Serum protein profile in sickle cell disease

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SUMMARY The total protein, albumin, globulin, and immunoglobulin levels of sera from 96 children with homozygous sickle cell disease were studied. A comparison of the results with the levels found in a control group of normal children of the same age shows that the sicklers have higher total protein, globulin, and IgM levels. The amounts of albumin and IgA seen were almost the same in both groups. The IgG levels differed considerably, the sicklers having only about half the quantity seen in normal children.

The low albumin/globulin ratio, which typifies the protein pattern in the normal African, has already been discussed extensively (Edozien, 1957, 1961; Edozien *et al.*, 1960).

The changes seen in the serum protein values, although not specific for the diagnosis of disease, have invariably yielded extremely valuable information regarding clinical conditions. There is scarcely any comprehensive work in the literature on serum protein patterns in sickle cell disease. Previous information on serum protein values in American Negroes with the homozygous disease were based on scanty evidence (Table 1). These studies, the results of which were rather inconsistent, were mostly case reports, and the information gathered regarding the serum proteins suffered from the very limited number of patients investigated. It must also be stated that all their values were compared with values commonly found in a normal population; in this study, however, the protein pattern of sickle cell patients was compared with that of normal children of identical age range. The only work done on African sicklers was based exclusively on the heterozygote traits (Edozien *et al.*, 1960).

A study of the protein pattern in a large number of these patients was, therefore, considered necessary in the hope that this would provide additional information. The work presented here discusses the serum protein pattern in 92 homozygous sickle cell patients.

Table 1 Results from previous investigations of mean protein values in homozygous sickle cell disease

Study	No. of patients	Age (years)	Protein (g/l)		
			Total	Albumin	Globulin
Murphy and Shapiro (1945)	1	21	88	42	46
Bogoch <i>et al.</i> (1955)	4	Not given	77	40	38
Owen <i>et al.</i> (1965)	5	12-23	92	41	51
Rosenblate <i>et al.</i> (1970)	12	Not given	72	42	30

Material and methods

In all, 92 children with the homozygous disease were investigated. Their ages ranged from 1 to 11 years with the exception of two patients who were 10 months old. These patients were divided for the purpose of clinical assessment into two groups: 46 children under 5 years and 46 children between 5 and 11 years. A control reference group of 46 healthy schoolchildren aged 5 to 11 years was also examined and assessed. It was not possible to provide suitable controls for those under 5 years owing to the difficulties involved in procuring blood samples from normal children in that age group.

The diagnosis of homozygous disease was made by means of haemoglobin electrophoresis on cellulose acetate according to the method described in the 4th edition of *Practical Haematology* (Dacie and Lewis, 1968). Other investigations done along with the electrophoresis to support the diagnosis of the disease included haemoglobin and packed cell volume estimation and blood film examination. The haemoglobin content of blood was estimated by the

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Table 2 A comparison of serum total protein, albumin, globulin, albumin/globulin ratio, and immunoglobulin concentrations in two age groups of sickle cell disease with levels seen in normal children

Group	Age (yr)	Protein (g/l)					
		Total		Albumin		Globulin	
		Mean	Range	Mean	Range	Mean	Range
Sickle cell disease	5-11 (n = 46)	80 SD 8	64-94	43 5.4	33-53	35 7.8	20-50
	1-4 (n = 46)	72 SD 6.5	59-85	45 5	35-55	27 5.1	17-37
Normal	5-11 (n = 46)	75 SD 5.6	63-87	45 4	35-53	30 5.6	19-41

photoelectric colorimetric method, and the packed cell volume by the microhaematocrit method. Both determinations were done according to the methods described by Dacie and Lewis (1968). Blood was also collected from each patient for quantitative serum protein and immunoglobulin determinations. The former was estimated by the Biuret method, and the latter by the single radial immunodiffusion technique¹. For the control group, haemoglobin electrophoresis was done on each healthy child to eliminate the possibility of including a homozygous or heterozygous subject in the control group. Blood was also collected from each healthy child for quantitative serum protein analysis and for quantitative immunoglobulin determination.

Results

Our laboratory at the University of Nigeria Teaching Hospital takes part in an International Quality Control Practice (Wellcome International). Our results during the 10-month period in which these investigations were carried out show a high degree of accuracy. The mean values for total protein and globulin were 71.7 g/l and 24 g/l respectively, compared with 70.8 g/l and 23 g/l derived from the Quality Control Laboratory. Suitable control specimens were included in the various tests to ensure accuracy. The results are shown in Table 2.

TOTAL PROTEIN

The mean total protein in the older homozygous sicklers was significantly higher than the value found in normal children of identical age range (difference between means: $t = 3.47$, degrees of freedom 82, $P < 0.001$). Table 2 also shows clearly that the older sicklers have a much higher mean total protein value than the younger sicklers. The amount of protein found in the sicklers appears to be related to age (see Fig. 4).

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ALBUMIN

The mean albumin value seen in normal children was only just significantly higher than the value found in sicklers of equivalent age ($t = 2.02$, df 85, $P < 0.025$).

GLOBULIN

As in the case of total protein, the mean globulin in the homozygous sicklers was very significantly greater than the values found in normal children in the same age group ($t = 3.53$, df 83, $P < 0.001$). Also, as with the total protein, age seemed to influence the globulin level (see Fig. 4).

Fig. 1 Serum total protein levels.

G ratio _G (mean)	IgG (g/l)		IgA (g/l)		IgM (g/l)	
	Mean	Range	Mean	Range	Mean	Range
2/1	7.40	(5.50-9.20)	2.10	(0.95-3.20)	1.90	(1.05-2.70)
5/1						
1/1	15.50	(10.30-20.70)	2.07	(0.80-3.40)	1.25	(0.80-1.70)

IMMUNOGLOBULIN

The IgG values differed considerably between the normal and abnormal groups, the normal children having a significantly higher value than the sicklers ($t = 10.8$, $df\ 27$, $P < 0.001$). The IgM levels were similarly affected but in the reverse direction, the sicklers having a significantly higher value than the normal children ($t = 5.6$, $df\ 27$, $P < 0.001$). IgA levels were almost the same in both groups.

Discussion

The protein patterns seen in the three groups in this study are interesting in many respects. A comparison of the serum protein values shows definite evidence of relative hyperproteinaemia as well as hyperglobulinaemia in sickle cell disease (Table 2; Figs 1 and 3). Whereas the albumin levels were almost the same in sicklers and normal children (Fig. 2), the globulin values in sicklers were significantly greater than in normal children of the same age (Table 2; Fig. 3), showing that the globulin fraction is largely accountable for the high total protein. It is interesting that the quantity of total protein and the amount of globulin found in sicklers appear to be linearly related to age. Figure 4 shows these relationships in 80 sicklers.

According to Edozien and his colleagues (1960), the African child does not attain the normal total protein of the adult until the age of 6 months. After that age, according to them, there is no further increase. This implies that any change that takes place in the total protein development six months after birth is a qualitative rather than quantitative change. The results of the present investigation show, however, that African sicklers do not follow this pattern of total serum protein development. On the contrary, there is a progressive gain in the serum protein concentration with age, and the larger amount seen in these patients compared with normal children of the same age is proof that sickling

stimulates protein production.

It was not possible, owing to reasons beyond one's control, to fractionate the globulin complex by

Fig. 2 Serum albumin levels.

Fig. 3 Serum globulin levels.

electrophoresis. It was, however, possible to carry out some immunoglobulin studies. It is particularly interesting that virtually all the sicklers, whose immunoglobulins were quantitatively estimated, had only about half the IgG (which constitutes about 75% of total serum immunoglobulin) levels seen in healthy children of the same age (Table 2), a finding that seems to agree with a previous conclusion by Schwartz and Pearson (1972) of impaired antibody response to antigenic stimulation in 90% of the children with sickle cell disease in their study. These workers attributed their findings to functional asplenia resulting from chronic splenic reticulo-endothelial dysfunction and progressive infarction due probably to a regular crisis in this disease leading to autosplenectomy. Furthermore, previous reports of fulminant pneumococcal septicaemia (Kabins and Lerner, 1970), pneumococcal meningitis (Robinson and Watson, 1966), chronic osteomyelitis (Hook *et al.*, 1957), and numerous comments on the frequency of infections in sickle cell patients all seem to suggest a defect in the immunological function in children with this disease. The relevance of this finding to the frequency of infection in this disease needs to be studied. Edozien *et al.* (1960), in their interesting work on children with the heterozygote trait, suggested that sickling protects against

malaria by enhancing the antibody response to the malarial parasite. It is now, however, a well-established fact that malaria, like other infections, happens to be the greatest killer in this disease. The finding of a deficiency of immunoglobulin in spite of an apparent hyperglobulinaemia raises certain pertinent questions. Does the relative hyperglobulinaemia seen in patients with this disease represent a state of true immune response? How protective are these proteins contained in the globulin fraction of these patients? Are these proteins other proteins, with non-immunological activity, which precipitate along with the globulin fraction during estimation in the laboratory? These questions cannot be answered until the nature of the high protein found in the globulin fraction in sickle cell disease is fully characterised and assessed.

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Fig. 4 Linear regression of total protein ($r^2 = 0.68$) and globulin ($r^2 = 0.69$) against age in children with sickle cell disease: () number of children investigated.

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