 to 70 months at the time of their first episode had 13 episodes of pneumococcal meningitis. One patient who had two episodes had undergone splenectomy, three patients had palpable spleensat the time of their episodes, and two patients who died had spleensof normal size for their age.

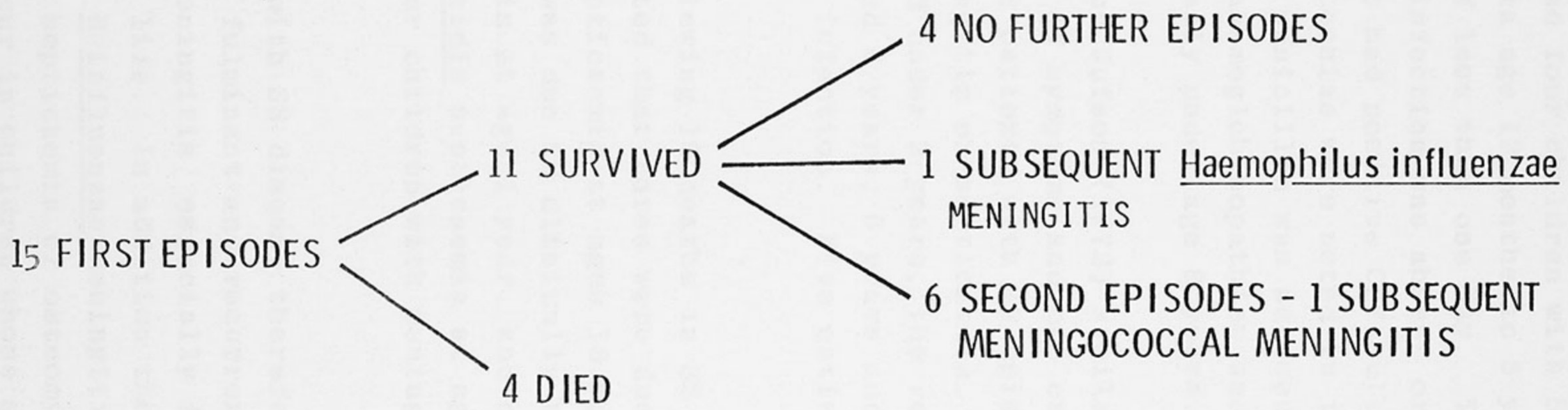
Bacterial meningitis was seen in only three other SS patients, with H influenzae and Streptococcus (unspecified) in two cases, and no organism identified in the third. The pneumococcus was thus responsible for 81% of cases of bacterial meningitis in SS children, compared with 26% of cases in other children.

Barrett-Connor (1971) reported 21 episodes of pneumococcal infection with the organism grown from either CSF or blood in 15 SS children out of a total of 166 SS children admitted to hospital over an 11 year period. Surviving patients experienced further episodes of meningitis due to H influenzae and Neisseria meningitidis as well as further episodes of pneumococcal infection (Figure 2). The greatest incidence of these severe infections was below age 2 years, with a dramatic fall in the incidence of infections after age 3 years. Pneumococcal infection was only seen in children aged over 3 years if they had had a previous infection before or at this age. While certain patients with recurrent severe bacterial infectons appeared to be at particularly high risk, over a third of patients had no documented bacterial infection whatsoever. This series also confirmed the increased risk of salmonella septicaemia and osteomyelitis in SS children.

FIGURE 2

PNEUMOCOCCAL INFECTION IN SS DISEASE

(Barrett-Connor, 1971)



The fulminant course of pneumococcal infection in sickle cell disease was stressed by Kabins and Lerner (1970) who reported four children with SS disease and sickle thalassaemia age 13 months to 5 years who died after illnesses of less than one day. The CSF white cell reaction to infection was absent or delayed in the three patients who had positive CSF cultures for the pneumococcus. Petechiae were noted in the skin of two patients. Benzyl penicillin was advised for all children with sickle cell haemoglobinopathies and acute febrile illnesses, especially under age 6 years.

Seeler, Metzger and Hufson (1972) similarly reported a rapid progression of symptoms and one case of purpura fulminans in 10 SS patients with 12 episodes of pneumococcal meningitis or septicaemia. Seven of these patients were aged under 2 years, the remaining three patients being aged 6 years, 6 years and 8 years at the time of the first infection. Five patients had palpable spleens.

Seeler (1972) reviewing 19 deaths in SS children over a 5 year period noted that three were due to pneumococcal meningitis and septicaemia at ages 18 months, 18 months and 4 years, one was due to clinically unsuspected purulent meningitis at age 1 year, and one was due to Salmonella enteritidis septicaemia at age 13 years. The spleens of the four children with meningitis were small but not fibrotic.

Certain children with SS disease therefore have an increased susceptibility to fulminant and recurrent pneumococcal septicaemia and meningitis, especially in the first two or three years of life. In addition they show an increased susceptibility to H influenzae meningitis or septicaemia and to Salmonella septicaemia or osteomyelitis. The infections may occur in children whose spleens have not yet fibrosed and are still palpably enlarged.

7. Bacterial infection and the asplenic state

This increased susceptibility of SS children to fulminant and recurrent bacterial, especially pneumococcal, infections is similar to the pattern of infection seen in children who have undergone splenectomy or who are born without a spleen.

King and Shumacker (1952) reported severe infections in five children in whom splenectomy had been performed under the age of 6 months. Two children had meningococcal meningitis and septicaemia with a petechial rash at ages 8 and 12 months. One child had purulent meningitis with no bacteria isolated (following suphadiazine therapy) at age 6 months, and one child had H influenzae meningitis at age 3 years. The fifth child died in a rapidly progressive febrile illness one month after splenectomy at age 2 months.

Subsequently Smith et al (1957) reported 13 bacterial infections in 12 patients who had undergone splenectomy at ages ranging from 14 months to 14 years, the majority of episodes of infection occurring within two years of splenectomy, although one episode of Escherichia coli meningitis occurred 11 years after splenectomy. The pneumococcus was responsible for seven of the 13 episodes. One patient who underwent splenectomy for traumatic rupture of the spleen had recurrent episodes of pneumococcal meningitis at 12 and 18 months after splenectomy, the pneumococci isolated being of different serotypes. H influenzae meningitis was seen three years after splenectomy performed at age 11 years, an age at which meningitis due to H influenzae is unusual. Further follow-up studies of patients who had undergone splenectomy (Lowdon et al, 1966, Eraklis et al, 1967) showed that severe bacterial, especially pneumococcal, infections were commonest in patients whose underlying disease was thought to predispose to infection, such as thalassaemia major,

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portal hypertension and hypersplenism, and less common in those who had undergone splenectomy for splenic rupture, and that the risk was greatest if splenectomy was performed in early life, the risk of fatal infection being at least twice as great if splenectomy was performed before age 5 years.

A review of 1413 children who underwent splenectomy under age 16 (Eraklis and Filler, 1972) confirmed that mortality from overwhelming infection was related to primary diagnosis, and that the risk of death from overwhelming infection was greatest if splenectomy was performed under age 2 years, 7.2% (16/223) of these patients dying subsequently from overwhelming infection, compared to 1.5% (18/1189) of those in whom splenectomy was performed later.

Pearson et al (1978) showed by radioisotope spleen scans that in children who had undergone splenectomy for trauma regeneration of splenic nodules occurred, presumably from seeding of splenic tissue shed from the ruptured spleen. They suggested that this finding explained the smaller increase in susceptibility to subsequent infection seen after splenectomy for splenic rupture.

Splenectomy in childhood thus predisposes to severe bacterial infections, especially fulminant pneumococcal septicaemia and meningitis, whatever the indication for splenectomy or the age at which it is performed, although the risk is greater the younger the age at splenectomy, in patients with a predisposing underlying disease, and when there is no peritoneal seeding by trauma or at operation.

Confirmation that absence of the spleen in early childhood predisposes to severe infection is provided by a review of organisms isolated from blood or CSF in 59 children with congenital asplenia (Waldman et al, 1977). Under age 6 months, E coli was isolated from four children, Klebsiella from three and Staphylococcus aureus from one.

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After age 6 months the pneumococcus was isolated from five children (ages 6 months to 5 years) and H influenzae from two (ages 10 months to 3 years). The overall frequency of isolation of pathogens from blood or CSF of these asplenic children was 90/1,000 patient-years. The frequency in a control group of children with cyanotic congenital heart disease (chosen because cardiac abnormalities were present in 88% of the asplenic children) was 8/1,000 patient-years.

8. Immune response after splenectomy

Rowley (1950a) injected sheep erythrocytes intravenously in splenectomised and control rats and found that splenectomised rats failed to produce circulating haemolysins in response to a dose which gave maximal haemolysin response in controls. Splenectomised rats failed to respond to repeated immunisation with the same dose of antigen but gave a reduced response after larger doses of antigen. Splenectomised rats similarly failed to respond to intravenous sheep red cells even if prior to splenectomy they had shown a good haemolysin response to the procedure. Intravenous injection of sheep red cells in 14 humans who had undergone splenectomy failed to produce a haemolysin response in all but one, compared with at least a four fold response in circulating antibody in 16 normal controls (Rowley 1950b). Similarly the primary IgM response to intravenous bacteriophage ØX 174 was reduced in 24 patients who had undergone splenectomy, despite normal clearance of the bacteriophage from the circulation (Sullivan et al, 1978). After a second intravenous injection of bacteriophage the quantitative antibody response was normal but the IgG contribution to this secondary response was reduced.

Intradermal, subcutaneous, intramuscular, intraperitoneal and intraportal injection of sheep red cells gave the same haemolysin responses in both splenectomised and control rats, although these responses were smaller than the response to intravenous injection in controls. Splenectomised rats failed to show a response to intravenous sheep red cells even after a normal response to intraperitoneal injection (Rowley, 1950a). Subcutaneous pneumococcal polysaccharide vaccine induced normal antibody responses in humans who had undergone splenectomy, except in four who had received immunosuppressive drugs for Hodgkin's disease (Sullivan et al, 1978). Previous findings of a normal response to subcutaneous antigen after splenectomy in individuals without other immunological deficits (Saslaw et al, 1959) were thus confirmed.

IgM levels have been found to be low in children who have undergone splenectomy (Schumacher, 1970), although this was not confirmed by Sullivan et al (1978). The activity of the peptide tuftsin, which is thought to stimulate phagocytosis by polymorphs, has also been found to be reduced in 18 of 23 patients who had undergone splenectomy (Constantopoulos et al, 1973).

Possible consequences of the impairment of the antibody response to intravenous particulate antigen after splenectomy are suggested by the experiments of Schulkind et al (1967) who studied the uptake of radio-isotope labelled pneumococci by the liver and spleen after intravenous injection in rabbits. The total uptake by the liver exceeded splenic uptake, but the spleen's capacity to take up pneumococci per unit weight was greater than that of the liver, and was greatest relative to that of the liver in the non-immune animal with no preformed humoral antibody. After immunisation, either active by prior injection of pneumococci, or passive by injection of type specific pneumococcal antibody, the liver's uptake per unit weight relative to the spleen's uptake was increased about ten times. These findings suggested that in the non-immune individual absence of the spleen or impairment of splenic function and a consequent failure to produce humoral antibody in response to intravenous antigen would result in a reduced rate of uptake of pneumococci by the liver. If clearance of pneumococci from the blood was thus impaired a transient bacteraemia might transform into a fulminant septicaemia.

9. Functional asplenia in SS disease

Several studies have demonstrated that the immune deficit in SS disease is similar to that found after splenectomy. Pearson et al (1969), noticing Howell-Jolly bodies in blood films from SS children with splenomegaly, performed radioactive (^{99m}Tc) colloid scans on 12 SS children aged 8 months to 14 years and found no colloid uptake in 11 even though the spleen was palpably enlarged in nine of the 12 children. This failure of reticuloendothelial function of the palpably enlarged spleen they described as functional asplenia. Splenic uptake of colloid could be restored by transfusions of red cells but not of fresh plasma alone in five children aged 1 to 4 years with palpable spleens (Pearson et al, 1970). Three SS children without palpable spleens aged 3, 4 and 14 years showed no splenic uptake even after red cell transfusion. Serial spleen scans in children with SS diagnosed at birth have shown that failure of collid uptake is an acquired defect (O'Brien et al, 1976). The spleen's ability to take up colloid was usually present until at least age 6 months, although one patient with a palpable spleen before age 2 months lost splenic uptake of colloid by age 5 months and died with pneumococcal meningitis at age 7 months (Pearson, 1973).

Three SS children, aged 2 to 6 years, with palpable spleens but no splenic uptake of colloid received intravenous injections of sheep red cells and showed a much reduced or absent heterophil antibody response when compared with AA and AS controls (Schwartz and Pearson, 1972). Compatible red cell transfusion did not improve the antibody response to sheep red cells, even when splenic radioactive colloid uptake was restored. However, intramuscular injection of sheep red cells to a 3 year old SS child with a palpable spleen but no splenic uptake of colloid resulted in a normal antibody response. Similarly four SS children, aged 2 to 10 years, showed decreased antibody response to intravenous bacteriophage ØX 174 and a reduced IgG response on repeat

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intravenous administration, but normal antibody responses to subcutaneously administered pneumococcal polysaccharide vaccine (Sullivan et al, 1978). SS children, even with palpable spleens, may thus have the same impaired response to intravenous immunisation as splenectomised individuals, and may then be described as functionally asplenic.

The mechanism of the failure of the immune response following loss of reticuloendothelial function is not clear. Winkelstein and Drachman (1968) studied sera from 14 SS children aged 8 months to 12 years, six of the children having palpable spleens. The opsonizing activity of the sera for polymorphs from normal individuals for phagocytosis of pneumococci was reduced for 12 children, the serum of two SS children aged 2 and 3 years having normal activity. Sera from 15 control children had normal opsonizing activity, apart from one serum with reduced activity from a child aged 2 years. The opsonizing activity was heat-labile, in contrast to opsonizing activity for salmonella which was not heat-labile and was not reduced in either normal or SS sera. As serum opsonizing activity for pneumococci reaches adult levels at about age 2 years, suggesting that antigenic stimulation is required, thedefect in heat labile opsonin production by SS children might be due to failure to respond to antigenic stimulation in the absence of a normally functioning spleen.

Further investigation of serum opsonization activity (Johnston et al, 1973) showed an abnormality in the alternative pathway of complement activation. Sera from 28 SS patients aged 1 to 30 years were compared with sera from 20 controls aged 1 to 35 years. Opsonizing activity for pneumococci prepared with type specific IgG was similar in SS and control sera if the pneumococci were prepared with a maximal amount of antibody, but was reduced in SS sera relative to controls if the amount of antibody was reduced. Little agglutinating activity for the Type 2 pneumococcus used could be found in either control or SS sera, so deficiency of antibody in SS patients seemed unlikely to be the explanation for this difference. Comparison of classical complement pathway

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activities in the control and SS sera showed no difference. However using a special assay in the presence of ethylene glycol tetracetic acid to chelate calcium, which is required for the classical pathway, a deficiency of alternative pathway activity for the phagocytosis of zymosan was demonstrated in SS sera and was shown by the use of C3 specific serum to be due to failure of C3 fixation to the surface of the antigen. As the alternative pathway, unlike the classical complement pathway, does not require preformed antibody, such a deficiency would be particularly significant on first encounter with a pathogenic organism. In a further study alternative pathway activity assessed by haemolytic radial diffusion was depressed in 8 out of 29 SS patients (Wilson et al, 1976) and in these patients factor B levels were significantly reduced.

Corry et al (1979) compared alternative pathway activity in patients with sickle cell disease, patients with agamma-globulinaemia, patients after splenectomy and normal controls. The patients with sickle cell disease were age 1 to 43 years; their haemoglobin genotypes were not stated. The ranges of activity seen in the four groups overlapped, but 15% of the sickle cell disease group, 10% of the splenectomy group, and 50% of the agammaglobulinaemia group showed activities reduced below the range seen in normal controls. Mean activities were also significantly reduced in the sickle cell disease and hypogammaglobulinaemia groups, but the mean activity in the splenectomy group was not significantly reduced below the mean activity in the control group. Differences in age, or in age at splenectomy, could not be associated with the differences in activities.

Opsonization and alternative pathway studies have thus shown defects in a proportion only of patients with sickle cell disease. So far these defects have not been shown to be associated with individual susceptibility to infection, nor have they been related to the age of the individual or the age of onset of functional asplenia.

10. Pitted red cells as an index of defective reticuloendothelial function

Differential interference contrast microscopy (Nomarski optics) can be used to give a three dimensional appearance by apparent lateral illumination, the image being formed by a beam of light split and recombined by prisms below and above the objective. When red cells from an individual who has undergone splenectomy are examined by this method 10% or more appear to have one or more round crater-like indentations. Red cells from newborn, especially premature, infants may show these indentations, which for simplicity are described as pits, but after two months of age less than 4% of red cells from normal individuals are pitted (Holroyde, Oski and Gardner, 1969). The exact nature of the pits is uncertain: electron microscope studies do not show corresponding indentations but instead vacuoles in the cells (Holroyde and Gardner, 1970). The pitted red blood cell count (abbreviated to pit count) is defined as the percentage of red blood cells appearing to have one or more circular indentations, regardless of the sizes of the indentations or the number of indentations per cell, when viewed by differential interference contrast microscopy.

Holroyde and Gardner transfused blood from a normal individual into a splenectomized patient with red cell asplasia and a pit count of 45%, and found that the patient's pit count, initially diluted to 25%, returned to 45% in about 20 days while the haematocrit was still elevated above baseline level. Normal erythrocytes are therefore capable of developing pits after tranfusion into a splenectomized individual. When blood with 50% pit count from an individual splenectomized after trauma was labelled with radiochromium and transfused into a normal recipient with intact spleen the pits disappeared from the blood over 72 hours, while the half life of the circulating radiochromium tagged red cells was 30 days. In the presence of an intact spleen the

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pits therefore appear to be removed from the pitted red cells.

Crosby (1959) in his review of the functions of the spleen refers to this removal of red cell inclusions as the 'pitting function'. Care must therefore be taken to avoid confusion in nomenclature, as the pits are removed by splenic 'pitting' and are not a consequence of it.

Pearson et al (1978) studied pit counts in normal children, children who had undergone splenectomy because of trauma, and children who had undergone splenectomy for haematological indications. Normal children had pit counts in the range of 0 - 0.8% (mean ± SD:0.08 ±0.003). Children with splenectomies performed for haematological indications all had pit counts greater than 12%, whereas a proportion of children with splenectomies performed for trauma had pit counts intermediate between these levels with range 2 - 7%. Spleen scans of five of these children showedextrahepatic uptake of colloid in what were presumed to be multiple small nodules of reticuloendothelial tissue on the peritoneum developing from seeding of splenic tissue at the time of splenic rupture. Pearson and colleagues suggested that the normal pit counts seen in children who underwent splenectomy for splenic rupture were a consequence of this regeneration of reticuloendothelial activity and described the phenomenon of splenosis as the 'born-again spleen'.

11. Pitted red cell counts in SS disease

Casper et al (1976) used Nomarski optics to examine red cells from patients with SS and SC disease aged 6 months to 39 years. They found that pitted red cell counts were elevated in the range 2% to 71% with mean 30% in these patients, compared with pit counts in the range 0 - 3% with mean 0.5% in normal controls. Spleen scans performed in 12 patients with pit counts ranging from 12 to 50% were found to be abnormal in all patients except one who had a pit count of 12%. Although Howell-Jolly bodies indicating splenic reticuloendothelial abnormality were seen in the peripheral blood films of some of these 12 patients the percentage counts of cells with Howell-Jolly bodies did not correlate with either pit counts or spleen scan results. Four SS patients and one SC patient with pit counts greater than 15% developed meningitis or other (unspecified) sepsis.

Pearson et al (1979) determined pit counts in 28 SS patients aged 3 months to 22 years and found that the pit count increased with age, exceeding 12%, the lower end of the range of pit counts in asplenic patients without splenic regeneration, after age 6 to 8 years. In one child in whom serial pit counts were performed the rise in pit count with age correlated with progressive loss of splenic uptake of labelled colloid. In another child age 10 months the rise in pit count was reversed by transfusion.

Sills and Oski (1979) found pit counts in the range 3 - 67% in 21 SS patients (mean $\pm$SD 31 $\pm$ 17) compared with the range 0 - 1.6% in 15 normal children (mean $\pm$SD 0.9 $\pm$ 0.4) and the range 16 - 56% in 14 post splenectomy patients (mean $\pm$SD 39 $\pm$ 13). .....

The mean pit count in all their patients with sickle haemoglobinopathies rose with age from 14% in the age group 0 - 3 years to 24% in the age group 3 - 7 years and 32% in the age group 7 - 11 years. They also demonstrated reversibility of the rise in pit count after transfusion in a 9 month old child.

Pit count estimation is thus an alternative measure of splenic function, particularly suitable for serial studies in SS children because of the small volume of blood required and the ease and speed with which the investigation can be performed.

12. Haematological changes in childhood SS disease

The haematological changes seen with age in the AA control children described in the present study have been reported by Serjeant et al (1980). Haemoglobin concentration (Hb) was found to fall rapidly in the first eight weeks of life and then to remain at a plateau from age 2 to 6 months (approximate mean value 11.3 gm/dl), before falling slowly to a minimum at about age 15 months (approximate mean value 9.9 gm/dl), and then rising again steadily towards adult levels. The initial rapid fall was associated with a rapid fall in red cell count, but from age 2 months red cell count rose to a peak at about age 6 months before falling slowly once again. The second, slow, fall in Hb was associated with falling mean cell volume (MCV), mean cell haemoglobin concentration (MCHC) and mean cell haemoglobin (MCH) which all reached minimum values at about age 15 months before slowly rising to reach adult values at about age 4 years. Girls tended to have higher Hb than boys, with significantly higher MCV and MCHC during the period of the second fall in Hb.

This second fall in Hb was thought to be due to dietary iron deficiency on the basis of the falling MCV, MCHC and MCH and of low serum iron levels at age 1 year. The higher Hb and red cell count seen in girls in the first two months of life were unexplained, but the later sex difference was ascribed to a possibly milder pattern of iron deficiency in girls, although no difference was found in serum iron levels between boys and girls at age 1 year.

The cohort of SS children described in the present study is included in the larger group (enlarged by inclusion of children born and diagnosed between July 1977 and April 1980) of 266 SS children whose haematological changes are reported by Serjeant et al (1981) in what is so far the only account of haematological development in an unselected group of SS children large enough for statistical analysis.

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In these children Hb was significantly lower than in AA controls from age 2 months onwards. The same pattern of early haematological development was seen with an initial rapid fall in Hb followed by a short plateau at age 3 to 6 months (mean values at age 6 months, boys and girls: 8.7 gm/dl) followed by a second fall to age 15 months (mean values at age 15 months: boys 7.4 gm/dl, girls 7.9 gm/dl). However, following the second fall Hb remained static in SS males and rose only slightly in SS females. The rise in red cell count from age 2 to age 6 months was much less marked in SS children than in AA children and after age 6 months the fall in red cell count was more rapid in SS children. In contrast SS children showed smaller falls in MCV, MCHC and MCH than AA children, and the rise in MCV began earlier at about age 8 months. Serum iron levels in SS children at ages 1 to 4 years were significantly higher than in AA children (with levels in SS girls higher than in SS boys although not significantly so). The main differences between SS and AA children could therefore be explained on the basis of the lower red cell count in the SS children falling almost continuously from birth (with the exception of the brief and small rise from age 2 to 6 months) until at least age 6 years, together with very much smaller changes in MCV, MCHC and MCH in the period age 4 months to 4 years. Serjeant et al suggest that these SS children, who like the AA children were not receiving any regular dietary supplementation with iron or folic acid, might be protected against the effects of a diet deficient in iron perhaps by increased intestinal iron absorption secondary to increased marrow activity or by increased iron availability as a result of continuing haemolysis. They speculate that this protection against iron deficiency may paradoxically be a disadvantage, since increasing the intracellular haemoglobin concentration may increase the tendency of the red cells to sickle during a critical phase of early development.

Haemoglobin F (HbF) levels in the SS children of the present study have been reported and correlated with clinical severity of disease by Stevens et al (1981). They found that at age 6 months HbF levels were approximately normally distributed with mean 24.7% and standard deviation 7.5%. HbF levels at age 6 months correlated significantly with levels at age 2 years in 65 individual children in whom levels at both ages were available. Significantly lower HbF levels at age 6 months were found in children who experienced dactylitis, acute splenic sequestration or death in the first two years of life compared with those who did not experience each of these events. Children in whom the spleen was first palpable at or before age 6 months had significantly lower HbF levels than those in whom the spleen was first palpable only after age 6 months or not at all, and a significant correlation was found between HbF level at age 6 months and the age of first palpable splenomegaly. The risks of dactylitis and of acute splenic sequestration during the first two years were shown by the survival curve method to be significantly related to HbF levels at age 6 months.

PATIENTS AND METHODS

1. Cord blood screening programme

Since June 1973 cord blood samples from Victoria Jubilee Hospital, the main government maternity hospital serving Kingston, Jamaica, have been screened by haemoglobin electrophoresis (Serjeant et al, 1974). Fourteen thousand deliveries a year, a quarter of all registered births in Jamaica, take place in this hospital. Cord blood samples from all non-operative deliveries are collected into lithium heparin tubes by the labour ward staff. The tubes are collected daily from the wards by a member of the MRC staff, who also takes heel prick specimens from any babies from whom cord bloods have not been collected. Specimens are in this way collected from 90% of all deliveries.

One drop of blood from each of these specimens is haemolysed with 0.5 ml tetrasodium ethylenediaminetetra-acetate (10 mg/dl) including potassium cyanide (82 mg/dl). The concentrations of the resulting haemolysates are adjusted visually before electrophoresis on cellulose acetate paper at pH 8.4, which is performed daily by MRC staff. Once a week haemoglobin electrophoresis on agar gel at pH 6.0 is performed on all specimens with electrophoretically abnormal haemoglobins. Specimens which show only haemoglobins (Hb) S, F and A_2 indicate SS genotype, or less commonly sickle cell beta^o thalassaemia or sickle cell hereditary persistence of HbF, which show the same electrophoretic pattern. Family studies to exclude these two genotypes are attempted, but are unreliable because of low paternal cooperation with a high impaternity rate. Children who have HbA_2 levels greater than 3.2% or HbF levels greater than 30% at age 1 year are assumed to belong to these genotypes and have been excluded from the present study. SS genotype is confirmed by repeat electrophoresis at yearly intervals.

From June 1973 to July 1977, 49,000 cord blood samples were screened and 160 cases of SS disease diagnosed according to the above criteria. The present study is based on the follow-up of these children from birth until 8th July 1978 when the oldest SS child was aged 5 years and the youngest aged 1 year.

2. Follow-up clinic

Mothers of babies whose cord blood specimens suggested SS genotype on cellulose acetate electrophoresis were given appointments to attend a special clinic at the University Hospital of the West Indies. This clinic was held from July 1973 to July 1974 by Dr G R Serjeant, from August 1974 to July 1976 by Dr J M Clarke, and from August 1976 to July 1978 by myself. Dr M F Lowry, Dr D Millard, and Dr A M Ramlal assisted in the clinic to provide cover for holiday, week-end and study periods. Children with other sickle cell haemoglobinopathies and variant haemoglobins identified in the screening programme were also followed in the clinic, together with 230 controls with normal haemoglobin genotype AA. These controls were selected by inviting the mothers of each of two children with presumed AA genotype born on the same day in the same hospital and of the same sex as each of the first 125 index cases of presumed SS genotype to bring their babies to the clinic. After exclusion of 14 children of mothers who declined to join the clinic, four AA children with HbA_2 levels greater than 4% at one year suggesting beta thalassaemia trait, one child who was abducted, and one child who emigrated, this control group consisted of 111 AA boys and 119 AA girls whose ages ranged from 2 years 6 months to 5 years on the 8th July 1978.

At the first visit to the clinic the child's probable genotype and its implications, as well as the service and research aims of the clinic, were explained to the mother, and to the father or grandmother if also attending. Full details were taken of the family, including at least one Kingston address and one country address of close relatives, for future follow-up. The baby was weighed on a balance scale, length was measured on an infant measuring scale and head circumference with a tape measure. The baby was examined by the doctor. A heel prick blood specimen was taken to confirm the diagnosis by haemoglobin electrophoresis on cellulose acetate and agar; single drops of blood were taken into brilliant cresyl blue for reticulocyte count and into 10% formol saline for pitted red cell count, 0.02 ml blood was collected by pipette for dilution for analysis in an electronic cell counter (Coulter Electronics), and a microhaematocrit tube was collected for packed cell volume, micromethod HbF and plasma bilirubin estimations. Packed cell volume was estimated on a microcentrifuge without correction for trapped plasma. HbF was estimated by a denaturation method which measured unaltered haemoglobin after a precisely timed period of denaturation (Serjeant et al 1975). Plasma bilirubin was estimated in an optical bilirubinometer (American Optical Corporation).

Follow-up appointments were given monthly to age 6 months, two monthly to age 1 year, and three monthly thereafter. When the screening programme began some SS children were assigned to a no intervention group and were given appointments six monthly only: this policy was abandoned when many of the mothers of these children brought their sick children to the clinic before their appointments were due. Each visit followed the same pattern with discussion of the child's health since the last visit, measurement of weight, length and head circumference, full physical examination and blood test.

Heel pricks or fingerpricks were performed at the two or three monthly appointments, and venepunctures, which permitted serum iron, serum bilirubin, serum folate and HbF and HbA_2 estimations, at yearly intervals. HbF was estimated from venepuncture specimens by the method of Betke (1959) and HbA_2 by the method of Weatherall et al (1971) as modified by Millard et al (1977).

Venepunctures were also performed if clinically indicated for blood cultures or for biochemical or serological investigations. Blood culture specimens were taken into brain heart infusion and thioglycollate medium, or into thiol broth if the child was already receiving antibiotics. Blood cultures were taken for all fevers greater than 101°F (38.3°C) except during epidemics of measles and dengue fever, and for all suspected episodes of acute splenic sequestration, as well as in all children in whom septicaemia, meningitis or osteomyelitis were suspected.

Every effort was made to provide nutritional, health and family planning advice, and in this the doctor was greatly assisted by the clinic nurse Mrs Lena Cupidore. Routine immunisations against diphtheria, pertussis, tetanus, polio and measles were given, and when available food supplements from the Ministry of Health were distributed.

Attendance was encouraged without appointment if the child was at all unwell. Children were seen from 8.30 am to 4.30 pm Mondays to Fridays and from 8.30 am to 12.30 pm on Saturdays. Outside these hours children were occasionally seen in the clinic if the mother was able to contact the clinic doctor by telephone; otherwise they were taken to the Casualty Departments at the University Hospital and at the Bustamante Children's Hospital. .....

Mothers were strongly advised that whenever they saw a doctor they should say that their child had sickle cell disease, and they were provided with appointment cards on which the child's genotype was recorded.

As access to free medical care (and often also to private medical care) in Kingston entails long delays, many relatives and friends were also brought to the clinic. This was welcomed as a means of strengthening links with families of the study children, but because of the pressure of numbers it was usually necessary to limit the clinic to siblings, parents, grand-parents and great grand-parents of the study children.

When a mother failed to keep her appointment she was visited at home by a field worker, or, during periods of civil unrest, contacted by telegram. Repeated efforts were made to trace long term defaulters. If children moved out of Kingston and its surroundings to remoter areas of Jamaica they were visited in the country by a doctor at least once a year and appropriate blood specimens taken.

3. In-patient care

Children requiring admission were referred to the Department of Paediatrics, University Hospital for in-patient care. While I was doctor to the follow-up clinic I held an Honorary Senior Registrar appointment in the Department, joining the on-call rota, which enabled me to maintain personal contact with staff in the Department and to follow the course of children in the study when admitted to hospital. Children were also admitted to the Bustamante Children's Hospital via Casualty, and by courtesy of Dr K McKenzie and Dr B Johnson, hospital notes of these admissions were examined by Dr Clarke, Dr Ramlal and myself.

4. Mortality

Case notes for children who died in hospital were examined for details of their terminal illnesses. If a child died at home or was dead on arrival at hospital details of the terminal illness were sought from the parents.

Blood cultures and blood specimens for haematology were taken immediately from patients brought to hospital dead. Post mortem examinations were performed in the Department of Pathology, University of the West Indies, by Dr B R Sparke or another member of the Department and were attended by myself or another M R C doctor. Cultures were taken from the middle ear, cerebrospinal fluid (C S F), bronchi, heart blood clot, and rectum at necropsy.

5. Splenomegaly

Whether or not the spleen was palpable was recorded at each clinic visit. The size of the spleen was measured from the costal margin in its long axis. In addition for easier comparison splenomegaly was graded according to the following scale:

0 Spleen impalpable
1 Spleen may be palpable
2 Definite spleen tip, less than 3 cm
3 Spleen 3 cm, but above umbilicus
4 Spleen to umbilicus
5 Spleen extends beyond umbilicus

6. Pitted red cell counts

Splenic reticuloendothelial function was assessed by estimating percentage pitted red cell counts. One drop of fresh blood was taken at heel prick, finger prick or venepuncture into a tube containing 10% formol saline. .....

The red cells settled to the bottom of the tube which was stoppered and stored at 4°C for periods ranging from one hour to eight weeks.

To perform a pit count the cells were resuspended by shaking the tube, and a small drop of the suspension was placed on a clean slide and gently covered with a coverslip. Counting was easier when the suspension did not extend to the edges of the coverslip. The preparation was examined under oil immersion on a standard Zeiss microscope with X100 objective using differential interference contrast microscopy (Nomarski optics) by which the three dimensional structure of the cell is shown as if by side illumination (Plate A). The pit count for a given specimen was defined as the percentage of red cells with one or more regular circular indentations irrespective of the size or number of the indentations. Five hundred cells were counted for each estimation, unless no pitted cells were seen, in which case only three hundred cells were counted.

All pit counts reported in this study were estimated by myself after initial instruction in the method by Mrs Beryl Serjeant, with the sole exception of the counts on patients after splenectomy. Repeat counts on the same specimen by different observers and before and after several months storage gave results within the range ± 2.5 of the original count.

7. Spleen scans

Radioactive isotope (Tc-99m) sulphur colloid spleen scans were performed by the staff of the Nuclear Medicine Laboratory, University Hospital of the West Indies, under the supervision of Dr Allele. Several of these investigations were attended by myself to give assistance if required in the care of the child.

8. Statistical analyses

Results were analysed using the life table method and logrank significance test described by Peto et al, (1976, 1977). These methods are suitable for the analysis of events occurring in groups of individuals followed for a period of time, as not only the number of individuals experiencing an event but also the times for which the individuals are at risk are taken into account. The expected number of events in each group assuming no differences between the groups calculated by the logrank method can be compared with the observed number using the chi-squared test, and if more than two groups are observed a chi-squared value for trend can be calculated. The method allows for individuals lost to follow-up if it can be assumed that these do not differ in outcome from individuals successfully followed-up: any error which might result from such an assumption is minimised by making every effort, as in the present study, to avoid losses to follow-up. Analyses for survival by genotype and for time to first severe infection by spleen group (grouped according to age at which the spleen was first felt) were first calculated by myself without computer assistance to confirm the usefulness of the method. These analyses were checked and further analyses performed using the SURV-C programme developed for the Medical Research Council's trials of the treatment of childhood leukaemia, described by Peto et al. The programme was adapted for use on the Government of Jamaica's Central Data Processing Centre's IBM 370/135VS computer by Miss Shubha Vaidya, who was responsible for all programming: the data were prepared for programming and the results from the computer print-outs assessed by myself.

For analysis of the haematological variables means and standard deviations were calculated for each variable for the SS children grouped by age, sex, age spleen first palpable and age of rise of pit count.

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Ages were defined as follows:

6 months:	5 months to 7 months
12 months:	11 months to 13 months
24 months:	22 months to 26 months
36 months:	34 months to 38 months

The variables were found to be normally distributed with the exception of reticulocyte counts which were therefore analysed after log transform. Calculations were performed on a Casio fx 48 scientific calculator accurate to 8 significant figures.

For life table analysis of time to spleen first felt, first ASS, first severe infection and death for the SS children grouped by haematological values at age 6 months the three variables selected were HbF percentage concentration, MCV and reticulocyte counts, as these were thought to be the variables most likely to indicate independent influences on the development of SS disease, HbF percentage because of HbF's inhibitory influence on sickling, MCV because of the possibility that smaller cells might negotiate the splenic circulation more easily, and reticulocyte count as a measure of red cell turnover.

RESULTS

1. Follow-up and survival

During the study 11 of 160 (7%) of the initial SS children were lost to follow-up by emigration, removal to remote areas of Jamaica or default. Seven were boys and four were girls. Six were lost to follow-up before age 1 year, another four before age 2 years, and another one before age 3 years. No more were lost to follow-up after age 3 years.

The survival curve (Figure 3) compares survival for the first five years of life in these 160 SS children and in the 230 AA children who were age and sex matched with the first 109 SS children. The percentages of the initial AA children lost to follow-up were 11% at age 1 year and 16% at age 2 years. The analysis is based on known survival on 8th July 1978. Comparison by the life table method of the matched groups shows that mortality was similar in the two genotypes in the first 6 months, but that after 6 months mortality rose significantly ($p<0.001$ to one year) in SS children while there were no further deaths among AA children. Infant mortality rates (deaths in the first year per thousand live births) calculated from the survival curves were 95 for SS disease and 13 for AA controls.

Survival curves for SS girls and SS boys separately (Figure 4) show the higher mortality among girls. Seventeen girls and five boys died. This sex difference in mortality is statistically significant (Chi-squared test 6.25, d.f. = 1, $p<0.02$).

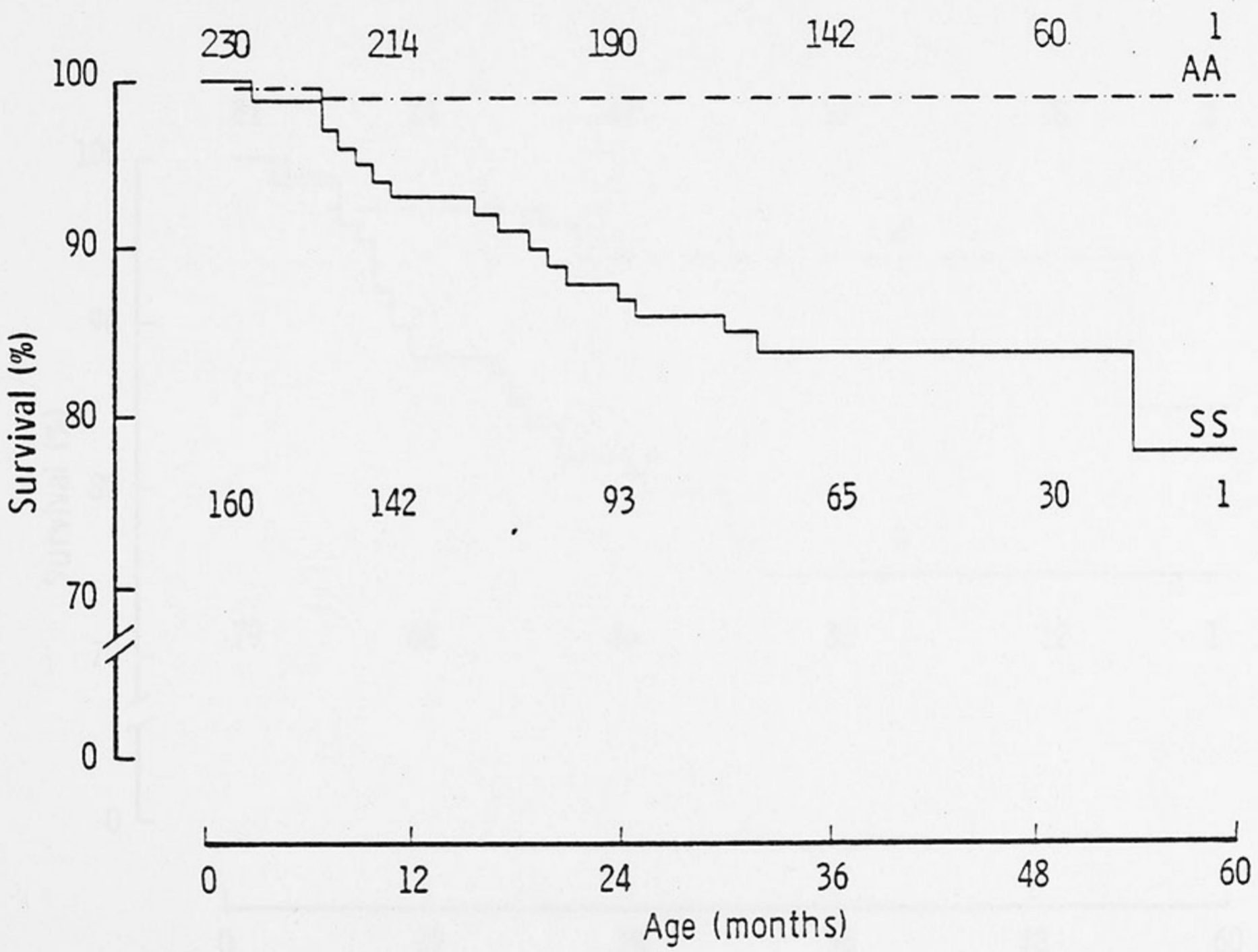

Figure 3 Survival in SS and AA children

Numbers are those at risk in each genotype at yearly intervals

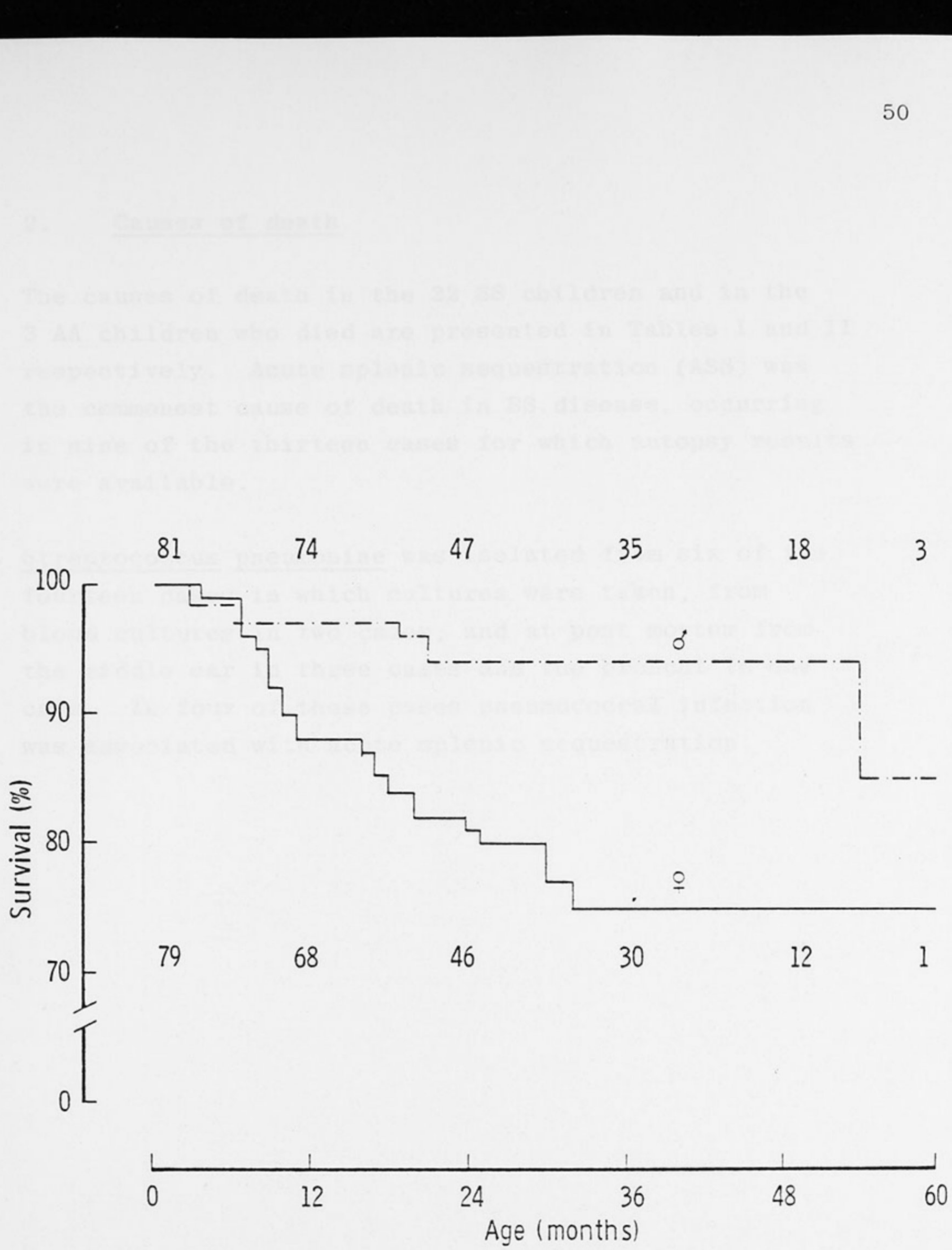

Figure 4 Survival in SS boys and girls

Numbers are those at risk in each sex at yearly intervals.

2. Causes of death

The causes of death in the 22 SS children and in the 3 AA children who died are presented in Tables I and II respectively. Acute splenic sequestration (ASS) was the commonest cause of death in SS disease, occurring in nine of the thirteen cases for which autopsy results were available.

Streptococcus pneumoniae was isolated from six of the fourteen cases in which cultures were taken, from blood cultures in two cases, and at post mortem from the middle ear in three cases and the bronchi in one case. In four of these cases pneumococcal infection was associated with acute splenic sequestration.

TABLE I

CAUSE OF DEATH IN SS CHILDREN

PATIENT NO	SEX	AGE IN MONTHS	CAUSE OF DEATH	BACTERIOLOGICAL FINDINGS
98	M	3	Gastroenteritis*	No cultures taken
143	F	4	Uncertain*	No cultures taken
2	M	7	Gastroenteritis	Streptococcus pneumoniae (Middle ear) Pathogenic Escherichia coli (rectum)
52	F	7	ASS, pneumonia	S pneumoniae (blood)
101	F	7	ASS, pneumonia	S pneumoniae (bronchi)
25	F	8	Cot death*	No cultures taken
65	F	9	ASS	S pneumoniae (middle ear)
20	F	9	Gastroenteritis*	No cultures taken
28	F	10	Pneumonia*	No cultures taken
120	F	10	ASS	No significant growth
102	F	11	Cardiac failure (?ASS ?septicaemia)	No cultures taken
122	F	16	Meningitis	S pneumoniae (middle ear) Salmonella typhimurium (middle ear)
41	F	17	ASS	No significant growth
135	F	18	Uncertain*	No significant growth
24	M	19	Uncertain*	No cultures taken
138	F	20	ASS	No significant growth
75	M	21	ASS	S pneumoniae (blood, middle ear and bronchus)
79	F	24	Uncertain*	No cultures taken
61	F	25	Pneumonia, malnutrition	No significant growth
97	F	30	?Meningitis*	No significant growth
70	F	32	ASS, gastroenteritis	No significant growth
12	M	54	ASS	No significant growth

* No necropsy performed

ASS - Acute splenic sequestration

TABLE II

CAUSE OF DEATH IN AA CHILDREN

PATIENT	SEX	AGE IN MONTHS	CAUSE OF DEATH	BACTERIOLOGICAL FINDINGS
1	F	2	Congenital heart disease*	No cultures taken
2	M	3	Gastroenteritis*	No cultures taken
3	M	6	Gastroenteritis*	No cultures taken

* No necropsy performed

3. Splenomegaly

The time to first clinical detection of splenomegaly in the SS children is shown as a survival curve in Figure 5. The percentages who have had palpable splenomegaly at some time are 37% at age 6 months, 66% at 12 months, 79% at 24 months and 85% at 36 months. Survival curves comparing the time to first detection of splenomegaly show no difference between the sexes. The pattern of splenomegaly is shown in detail in Figure 6, which demonstrates the wide variation in the degree and persistence of splenomegaly from patient to patient. This figure was prepared by arranging the SS children in order of age of spleen appearing and then, to reduce the size of the figure, selecting every third child for inclusion.

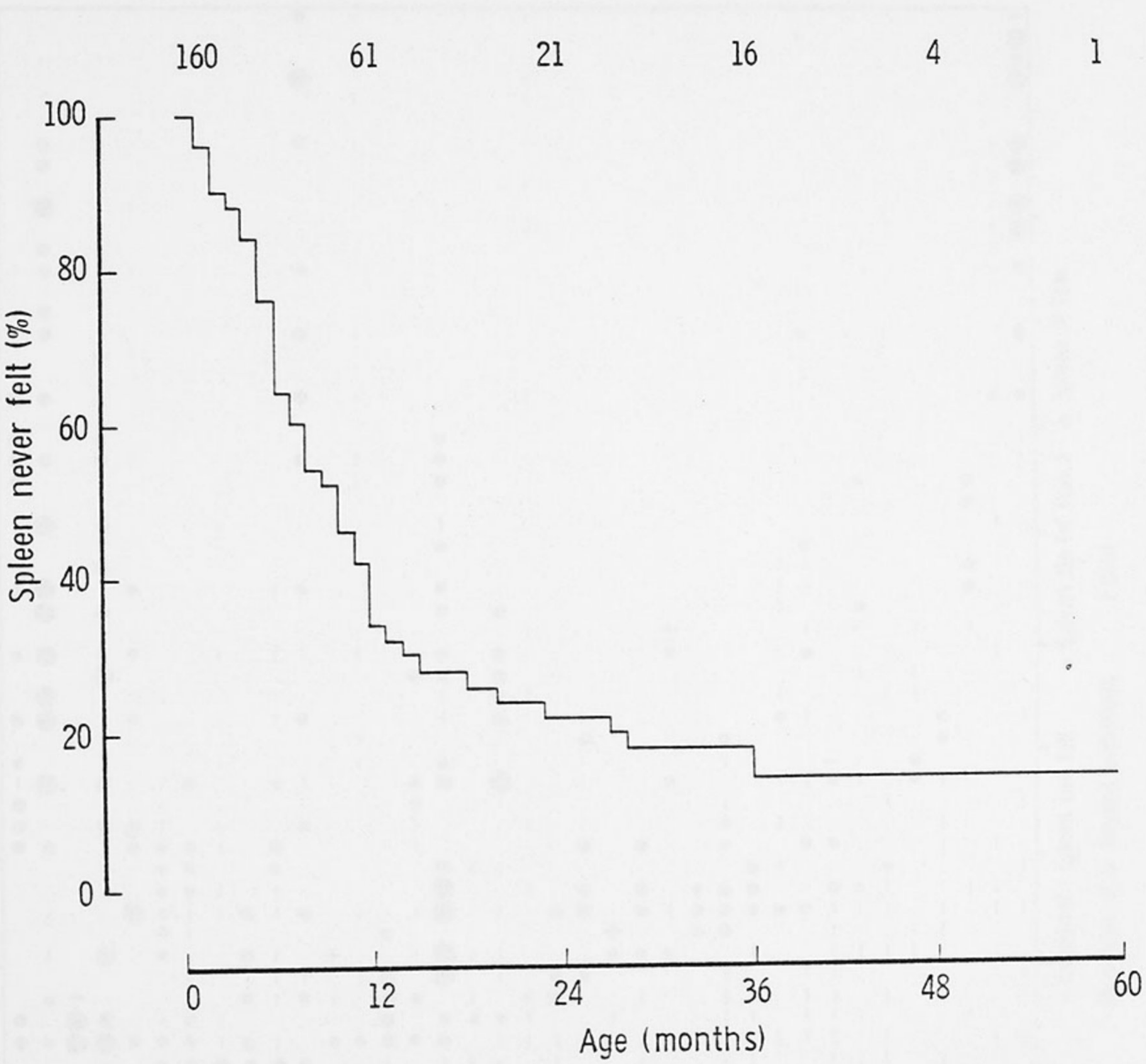

Figure 5 Survival curve indicating first appearance of splenomegaly

Sharp increases in splenomegaly at 6 and 12 months represent artefacts from appointment system

Numbers are those at risk at yearly intervals

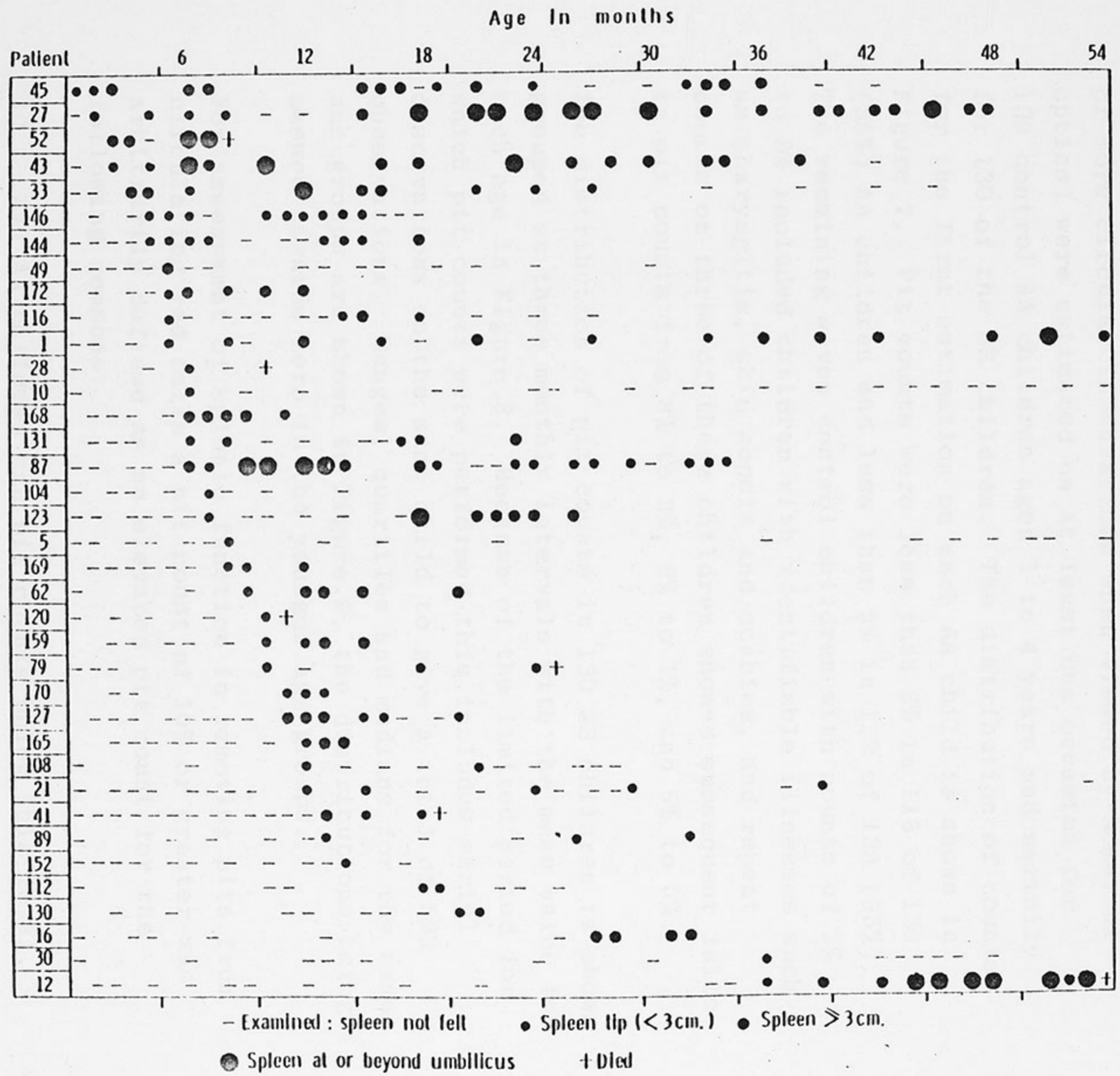

Figure 6: Patterns of Splenomegaly in 30 SS Children

4. Pitted red cell counts

Between November 1976 and July 1978 pit counts (percentages of red blood cells appearing to have one or more circular indentations when viewed by Nomarski optics) were estimated on at least one occasion for 139 control AA children aged 1 to 4 years and serially for 130 of the SS children. The distribution of counts for the first estimation on each AA child is shown in Figure 7. Pit counts were less than 2% in 118 of 139 (85%) AA children and less than 5% in 132 of 139 (95%). The remaining seven control children with counts of 5% to 8% included children with identifiable illnesses such as pharyngitis, skin sepsis and scabies, and repeat counts on three of these children showed subsequent falls in pit counts from 8% to 3%, 8% to 1%, and 5% to 0%.

The distribution of pit counts in 130 SS children is shown grouped at three monthly intervals with the mean value for each age in Figure 8. Because of the limited period for which pit counts were performed this includes serial observations on the same child to give a total of 529 observations. Ranges, quartiles and medians for the same age groups are shown in Figure 9, the distributions being skewed towards zero in the younger age groups.

For assessment of splenic function in removing pits from circulating red cells a pit count of 10% or greater was arbitrarily defined as an elevated pit count for the following reasons:

1) All AA children had pit counts below this level.

2) Spleen scans in eleven SS children with palpable spleen showed no splenic uptake of colloid at pit counts of 9% or greater (Table III).

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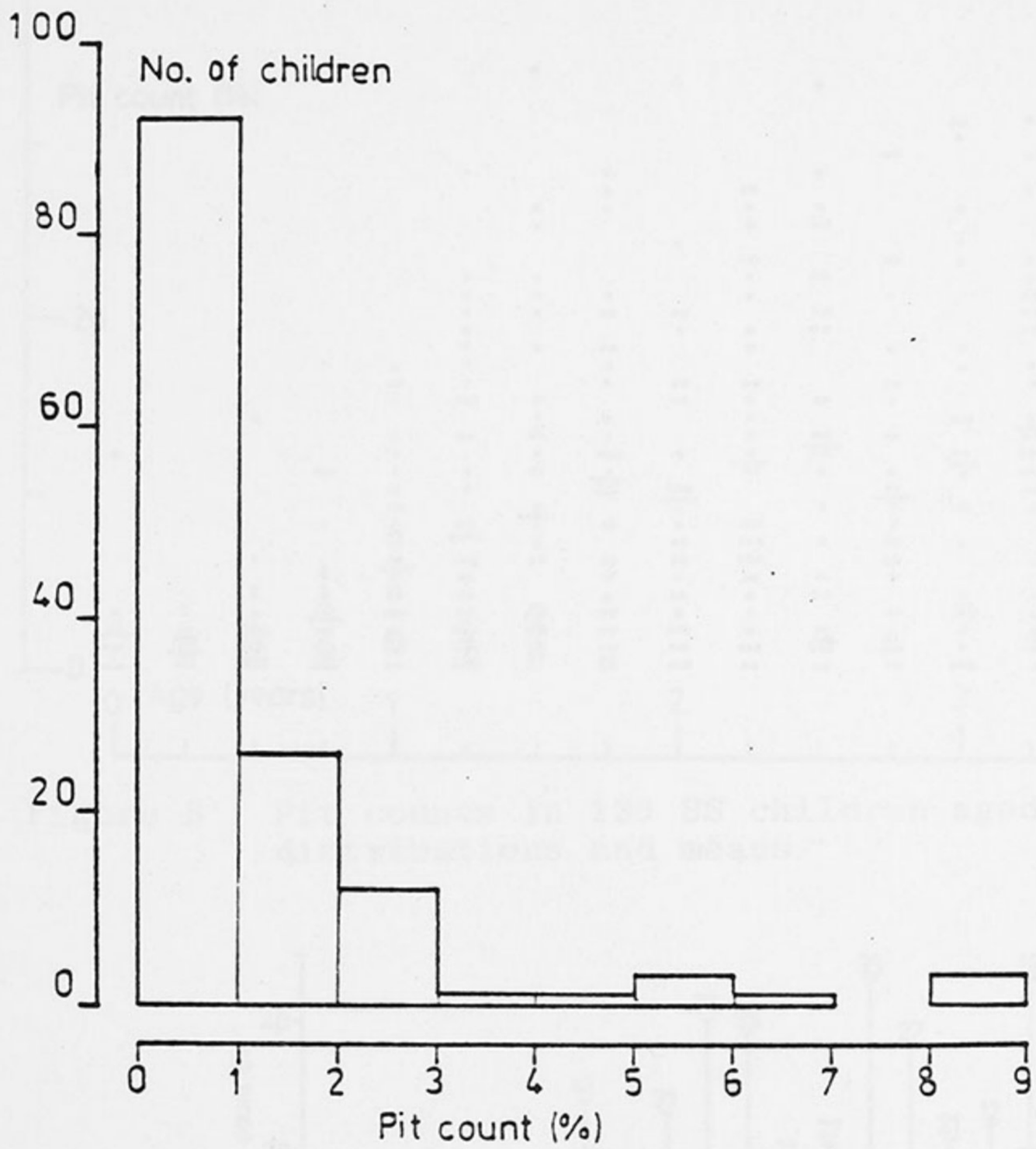


Figure 7: Pit counts in 139 AA children age 1 to 4.

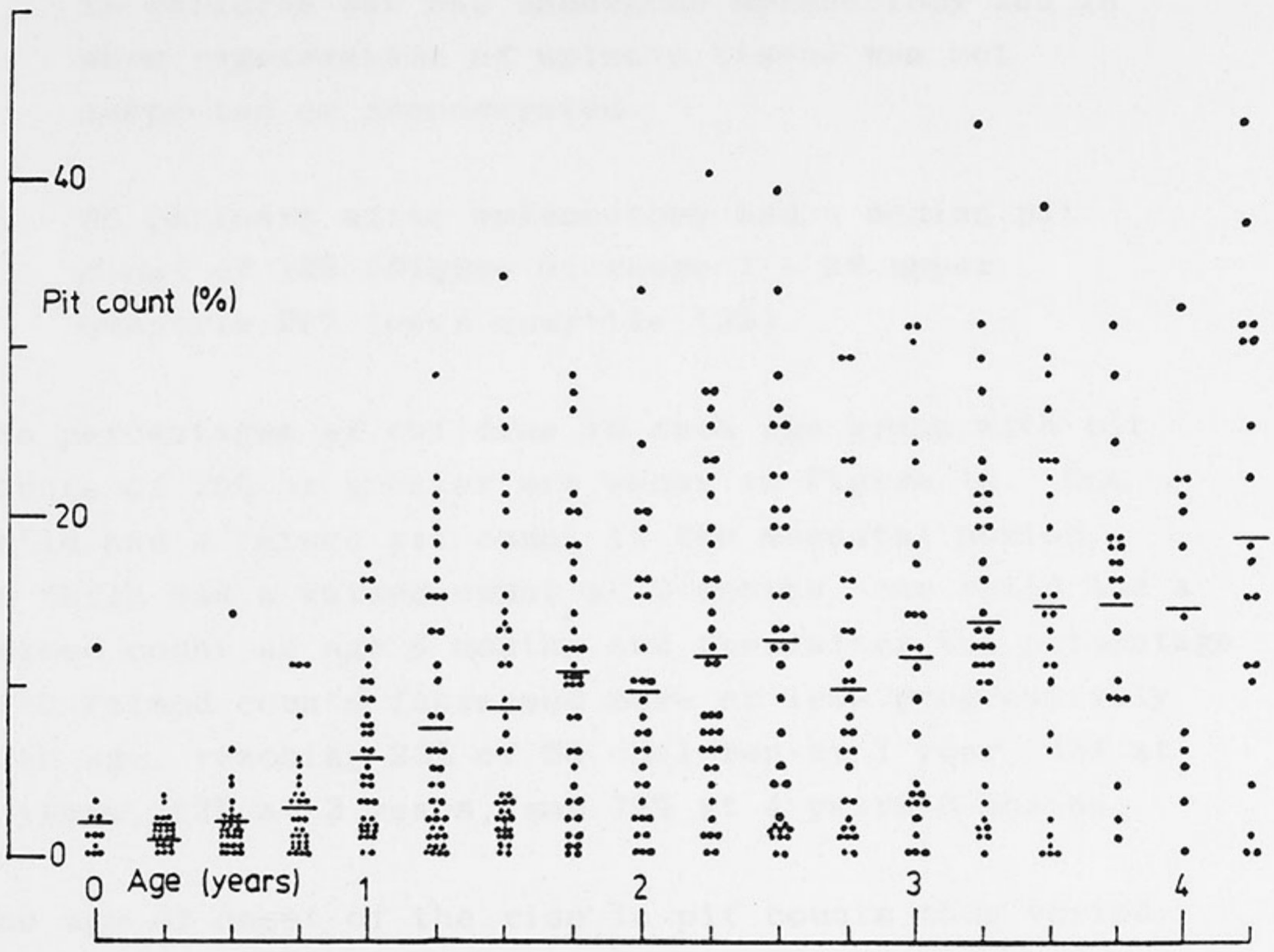


Figure 8: Pit counts in 130 SS children aged 0 to 4: distributions and means

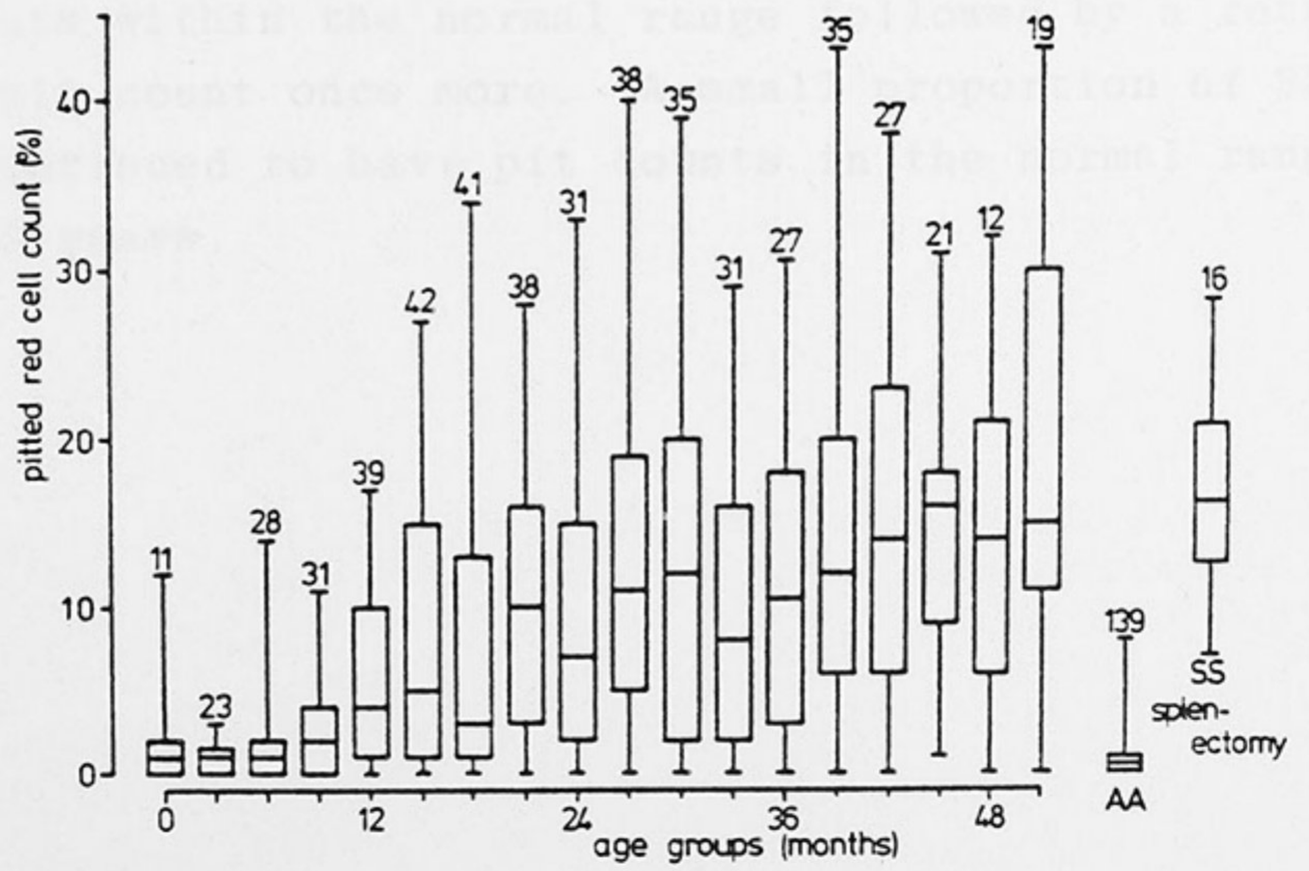


Figure 9: Pit counts in 130 SS children aged 0 to 4, 139 AA children age 1 to 4 and 16 splenectomized SS children and adults: ranges, quartiles and medians.
Numbers are total observations in each age group.

3) Pearson (1978) found pit counts of 12% or greater in children who had undergone splenectomy and in whom regeneration of splenic tissue was not suspected or demonstrated.

4) SS patients after splenectomy had a median pit count of 16% (Figure 9: range 7 - 28 upper quartile 20% lower quartile 12%).

The percentages of children in each age group with pit counts of 10% or greater are shown in Figure 10. One child had a raised pit count in the neonatal period, no child had a raised count at 3 months, one child had a raised count at age 6 months and thereafter the percentage with raised counts increased more or less progressively with age, reaching 23% of SS children at 1 year, 42% at 2 years, 52% at 3 years, and 79% at 4 years 3 months.

The age of onset of the rise in pit counts thus varied from patient to patient. In some patients the rise appeared to be reversible for a period after the initial rise, as the rise wasfollowed by a period of one or two months with pit counts within the normal range followed by a return to raised pit count once more. A small proportion of SS children continued to have pit counts in the normal range up to age 5 years.

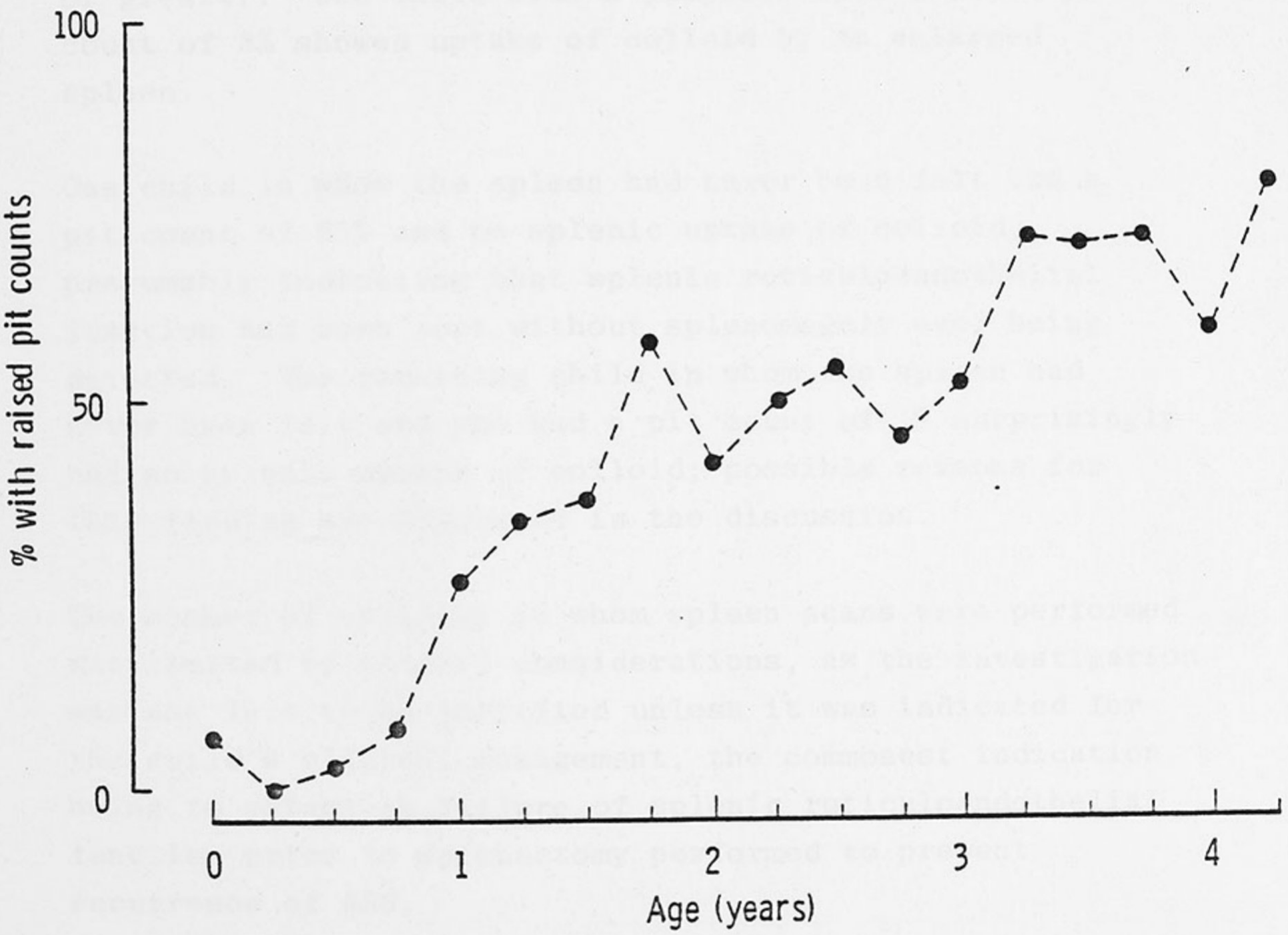


Figure 10: Percentages of SS children with pit counts greater than 9% at three monthly intervals

5. Spleen scans and pitted red cell counts

Spleen scans were performed on 12 SS children with palpable spleens and two further SS children in whom the spleen had never been felt. Spleen size and pit count for each child at the time of the scan are shown in Table III. No splenic uptake of colloid was seen in 12 children with palpable spleens and pit counts of 9% or greater. One child with a palpable spleen and a pit count of 3% showed uptake of colloid by an enlarged spleen.

One child in whom the spleen had never been felt had a pit count of 25% and no splenic uptake of colloid, presumably indicating that splenic reticuloendothelial function had been lost without splenomegaly ever being detected. The remaining child in whom the spleen had never been felt and who had a pit count of 1% surprisingly had no splenic uptake of colloid; possible reasons for this finding are suggested in the discussion.

The number of children in whom spleen scans were performed was limited by ethical considerations, as the investigation was not felt to be justified unless it was indicated for the child's clinical management, the commonest indication being to establish failure of splenic reticuloendothelial function prior to splenectomy performed to prevent recurrence of ASS.

TABLE III

RADIOISOTOPE SPLEEN SCANS

PATIENT	NO	SEX	AGE IN MONTHS	CLINICAL SPLEEN SIZE (cm)	PIT COUNT %	SPLEEN	LIVER
1	162	M	18	5	16	No activity	Normal
2	123	M	29	5	15	No activity	Normal
3	121	M	30	6	14	No activity	Normal
4	119	F	32	2	15	No activity	Small cold nodules
5	99	M	35	0	25	No activity	Normal
6	92	M	36	2	11	No activity	Normal
7	47	M	43	3	9	No activity	Patchywith cold nodules
8	56	M	43	6	3	Activity present: splenomegaly	Normal
9	53	F	44	8	11	No activity	Normal
10	27	M	49	5	18	No activity	Patch with cool nodules
11	29	F	50	6	11	No activity	Normal
12	8	M	53	6	14	No activity	Many cool nodules
13	23	M	54	0	1	No activity	Patchy activity
14	1	F	60	6	15	No activity	Minimal left lobe activity with irregular margin

6. Severe bacterial infections

Severe bacterial infections were defined as isolation of a pathogenic organism from blood culture or cerebrospinal fluid (CSF), purulent meningitis from which no organism was identified, or isolation at necropsy of an organism known to be responsible for post splenectomy infection.

Nineteen episodes were seen in 14 SS children (5 boys and 9 girls) (Table IV). S pneumoniae was isolated from 15 episodes, the other organisms isolated being H influenzae (one septicaemia and one osteomyelitis) and Salmonella aberdeen (one septicaemia). Recurrent infection were seen in two patients. One survived H influenzae septicaemia but died with pneumococcal septicaemia and ASS three months later. The other survived one episode of H influenzae osteomyelitis and four episodes of pneumococcal meningitis. No severe bacterial infections were seen in the AA control children. The survival curve for time to first severe infection for 160 SS children is shown in Figure 11. The risk of first infection ± standard error is 6% ± 2% by age 1 year, 8% ± 2% by age 2 years, and 11% ± 3% by age 3 years. For pneumococcal infections the estimated risk is 6% by age 1 year, 8% by age 2 years, and 9% by age 3 years.

Two further episodes of severe infection were seen in the SS children after the end of the study period, one successfully treated Salmonella californiae osteomyelitis and septicaemia and one fatal E coli pneumonia and septicaemia in a survivor of pneumococcal septicaemia. For comparison with Figure 2 the pattern of recurrent infection is shown as a flow diagram in Figure 12.

TABLE IV

SEVERE BACTERIAL INFECTIONS

PATIENT	NO	SEX	AGE SPLEEN FIRST FELT (MONTHS)	AGE AT INFECTION (MONTHS)	DIAGNOSIS	ORGANISMS	SITE	OUTCOME
1	111	M	4	4	Meningitis	Streptococcus pneumoniae	Blood, CSF	Survived
2	2	M	5	7	Gastroenteritis	S pneumoniae Pathogenic Escherichia coli	Middle ear* Rectum*	Died
3	52	F	2	7	Septicaemia, ASS	S pneumoniae	Blood	Died
4	101	F	6	7	Pneumoniae, ASS	S pneumoniae	Bronchus*	Died
5	168	F	6	8	Septicaemia	S pneumoniae	Blood	Survived
6	6	F	3	9	Meningitis	S pneumoniae	CSF	Survived
7	65	F	8	9	ASS	S pneumoniae	Middle ear*	Died
8	62	M	9	9	Meningitis	Nil isolated (on penicillin)		Survived
9	43	F	2	12	Meningitis	S pneumoniae	CSF	Survived
10	164	M	4	15	Septicaemia	S pneumoniae	Blood	Survived
11	122	F	3	16	Meningitis	S pneumoniae Salmonella typhimurium	Middle ear*	Died
12	75	M	2	19	Septicaemia	Haemophilus influenzae	Blood	Survived
				21	Septicaemia, ASS	S pneumoniae	Blood, middle ear* and bronchus*	Died
13	10	F	6	29	Meningitis	S pneumoniae	CSF	Survived
				32	Osteomyelitis	H influenzae	Blood	Survived
				42	Meningitis	S pneumoniae	CSF	Survived
				43	Meningitis	S pneumoniae	CSF	Survived
				57	Meningitis	S pneumoniae	CSF	Survived
14	87	F	6	30	Septicaemia	Salmonella aberdeen	Blood	Survived

* Isolated at necropsy

ASS Acute splenic sequestration

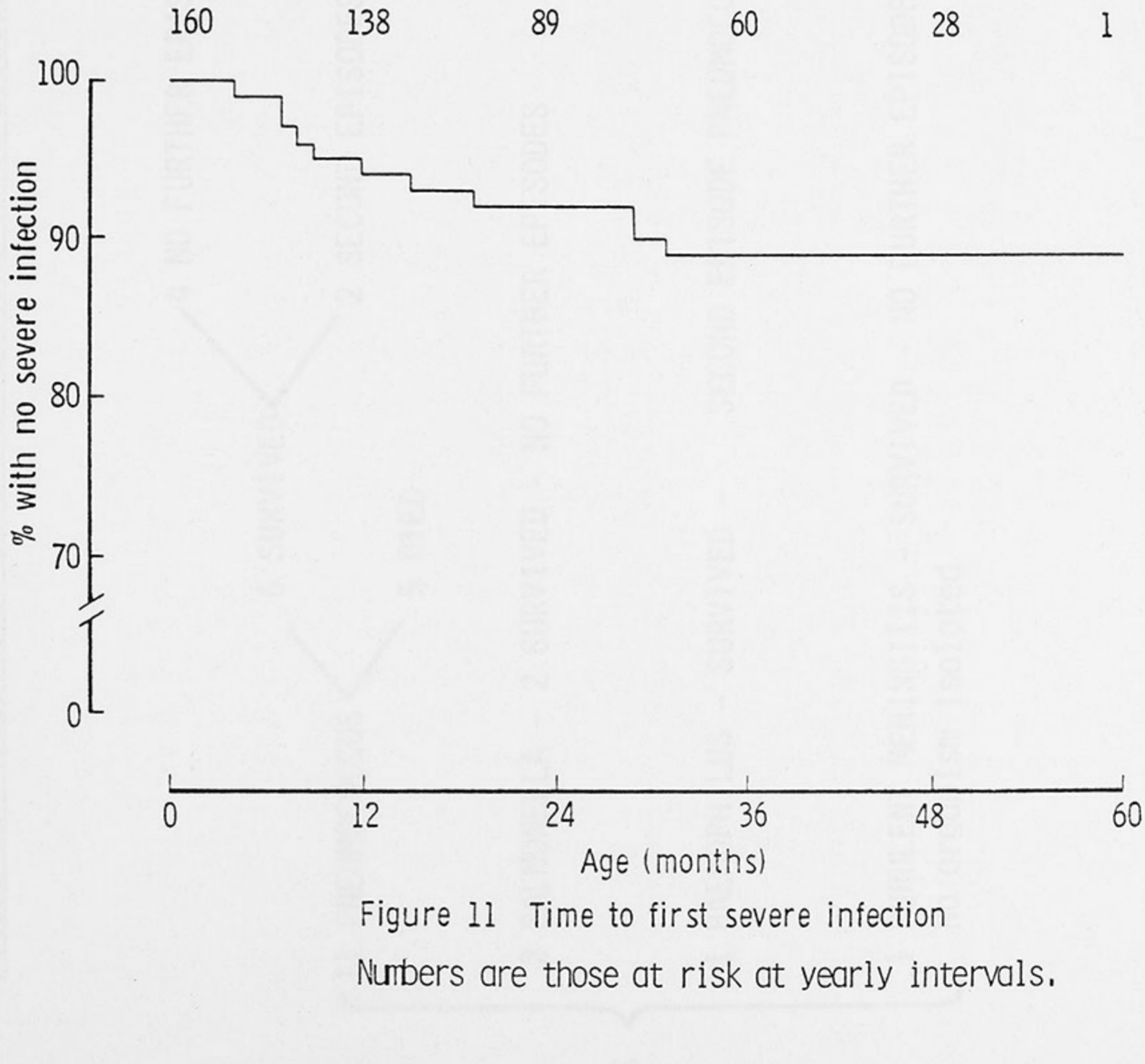
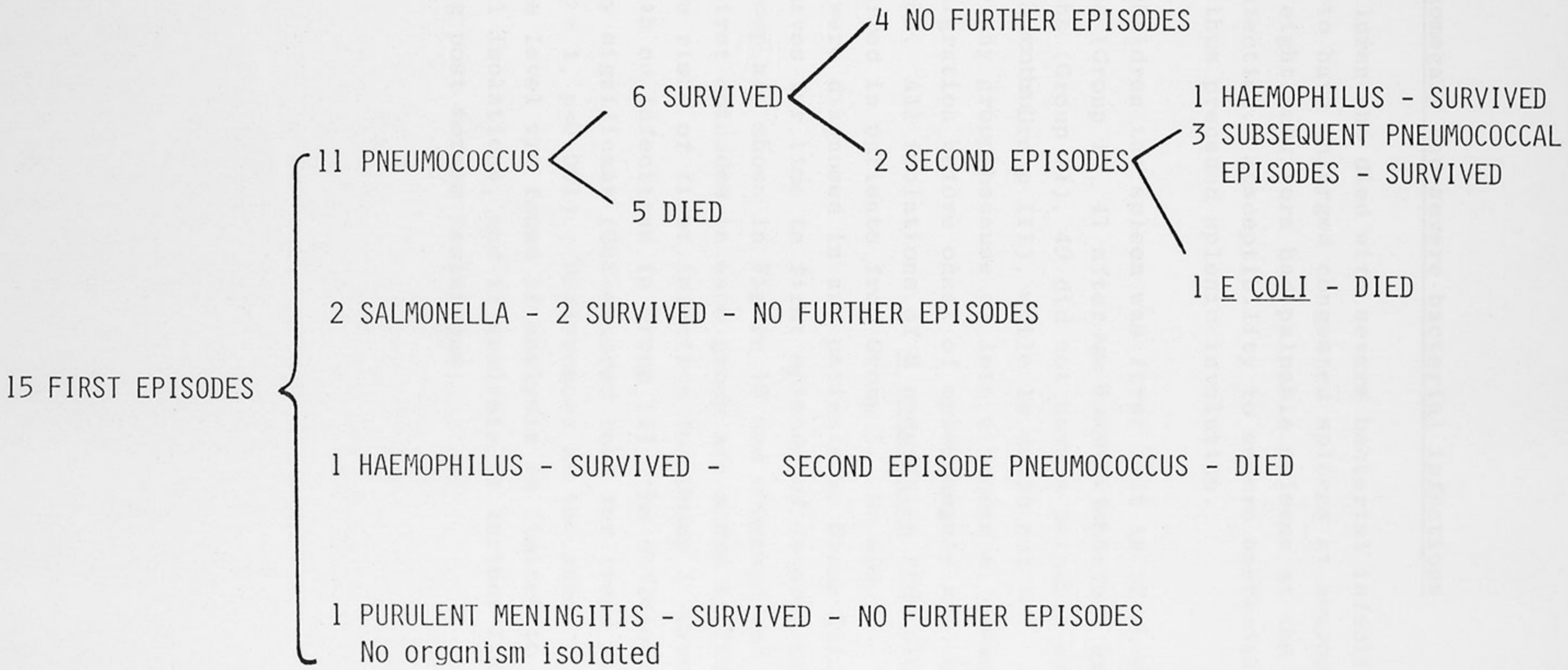


Figure 11 Time to first severe infection

Numbers are those at risk at yearly intervals.

FIGURE 12 Outcome of severe infections in SS disease



7. Splenomegaly and severe bacterial infections

All six children who died with severe bacterial infections were found to have enlarged congested spleens at necropsy. Six of the eight survivors had palpable spleens at the time of their infection. Susceptibility to severe bacterial infections thus preceded splenic involution.

Of 160 SS children the spleen was first felt in 52 at or before age 6 months (Group I), 41 after age 6 months but before or at age 12 months (Group II), 49 did not have a palpable spleen before age 13 months (Group III), while 18 could not be assigned to any group because of late attendance, default, death or emigration before onset of splenomegaly and before age 13 months. All isolations of S pneumoniae from blood or CSF occurred in patients from Group I. No severe infections were diagnosed in any patients in Group III. Survival curves for time to first episode of severe infection for each group are shown in Figure 13 and observed and expected number of first episodes in each group are shown in Table V. The relative risks of first infection for Group I: Group II were 5:1 with no infections in Group III, the differences being highly significant (Chi-squared test for trend yielded 14.15, d.f. = 1, $p<0.001$). Differences at the same significance level are found if analysis is limited to pneumococcal isolations, and if analysis is further limited by excluding post mortem isolations.

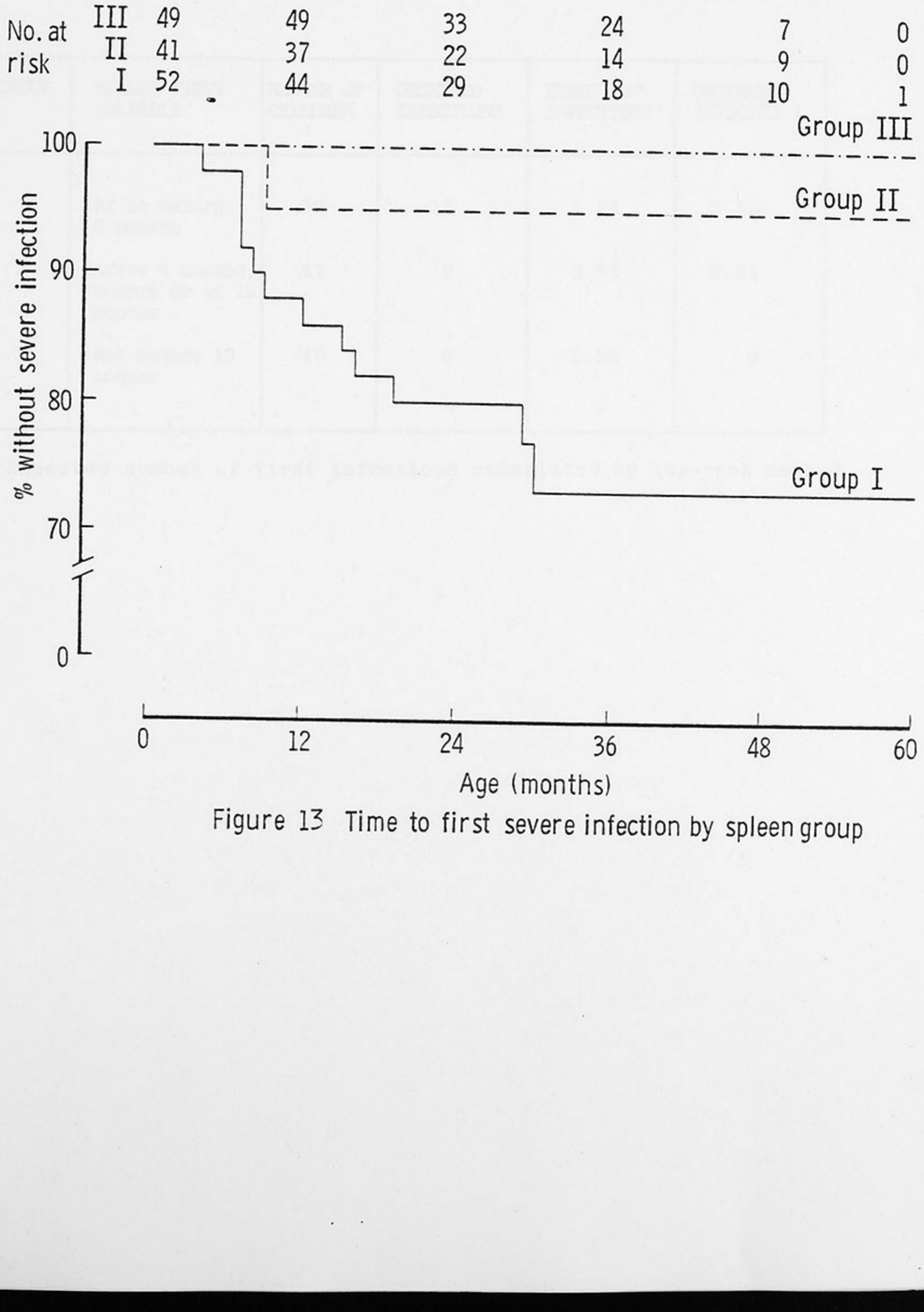


Figure 13 Time to first severe infection by spleen group

TABLE V

FIRST SEVERE INFECTIONS BY SPLEEN GROUP

GROUP	SPLEEN FIRST PALPABLE	NUMBER OF CHILDREN	OBSERVED INFECTIONS	EXPECTED * INFECTIONS	OBSERVED / EXPECTED
1	At or before 6 months	52	12	4.84	2.48
2	After 6 months, before or at 12 months	41	2	3.94	0.51
3	Not before 13 months	49	0	5.22	0

* Expected number of first infections calculated by log-rank method.

8. Pit counts and severe bacterial infections

Nine episodes of severe bacterial infection have been seen in seven SS children in whom pit counts have been performed serially. One of these episodes occurred in August 1978 after the end of the study period. Details are given in Table VI. The pit counts in relation to the infections are plotted for three of these children in Figure 14. The pit count had been raised before the first episode in six patients, and was raised at the time of the episode in the seventh.

Of the eight patients who experienced severe bacterial infections before pit counts were available, four had fatal episodes. Of the remaining four, three who had pneumococcal meningitis had raised pit counts recorded subsequently, while one who had purulent meningitis with no organism isolated had a subsequent maximum pit count of 7%.

Four children who experienced severe bacterial infections had serial pit counts performed from one year or earlier. All had raised pit counts before age one year. Life table and logrank test analysis for all 52 children with pit counts followed from one year or earlier shows that this increased risk of severe infection among those with raised pit counts at or before 1 year is significant (Table VII chi squared 5.32 d.f. = 1 $p<0.01$).

TABLE VI

PIT COUNTS AND SEVERE BACTERIAL INFECTIONS

PATIENT	NO	AGE SPLEEN FIRST FELT (MONTHS)	AGE PIT COUNT ELEVATED (MONTHS)	AGE AT INFECTION (MONTHS)	INFECTIONS	ORGANISMS	OUTCOME	PREVIOUS PIT COUNT	PIT COUNT AT INFECTION	SUBSE-QUENT PIT COUNT	PREVIOUS INFECTIONS
1	168	6	8	8	Septicaemia	Streptococcus pneumoniae	Survived	4%	13%	15%	
2	182	7	10	14	Osteomyelitis	Salmonella californiae	Survived	14%	Unknown	11%	
3	164	4	7	15	Septicaemia	S pneumoniae	Survived	16%	Unknown	34%	
4	122	3	< 12	16	Meningitis	S pneumoniae Salmonella typhimurium	Died	18%	Unknown	-	
5	75	2	< 21	21	Septicaemia	S pneumoniae	Died	24%	Unknown	-	Haemophilus influenzae septicaemia
6	87	6	< 19	30	Septicaemia	Salmonella aberdeen	Survived	24%	24%	14%	
7	10	6	< 36	42	Meningitis	S pneumoniae	Survived	12%	23%	Unknown	S pneumoniae meningitis
				43	Meningitis	S pneumoniae	Survived	Unknown	Unknown	15%	H influenzae osteomyelitis
				57	Meningitis	S pneumoniae	Survived	21%	12%	10%	

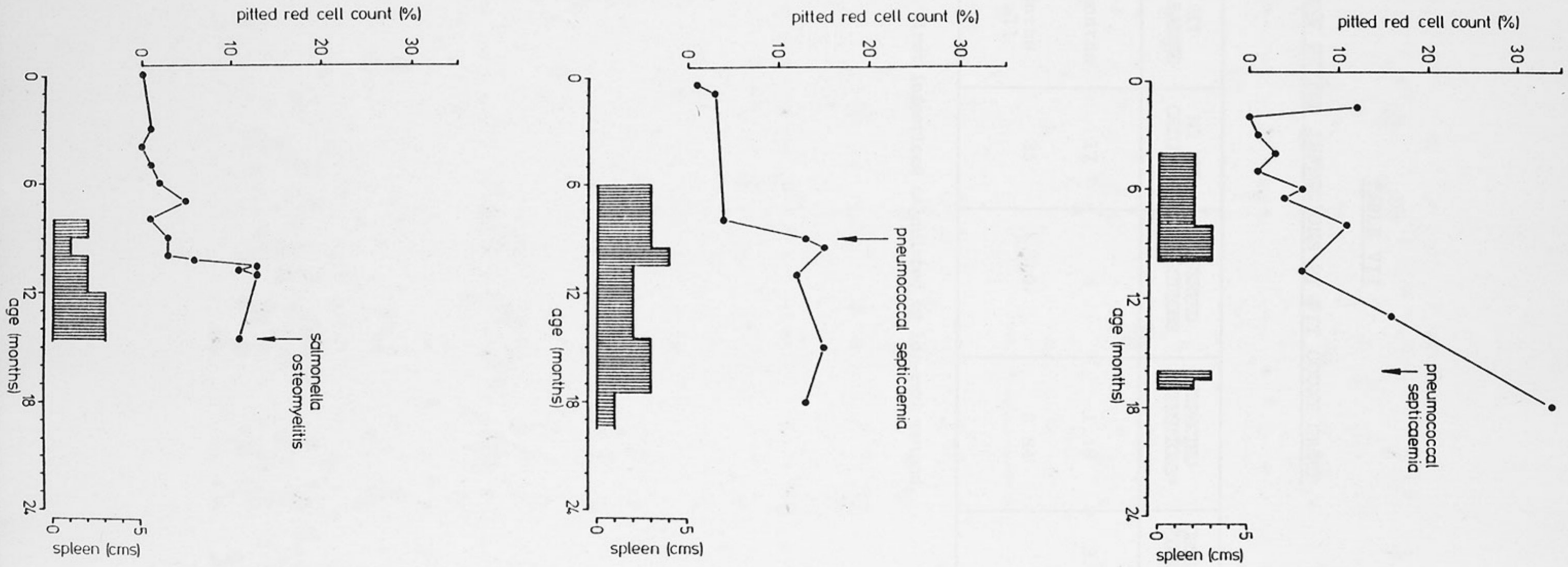


Figure 14: Pit counts and splenomegaly in relation to infections

Plate B. Necropsy after acute splenic sequestration (Patient 12)

9. Acute splenic sequestration

Acute splenic sequestration (ASS) was defined as an episode of acute enlargement of the spleen, associated with a fall in haematocrit or haemoglobin of at least 25% below steady state values, maintained or increased reticulocytosis, and circulatory failure as evidenced by tachypnoea, tachycardia, peripheral oedema or hepatomegaly. At necropsy the characteristic findings were an enlarged congested spleen with hypercellular marrow and pallor of the other organs (plate B) sometimes with peripheral oedema or pleural effusion.

Twenty-four episodes of ASS were seen during the study period in 18 SS children (7 boys and 11 girls). Recorded clinical features in each episode are shown in Table VIII and haematological changes for the 17 episodes for which data is available in Table IX. Time to first episode of ASS is shown as a survival curve in Figure 15. The risk of first episode of ASS ± standard error is 6% ± 2% by age 1 year, 12% ± 3% by age 2 years, and 14% ± 3% by age 3 years.

The outcome and subsequent course following the first episode of ASS is shown in the flow diagram in Figure 16. Seven of the 18 (39%) children died in their first episode. Five of the 11 (45%) survivors of the first episode had further episodes of which two proved fatal. Overall mortality for all 24 episodes was 38%. Necropsy findings in 9 children are summarised in Table X.

TABLE VIII

CLINICAL FEATURES OF ACUTE SPLENIC SEQUESTRATION

PATIENT	NO	SEX	EPI-SODE	AGE (MTHS)	INCREASE IN SPLEEN SIZE	DURATION OF SYMPTOMS (HOURS)	TEMP (°F)	RESP RATE	HEART RATE	CLINICAL STATE	BACTERIOLOGY	OUTCOME	SUBSEQUENT EVENTS
1	52	F	1	3	+++	NR	100	36	180	Anorexic	No cultures	Survived	-
			2	7	++	36	104	80	NR	Opisthotonic	S pneumoniae (blood)	Died	-
2	117	M	1	3	++	96	+	+	NR	Diarrhoea	No cultures	Survived	-
3	75	M	1	5	+++	12	NR	+	170	NR	No cultures	Survived	H influenzae septicaemia
			2	21	++	12	NR	NR	NR	Groaning, vomiting, diarrhoea	S pneumoniae (blood, bronchi and middle ear)	Died	-
4	43	F	1	6	+++	NR	NR	+	120	Unconscious	No cultures	Survived	S pneumoniae meningitis
5	87	F	1	6	++	120	100	NR	140	Listless	No cultures	Survived	
			2	10	++	156	100	42	180	Irritable	No cultures	Survived	
			3	12	+	144	103	58	160	Drowsy, vomiting	No cultures	Survived	Salmonella aberdeen septicaemia
6	101	F	1	7	+	6	NR	NR	NR	NR	S pneumoniae	Died	-
7	65	F	1	9	+	24	+	+	NR	NR	S pneumoniae (middle ear)	Died	-
8	120	F	1	10	+	6	NR	NR	NR	Anorexic	No growth	Died	-
9	160	M	1	10	+	NR	103	28	150	NR	No cultures	Survived	-
10	41	F	1	17	++	12	+	NR	NR	Vomiting	No growth	Died	-
11	92	M	1	18	++	156	104	+	160	Vomiting	No growth	Survived	-
12	123	M	1	17	+++	6	103	48	140	Lethargic	No growth	Survived	-
			2	28	+	24	98	28	120	NR	No growth	Survived	Splenectomy
13	138	F	1	20	+++	3	NR	NR	120	Vomiting	No growth	Died	-
14	119	F	1	22	+++	24	104	+	180	Lethargic, diarrhoea	No growth	Survived	-
15	8	M	1	23	++	8	104	88	168	Lethargic	No growth	Survived	Splenectomy
16	53	F	1	24	++	72	100	68	160	Vomiting	No growth	Survived	-
			2	28	++	16	100	+	NR	NR	No growth	Survived	-
17	70	F	1	32	+++	24	NR	NR	NR	Drowsy, vomiting, diarrhoea	No growth	Died	-
18	12	M	1	54	++	10	NR	++	+	Back pain	No growth	Died	-

NR = Not recorded

+ = increased

TABLE IX

HAEMATOLOGICAL FINDINGS IN ASS

PATIENT	NO	EPISODE	Hb gm/dl	FALL IN Hb FROM STEADY STATE (gm/dl)	RETICS %	PLATELETS x 10^9/litre
1	52	1	3.0	8.5	NK	NK
		2	5.0	2.1	20	NK
2	117	1	4.2	2.7	7	NK
3	75	1	4.5	3.7	30	NK
		2	2.4	4.1	16	113
4	43	1	1.5	6.1	NK	NK
5	87	1	4.5	3.0	26	NK
		2	3.5	3.1	33	217
		3	4.6	2.3	26	211
6	160	1	4.0	4.5	NK	NK
7	123	1	4.0	5.1	10	NK
8	92	1	4.0	3.4	14	290
9	119	1	5.2	3.7	14	NK
10	8	1	3.4	2.1	29	NK
11	53	1	4.4	1.7	16	171
		2	6.1	1.8	16	NK
12	12	1	2.5	4.0	38	NK

NK = Not known

TABLE X

NECROPSY FINDINGS IN ASS

PATIENT	NO	SEX	AGE IN MONTHS	SPLEEN WEIGHT IN GM	EXPECTED SPLEEN WEIGHT IN GM	ORGAN PALLOR	HYPERCELLULAR MARROW •	OEDEMA	BACTERIOLOGY
1	52	F	7	60	20				Streptococcus pneumoniae (blood)
2	101	F	7	122	20	+	+	Pleural effusion	S pneumoniae (bronchus)
3	65	F	9	92	20	+	+	Pulmonary oedema	S pneumoniae (middle ear)
4	120	F	10	60	25	+	+		No significant growth
5	41	F	17	172	30	+	+	Pleural effusion and peripheral	No significant growth
6	138	F	20	160	30	+	+		No significant growth
7	75	M	21	61	33	+	+	Pleural effusion and pulmonary oedema	S pneumoniae (middle ear, blood and bronchi)
8	70	F	32	140	37		+		No significant growth
9	12	M	54	400	42	+	+	Pleural effusions	No significant growth

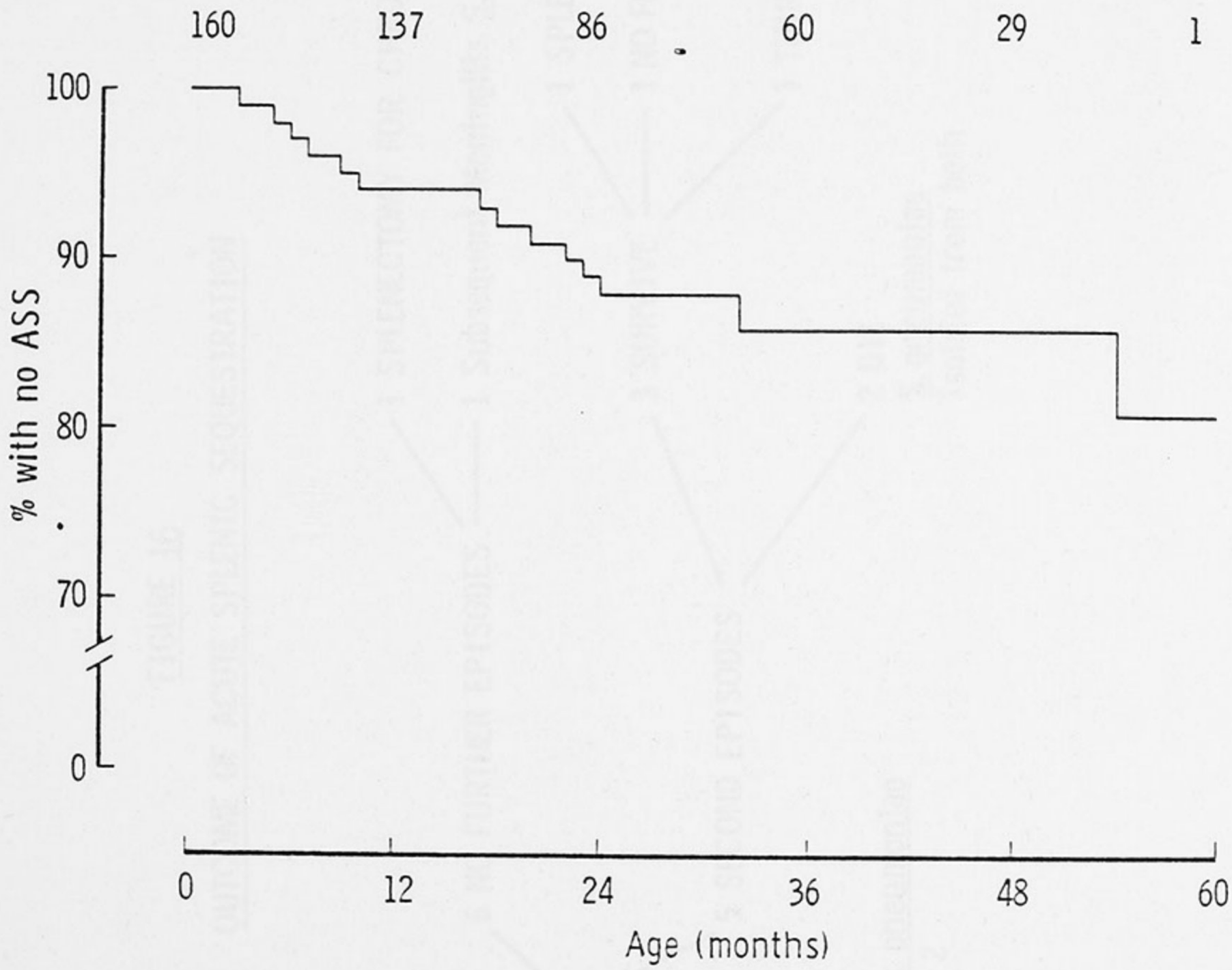
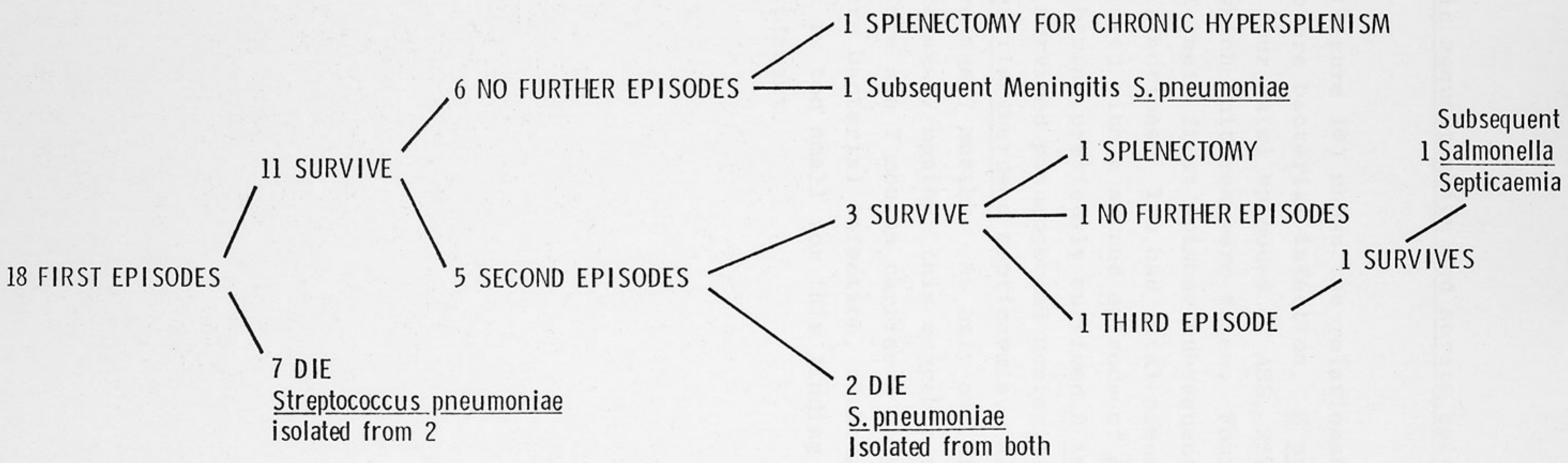


Figure 15 Time to first episode of ASS
Numbers are those at risk at yearly intervals.

FIGURE 16

OUTCOME OF ACUTE SPLENIC SEQUESTRATION



10. Acute splenic sequestration and severe bacterial infections

The flow diagram (Figure 16) shows the relationship between ASS and severe bacterial infection. S pneumoniae was isolated from four fatal episodes of ASS, 25% of the 16 episodes from which cultures were taken. Four of 11 (36%) survivors of their first episode subsequently had severe bacterial infections. Two had fatal pneumococcal septicaemia associated with a second episode of ASS, one of these children having previously survived H influenzae septicaemia. One survived pneumococcal meningitis and one survived Salmonella aberdeen septicaemia. All four had suffered ASS before age 7 months. As only one other child suffered ASS before age 7 months, this suggests that an episode of ASS before age 7 months carries a very high risk of subsequent severe bacterial infection, although the number of children is too small for this finding to be statistically significant.

11. Splenomegaly and acute splenic sequestration

In 14 of the 18 children who experienced ASS the spleen had been felt before their first episode. The time from onset of palpable splenomegaly to first episode of ASS ranged from 0 to 23 months. No relationship between risk of ASS and age of onset of palpable splenomegaly has been demonstrated, as survival curves for time to first episode of ASS for the three spleen groups showed no signficant difference using the log rank test.

12. Pit counts and acute splenic sequestration

Serial pit counts are available for nine SS children who experienced eleven episodes of ASS. Four children died in the acute episode, two with pit counts previously in the normal range and two with pit counts previously raised, one of these who had had previous episodes of ASS and Haemophilus influenzae septicaemia dying with associated pneumococcal septicaemia. The five surviving children had pit counts in the normal range at or soon after their first episodes, but their pit counts subsequently became raised at 3 months, 3 months, 5 months, 15 months, and 18 months (mean 9 months) after their first episode. Pit counts in relation to these episodes of ASS are plotted for two of these patients in Figure 17.

Four other children who had survived episodes of ASS prior to the availability of pit counts had raised pit counts when tested at 9 months, 13 months, 18 months, and 20 months after their first episode.

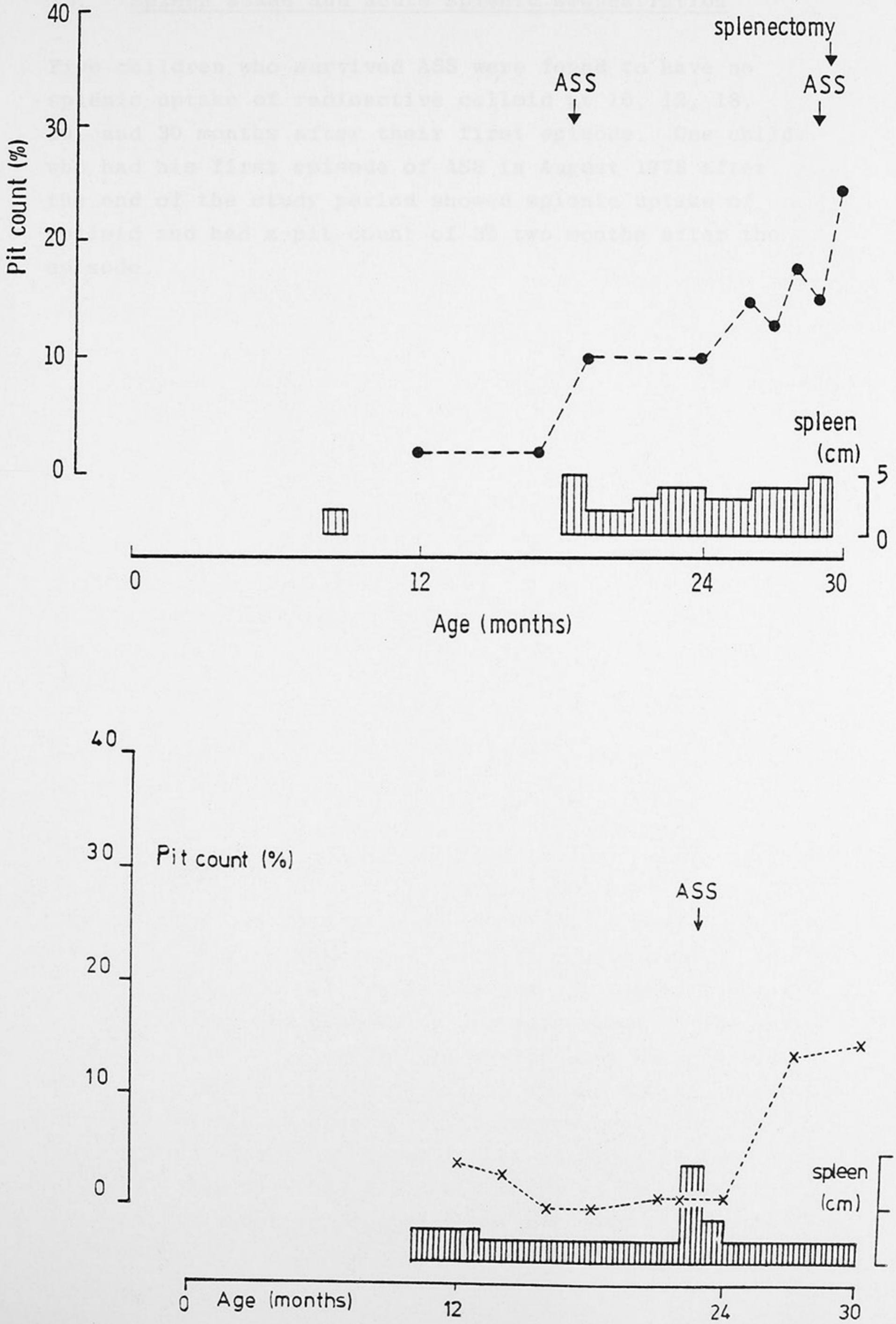


Figure 17: Pit counts and splenomegaly in relation to ASS in 2 SS children

13. Spleen scans and acute splenic sequestration

Five children who survived ASS were found to have no splenic uptake of radioactive colloid at 10, 12, 18, 20, and 30 months after their first episode. One child who had his first episode of ASS in August 1978 after the end of the study period showed splenic uptake of colloid and had a pit count of 3% two months after the episode.

14. Haematological differences between the spleen groups

Means and standard deviations for haemoglobin concentration (Hb), red cell count, reticulocyte count, mean cell volume (MCV), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), percentage foetal haemoglobin (HbF), serum iron concentration and platelet count are given for the spleen groups subgrouped for age and sex in Appendix II. The spleen groups are the same as those used for the survival analysis (Results section 7):

Group I	spleen first felt at or before age 6 months
Group II	spleen first felt after 6 months but before or at age 12 months
Group III	spleen not felt before age 13 months

The findings are summarised in Table XI, significant differences between the groups being found only for Hb, red cell count, reticulocyte count, MCV and percentage HbF. The changes in these five variable with age are shown in Figure 18.

The spleen groups vary in the size and timing of the changes associated with the development of anaemia.

The fall in Hb is both greater and earlier in spleen Group I than in Groups II and III which at 6 months have similar mean Hb; only at 12 months does Group II's mean Hb follow Group I's mean Hb downwards reaching levels similar to Group I at 24 months. These changes are almost exactly parallelled by the changes in red cell count in the three groups, while no relationships can be seen between MCHC or MCH and spleen group. The fall in Hb and RBC in accompanied in each group by a rise in reticulocyte count, counts being consistently higher in Group I than in Group II and in Group II than in Group III. MCV tends to be higher in Group I and lower in Group III but the differences are not consistent.

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TABLE XI SUMMARY OF DIFFERENCES IN HAEMATOLOGICAL VARIABLES BETWEEN SPLEEN GROUPS

Romans numerals indicate spleen group.
Subscript numbers indicate significance (Student's t-test) of differences indicated, or between Groups I and III if centrally placed.
= indicates no consistent difference

AGE IN MONTHS	6	12	24	36
Hb	I<II=III 0.01	I<II<III 0.01	I=II<III NS	I=II<III 0.05
Red cell count	I<II=III 0.01	I<II<III 0.01	I=II<III 0.01	I=II<III 0.02
Reticulocyte count	I>II=III 0.01	I>II>III 0.001	I>II>III 0.02	I>II>III NS NS
MCV	I>II=III NS	I>II>III NS	I=II>III 0.01	I=II=III
MCHC	I=II=III	I=II=III	I=II=III	I=II=III
MCH	I=II=III	I=II=III	I=II=III	I=II=III
Fe	-	I=II=III	I=II=III	I=II=III
HbF	I<II=III 0.05	I<II=III NS	I<II<III NS NS	I<II<III NS NS
Platelets	-	I=II=III	I=II=III	I=II=III

Figure 18 Haematological differences between the spleen groups

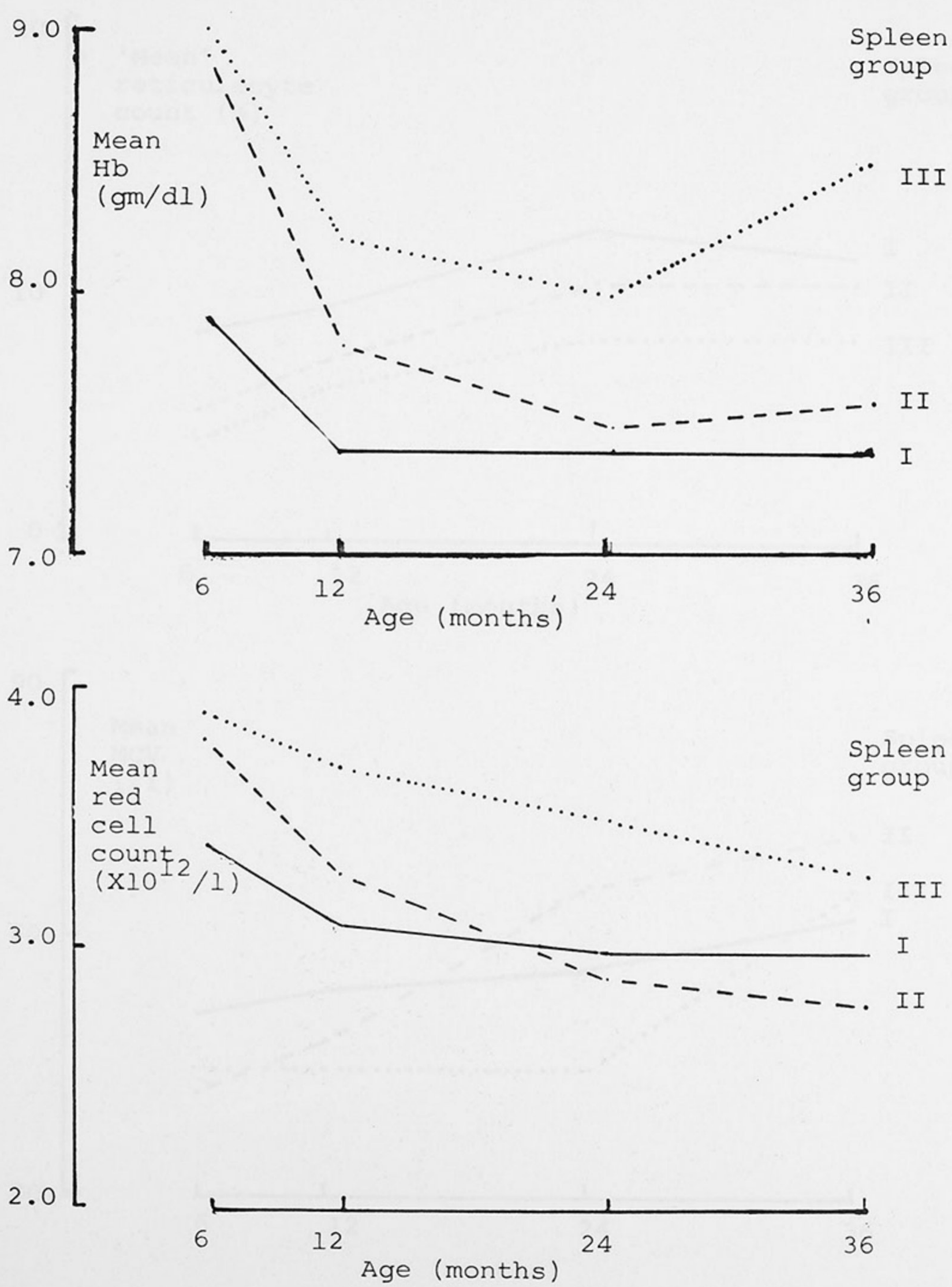


Figure 18 continued

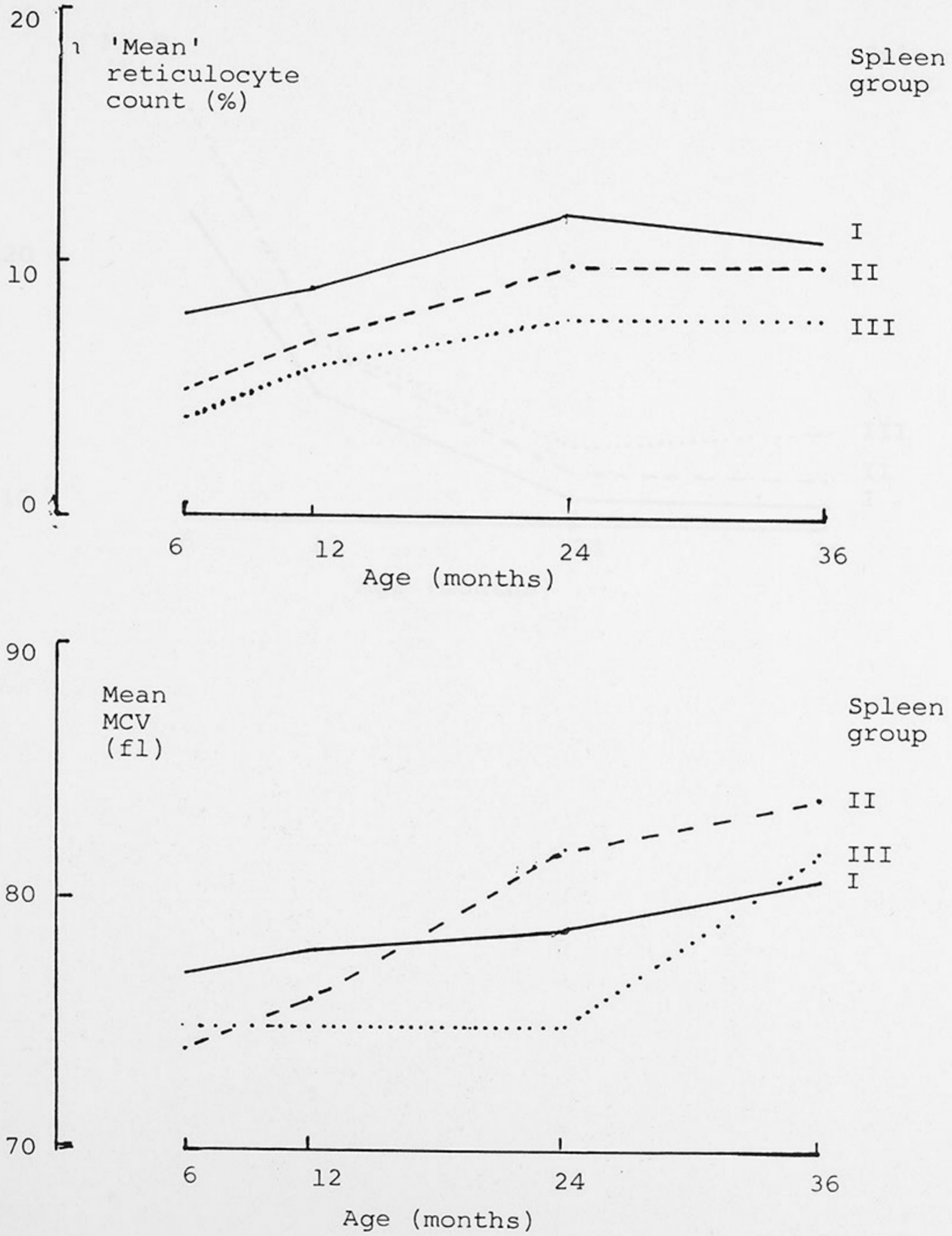
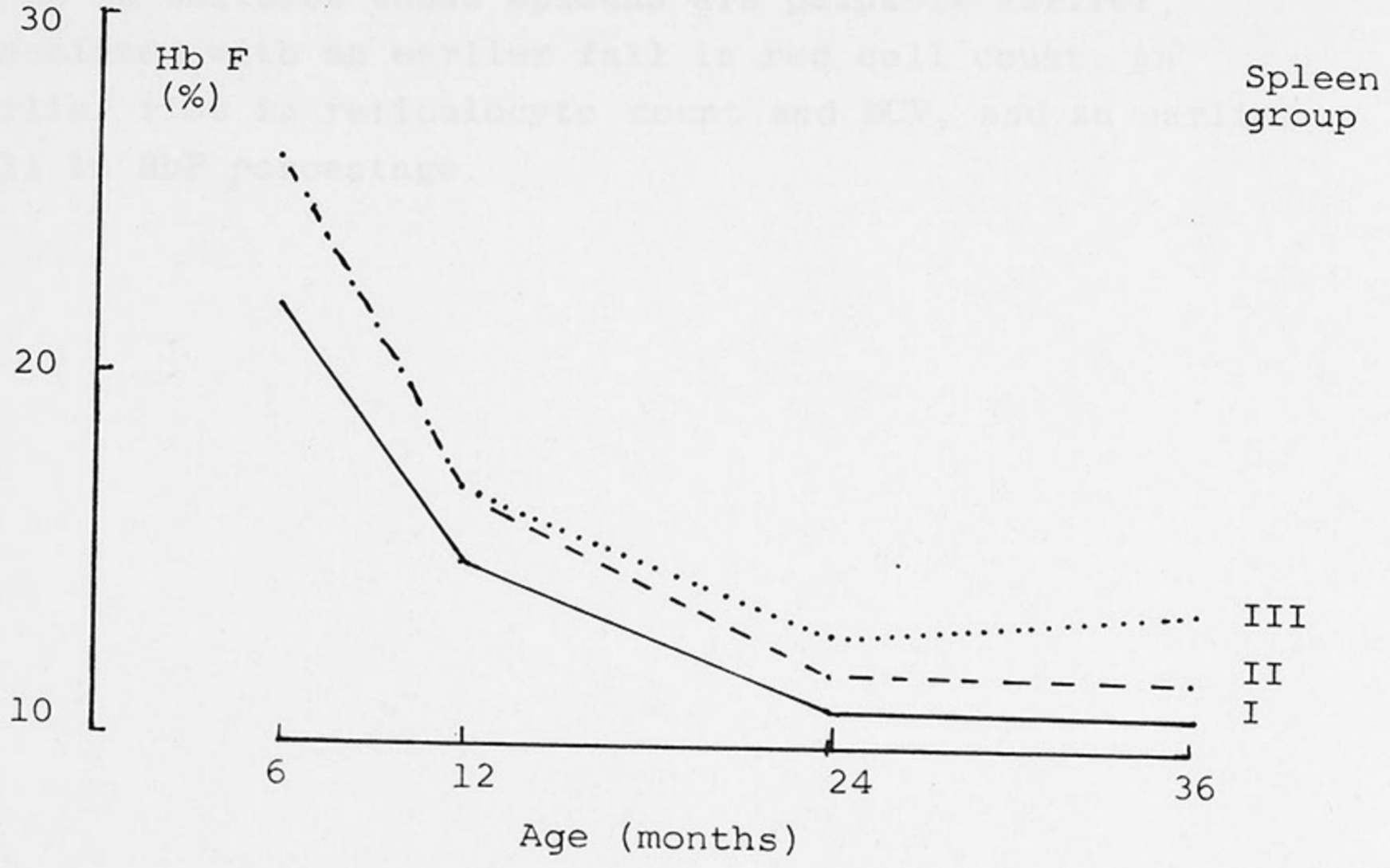


Figure 18 continued



HbF percentage is significantly lower in Group I than in Group II and III at 6 months and remains lower at ages 12, 24 and 36 months.

The pattern is therefore of an earlier and greater fall in Hb in children whose spleens are palpable earlier, associated with an earlier fall in red cell count, an earlier rise in reticulocyte count and MCV, and an earlier fall in HbF percentage.

15. Further clinical differences between the spleen groups

Table XII and XIII show the differing proportions of SS children in each spleen group affected by dactylitis and pneumonic episodes respectively at different age groups with significant differences recorded in the text beneath each table.

TABLE XII DACTYLITIS IN THE FIRST TWO YEARS OF LIFE

AGE (Months)	SPLEEN GROUP	NUMBER EXPERIENCING ONE OR MORE ATTACKS	NUMBER AT RISK	PERCENTAGE AFFECTED	SIGNIFICANCE GROUP I v GROUP II+III (chi-squared, DF=1)
0 - 5	I	4	52	8	$p < 0.05$
	II	0	40	0	
	III	1	49	2	
6 - 11	I	9	48	18	NS
	II	4	38	11	
	III	8	49	16	
12 - 23	I	11	33	33	$p < 0.05$
	II	4	28	14	
	III	6	40	15	

Dactylitis affects significantly more children in spleen Group I in the age groups 0-5 months and 12-23 months.

TABLE XIII PNEUMONIC EPISODES IN THE FIRST TWO YEARS OF LIFE

AGE (Months)	SPLEEN GROUP	NO. EXPERIENCING ONE OR MORE ATTACKS	NO. AT RISK	PERCENTAGE AFFECTED	SIGNIFICANCE GROUP I v GROUP II+III (chi-squared, DF=1)
0 - 5	I	1	52	2	NS
	II	0	40	0	
	III	0	49	0	
6 - 11	I	8	48	17	$p < 0.05$
	II	1	38	3	
	III	4	49	8	
12 - 23	I	11	33	33	NS
	II	7	28	25	
	III	8	40	20	

Pneumonic episodes affect significantly more children in spleen Group I in the age group 6 - 11 months.

16. Haematological differences between the pit groups

Appendix III gives tables of means and standard deviations for the haematological variables at ages 6 months and 12 months in the two pit groups, defined for statistical purposes as:

Group I	Count greater than 9% before or at age 12 months.
Group II	Count greater than 9% after age 12 months or not at all.

The differences for each variable are summarised in Table XIV. Significant differences are seen for Hb, red cell count, reticulocyte count, MCV and HbF percentage, the children with earlier rise in pit count having significantly lower Hb, higher red cell counts, reticulocyte counts, and MCV, and lower HbF percentage.

TABLE XIV

SUMMARY OF DIFFERENCES IN HAEMATOLOGICAL VALUES BETWEEN PIT GROUPS

Roman numerals indicate pit group. Subscript numbers indicate significance level (Student's t-test).= indicates no consistent difference.

AGE IN MONTHS	6	12
Hb	I<II	I<II
	0.05	0.01
Red cell count	I<II	I<II
	0.05	0.02
Reticulocyte count	I>II	I>II
	0.02	0.001
MCV	I>II	I>II
	0.05	NS
MCHC	I=II	I=II
MCH	I>II	I>II
	NS	NS
Fe	I=II	I=II
HbF	I<II	I<II
	NS	0.05
Platelets	I=II	I=II

17. Further clinical differences between the pit groups

Tables XV and XVI show the differing proportions of SS children in each pit group affected by dactylitis and pneumonic episodes respectively at different age groups. No significant differences are seen.

TABLE XV DACTYLITIS IN THE FIRST YEAR OF LIFE

AGE (Months)	PIT GROUP	NO. EXPERIENCING ONE OR MORE EPISODES	NO. AT RISK	PERCENTAGE AFFECTED
0 - 5	I	0	17	0
	II	2	34	6
6 - 11	I	3	17	18
	II	2	34	6

No significant relationship is seen between pit group and dactylitis in the first year of life.

TABLE XVI PNEUMONIC EPISODES IN THE FIRST YEAR OF LIFE

AGE (Months)	PIT GROUP	NO. EXPERIENCING ONE OR MORE EPISODES	NO. AT RISK	PERCENTAGE AFFECTED
0 - 5	I	0	17	0
	II	0	34	0
6 - 11	I	2	17	12
	II	2	34	6

No significant relationship is seen between pit group and pneumonic episodes in the first year of life.

18. Haemoglobin F levels, mean cell volume, and reticulocyte count in relation to survival curve analysis for clinical events

The 160 SS children were grouped according to haemoglobin F percentage (HbF), mean cell volume (MCV) and reticulocyte percentage count at age 6 months. The numbers in each subgroup are shown in Table XVII together with the haematological values chosen for each grouping. Survival curves were calculated for each of the three haematological variables for time to spleen first felt, time to first ASS, time to first severe infection and time to death. Tables of observed and expected values with chi^2 tests for trend are presented in Appendix IV and are numbered in lower case Roman numerals.

Significant relationships were seen between time to spleen first palpable and two haematological variables as follows (all chi-squared tests for trend on one degree of freedom).

HbF : chi^2 5.75, d.f. = 1, $p < 0.02$ (table i)

Reticulocytes : chi^2 5.79, d.f. = 1, $p < 0.02$ (table iii)

but no relationship was detected between time to spleen first palpable and MCV (table ii).

Early appearance of splenomegaly is therefore associated with a high reticulocyte count and a low haemoglobin F level at age 6 months, confirming by another method the similar conclusions in Section 14.

No significant relationships were seen between time to first ASS and any of the haematological variables .

TABLE XVII SS CHILDREN SUBDIVIDED BY HAEMATOLOGICAL VARIABLES AT AGE 6 MONTHS

	GROUP I	GROUP II	GROUP III	TOTAL
HbF (%)	<21	21-27.5	>27.5	
No	35	40	48	123
MCV (fl)	>79	70-79	<70	
No	38	68	26	132
Reticulocytes(%)	>9	5-9	<5	
No	28	54	50	132

Significant relationships were found between time to first severe infection and each haematological variable as follows:

HbF : chi^2 6.89, p <0.01 (table i)
MCV : chi^2 5.63, p <0.02 (table ii)
Reticulocytes : chi^2 11.36, p <0.001 (table iii)

If the relationship between spleen group and time to first infection (Results section 7 page 68) is examined allowing for each haematological variable in turn the relationship remains significant with the following significance levels:

Spleen group allowing for HbF : chi^2 5.04, p <0.03 (table iv)
Spleen group allowing for MCV : chi^2 10.41, p <0.02 (table v)
Spleen group allowing for reticulocytes : chi^2 6.83, p <0.01 (table vi)

Repeating the analysis for each haematological variable allowing for spleen group the relationship between HbF and time to first infection is no longer significant:

HbF allowing for spleen group: chi^2 3.00
p <0.09 (table vii)

but the relationships between the other two haematological variables and time to first severe infection remain significant:

MCV allowing for spleen group: chi^2 5.49, p <0.02 (table viii)
Reticulocytes allowing for spleen group: chi^2 5.31,
p <0.03 (table ix)

The analysis therefore suggests that time to first severe infection is related to age spleen first palpable independently of HbF percentage, MCV and reticulocyte count, and to MCV and reticulocyte count independently of age spleen first palpable, but that the relationship between time to first severe infection and low HbF at 6 months may be dependent on the relationship between low HbF and early appearance of splenomegaly.

No significant relationships were seen between time to death and any of the haematological variables.

DISCUSSION

1. Mortality

The comparison of survival between SS and AA Jamaican children shows that the excess mortality in SS disease begins after age 6 months. The SS infant mortality rate (95 deaths in the first year of life per thousand live births) is seven times the rate among the control infants (13). The infant morality rate quoted for the control infants is valid for comparison with the SS infants but is not a valid sample of Jamaican infant mortality as both the control and the SS infants were selected after the first day of life with the exclusion of operative deliveries, premature infants and infants with obvious severe congenital abnormalities. The most recent published figures of overall infant mortality are 26 for Jamaica as a whole, and 31 for Kingston and St Andrew Corporation area where most of these children lived (Ashworth and Picou, 1976). These figures are probably underestimates as a 10% deficit in registration of infant deaths has been reported for Kingston and St Andrew (Puffer and Serano, 1973).

The significantly higher mortality among the SS girls (three times the mortality in the SS boys) is unexpected as life tables calculated from Jamaican census data for 1959 - 1961 (University of the West Indies, 1966) show that in Jamaica, as in most populations, overall mortality is higher in boys, 5% girls and 6% boys dying by age 1 year and 7.5% girls and 8.5% boys dying by age 5 years.

Interestingly the Lambotte-Legrands (1955) reported a similar reversed sex difference in SS mortality, 52% of girls and 43% of boys dying before age 5 years. No cause could be found for the sex difference in mortality in the present study for there was no significant sex difference for time to appearance of splenomegaly, time to raised pit

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count, time to first ASS or time to first severe infection, although both ASS and severe infections were commoner in girls than in boys.

The high mortality in SS children in Jamaica shown in this study is similar to estimates based on North American experience (Porter and Thurman, 1963; Powars, 1975). The two principal causes of death, acute splenic sequestration and pneumococcal infection, are known to be responsible for many childhood deaths in SS disease (Seeler, 1972); this study demonstrates their contribution to mortality under age 1 year. The nine cases of ASS and the six isolations of S pneumoniae among the 22 SS children who died probably underestimate the true incidence of both ASS and severe pneumococcal infections because full necropsies were performed in only 12 patients, and bacteriological studies were available for only 14. That mortality from these conditions remains so high despite careful follow-up of the children and free access to emergency medical care reflects the rapid course of both in young SS children, often with no warning signs of imminent collapse (Pearson, 1973; Powars, 1975).

2. Pitted red cell counts

Pit counts in AA controls (Mean 0.73%; SD 1.54%; range 0 - 8%) were of the same order as those reported by Holroyde and Gardner (1970) (mean 2.6%; SD 1.1%; range not quoted), Casper et al (1976) (mean 0.5%; SD 0.5%; range 0 - 2.6%) and Sills and Oski (1979) (mean 0.9%; SD 0.35%; range 0 - 1.6%), but were higher than those reported by Pearson et al (1978) (mean 0.08%; SD 0.003%; range 0 - 0.8%). The relatively high pit counts seen in a few AA children in the present study, which were shown in three to be transitory only, might have been due to coincidental illness.

Pit counts in SS children in this study rose with age above the normal range of controls, reaching at about age 4 years levels similar to those of 15 SS patients post splenectomy (range 7 - 28%, median 16%) and of Pearson's post splenectomy patients (range 12 - 33%, mean 20%, SD 6.4%). The age at which pit counts rose out of the normal range varied from child to child, but was usually after age 6 months. About a quarter of the children still had pit counts within the normal range even after age 4 years. The distributions of pit counts at each age group are similar to the distributions found by Pearson et al (1979) and by Sills and Oski (1979), although the small number (16) of counts under age 5 in Pearson's study and the failure to subdivide children by age and genotype together in Sills and Oski's study make statistical comparison unhelpful.

The radioactive colloid spleen scans confirmed that the elevated pit counts indicated the same state of acquired functional asplenia as described by Pearson et al (1969). Twelve SS children with palpable spleens and pit counts in the range 9 - 25% had no splenic uptake of colloid, while one patient with a palpable spleen and a pit count of 3% showed splenic uptake. One child in whom the spleen had never been felt had a normal pit count but unexpectedly no splenic activity detected on spleen scan, perhaps because of an ectopic spleen or compensatory activity in the remainder of the reticuloendothelial system.

3. Severe bacterial infections

The high incidence of severe bacterial infections in the SS children, contrasted with no severe infections in the AA controls, confirms the well-known association of SS disease with septicaemia or meningitis. This susceptibility to infection resembles that seen after splenectomy and in congenitally asplenic children in three ways; the organisms principally responsible (Streptococcus pneumoniae and Haemophilus influenzae), the tendency of the severe infections to recur, and the fulminant course of the infections, all of which were seen in the present study.

Twelve of the 14 children who had severe infections had splenomegaly at the time of infection. Susceptibility to severe infections may thus precede splenic involution, justifying the use of the term functional asplenia, defined by Pearson et al (1969) as failure of reticuloendothelial function of the palpably enlarged organ (although splenic function may of course also be absent if the spleen is impalpable).

As the failure of splenic reticuloendothelial function usually follows the onset of palpable splenomegaly, those children in whom the spleen enlarges earliest might be those at greatest risk of infection, for in children who have undergone splenectomy the risk of infection is greater the younger splenectomy is performed. In the present study the risk of severe infection was five times as great among those children whose spleens were first felt at or before age 6 months as in those whose spleens were felt after 6 months but at or before age 12 months, with no severe infections occurring in those whose spleens were not palpable before age 13 months. Diagnosis of SS disease at birth and subsequent careful follow up can thus identify from early appearance of the spleen those at greatest risk of severe bacterial infections, without the necessity for specialised radioisotope or microscope techniques.

Pit counts were only performed for the last 21 months of the study. Nine episodes of severe infection occurred in six SS children during this period, and one episode occurred in a seventh child after the end of the study period. Pit counts were raised before the first episode in six children and at the first episode in one child. A pit count at the time of a possible infection is therefore of value in determining whether the child is at risk of developing septicaemia. However as the child who had her first raised pit count at the time of her septicaemia had a pit count of 4% three weeks before, a normal pit count does not exclude a rise in pit count and severe infection in the near future.

Of the 41 children whose pit counts were followed from age 12 months or earlier all four who experienced severe infections were among the 17 who had raised pit counts at or before age 12 months. This suggests that the earlier the rise in pit count the greater the risk of subsequent infection. An early rise in pit count in an SS child followed with serial pit counts would therefore have the same significance as an indicator of susceptibility as early appearance of splenomegaly, but with three advantages.
1) The rise in pit count would define more precisely the age of onset of functional asplenia and so the risk of subsequent infection.
2) The age at which pit counts rise is likely to be a more sensitive indicator of susceptibility to infection, about a quarter of SS children having raised pit counts at or before age 1 year, as opposed to about two thirds of SS children having a palpable spleen at or before age 1 year.
3) Splenic enlargement is likely to disappear with time, and if the period of splenomegaly is brief may be missed in the clinic, while the tendency for pit counts to rise with age and thereafter to remain raised makes the pit count a more permanent indicator of susceptibility to infection.

For these reasons pit count estimations should be considered for inclusion in the routine investigation of SS children, for the ease and speed with which they can be performed on one drop of blood from a routine finger prick blood test makes them suitable for ward or clinic use. In any study of the natural history of SS disease or trial of therapy serial pit counts are useful investigations for determining the state of splenic reticuloendothelial function in each child under study.

4. Prophylaxis against severe bacterial infection

As the majority of severe infections in SS disease are due to the pneumococcus, vaccination against this organism would seem a logical prophylaxis, especially as there is good evidence that asplenic individuals can respond to intramuscular (as opposed to intravenous) antigen (Sullivan *et al*, 1978). A vaccine for *H influenzae* is also available. However there is as yet no evidence that vaccines provide adequate immunity if administered under age 2 years, and this study has shown that the risk of death from these infections is greatest under age 2 years. Regular penicillin is an additional prophylaxis as almost all pneumococci are still sensitive to this antibiotic, but compliance failures with both oral and intramuscular routes of administration limit its dependability.

A double blind randomized trial comparing vaccination and penicillin prophylaxis is at present under way for the surviving children in this study and for SS children subsequently diagnosed in the cord blood screening programme. The eventual analysis of this trial should be helped in two ways by the current study. Firstly the historical risks of severe infection without prophylaxis have been calculated. Secondly using time of first palpable splenomegaly and time of rise in pit counts, children at greater risk of infection can be identified and the retrospective analysis made more sensitive (Peto *et al*, 1976).

5. Acute splenic sequestration

The rapidity with which ASS occurs has made it a difficult condition to characterise clinically or haematologically, but the clinical, haematological and necropsy data presented in tables VIII, IX and X confirm that the spleen of the SS child can sequester red cells to such an extent that the resulting acute anaemia causes circulatory failure. The high mortality (9 deaths in 24 episodes) in this study emphasises the need for rapid diagnosis and treatment. The short duration of any symptoms of ill health (12 hours or less in 9 of the 21 episodes in which the period for which the child had been unwell was recorded) means that the mother must seek medical attention at once if she feels that the child is sick. Mothers of children with palpable spleens have been taught to check daily for enlargement of the spleen and for conjunctival pallor, and on this basis episodes of ASS have been diagnosed by mothers and treatment has been given in time. However as in 4 of 18 first episodes of ASS the spleen had never previously been palpable, and as the youngest age at which ASS occurred in this study was at 2 months 23 days, some instruction should probably be given to mothers about this complication at the first or second clinic visit. Medical staff also need to be more aware of the syndrome and its rapid progression, as four of the deaths from ASS in the present series occurred after the patient had been seen by a doctor. Neither age of onset of splenomegaly nor age of first raised pit count appear to be of any use in predicting susceptibility to ASS.

Emergency treatment of established ASS is immediate red blood cell transfusion, which may have to be given uncrossmatched to avoid delay. Other intravenous infusions may do more harm than good, the death of one patient in the present series having been precipitated by pulmonary oedema from the intravenous infusion given while awaiting crossmatched blood. In contrast one unconscious patient was successfully resuscitated by uncrossmatched blood given through a cut down on the femoral vein. In view of the possible need for

emergency transfusion the mother of every SS child should carry a card with details of the child's blood group.

The pneumococcus was isolated from four of the nine children who died with ASS. In view of this parenteral antibiotics should be given to all cases of ASS, as septicaemia may be a precipitating cause. The choice of antibiotics depends on the organism thought to be responsible. Penicillin should be used because of the high incidence of pneumococcal septicaemia, but in view of the possibilities of haemophilus and salmonella septicaemias the use of chloramphenicol should also be considered in the severely ill child, ampicillin being inappropriate because of the significant proportion of these bacteria now resistant to this drug.

In 12 of the 16 ASS episodes for which cultures were taken no organism was isolated. In 7 of the 12 ASS episodes in which the temperature was measured the child's temperature was 103°F (38.2°C) or greater and in four of these episodes the cultures taken showedno significant growth suggesting that a viral infection might be responsible. Vomiting was reported in seven ASS episodes and diarrhoea in four, perhaps representing an underlying gastroenteritis as the precipitating cause. These clinical observations provide a framework within which possible mechanisms of ASS can be suggested, and one such mechanism is proposed in Section 8 of the Discussion.

6. Prophylaxis against acute splenic sequestration

With a 45% recurrence rate among survivors of the first episode of ASS prophylaxis against a second episode is urgently required. Theoretically regular transfusion would prevent recurrence, but a complicated trial would be required to determine the minimum level of Hb S at which ASS could recur and the age to which transfusion should be continued, while the complications of infection and iron-overload inherent in a transfusion programme might be exacerbated in children who are excessively susceptible to infection and who already have higher serum iron than normal controls.

Splenectomy is a sure prophylaxis, but its use has been discouraged because of the assumption that given time the spleen would involute spontaneously and because of fear of the complications of the operation. However the spleen continued to be palpable for at least a year in all eight patients followed for more than a year after the first episode, and continued to be palpable in all nine continuing survivors of ASS at ages ranging from 1 year 3 months to 4 years 6 months. With optimal surgical and anaesthetic care the operative risks for SS children are unlikely to be much greater than for normal individuals (Homi et al, 1979). Splenectomy in this age group will render children more susceptible to severe bacterial infections, but the following evidence from this study suggests that survivors of ASS already have this increased susceptibility.

1) Four of 11 survivors of first episodes of ASS (four out of five survivors of ASS before age 7 months) subsequently experienced pneumococcal (3) or salmonella (1) septicaemia or meningitis.

2) Radioactive colloid spleen scans in five survivors of ASS showed no uptake of colloid by their palpably enlarged spleens.

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3) Pit counts have become raised in ten of the eleven survivors of a first episode of ASS, the eleventh survivor having died in a second ASS episode with pneumococcal septicaemia before pit counts were available.

For these reasons this study supports the advice of Pearson (1973) and Robinson (1975) that one episode of ASS should be a sufficient indication for splenectomy.

7. The immune defect in SS disease

This study has demonstrated that the failure of splenic reticuloendothelial function, as shown by increased incidence of severe infections, raised pitted red cell counts, and failure of radioactive colloid uptake, follows the onset of palpable splenomegaly. The risk of severe infections as a consequence of this functional asplenia is related to the age of onset of palpable splenomegaly and to the age at which the pit count becomes elevated, these relationships resembling that between the risk of severe infection and the age at splenectomy. A possible explanation for these relationships is as follows.

From birth until about age 6 months immunity against the organisms principally responsible for severe infections, S pneumoniae and H influenzae, is probably achieved by humoral antibody acquired passively from the mother. In congenitally asplenic individuals no cases of infection due to these organisms were seen before age 6 months (Waldman et al, 1977) and in the present study only one episode was seen in SS children before age 6 months.

From about age 6 months the level of maternally derived antibody has fallen too low to give this protection. On subsequent first encounter with one of these organisms the now non-immune individual with intact spleen clears the organism from the blood stream most effectively on a unit weight of organ basis in the spleen (Schulkind et al, 1967) and the normal production of antibody appears to be dependent on a functioning spleen. Once antibody has been formed the importance of splenic phagocytosis is diminished in favour of the liver and other sites of reticuloendothelial activity, which will play a dominant role in clearing the organisms if a normal secondary humoral antibody response occurs.

In the absence of a functioning spleen the individual is unable to respond to first encounter with blood borne organisms with an adequate humoral antibody response (Rowley, 1950 a and b) and phagocytosis at other sites may be impaired as a result. The consequence may be a fulminant septicaemia. However if preformed antibody to the organism is present, as may occur if splenic function was lost only after first encounter with the organism, or in immunised individuals, extrasplenic sites of phagocytosis may be adequate, so that transient bacteraemia does not develop into septicaemia. Loss of splenic function therefore appears to be of greatest significance in the immunologically inexperienced individual, and individuals at greatest risk of severe infections are those who are congenitally asplenic, those who undergo splenectomy at an early age and those SS children who develop functional asplenia earliest.

The role of the defect in opsonisation and in complement alternative pathway activity seen in post splenectomy and SS children have not yet been related directly to susceptibility to infection. If such a relationship could be demonstrated for the alternative pathway defect it would be particularly significant on first encounter with a pathogenic organism as the alternative pathway, unlike the classical pathway, appears to operate before specific antibody is available.

8. Haematological changes and a model of splenic pathophysiology

This study of the natural history of SS disease has shown a wide variation in severity of disease, from the children who die in infancy to those who have still experienced no symptoms at age 5 years. Only 26 of 160 (16%) of the original SS children are known to have experienced the two major complications of SS disease in this age group, severe bacterial infections and ASS. The aims of the study were to identify these children at particular risk and to make recommendations for their management. Identification of such risk factors as early splenomegaly and early rise in pit count does not define a discrete group of children since the age of first palpable splenomegaly and the age of rise of pit count are both continuous variableswhich in this study have been used to divide the children into arbitrary groups for statistical purposes. Nonetheless it is possible to examine the haematological data and the clinical data using the same statistical groupings together with subgrouping by sex and age to see if this sheds any light on the underlying splenic pathology and its relation to the biochemical process of sickling.

Examination of the haematological variables grouped according to the age at which the spleen is first palpable shows an earlier and greater fall in Hb in the SS children whose spleens are palpable earlier, associated with earlier falls in red cell count and HbF concentration and earlier rises in reticulocyte count and MCV, suggesting earlier onset and greater severity of haemolysis in these children. Such an earlier onset of haemolysis might be an explanation for the earlier onset of splenomegaly, although of course conversely earlier splenomegaly by increasing red cell sequestration and sickling within the spleen would be expected to result in a lower Hb and red cell count and an increased reticulocyte count. Similarly it is attractive to suggest that the lower HbF concentrations seen in the children with earlier

splenomegaly indicate an earlier increase in the proportion of red cells with low HbF concentrations and so shorter delay time, resulting therefore in an increase in the proportion of cells sickling and causing obstruction within the spleen and hence in splenomegaly. The consistent differences in HbF levels between the spleen groups would support this hypothesis. However the HbF percentage concentrations must be interpreted with caution for an enlarging spleen by increasing red cell sequestration and thus red cell turnover might cause an earlier fall in HbF percentage, HbF containing cells being more rapidly replaced than in children with less rapid red cell turnover.

SS girls have a higher Hb associated with higher MCV and MCHC levels than SS boys, a similar sex difference to that seen in the AA controls. The higher MCV in girls does not appear to be the result of a higher proportion of immature cells as there are no significant differences in reticulocyte count between boys and girls. The higher MCV and MCHC in girls might conceivably be a disadvantage, the higher MCV increasing the risk of obstruction in the microcirculation and the higher MCHC increasing the risk of vaso-occlusive sickling by reducing the delay time. Certainly these are the only adverse haematological findings which might explain the higher mortality in girls, for girls tend to have higher HbF levels than boys.

Examination of the haematological variables grouped according to the age of rise in pit count shows, in a smaller population over a shorter age range, approximately the same pattern of differences as that seen between the spleen groups. Early rise in pit count (before or at one year) is associated with an earlier and significantly greater fall in Hb and red cell count, with an earlier rise in reticulocyte count and a higher MCV. No significant differences are found in MCHC or MCH, but HbF is lower in pit Group I than in pit Group II, significantly so at age 12 months. The similarity of these differences to those seen in relation to the age of appearance

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of the spleen lends weight to the assumption that the development of splenic enlargement and of functional asplenia have the same underlying pathological basis.

Survival curve analyses of time to appearance of the spleen, ASS, severe infection and death, in relation to HbF, MCV and reticulocytes confirm the close associations between the changes in spleen size and function and these haematological variables. However, no significant relationships were found between any of the variables and ASS or death. This finding contrasts with the findings of Stevens et al, (1981) studying the same population, who found an increased risk of ASS associated with low HbF at age 6 months. This may be because Stevens et al used a wider definition of ASS to include subacute episodes with haematological changes but without clinical symptoms (Topley et al, 1981).

The survival curve analysis shows that early appearance of splenomegaly is associated with low HbF and high reticulocyte count at age 6 months, but shows no significant relationship to MCV at age 6 months. The age of first severe infection is related to these three haematological variables, but remains related to age of appearance of splenomegaly even allowing for these variables.

In contrast if allowance is made for age of appearance of splenomegaly only MCV and reticulocyte count remain significantly related to time to first severe infection, and of these the relationship with MCV may be dependent on the relationship with reticulocyte count, for a high reticulocyte count will itself raise the MCV. On this basis early appearance of splenomegaly and early rise in reticulocyte count, together with early rise in pit count, appear to be the most important indicators of susceptibility to infection.

These haematological findings are compatible with a unified model of the development of splenomegaly, acute splenic sequestration and functional asplenia. In the first months of life the rate of sickling of red cells both within the spleen and in the circulation is likely to be determined by a number of factors, the rate of decline of HbF synthesis playing an important part by determining the proportion of red cells with intracellular HbF concentrations low enough to permit sickling to occur. As increasing numbers of sickled cells reach the fine slits through which they need to pass to enter the splenic sinusoids (Chen and Weiss, 1973, previously cited) and are delayed in their passage by their rigidity, so a mechanical block might arise at this point in the splenic circulation, resulting in accumulation of red cells within the spleen, and so in splenic enlargement, falling red cell count and haemoglobin concentration, and a rising reticulocyte count.

This chronic red cell accumulation within the spleen might accelerate with a sudden increase in sickling rate to cause acute splenic sequestration. Such a sudden increase in sickling rate might have a number of different causes, and the clinical conditions under which ASS is known to occur provide some indication of what such causes might be. Septicaemia and gastroenteritis in young children can both result in a rapid fall in blood pH, associated with vasoconstriction and tissue hypoxia, all conditions which could theoretically by lowering intracellular pH and pO_2 decrease the delay time for the sickling process at a moment when capillary transit time (especially in the case of gastroenteritis, with haemoconcentration and increased blood viscosity) is likely to be prolonged. High fever (greater than $38^{o}C$) is also seen in association with some cases of acute splenic sequestration in the absence of bacterial infection, and as the delay time for the sickling process is reduced by an increase in temperature, this too might be a stimulus for ASS to occur. Once a critical number of slits into the splenic sinuses had become blocked rapid accumulation of red cells within the spleen pulp might occur, the

resulting tissue hypoxia and delayed transit time further increasing the rate of sickling within the spleen. In the general circulation the sudden loss of circulating red cells would then result in circulatory failure, with a hypoxic heart unable to maintain adequate cardiac output and an increasing left atrial filling pressure from the maintained plasma volume resulting in pulmonary oedema. Reversal of this situation by transfused AA red cells would then result firstly from restoring an adequate oxygen supply to the heart and secondly from improving red cell oxygenation and circulation in the spleen.

The failure of splenic reticuloendothelial function could also be explained on the basis of mechanical blockade, as first suggested by Robinson and Watson (1966, previously cited). Engorgement of the spleen with sickled red cells might make it physically impossible for the splenic reticuloendothelial cells to phagocytose bacteria in blood-borne infections and so might prevent the development of the normal antibody response. The earlier the onset of this blockage the greater the subsequent susceptibility to severe infection. This explanation is consistent with the observed development of functional asplenia following the onset of splenomegaly and also with the development of functional asplenia in survivors of ASS, most of whom show continuing hypersplenism indicating chronic red cell sequestration within the spleen. The failure of the enlarged spleens to take up radioactive colloid supports this mechanical explanation. The rise in pit count is also explicable as the inclusion bodies giving the pitted appearance to the red cells would be expected like Heinz bodies to be removed from the red cell at the slits in the endothelial walls of the splenic sinuses: should these slits be blocked by rigid sickled cells no pits would be removed.

This integrated mechanical model of splenic pathophysiology in SS children is attractive in its simplicity. However it remains only a hypothesis which is compatible with the clinical and laboratory data presented in this study but which is not in any way proved by that data. In particular the development of splenomegaly and the changes in the haematological features are so closely connected in terms of possible cause and effect that great care must be taken not to assume evidence of causation from what is merely evidence of close association.

9. The natural history of sickle cell disease in early childhood

Throughout this study emphasis has been placed on the variable rate of evolution of the sickling disorders, especially SS disease, with age. The findings have confirmed the observation of the Lambotte-Legrands (1955) that early symptomatic presentation carries a poor prognosis.

In part the poor prognosis is likely to be due to the greater difficulty in diagnosing and treating the life-threatening complications, ASS and severe infections, in the younger child. In addition this study has demonstrated the poor prognostic implications of early splenomegaly and early functional asplenia. The analyses for the other significant clinical events of the first two years of life, dactylitis and pneumonic episodes, show that these affect significantly more of the children with early splenomegaly in certain age groups during the first two years of life. Whatever the mode of presentation the age of presentation is likely to be a useful guide to subsequent severity of disease, assuming of course that the child survives the initial presenting event.

Early presentation may be of use in determining prognosis for the majority of children with SS disease who live where medical services are only beginning to be developed. In countries with more developed medical services the possibility of neonatal diagnosis should make it unnecessary to await presentation. This study has shown that neonatal diagnosis and careful surveillance with regular haemoglobin, reticulocyte and pit count estimation should identify the children at high risk. Concentration of medical care, including appropriate prophylaxis and parental education, on these high risk children and their parents might well reduce mortality and morbidity from childhood SS disease while avoiding unnecessary expenditure, concern and anxiety for those at lower risk.

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APPENDIX I

Survival data used in the life table calculations.

Ages in months

- indicates known survival without event

0 indicates no data

PATIENT	SEX	SPLEEN GROUP	SPLEEN APPEARS	FIRST ASS	FIRST INFECTION	DEATH
1	F	1	5	-60	-60	-60
2	M	1	5	-7	7	7
3	M	3	-59	-59	-59	-59
4	M	1	6	-59	-59	-59
5	M	2	8	-59	-59	-59
6	F	1	3	-58	9	-58
6A	M	2	11	-58	-58	-58
7	F	2	7	-51	-51	-51
8	M	1	5	23	-57	-57
9	M	3	-52	-52	-52	-52
10	F	1	6	-58	29	-58
11	M	2	12	-58	-58	-58
12	M	3	36	54	-54	54
13	F	2	12	-57	-57	-57
14	M	1	5	-57	-57	-57
15	M	2	12	-51	-51	-51
16	M	3	27	-32	-32	-32
17	M	3	36	-56	-56	-56
18	F	1	1	-56	-56	-56
19	F	0	0	-51	-51	-51
20	F	1	2	-9	-9	9

PATIENT	SEX	SPLEEN GROUP	SPLEEN APPEARS	FIRST ASS	FIRST INFECTION	DEATH
21	M	2	12	-50	-50	-50
22	M	0	0	-54	-54	-54
23	M	3	-54	-54	-54	-54
24	M	1	6	-19	-19	19
25	F	0	0	-8	-8	8
26	M	3	-53	-53	-53	-53
27	M	1	1	-53	-53	-53
28	F	1	6	-10	-10	10
29	F	2	11	-50	-50	-50
30	F	3	36	-52	-52	-52
32	F	1	6	-50	-50	-50
33	F	1	3	-50	-50	-50
34	M	1	6	-50	-50	-50
35	M	0	0	-43	-43	-43
37	F	2	8	-49	-49	-49
40	M	1	1	-48	-48	-48
41	F	3	13	17	-17	17
42	F	2	12	-42	-42	-42
43	F	1	2	6	12	-47
44	F	3	15	-42	-42	-42
45	F	1	1	-47	-47	-47
46	M	2	9	-42	-42	-42
47	M	2	12	-47	-47	-47
49	F	1	5	-44	-44	-44
50	F	3	-43	-43	-43	-43

PATIENTS	SEX	SPLEEN GROUP	SPLEEN APPEARS	FIRST ASS	FIRST INFECTION	DEATH
51	F	1	5	-45	-45	-45
52	F	1	2	3	7	7
53	F	1	1	24	-45	-45
54	F	3	-45	-45	-45	-45
55	F	3	-44	-44	-44	-44
56	M	1	6	-44	-44	-44
57	M	3	28	-44	-44	-44
58	F	3	24	-44	-44	-44
59	F	2	8	-12	-12	-12
60	M	3	28	-43	-43	-43
61	F	1	4	-25	-25	25
62	M	2	9	-36	9	-36
63	F	3	-15	-15	-15	-15
64	F	3	-41	-41	-41	-41
65	F	2	8	9	9	9
66	M	2	12	-42	-42	-42
69	M	0	0	0	0	0
70	F	2	10	32	-32	32
71	F	2	10	-32	-32	-32
72	M	2	9	-37	-37	-37
74	F	3	-41	-41	-41	-41
75	M	1	2	5	19	21
76	F	3	-41	-41	-41	-41
78	M	3	14	-40	-40	-40
79	F	2	10	-24	-24	24
80	F	3	16	-36	-36	-36

PATIENTS	SEX	SPLEEN GROUP	SPLEEN APPEARS	FIRST ASS	FIRST INFECTION	DEATH
81	F	0	0	0	0	0
83	F	1	2	-39	-39	-39
84	M	1	6	-36	-36	-36
85	M	1	5	-39	-39	-39
87	F	1	6	6	30	-39
89	F	3	13	-38	-38	-38
90	M	0	0	-38	-38	-38
91	M	3	-38	-38	-38	-38
92	M	3	18	18	-37	-37
93	M	3	-36	-36	-36	-36
95	F	0	0	0	0	0
97	F	3	15	-30	-30	30
98	M	0	0	-3	-3	3
99	M	3	-36	-36	-36	-36
101	F	1	6	7	7	7
102	F	2	11	-11	-11	11
103	F	1	6	-30	-30	-30
104	M	2	7	-14	-14	-14
105	F	1	6	-33	-33	-33
107	F	3	20	-30	-30	-30
108	M	2	12	-33	-33	-33
109	M	0	0	-1	-1	-1
111	M	1	4	-30	4	-30
112	M	3	18	-25	-25	-25
114	F	1	1	-28	-28	-28
115	F	3	14	-32	-32	-32
116	F	1	5	-31	-31	-31
117	M	1	3	3	-31	-31

PATIENTS	SEX	SPLEEN GROUP	SPLEEN APPEARS	FIRST ASS	FIRST INFECTION	DEATH
118	F	0	0	-29	-29	-29
119	F	2	10	22	-30	-30
120	F	2	10	10	-10	10
121	M	1	5	-27	-27	-27
122	F	1	3	-16	16	16
123	M	2	7	17	-30	-30
124	F	3	23	-30	-30	-30
125	F	3	-30	-30	-30	-30
127	M	2	11	-28	-28	-28
128	M	0	0	-2	-2	-2
129	M	0	0	-6	-6	-6
130	M	3	20	-27	-27	-27
131	F	1	6	-27	-27	-27
133	M	1	4	-26	-26	-26
134	M	3	13	-26	-26	-26
135	F	1	5	-18	-18	18
136	M	2	12	-24	-24	-24
137	F	3	-23	-23	-23	-23
138	F	3	16	20	-20	20
139	M	3	-23	-23	-23	-23
140	M	0	0	0	0	0
141	F	3	21	-23	-23	-23
142	M	2	12	-23	-23	-23
143	F	0	0	-4	-4	4
144	M	1	4	-22	-22	-22
145	F	3	-18	-18	-18	-18
146	M	1	4	-22	-22	-22
147	M	2	10	-21	-21	-21

PATIENTS	SEX	SPLEEN GROUP	SPLEEN APPEARS	FIRST ASS	FIRST INFECTION	DEATH
148	F	3	-21	-21	-21	-21
149	F	3	-21	-21	-21	-21
151	M	0	0	-21	-21	-21
152	F	3	14	-21	-21	-21
153	F	3	-21	-21	-21	-21
154	M	2	9	-21	-21	-21
155	M	3	-20	-20	-20	-20
156	F	3	-20	-20	-20	-20
159	M	2	10	-19	-19	-19
160	M	0	0	10	-16	-16
161	F	1	6	-19	-19	-19
162	M	1	4	-19	-19	-19
163	M	2	7	-16	-16	-16
164	M	1	4	-18	15	-18
165	M	2	12	-18	-18	-18
166	F	2	11	-18	-18	-18
167	M	1	6	-18	-18	-18
168	F	1	6	-17	8	-17
169	M	2	8	-17	-17	-17
170	F	0	0	-15	-15	-15
171	M	1	2	-17	-17	-17
172	F	1	5	-16	-16	-16
173	M	2	10	-15	-15	-15
174	M	2	12	-15	-15	-15
175	F	3	-15	-15	-15	-15
177	M	3	-13	-13	-13	-13
178	F	2	8	-13	-13	-13
179	F	3	-13	-13	-13	-13

PATIENTS	SEX	SPLEEN GROUP	SPLEEN APPEARS	FIRST ASS	FIRST INFECTION	DEATH
180	M	2	7	-13	-13	-13
181	M	1	2	-12	-12	-12
182	M	2	7	-12	-12	-12
183	M	1	6	-12	-12	-12

APPENDIX II

Haematological tables for the spleen groups

HAEMOGLOBIN CONCENTRATION (gm/dl)

AGE (months)	SPLEEN GROUP	MEAN ± STANDARD DEVIATION (number in subgroup) MALE	FEMALE	COMBINED
6	I	8.3 ± 1.4 (24)	7.5 ± 1.7 (27)	7.9 ± 1.6 (51)
	II	8.8 ± 1.2 (24)	9.2 ± 1.0 (13)	8.9 ± 1.2 (37)
	III	9.0 ± 1.1 (16)	9.0 ± 1.3 (25)	9.0 ± 1.3 (41)
12	I	7.4 ± 1.1 (20)	7.4 ± 1.4 (23)	7.4 ± 1.3 (43)
	II	7.8 ± 1.6 (25)	7.8 ± 1.3 (11)	7.8 ± 1.5 (36)
	III	8.1 ± 1.4 (18)	8.3 ± 1.7 (25)	8.2 ± 1.6 (43)
24	I	7.2 ± 1.5 (12)	7.5 ± 1.4 (18)	7.4 ± 1.4 (30)
	II	6.7 ± 1.2 (12)	8.9 ± 1.1 (7)	7.5 ± 1.6 (19)
	III	7.7 ± 1.3 (14)	8.3 ± 1.1 (14)	8.0 ± 1.2 (28)
36	I	6.7 ± 1.1 (9)	8.0 ± 1.4 (11)	7.4 ± 1.4 (20)
	II	7.2 ± 0.6 (9)	8.6 ± 2.3 (4)	7.6 ± 1.4 (13)
	III	8.3 ± 0.8 (13)	8.8 ± 1.0 (10)	8.5 ± 0.9 (23)

At age 6 months Hb levels show no difference between boys and girls. Levels in spleen Group I are significantly lower than those in Group II ($p < 0.01$) and Group III ($p < 0.001$), but levels in Group II and III do not differ significantly.

At age 12 months Hb levels within each subgroup are lower than the corresponding levels at age 6 months. Again there is no difference between boys and girls. Levels in spleen Group I are significantly lower than those in Group III ($p < 0.01$) with levels in Group II lying between with no significant difference from either Group I or Group III.

At age 24 months girls have significantly higher Hb levels than boys ($p < 0.01$), levels in girls being higher than at age 12 months while those in boys are lower. There are no significant differences between the spleen groups.

At age 36 months all subgroups show a rise in levels above those at age 24 months, except boys in spleen Group I and girls in Group II. Girls again have significantly higher levels than boys ($p < 0.02$), and levels in spleen Group III are significantly higher than those in either Group II ($p < 0.05$) or Group I ($p < 0.01$). Levels in Group I are lower than in Group II, but the difference is not statistically significant.

RED CELL COUNT ($x10^{12}/l$)

MEAN ± STANDARD DEVIATION (NUMBER IN SUBGROUP)

AGE (Months)	SPLEEN GROUP	MALE	FEMALE	COMBINED
6	I	3.63 ± 0.77(24)	3.19 ± 0.78(25)	3.41 ± 0.77(49)
	II	3.75 ± 0.56(23)	3.91 ± 0.41(13)	3.81 ± 0.50(36)
	III	4.05 ± 0.60(15)	3.75 ± 0.67(25)	3.86 ± 0.64(40)
12	I	3.16 ± 0.73(20)	3.02 ± 0.81(23)	3.09 ± 0.77(43)
	II	3.31 ± 0.76(25)	3.27 ± 0.89(11)	3.30 ± 0.79(36)
	III	3.84 ± 0.72(18)	3.51 ± 0.84(25)	3.65 ± 0.80(43)
24	I	3.17 ± 0.70(12)	2.95 ± 0.69(18)	3.04 ± 0.69(30)
	II	2.79 ± 0.54(12)	3.21 ± 0.42(7)	2.94 ± 0.53(19)
	III	3.38 ± 0.74(14)	3.68 ± 0.57(14)	3.52 ± 0.66(28)
36	I	2.94 ± 0.89(9)	2.99 ± 0.55(11)	2.97 ± 0.70(20)
	II	2.73 ± 0.42(9)	2.91 ± 0.77(4)	2.78 ± 0.52(13)
	III	3.15 ± 0.61(13)	3.58 ± 0.66(10)	3.33 ± 0.65(23)

No significant differences in red cell counts are seen between boys and girls.

At age 6 months counts in spleen Group I are significantly lower than in Group II ($p<0.01$) or Group III ($p<0.01$). Counts are lower in Group II than in Group III but the difference is not significant.

At age 12 months counts are lower than at age 6 months in each subgroup. Counts rise from spleen Group I through Group II to Group III, the difference between counts in Group I and Group III being significant ($p<0.01$).

At age 24 months counts are similar to or slightly lower than those at age 12 months. Counts in spleen Groups I and II are similar and in both are significantly lower than in Group III ($p<0.01$).

At age 36 months counts are similar to or only slightly lower than those at age 24 months. Counts in spleen Group I and II are again similar and lower than those in spleen Group III, but at this age only the difference between Groups II and III is significant ($p<0.02$).

RETICULOCYTE COUNT (%)
'MEAN' ('MEAN ± SD' : NUMBER IN SUBGROUP)

AGE (months)	SPLEEN GROUP	MALE	FEMALE	COMBINED
6	I	6 (3-13:23)	9 (4-20:25)	8 (4-16:48)
	II	5 (2-10:23)	5 (3- 8:13)	5 (3- 9:36)
	III	4 (2- 7:16)	5 (2- 9:25)	4 (2- 8:41)
12	I	8 (4-15:20)	11 (7-18:23)	9 (4;21:53)
	II	8 (4-15:25)	6 (3-12:11)	7 (4-14:36)
	III	5 (3-10:18)	6 (3-10:24)	6 (3-10:42)
24	I	13 (7-25:12)	11 (6-22:18)	12 (6-23:30)
	II	11 (7-16:12)	9 (4-17: 7)	10 (5;13:19)
	III	10 (6-16:14)	6 (3-12: 6)	8 (5-14:28)
36	I	12 (4-34: 8)	11 (7-16:11)	11 (5-24:19)
	II	11 (6-21: 9)	7 (5-11: 4)	10 (5-18:13)
	III	10 (6-16:13)	7 (5-11:10)	8 (5-13:23)

No significant differences in reticulocyte count are seen between boys and girls at 6 months, 12 months, 24 months or 36 months.

At age 6 months reticulocyte counts are significantly higher in spleen Group I than in spleen Group II and III ($p < 0.01$). Counts are similar in Groups II and III.

At age 12 months mean reticulocyte counts in all subgroups are higher than at age 6 months. Boys in spleen Group I and II have similar counts, but otherwise counts decrease from spleen Group I through Group II and Group III. Only the difference between spleen Group I and III is significant ($p < 0.001$).

At age 24 months mean reticulocyte counts are higher than at age 12 months in all subgroups except girls in spleen Groups I and III. Counts again decrease from spleen Group I through Group II to Group III with only the difference between Groups I and III being significant ($p < 0.02$).

At age 36 months mean reticulocyte counts are similar to those at age 24 months. Counts in girls in spleen Groups II and III are similar but otherwise counts decrease from spleen Group I through Group II to Group III. No significant differences between the subgroups are found.

* Log transform: 'Mean'= $_e$ mean $\ln x$, 'Mean ± SD'= $_e$ mean $\ln x \pm$ SD $\ln x$.

MEAN CELL VOLUME (fl)

AGE (months)	SPLEEN GROUP	MEAN ± STANDARD DEVIATION (number in each subgroup) MALE	FEMALE	COMBINED
6	I	75 ± 8 (24)	79 ± 8 (26)	77 ± 8 (50)
	II	74 ± 7 (23)	75 ± 6 (13)	74 ± 6 (36)
	III	71 ± 8 (16)	77 ± 6 (25)	75 ± 7 (41)
12	I	76 ± 10 (20)	80 ± 10 (23)	78 ± 10 (43)
	II	75 ± 8 (25)	79 ± 10 (11)	76 ± 9 (36)
	III	71 ± 12 (18)	77 ± 9 (25)	75 ± 11 (43)
24	I	75 ± 12 (12)	82 ± 10 (18)	79 ± 11 (30)
	II	80 ± 8 (12)	84 ± 7 (7)	82 ± 8 (19)
	III	75 ± 9 (14)	74 ± 10 (14)	75 ± 10 (28)
36	I	78 ± 12 (9)	83 ± 7 (11)	81 ± 9 (20)
	II	83 ± 7 (9)	87 ± 5 (4)	4 ± 6 (13)
	III	83 ± 9 (13)	80 ± 11 (10)	82 ± 10 (23)

At age 6 months MCV levels are significantly higher in girls than in boys ($p < 0.01$). Levels are higher in spleen Group I than in Groups II or III, but the differences between the spleen groups are not statistically significant.

At age 12 months MCV levels are again significantly higher in girls than in boys ($p < 0.02$). Levels are higher than at age 6 months in all subgroups except in boys in spleen Group III where there is no change. Levels fall progressively from spleen Group I through spleen Group II to spleen Group III, but there are no significant differences between the subgroups.

At age 24 months no significant difference is found between MCV levels in boys or girls. Levels in spleen Groups I and II are raised when compared to levels at age 12 months, but levels in Group III remained unchanged, being significantly lower than levels in Group I ($p < 0.01$).

At age 36 months there is again no significant difference between boys and girls. MCV levels within each subgroup are raised when compared to levels at age 24 months. Little if any difference is now apparent between the levels in the three spleen groups.

MEAN CELL HAEMOGLOBIN CONCENTRATION (g/l)

MEAN ± STANDARD DEVIATION (NUMBER IN SUBGROUP)

AGE (Months)	SPLEEN GROUP	MALES	FEMALES	COMBINED
6	I	0.32 ± 0.03(23)	0.31 ± 0.02(24)	0.32 ± 0.02(47)
	II	0.33 ± 0.02(21)	0.33 ± 0.01(13)	0.33 ± 0.02(34)
	III	0.32 ± 0.02(15)	0.32 ± 0.02(24)	0.32 ± 0.02(39)
12	I	0.32 ± 0.03(21)	0.32 ± 0.02(23)	0.32 ± 0.02(44)
	II	0.31 ± 0.03(25)	0.31 ± 0.02(11)	0.31 ± 0.02(36)
	III	0.31 ± 0.02(18)	0.32 ± 0.03(25)	0.31 ± 0.02(43)
24	I	0.31 ± 0.03(12)	0.32 ± 0.03(18)	0.32 ± 0.03(30)
	II	0.31 ± 0.03(12)	0.33 ± 0.01(7)	0.31 ± 0.02(19)
	III	0.32 ± 0.02(14)	0.32 ± 0.02(14)	0.32 ± 0.02(28)
36	I	0.31 ± 0.03(9)	0.33 ± 0.02(11)	0.32 ± 0.03(20)
	II	0.33 ± 0.01(9)	0.33 ± 0.02(4)	0.33 ± 0.01(13)
	III	0.34 ± 0.01(13)	0.34 ± 0.02(9)	0.34 ± 0.02(22)

Mean cell haemoglobin concentrations appear to fall slightly from age 6 months to age 12 months and then to increase again. Girls may have slightly higher concentrations than boys from age 12 months on but the difference is not significant. No consistent relationships with spleen groups are seen.

MEAN CELL HAEMOGLOBIN (pg)

MEAN ± STANDARD DEVIATION (NUMBER IN SUBGROUP)

AGE (Months)	SPLEEN GROUP	MALE	FEMALE	COMBINED
6	I	23 ± 2 (24)	24 ± 3 (25)	24 ± 3 (49)
	II	24 ± 2 (22)	24 ± 3 (13)	24 ± 2 (35)
	III	23 ± 3 (15)	24 ± 2 (25)	23 ± 3 (40)
12	I	23 ± 3 (20)	25 ± 4 (23)	24 ± 4 (43)
	II	23 ± 3 (25)	24 ± 4 (11)	24 ± 3 (36)
	III	22 ± 4 (17)	24 ± 4 (25)	23 ± 4 (42)
24	I	23 ± 4 (12)	27 ± 4 (18)	25 ± 4 (30)
	II	25 ± 3 (12)	25 ± 5 (7)	25 ± 4 (19)
	III	24 ± 3 (14)	23 ± 3 (14)	24 ± 3 (28)
36	I	23 ± 4 (11)	27 ± 3 (11)	25 ± 4 (22)
	II	27 ± 3 (9)	24 ± 8 (4)	26 ± 5 (13)
	III	26 ± 3 (13)	25 ± 4 (10)	26 ± 4 (23)

Mean cell haemoglobin is similar at ages 6 months and 12 months and then rises slightly at age 24 months and again slightly at age 48 months. No consistent relationships are seen with sex or spleen group.

SERUM IRON CONCENTRATION (mg/dl)

MEAN ± STANDARD DEVIATION (NUMBER IN SUBGROUP)

AGE (Months)	SPLEEN GROUP	MALE	FEMALE	COMBINED
12	I	67 ± 32 (14)	76 ± 29 (19)	72 ± 30 (33)
	II	61 ± 38 (20)	53 ± 38 (9)	59 ± 38 (29)
	III	79 ± 52 (17)	84 ± 43 (22)	82 ± 47 (39)
24	I	73 ± 30 (11)	92 ± 35 (16)	85 ± 34 (27)
	II	42 ± 14 (9)	72 ± 42 (6)	54 ± 32 (15)
	III	64 ± 38 (14)	75 ± 63 (12)	69 ± 50 (26)
36	I	56 ± 30 (7)	77 ± 49 (11)	69 ± 43 (18)
	II	89 ± 32 (9)	81 ± 53 (4)	86 ± 38 (13)
	III	87 ± 29 (11)	67 ± 38 (9)	78 ± 34 (20)

Serum iron levels in the population under study show no consistent relationship to age, sex or spleen group.

PLATELET COUNT ($x10^9$/litre)

AGE (months)	SPLEEN GROUP	MEAN ± STANDARD DEVIATION (no in subgroup) MALE	FEMALE
12	I	401 ± 206 (12)	352 ± 145 (20)
	II	329 ± 151 (20)	277 ± 218 (8)
	III	481 ± 131 (14)	378 ± 197 (18)
24	I	279 ± 192 (9)	455 ± 197 (15)
	II	399 ± 117 (7)	366 ± 166 (7)
	III	391 ± 140 (8)	380 ± 188 (22)
36	I	383 ± 157 (8)	436 ± 189 (11)
	II	466 ± 112 (6)	649 ± 217 (4)
	III	401 ± 102 (8)	336 ± 117 (6)

These counts show no relationships to age, sex or spleen group.

FOETAL HAEMOGLOBIN (%)

AGE (months)	SPLEEN GROUP	MEAN ± STANDARD DEVIATION (number in subgroup) MALE	FEMALE	COMBINED
6	I	23 ± 9 (21)	21 ± 8 (23)	22 ± 8 (44)
	II	25 ± 8 (20)	27 ± 5 (13)	26 ± 7 (33)
	III	27 ± 9 (15)	26 ± 6 (24)	26 ± 7 (39)
12	I	15 ± 8 (21)	15 ± 5 (23)	15 ± 7 (44)
	II	17 ± 6 (25)	18 ± 7 (11)	17 ± 7 (36)
	III	16 ± 7 (18)	18 ± 5 (25)	17 ± 6 (43)
24	I	10 ± 7 (12)	12 ± 5 (18)	11 ± 6 (30)
	II	11 ± 6 (12)	15 ± 4 (7)	12 ± 6 (19)
	III	13 ± 6 (14)	14 ± 7 (14)	13 ± 7 (28)
36	I	10 ± 6 (9)	12 ± 6 (10)	11 ± 6 (19)
	II	11 ± 6 (12)	13 ± 4 (4)	12 ± 6 (16)
	III	13 ± 5 (12)	15 ± 7 (9)	14 ± 6 (21)

At age 6 months there are no significant differences in HbF levels between the sexes. Taking both sexes together HbF levels are significantly lower in spleen Group I than in Group II ($p < 0.05$) or Group III ($p < 0.01$). HbF levels do not differ significantly between Group II and Group III.

At age 12 months HbF levels are lower than at age 6 months. Girls tend to have higher HbF levels than boys in both spleen groups II and III, although the differences are not statistically significant. HbF levels in spleen Group I are again lower than in Groups I or II but this difference is not statistically significant.

At age 24 months HbF levels are again lower but levels at age 36 months differ little if at all from these. No significant differences exist between the subgroups at either of these ages, but mean HbF levels are higher for girls than for boys within each spleen group, and between the spleen groups mean HbF levels rise progressively from Group I through Group II to Group III.

APPENDIX III

Haematological tables for the pit groups
HAEMOGLOBIN CONCENTRATION (gm/dl)

AGE (months)	PIT GROUP	MEAN MALE	STANDARD DEVIATION (number in subgroup) FEMALE	COMBINED
6	I	8.6 ± 1.3 (11)	7.8 ± 1.9 (4)	8.4 ± 1.4 (15)
	II	9.0 ± 0.9 (16)	9.6 ± 1.0 (11)	9.2 ± 1.0 (27)
12	I	6.7 ± 1.2 (11)	7.5 ± 0.9 (6)	7.0 ± 1.1 (17)
	II	8.4 ± 1.3 (16)	9.1 ± 1.7 (17)	8.7 ± 1.5 (33)

Hb levels show no significant differences between boys and girls at either 6 months or 12 months. Levels are significantly lower in pit Group I than pit Group II both at age 6 months ($p < 0.05$) and at age 12 months ($p < 0.01$).

RED CELL COUNT ($x10^{12}/l$)

MEAN ± STANDARD DEVIATION (NUMBER IN SUBGROUP)

AGE (Months)	PIT GROUP	MALE	FEMALE	COMBINED
6	I	3.64 ± 0.72(11)	3.21 ± 0.60(4)	3.53 ± 0.69(15)
	II	4.02 ± 0.53(16)	3.97 ± 0.65(11)	4.00 ± 0.57(27)
12	I	2.94 ± 0.84(11)	2.98 ± 0.68(6)	2.95 ± 0.76(17)
	II	3.70 ± 0.67(18)	3.64 ± 0.91(15)	3.67 ± 0.78(33)

No significant differences are seen between boys and girls. Red cell counts are lower at age 12 months than at age 6 months in all subgroups, the fall being greater in pit Group I. Counts are significantly lower in pit Group I than in pit Group II at both 6 months ($p<0.05$) and 12 months ($p<0.02$).

RETICULOCYTE COUNT (%)

'MEAN' ('Mean ± SD': number in subgroup)*

AGE (months)	PIT GROUP	MALE	FEMALE	COMBINED
6	I	6 (3-12:11)	8 (6-11: 4)	6 (3-12:15)
	II	4 (2- 7:16)	3 (2- 7:11)	4 (2- 7:27)
12	I	12 (6-25:11)	12 (10-15:6)	12 (6-22:17)
	II	5 (3- 9:18)	5 (3- 8:15)	5 (3- 9:33)

Reticulocyte counts show no significant differences between boys and girls at either 6 months or 12 months. Counts are higher in all subgroups at 12 months than at 6 months, the difference being most marked in pit group I. Counts are significantly higher in pit Group I than in pit Group II at both 6 months ($p < 0.02$) and 12 months ($p < 0.001$).

* Log transform: 'Mean' = $_e$ mean $\ln x$, 'Mean ± SD' = $_e$ mean $\ln x \pm$ SD $\ln x$.

MEAN CELL VOLUME (H)

Mean ± Standard Deviation

(number in each subgroup)

AGE (months)	PIT GROUP	MALE	FEMALE	COMBINED
6	I	79 ± 9 (11)	78 ± 3 (4)	79 ± 8 (15)
	II	72 ± 7 (17)	77 ± 7 (11)	74 ± 7 (28)
12	I	78 ±11 (11)	84 ± 4 (6)	80 ± 9 (17)
	II	74 ± 9 (18)	78 ± 9 (15)	76 ± 9 (33)

At age 6 months MCV levels are similar in girls and boys in pit Group I, but are significantly higher in girls than in boys in pit Group II ($p < 0.05$). Levels in pit Group I are significantly higher than in pit Group II ($p < 0.05$).

At age 12 months MCV levels are higher than at age 6 months in all subgroups except boys in pit Group I in whom levels are little changed. Levels in girls are higher than in boys within each pit group, and levels in pit group I are higher than in pit Group II. None of these differences reach statistical significance.

MEAN CELL HAEMOGLOBIN CONCENTRATION (g/l)

MEAN ± STANDARD DEVIATION (NUMBER IN SUBGROUP)

AGE (Months)	PIT GROUP	MALES	FEMALES	COMBINED
6	I	0.33 ± 0.02(9)	0.33 ± 0.02(4)	0.33 ± 0.02(13)
	II	0.33 ± 0.02(16)	0.32 ± 0.03(10)	0.33 ± 0.02(26)
12	I	0.31 ± 0.02(11)	0.35 ± 0.02(6)	0.33 ± 0.03(17)
	II	0.33 ± 0.02(18)	0.33 ± 0.03(13)	0.33 ± 0.02(31)

At age 6 months mean cell haemoglobin concentrations show no difference between boys and girls or between pit groups. At age 12 months a small fall in the pit group I boys is balanced by a small rise in the pit group I girls leading to a significant difference between boys and girls within the group ($p<0.01$) but no change in the combined mean value which again shows no difference from the mean value in pit group II. In pit group II no difference is seen between boys and girls.

MEAN CELL HAEMOGLOBIN (pg)

MEAN ± STANDARD DEVIATION (NUMBER IN SUBGROUP)

AGE (Months)	PIT GROUP	MALES	FEMALES	COMBINED
6	I	24±3 (11)	24±2 (4)	24±3 (15)
	II	23±2 (15)	24±2 (11)	23±2 (26)
12	I	24±4 (11)	26±3 (6)	24±4 (17)
	II	23±3 (18)	25±3 (15)	24±3 (33)

Girls tend to have a slightly higher mean cell haemoglobin than boys and to have a higher mean cell haemoglobin at 12 months than at 6 months. Neither of these differences is statistically significant. Levels of mean cell haemoglobin may be slightly higher in pit Group I than in pit Group II, but the difference is not statistically significant.

SERUM IRON (micro gm/dl)

MEAN ± STANDARD DEVIATION (NUMBER IN SUBGROUP)

AGE (Months)	PIT GROUP	MALES	FEMALES	COMBINED
12	I	52 ± 17(7)	78 ± 25(4)	61 ± 23(11)
	II	63 ± 19(14)	59 ± 30(12)	61 ± 24(26)

No significant relationships are seen between serum iron and either sex or pit group.

FOETAL HAEMOGLOBIN (%)

Mean ± standard deviation (number in subgroup)

AGE (months)	PIT GROUP	MALE	FEMALE	COMBINED
6	I	20 ± 7 (8)	20 ± 7 (4)	20 ± 7 (12)
	II	22 ± 7 (16)	23 ± 6 (9)	23 ± 7 (25)
12	I	13 ± 6 (11)	15 ± 4 (6)	14 ± 6 (17)
	II	18 ± 8 (18)	18 ± 7 (15)	18 ± 7 (33)

No significant differences are found between boys and girls. Foetal haemoglobin levels are lower in pit Group I than pit Group II; the difference is not statistically significant at age 6 months, but just achieves statistical significance at age 12 months ($p < 0.05$).

PLATELET COUNT ($\times 10^9$/l)

MEAN ± STANDARD DEVIATION (NUMBER IN SUBGROUP)

AGE (Months)	PIT GROUP	MALES	FEMALES	COMBINED
12	I	328 ± 125(10)	314 ± 138(5)	323 ± 124(15)
	II	417 ± 216(13)	365 ± 180(9)	396 ± 199(22)

No significant relationships are seen between platelet count and either sex or pit Group.

APPENDIX IV Survival curve analyses for clinical events according to haematological group.

TABLE i TRENDS OF OBSERVED/EXPECTED RATIOS OF CLINICAL EVENTS ACCORDING TO HbF GROUP

	HbF GROUP HbF range (no. in group) I < 21% (35)	II 21-27.5(40)	III >27.5 (48)	Chi^2 for trend DF = 1	Significance
EVENT					
Spleen appears					
Observed	28	34	35		
Expected	21.38	29.02	46.6		
Ratio $^{O}/_{E}$	1.31	1.17	0.75	5.75	p< 0.02
ASS					
Observed	4	7	5		
Expected	3.97	5.02	7.01		
Ratio $^{O}/_{E}$	1.01	1.40	0.71	0.24	NS
Severe infection					
Observed	7	3	1		
Expected	2.94	3.65	4.42		
Ratio $^{O}/_{E}$	2.38	0.82	0.23	6.89	p< 0.01
Death					
Observed	4	6	5		
Expected	3.84	4.78	6.38		
Ratio $^{O}/_{E}$	1.04	1.25	0.78	0.12	NS

TABLE ii TRENDS OF OBSERVED/EXPECTED RATIOS OF CLINICAL EVENTS ACCORDING TO MCV GROUP

	MCV GROUP MCV RANGE (no. in group) I >79 (38)	II 70 - 79 (68)	III <70 (26)	Chi^2 for trend DF = 1	Significance
EVENT					
Spleen appears					
Observed	30	52	22		
Expected	25.73	56.82	21.45		
Ratio $^{O}/_{E}$	1.17	0.92	1.03	0.24	NS
ASS					
Observed	6	6	4		
Expected	4.17	8.24	3.59		
Ratio $^{O}/_{E}$	1.44	0.73	1.11	0.74	NS
Severe infection					
Observed	8	4	1		
Expected	3.39	6.91	2.7		
Ratio $^{O}/_{E}$	2.36	0.58	0.37	5.63	p< 0.02
Death					
Observed	7	5	4		
Expected	4.16	8.38	3.46		
Ratio $^{O}/_{E}$	1.68	0.60	1.16	0.44	NS

TABLE iii TRENDS OF OBSERVED/EXPECTED RATIOS OF CLINICAL EVENTS ACCORDING TO RECTICULOCYTE COUNT GROUP

	RETIC GROUP Range of retic count (no. in group) I >9 (28)	II 5-9 (54)	III >5 (50)	Chi^2 for trend DF = 1	Significance
EVENT					
Spleen appears					
Observed	26	41	37		
Expected	15.23	45.78	43.0		
Ratio $^{O}/_{E}$	1.71	0.90	0.86	5.79	p< 0.02
ASS					
Observed	6	6	4		
Expected	4.17	8.24	3.59		
Ratio $^{O}/_{E}$	1.44	0.73	1.11	0.11	NS
Severe infection					
Observed	8	4	1		
Expected	2.55	5.46	4.99		
Ratio $^{O}/_{E}$	3.13	0.73	0.20	11.36	p< 0.001
Death					
Observed	6	6	4		
Expected	3.40	6.46	6.14		
Ratio $^{O}/_{E}$	1.76	0.93	0.65	1.99	NS

TABLE iv TRENDS OF OBSERVED/EXPECTED RATIOS OF SEVERE INFECTIONS IN RELATION TO SPLEEN GROUP ALLOWING FOR HbF GROUP

	SPLEEN GROUP Age in months spleen appears (total no. in group) I 0-6 (45)	II 7-12 (34)	III 13+ (39)	Chi^2 for trend DF = 1	Significance
HbF Group I					
Observed	7	0	0		
Expected	4.00	1.54	1.47		
Ratio $^{O}/_{E}$	1.75	0	0		
HbF Group II					
Observed	1	2	0		
Expected	1.14	0.89	0.97		
Ratio $^{O}/_{E}$	0.88	2.25	0		
HbF Group III					
Observed	1	0	0		
Expected	0.24	0.33	0.43		
Ratio $^{O}/_{E}$	4.18	0	0		
Adjusted values					
Observed	9	2	0		
Expected	5.37	2.75	2.88		
Ratio $^{O}/_{E}$	1.68	0.73	0	5.04	p< 0.03

TABLE v TRENDS OF OBSERVED/EXPECTED RATIOS OF SEVERE INFECTIONS IN RELATION TO SPLEEN GROUP ALLOWING FOR MCV GROUP

	SPLEEN GROUP Age in months spleen appears (total no. in group) I 0-6 (49)	II 7-12 (37)	III 13+ (41)	Chi^2 for trend DF = 1	Significance
MCV Group I					
Observed	8	0	0		
Expected	3.36	1.76	2.88		
Ratio $^{O}/_{E}$	2.38	0	0		
MCV Group II					
Observed	3	1	0		
Expected	1.38	1.19	0.84		
Ratio $^{O}/_{E}$	2.17	0.84	0		
MCV Group III					
Observed	0	1	0		
Expected	0.38	0.35	0.27		
Ratio $^{O}/_{E}$	0	2.89	0		
Adjusted values					
Observed	11	2	0		
Expected	5.13	3.29	4.58		
Ratio $^{O}/_{E}$	2.14	0.61	0	10.41	p <0.002

TABLE vi TRENDS OF OBSERVED/EXPECTED RATIOS OF SEVERE INFECTIONS IN RELATION TO SPLEEN GROUP ALLOWING FOR RETIC GRO

	SPLEEN GROUP Age in months spleen appears (total no. in group) I 0-6 (49)	II 7-12 (37)	III 13+ (41)	Chi^2 for trend DF = 1	Significance
Retic Group I					
Observed	8	0	0		
Expected	5.19	1.80	1.00		
Ratio $^{O}/_{E}$	1.54	0	0		
Retic Group II					
Observed	3	1	0		
Expected	1.03	1.31	1.66		
Ratio $^{O}/_{E}$	2.92	0.76	0		
Retic Group III					
Observed	0	1	0		
Expected	0.33	0.29	0.39		
Ratio $^{O}/_{E}$	0	3.5	0		
Adjusted values					
Observed	11	2	0		
Expected	6.55	3.4	3.05		
Ratio $^{O}/_{E}$	1.68	0.59	0	6.83	p <0.01

TABLE viiTRENDS OF OBSERVED/EXPECTED RATIOS OF SEVERE INFECTIONS IN RELATION TO HbF GROUP ALLOWING FOR SPLEEN GROUP

	HbF GROUP HbF range (no. in group) I	II	III	Chi^2 for trend	Significance
	<21% (34)	21-27.5(38)	>27.5 (46)	DF = 1	
Spleen Group I					
Observed	7	1	1		
Expected	3.71	3.04	2.25		
Ratio $^{O}/_{E}$	1.89	0.33	0.44		
Spleen Group II					
Observed	0	2	0		
Expected	0.41	0.71	0.88		
Ratio $^{O}/_{E}$	0	2.83	0		
Spleen Group III					
Observed	0	0	0		
Expected	0	0	0		
Ratio $^{O}/_{E}$	0	0	0		
Adjusted values					
Observed	7	3	1		
Expected	4.12	3.75	3.13		
Ratio $^{O}/_{E}$	1.70	0.80	0.32	3.00	NS (p = 0.08)

TABLE viii TRENDS OF OBSERVED/EXPECTED RATIOS OF SEVERE INFECTIONS IN RELATION TO MCV GROUP ALLOWING FOR SPLEEN GROU

	MCV GROUP MCV range (no. in group) I	II	III	Chi^2 for trend	Significance
	>79 (35)	70-79 (66)	>70 (26)	DF = 1	
Spleen Group I					
Observed	8	3	0		
Expected	3.09	5.53	2.38		
Ratio $^{O}/_{E}$	2.59	0.54	0		
Spleen Group II					
Observed	0	1	1		
Expected	0.43	1.08	0.49		
Ratio $^{O}/_{E}$	0	0.92	2.06		
Spleen Group III					
Observed	0	0	0		
Expected	0	0	0		
Ratio $^{O}/_{E}$	0	0	0		
Adjusted values					
Observed	8	4	1		
Expected	3.52	6.61	2.86		
Ratio $^{O}/_{E}$	2.27	0.60	0.35	5.49	p< 0.02

TABLE ix TRENDS OF OBSERVED/EXPECTED RATIOS OF SEVERE INFECTIONS IN RELATION TO RETIC GROUP ALLOWING FOR SPLEEN GROUP

	RETIC GROUP Range of retic count (no. in group)				
	I	II	III	Chi^2 for trend	Significance
	>9 (28)	5-9 (50)	<5 (49)	DF = 1	
Spleen Group I					
Observed	8	3	0		
Expected	3.95	3.28	3.77		
Ratio $^{O}/_{E}$	2.02	0.92	0		
Spleen Group II					
Observed	0	1	1		
Expected	0.32	0.92	0.76		
Ratio $^{O}/_{E}$	0	1.09	1.32		
Spleen Group III					
Observed	0	0	0		
Expected	0	0	0		
Ratio $^{O}/_{E}$	0	0	0		
Adjusted values					
Observed	8	4	1		
Expected	4.28	4.20	4.52		
Ratio $^{O}/_{E}$	1.87	0.95	0.22	5.31	$p < 0.03$