field and Smith, 1955), and eluted. Following starch electrophoresis the haemoglobin fractions themselves were eluted. On measuring the colour of the eluates it was found that the abnormal component formed 28% of the total haemoglobin. The proportion of haemoglobin A_2 (Kunkel and Wallenius, 1955) was within normal limits.

On paper electrophoresis in phosphate buffer of pH 6.5 no separation occurred. The difference in appearance from haemoglobins A+G mixtures was insignificant, but a difference could always be discerned between the patient's haemoglobin and haemoglobin A or mixtures of haemoglobins
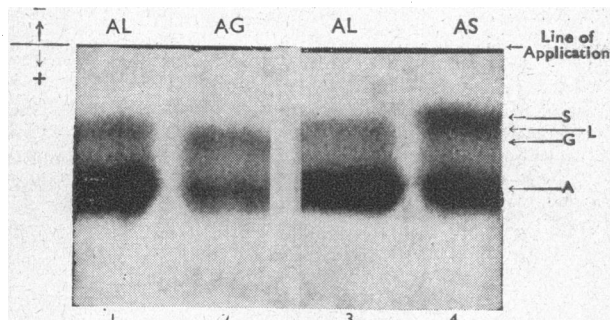
Comparison of patient's haemoglobin (1 and 3) with haemoglobin mixtures AG (2) and AS (4) respectively. The cells were washed in isotonic saline solution, packed, lysed with water and toluene, and centrifuged to separate the haemoglobin from the stroma. Paper electrophoresis was carried out by the hanging-strip method: Whatman filter paper No. 3 "for chromatography," barbiturate buffer pH 8.6. At the end of the electrophoretic run the strips were dried and the haemoglobin bands were stained with light green. It will be seen that the abnormal component in the patient's haemoglobin (haemoglobin L) migrates faster than haemoglobin S and more slowly than haemoglobin G.

A+S, A+E, and A+C. On paper electrophoresis in cacodylate buffer of pH 6.5 again no separation occurred, but with this buffer a difference could be seen in the appearance of the patient's haemoglobin and haemoglobin A+G mixtures.

The patient's haemoglobin mixture was rapidly denatured by alkali (Singer, Chernoff, and Singer, 1951), and it was resistant to cold denaturation (Rigas, Koler, and Osgood, 1956). The absorption spectra of his carboxyhaemoglobin and of a normal control showed identical peaks of extinction in the visible and ultra-violet ranges.

The solubility of his haemoglobin was within normal limits (Itano, 1953). We repeatedly found a slightly lower solubility of the ferrohaemoglobin than 5 g./l. when using a 2.24 M phosphate buffer. In Itano's solubility test all haemoglobin mixtures other than those containing haemoglobin S have a solubility of at least 5 g./l. at this buffer concentration. We thank Dr. H. A. Itano for measuring the solubility of our patient's haemoglobin. He found a normal solubility in 2.58 M phosphate buffer (1.8 ; 1.9 g./l.) and a slightly lowered solubility in 2.24 phosphate buffer (4.6 ; 4.7 g./l). Dr. Itano informs us that he does not place much confidence in such a small reduction in solubility approaching 5 g./l. in 2.24 M phosphate buffer, since that figure represents all the haemoglobin added in his test. A small amount of precipitation might occur before the concentrated buffer (2.8 M) is completely mixed. Dr. T. H. J. Huisman obliged us by testing the solubility of our patient's haemoglobin by a salting-out technique ; he also found it to be normal. We are grateful to Dr. Huisman for his permission to quote that the abnormal component of this haemoglobin moves on chromatography (Huisman and Prins, 1955) between haemoglobins S and C, whereas haemoglobin G moves between haemoglobins A and S, and to Dr. Itano for informing us of differences seen on open boundary electrophoresis between the haemoglobin of our patient and mixtures of haemoglobins A+S and A+G respectively.

Our patient was born a Hindu of the Khashtri caste in the Mianwali District, Pakistan. He is 27 years old and is now a citizen of India temporarily resident in London. He is not anaemic and there is nothing remarkable in his blood picture, excepting a slight eosinophilia. The osmotic fragility of his red cells is within normal limits. By the kindness of Dr. Ishwar Chandar we have examined the blood of our patient's parents and of one brother and one sister. The mother's haemoglobin consisted of haemoglobin A and of a component moving more slowly than haemoglobin A, but faster than haemoglobin S or D. The other three bloods contained haemoglobin A only.

New haemoglobins are allotted letters which are in general in alphabetical order of discovery (Statement on Hemoglobin Nomenclature, 1953). The accepted symbols for the haemoglobins so far identified are A, C, D, E, F, G, H, I, J, K, and S. The abnormal component in our patient's haemoglobin should therefore be called haemoglobin L.

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RELATIONSHIP BETWEEN HAEMOGLOBINS C AND S AND MALARIA IN GHANA

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Haemoglobin C was first described in a North American negro by Itano and Neel (1950) and was later found in high incidence in Ghana by Edington and Lehmann (1954). Allison (1956) and Edington and Lehmann (1956) discussed its incidence and distribution in West Africa. It would appear that the gene responsible for haemoglobin C occurs in high frequency in the tribes of Northern Ghana and declines in frequency from this focus to Nigeria and Sierra Leone, where low frequencies are recorded. Haemoglobin S is also found in Ghana, the incidence of the trait being high (19%) in the Southern regions, where the incidence of the C trait is low (10%), and low in the Northern regions (7%), where the incidence of C is high (21%). There are thus present in the country in high frequency two genes which in their homozygous and combined heterozygous expression are disadvantageous to their bearers. Other abnormal haemoglobins have been detected in Ghana, but their incidence is low. The common genotypes seen are listed in Table I.

The S gene is thus being eliminated from the population at large by individuals suffering from sickle-cell anaemia, sickle-cell haemoglobin C disease, microdrepanocytic-like disease (Edington and Lehmann, 1955), and perhaps in certain unspecified instances by bearers of the sickle-cell trait. It is difficult under these circum-

TABLE I.—*Abnormal Haemoglobin Genotypes Commonly Seen in Ghana*

Condition	Genotype	Clinical Effects
Sickle-cell trait	AS	Nil*
,, anaemia	SS	Disadvantageous + + + +
,, haemoglobin C disease	SC	Disadvantageous + +
Microdrepanocytic-like disease	AS + a thalas-saemia-like gene	Disadvantageous ±
Haemoglobin C trait	AC	Nil
Pure haemoglobin C disease ..	CC	Disadvantageous ±

* Although the sickle-cell trait is said to be innocuous, a characteristic pathology has been described at post-mortem examination in which the red blood cells sickle and the electrophoretic pattern of the haemoglobin is AS (Edington, 1957).

stances to explain the high incidence of this gene in Ghana and elsewhere in Africa, and there is evidence to show that this high incidence is due to the protection it affords the bearer against the lethal effects of *P. falciparum* malaria in holoendemic areas. The gene responsible for haemoglobin C is similarly being eliminated, to a much lesser extent, by individuals suffering from pure haemoglobin C disease and sickle-cell haemoglobin C disease. If the A, C, and S genes are in stable equilibrium it must be postulated that bearers of the haemoglobin C trait (AC) have a positive survival value, to account for its presence in high incidence in certain Northern tribes.

In a previous publication the relationship between sickling and malaria in Ghana was discussed (Colbourne and Edington, 1956). It was found that, in Southern Ghana, the sickle-cell trait appeared to protect the bearer partially against *P. falciparum* malaria in all age groups, whereas no protection over the age of 1 year was noted in sicklers in the north. The lack of protection exhibited by the trait in older children and adults in the north was thought to be due to the intense malarial transmission occurring in that district, the immunity thus rapidly acquired in the surviving non-sickler balancing the protection afforded by the trait plus the acquired immunity in the sickler.

The possibility of haemoglobin C being present in high incidence in the northern survey and interfering with the results had, however, to be considered. With the information available, no definite conclusions could be reached, although it was felt that the varying intensity of malarial transmission was probably responsible for the differing findings.

A second investigation has therefore been made in order to discover (1) if the presence of haemoglobin C in the north interfered with the findings in the original investigation, and (2) if the haemoglobin C trait protected the bearer against the effects of *P. falciparum* malaria.

Method of Investigation

The inhabitants of three villages in the Yendi (north-eastern) district of Ghana have been examined. The villagers were mainly of the Dagomba tribe, but a few Konkomba

had intermarried and settled permanently in the communities. The majority of the technical procedures employed have already been described (Colbourne and Edington, 1956). Sickling tests were repeated and electrophoretic analysis of the haemoglobin was performed on finger-prick blood in citrate saline sent by air to Accra. Four 90-volt dry batteries in series, with an output of 10 mA for five hours in barbitone buffer *p*H 8.6, gave satisfactory patterns on Whatman's filter paper No. 1 when utilized in an air-conditioned room.

In addition, as one of us (G. M. E.) has always considered that the protection afforded by the sickle-cell trait against *P. falciparum* malaria could be explained on physico-chemical grounds, particular attention was paid to the gametocyte count in thick films. It is known that in bearers of the sickle-cell trait the red blood cell sickles when deprived of oxygen. As the malarial parasite utilizes oxygen for growth it was thought that this reduction in oxygen within the cell would induce sickling and thus the destruction of both the parasite and the red cell by the reticulo-endothelial system. Advanced developmental forms (gametocytes) therefore should not be found in high rates in the peripheral blood of sicklers if this theory is correct.

Results

The blood of 1,055 villagers was examined. In nine the transmitted specimens were unsuitable for electrophoresis, in three the ages had been omitted from the record cards (genotype in all instances AA), and three haemoglobins on electrophoresis showed a second component moving faster than normal haemoglobin. These three specimens were sent to Dr. H. Lehmann, St. Bartholomew's Hospital, London, for identification, and the results will be published separately. Table II illustrates the genotypes seen in the remaining 1,040 villagers. It should be noted that the genotypes shown are based on the paper electrophoretic pattern of the haemoglobins and not on genetical studies. It has

TABLE II.—*Genotypes Seen in 1,040 Dagomba Villagers Shown in Age Groups*

Age Group	Genotypes					
	AA	AC	CC	AS	SC	SS
Under 1 year ..	46	10	1	6	—	—
1–4 years ..	81	27	1	12	1	—
5–10 ,, ..	215	52	6	24	4	—
11 + ,, ..	384	101	13	47	9	—
Total	726	190	21	89	14	—

TABLE III.—*Observed and Expected Genotype Frequencies in 1,040 Dagomba Villagers*

Genotype	Under 1 Year Old			1 Year and Over		
	No.	Observed Frequency	Expected Frequency	No.	Observed Frequency	Expected Frequency
AA	46	0·7302	0·7346	680	0·6960	0·7346
AC	10	0·1587	0·1632	180	0·1842	0·1632
AS	6	0·0952	0·0816	83	0·0850	0·0816
CC	1	0·0159	0·0091	20	0·0205	0·0091
SC	0	0·0000	0·0090	14	0·0143	0·0090
SS	0	0·0000	0·0023	0	0·0000	0·0023
Total ..	63	1·0000	0·9998	977	1·0000	0·9998

TABLE IV.—*Comparison of P. falciparum Rates and Densities Shown in Age Groups in 726 Dagomba Villagers with Normal, 211 with C, and 103 with S Haemoglobin*

Age Group	Total	Normal Haemoglobin						Haemoglobin S (Genotypes AS and SC)						Haemoglobin C (Genotypes AC and CC)					
		No.	P.f.+	Rate	Mean* Density	Mean Log Density	Gametes	No.	P.f.+	Rate	Mean* Density	Mean Log Density	Gametes	No.	P.f.+	Rate	Mean* Density	Mean Log Density	Gametes
Under 1 year	63	46	39	85%	7·9	3·19038	35%	6	4	67%	2·3	2·99301	0	11	10	91%	7·6	3·07051	45%
1–4 years..	122	81	70	86%	5·8	2·89102	35%	13	9	69%	14·8†	2·38040	31%	28	26	93%	4·6	2·95651	36%
5–10 ,, ..	301	215	173	80%	1·1	2·38503	38%	28	23	82%	4·3	2·61767	25%	58	47	81%	1·7	2·51683	34%
11 + ,, ..	554	384	151	40%	0·2	1·85129	15%	56	26	46%	0·7	1·84498	19%	114	46	40%	0·3	1·80000	20%
Total ..	1,040	726	433	60%	2·2	10·31772	23%	103	62	60%	4·2	9·83606	22%	211	129	61%	2·3	10·34385	27%

* In thousands per c.mm. † Heavily weighted by one child with a density of 132,000 parasites per c.mm.

been assumed in this study that the haemoglobin pattern gives a direct expression of the genotype.

From the observed genotype frequencies in the group under 1 year the expected genotype frequencies have been calculated and the results are shown in Table III. We are indebted to Mr. M. J. Hollingsworth, Zoology Department, University College of Ghana, for his help in compiling these frequencies and for the statistical analysis shown in Table IV.

Although there were greater numbers of the genotypes AC, AS, CC, and SC observed in those aged 1 year or more than would have been expected and fewer AA and SS, none of these differences reach an accepted level of statistical significance and no conclusions can be drawn from Table III.

The observed parasite rates and densities in individuals with normal C and S haemoglobins are shown in Table IV. The malarial parasite rates and densities in individuals suffering from pure haemoglobin C disease and sickle-cell haemoglobin C disease were compared in their respective age groups with those in normal, C, and S trait individuals. No significant differences were noted. These figures are not shown separately in Table IV, and AC and CC are considered together in the haemoglobin C column and AS and SC in the haemoglobin S.

A comparison of the parasite rates and densities in those with normal haemoglobin and haemoglobin C as shown in Table IV indicates that there is no evidence that haemoglobin C protects the bearer against $P.$ $falciparum$ malaria ($t = 0.1071$; $P > 0.5$).

The figures for haemoglobin S, although not definitely significant, are suggestive that S may partially protect the bearer against $P.$ $falciparum$ malaria ($t = 1.475$; $P < 0.2 > 0.1$). The findings are similar to those already recorded (Colbourne and Edington, 1956), the most marked differences being in the younger age groups. It can be concluded that the differing type of protection afforded by haemoglobin S against malaria in Northern and Southern Ghana is due to the variation in intensity of malarial infection and that the presence of haemoglobin C did not interfere with the results recorded in the paper quoted above.

It was disappointing to note that the incidence of gametocytes was similar in sicklers and non-sicklers, suggesting that the protection afforded by sickle-cell haemoglobin against malaria is not explicable by the physico-chemical mechanism mentioned above.

Discussion

We have been unable to show any protective relationship between haemoglobin C and malaria in this survey, and from our results we consider it most unlikely that any protection exists. It must be remembered, however, that the important question is whether children with the C trait die of cerebral malaria. In the future our survey results should be confirmed by a clinical investigation on the lines described by Raper (1956), who demonstrated that the incidence of cerebral malaria was significantly less in children with the sickle-cell trait and that non-sickling children were at a disadvantage. Unfortunately haemoglobin C occurs in its highest incidence in Ghana in areas where medical facilities are limited and necropsies are difficult to obtain.

In Accra the electrophoretic pattern of the haemoglobin is being determined in many necropsies, in particular in all children in whom the cause of death is suspected to be cerebral malaria. So far 10 children have died of histologically proved cerebral malaria. In nine the haemoglobin has been of the genotype AA and the non-sickling haemoglobin of the tenth exhibited a pattern which was considered to be AG. Neither C nor S haemoglobin has been noted. Necropsies on children dying of cerebral malaria are few, but eventually a sufficient number should be obtained to give statistically significant results. The pattern AC has been seen in 18 subjects coming to necropsy and the pattern CC in two. In one child with the haemoglobin C trait the cause of death was considered to be malaria and

bronchopneumonia, and in a second child who was killed in a car accident there were heavy deposits of malarial pigment in the liver and spleen—further indications that C does not protect against malaria. The two individuals suffering from pure haemoglobin C diseases were men aged 20 and 55, and both died of widespread miliary tuberculosis.

It is, of course, possible that haemoglobin C may protect its bearer against some other condition apart from malaria, thus accounting for its high incidence in Northern Ghana.

The following conditions have been responsible for death in the 18 necropsies performed on subjects exhibiting the C trait.

TABLE V.—Causes of Death in 18 Subjects With the Haemoglobin C Trait

Injury	..	..	.. 7	Carcinoma of lung 1	
Cellulitis	..	..	.. 2	Malarial anaemia and broncho-	
Nephritis and hypertension	..	2	pneumonia 1	
Appendicitis	..	..	.. 1	Pneumococcal meningitis .. 1	
Bronchopneumonia	..	.. 1	Schistosomiasis 1	
				Strangulated hernia 1	

Thus, in addition to malaria, haemoglobin C is unlikely to protect the bearer against pyogenic and pneumococcal infections, schistosomiasis, and tuberculosis.

Summary

The haemoglobin genotypes of 1,040 Dagombas have been determined and correlated with malarial parasite rates and densities. Haemoglobin S may partially protect the bearer against $P.$ $falciparum$ malaria in the younger age groups in Northern Ghana, but there was no evidence that haemoglobin C acted in a similar manner. The results of necropsy findings in Accra are briefly discussed.

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Postgraduate medical teaching is an important part of the work of the Royal Institute of Public Health. Courses are organized for the diploma in public health and the diploma in industrial health. These courses are held twice yearly, and may be taken either full-time or part-time. Details of the results for 1956 are given in the institute's annual report, which was issued at the annual general meeting last month. The chemical and bacteriological laboratories of the institute are available for the examination and investigation of pathological specimens, water, milk, sewage, and other types of analysis. During 1956 some 2,318 samples were submitted for examination, this being an increase of 81 over the previous year's total. Fourteen public addresses were given during the year, related to the following aspects of public health: social work, mothers, children, air, food, teeth, nursing, nutrition, and industry. These addresses were subsequently published in the journal of the institute. Membership of the institute is generally by examination, and includes medical practitioners, dental surgeons, State-registered nurses, registered medical auxiliaries, bacteriologists, and others whose duties entail a knowledge of public health and hygiene. Full details of the work of the institute may be obtained from the Secretary, 28, Portland Place, London. W.1.