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A New Look at the Protection of Hemoglobin *AS* and *AC* Genotypes against Plasmodium Falciparum Infection: A Census Tract Approach

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The suggestion that the *AS* genotype protects the carrier against *Plasmodium falciparum malariae* infection was presented 20 years ago by Allison [1, 2] and later by others [3–11]. Walters and Lehmann [12] assumed that if Hb C provides a selective advantage similar to Hb S, it would replace it because of fewer disadvantages.

Evidence supporting the theory of selection against malaria includes differential survival of the heterozygotes (AS) [1, 8, 10, 11, 13–15], lower parasite densities in Hb S carriers than Hb A subjects [2, 6, 9, 11, 16, 17], and the absence of Hb AS children with severe malarial infection [4, 6, 9]. Microgeographic mapping showing correspondence between areas of high malarial endemicity and high gene frequency on the one hand, and low endemicity and low gene frequency within a restricted geographical unit (i.e., an island or a country) on the other, also supports the theory of selection [1, 18]. Finally, the suggested mechanism of protection assumes that with high fever and malarial infection there is enhanced sickling of parasitized red cells, and that these cells are more likely to be eliminated by the reticuloendothelial system [19].

Large scale studies in tropical countries are difficult. Investigators who wish to study the protection enjoyed by carriers of Hb S or Hb C against falciparum malaria have to limit their investigations to small population samples which often do not represent that section of the population where protection is probably most efficient (i.e., young and healthy children). Evidence is meager that Hb C protects against falciparum infections; there are only three studies and one reappraisal

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with rather controversial conclusions [6, 16, 20, 21]. Investigations on a large unselected healthy population under 5 years of age are not available.

In 1964 a malariometric survey was initiated in Accra, Ghana by the National Institute of Health and Medical Research, Ghana Academy of Sciences, to collect basic information on selected aspects of malaria before launching an eradication program. The Central Bureau of Statistics of Ghana planned the sample and collaborated in the organization and supervision of the field operation. The survey was to derive data by a census tract approach on an age stratified population under 5 years of age [10].

MATERIALS AND METHODS

The 128 enumerated areas created for the 1960 Ghana population census provided a convenient sampling frame for random selection. A sample design following the model of Deming [22] was adopted. Two independent random subsamples were created to facilitate the estimation of variance. Three "domains" of study, Accra East, Accra West, and Accra Central were selected. The population of the Accra metropolitan area is heterogeneous ethnically and reflects diverse tribal, economic, and domiciliary characteristics. Accra Central is inhabited by fishermen and their families of the southern Ga tribe who live in three to four story buildings. In Accra East, there are many northerners usually living in one room wooden shacks with very limited amenities. Accra West is inhabited mainly by employed workers of various tribal origins where living standards are higher than those encountered in Accra East. There was a total of 4,097 children (table 1).

TABLE 1

DISTRIBUTION OF 4,097 CHILDREN INVESTIGATED

Area	Subsample 1	Subsample 2
Accra East	1,034	785
Accra West	524	670
Accra Central	570	514
Total	2,128	1,969

A census officer, a social worker, and a person trained to collect blood samples visited every house in each area. After questioning the head of the household, blood samples were collected from every child under 5 years of age. Two heparin capillary tubes were filled and sealed. One thick drop and four thin smears were prepared on prelabeled slides.

After centrifugation, the hematocrit was read, and the capillary tubes were broken above the red cell column. The packed red cells were blown into a microtube, washed, and the lipid removed with carbon tetrachloride. A modified Shandon microelectrophoretic cell was used for electrophoresis on cellulose acetate with Tris-EDTA-boric acid buffer, pH 8.9. The electrophoresis was run for 4 hr and the results read independently by two observers. An AS control sample was run with every series. In case of disagreement regarding the phenotype, the specimen was rerun. If disagreement still existed the strip was discarded.

The slides for malaria parasites were surveyed by three independent groups each headed

by an expert in malariology. The thick drop was hemolyzed and stained with Leishman stain. The thin smears were similarly stained. An average of 400 fields were surveyed for parasite type and density. A total of 300 unselected smears were sent to the Liverpool Tropical Medical School and to the Malaria Laboratory at Ho, Ghana Ministry of Health, for controls. The results of the controls agreed very well with those found by our teams.

Results were coded and punched on cards. A computer program was written and the data processed at the Data Processing Center of the National Planning Bureau, Budapest. Estimations of totals, variables, ratios, and variance computations* were carried out according to the method of Deming [22].

RESULTS

Age Stratification, Sex Distribution, and Death Rate

In 9,262 children under 1 year of age males outnumbered females 54.12:45.88 (figs. 1 and 2). This sex difference declined with age, and the male to female ratio in the 4–5-year-old group was 50.66:49.34.

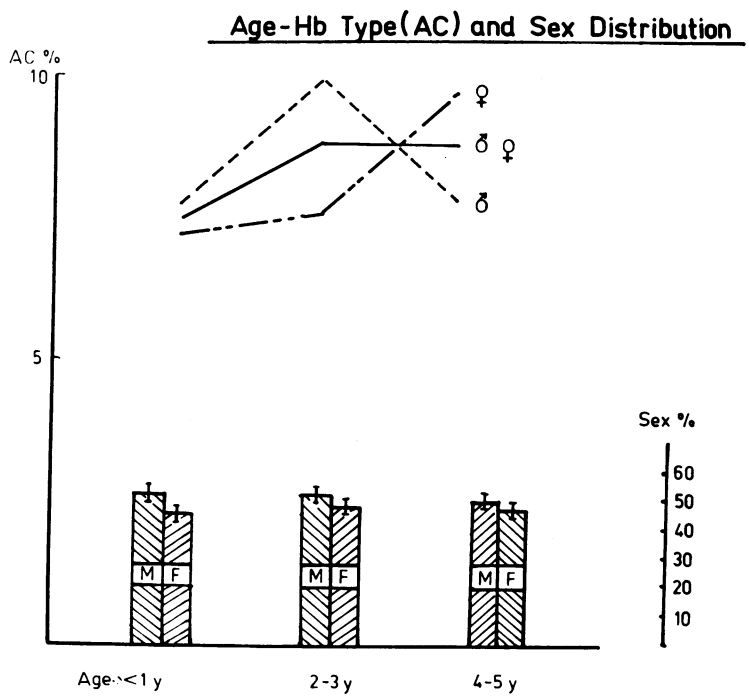

FIG. 1.—Columns show age and sex distribution of population under 5-years-old. Lines indicate rate of AC males and females in three age groups.

Data from a demographic study in Accra in 1966 by Miltényi [23] shows the overall mortality under 1 year is 22.5%, (52.5% males, 47.5% females); between 1–4 years, 24.5% (51.7% males, 48.3% females). Males have a higher mortality rate in both age groups.

* The description of the equations used for these calculations will be published separately.

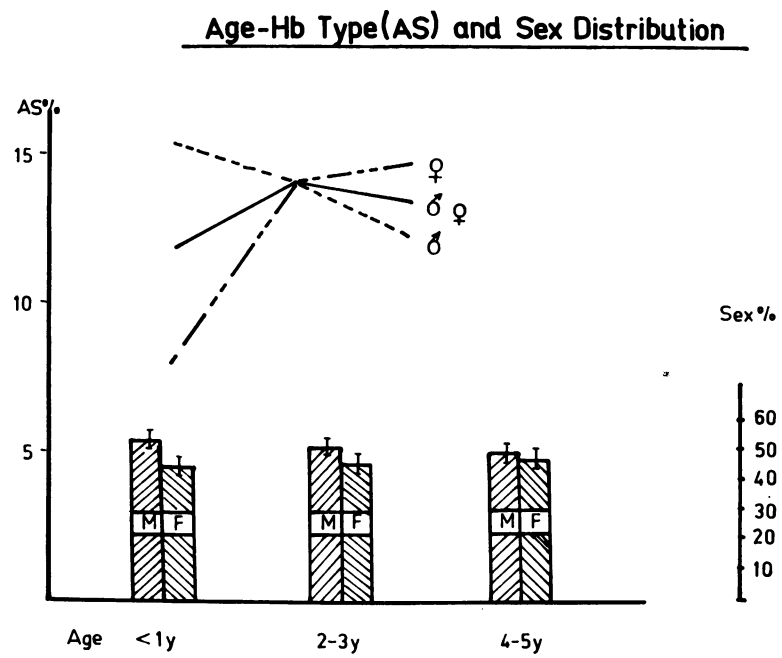

FIG. 2.—This graph is similar to figure 1. Lines show percentage of AS males and females in three age groups.

Age, Place of Birth, and Hb Type

In table 2 the prevalence of the six Hb types most frequently found is shown in three age groups under 5 years. The percentage of AA carriers decreases from 80.63% to 77.52% between the ages of under 1 year and 2–3 years. During this period the number of AS carriers rises from 10.39% to 12.48% and AC from 7.56% to 8.95%. No significant changes were noted between 3–5 years.

SS and *CC* genotypes are decreasing continuously with age whereas the per-

TABLE 2

AGE DISTRIBUTION OF HB TYPE

HB TYPE	AGE (YR)		
	1	2–3	4–5
AA	80.63 ± 2.04	77.52 ± 1.17	77.90 ± 0.93
AS	10.39 ± 1.34	12.48 ± 1.00	11.95 ± 0.77
AC	7.56 ± 1.04	8.95 ± 0.83	8.94 ± 0.78
SS	0.63 ± 0.37	0.46 ± 0.23	0.43 ± 0.25
SC	0.47 ± 0.27	0.33 ± 0.11	0.57 ± 0.19
CC	0.31 ± 0.22	0.20 ± 0.11	0.21 ± 0.12

NOTE.—Numbers indicate percent ± SD.

centage of *SC* first fell and then rose. Since individuals with Hb SS, CC, and SC were found in the expected small numbers, it is impossible to comment on these changes.

There was no difference in percentage of Hb AS and AC types between children born in Accra (83.08%) and those born elsewhere in Ghana (17%). A negligible number of immigrants were found from other African countries.

Hb AS and Hb AC among Males and Females in Three Age Groups

Although the sex distribution of type Hb AC was evenly distributed in the youngest age group (7.2% males, 7.8% females), there was an uneven distribution of Hb AS in this age group (14.9% males, 6.3% females).

Figure 1 shows that the frequency of males with Hb AC rose to 10.6% in the 2–3 year group and then fell to 7.9% in the 4–5 year group. Females with Hb AC showed little change in the 2–3 year group but an increase to 10% in the 4–5 year group.

Figure 2 shows a decreased frequency of Hb AS males with age. An opposite trend was noted in females with an increase to 12.5% at 2–3 years and 13.1% at 4–5 years. The difference between the youngest and oldest group is significant ($P < .01$) in females but not males.

Infection Rate, Parasite Density, Age, and Sex

Malarial infection rates were relatively low (1 year, 10.1%; 2–3 years, 18.7%; 4–5 years, 23.9%), although they increased with age.

Cases with parasite densities under 10,000 parasites/ml were more numerous in older children. Females in all three age groups displayed a higher infection rate with less than 1,000 parasites than males in the same age group. The difference is significant in the 2–3 and 4–5 year group ($P < .01$). At parasite densities between 1,000–10,000 more females were found in the two younger age groups, but these differences were not statistically significant. There were very few cases with a parasite count of 100,000/ml and over; none were found among males and only four among females.

Infection Rate, Parasite Species, and Hb Type

The highest infection rate, 21.9%, was seen in AS individuals. In AC and AA individuals the rates were 19.03% and 18.95%, respectively (fig. 3).

P. falciparum was found in 12.7% of Hb AS individuals, 11.9% of Hb AC, and 12.9% of Hb AA. *P. falciparum* gametocytes were present in 4.8% of the Hb AC individuals, 3.8% of Hb AS, and 2.5% of Hb AA, the difference between the Hb AC and Hb AA classes being marginally significant ($P < .05$). *P. malariae,* the second most frequently detected parasite, was seen twice as often in Hb AS than Hb AA and Hb AC children. Infections with both *P. falciparum* and *P. malariae* were present occasionally.

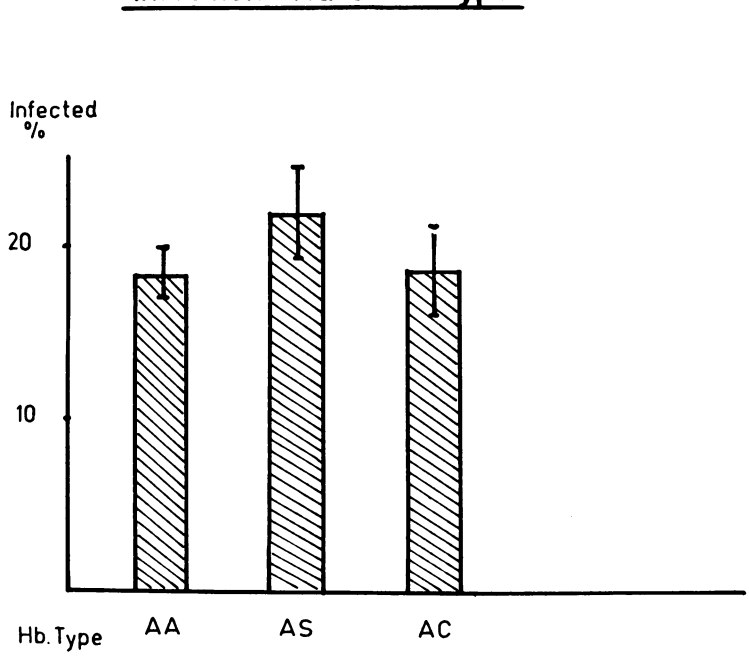

FIG. 3.—Percent infected of Hb genotype. Lines at top indicate 1 SD.

Parasite Density, Hb Type, and Sex

Parasite density related to sex and Hb type is shown in table 3. The infection rates with a low parasite density were almost the same in Hb AA and AC males and females. However, female Hb AS carriers had a higher rate of infection than children with AA and AC Hb type ($P < .05$). Individuals with a parasite density between 10,000–50,000 are more numerous among heterozygotes than Hb AA

TABLE 3

HB TYPE AND PARASITE COUNT

PARASITE COUNT	HB AS		HB AC		HB AA	
	Male	Female	Male	Female	Male	Female
<1,000	11.2 ± 1.4	17.8 ± 3.14	9.9 ± 2.7	8.4 ± 1.77	8.9 ± 0.9	11.5 ± 1.0
1,000–10,000 ...	9.3 ± 1.2	14.8 ± 2.15	10.0 ± 2.8	6.4 ± 2.26	7.5 ± 0.7	8.0 ± 0.8
10,000–50,000 ...	12.5 ± 4.4	11.7 ± 3.7	6.3 ± 3.2	11.7 ± 4.8	1.4 ± 0.3	1.9 ± 0.3
50,000–100,000 ..	...	...	...	...	0.1 ± 0.06	0.1 ± 0.05
≧100,000	...	...	...	...	...	0.15 ± 0.07

NOTE.—Figures represent percent of total sample.

individuals. There is a marginally significant difference in infection rate between AS and AA males with a parasite density of 10,000–50,000 ($P < .05$). No AS or AC individual was seen with more than 50,000 parasites.

Hb Type and Infection Rate in Three Domains of Study

Hb AS and AC individuals were present in a similar frequency in the populations of Accra East (10.7% and 8.8%, respectively) and West (11% and 9.6%, respectively). Hb AS individuals were more frequent (14.9%), and Hb AC less frequent (7.6%) in Accra Central. The overall infection rate in Accra Central is about half of that seen in the other two domains (East, 21.5%; West, 22.9%; Central, 11.8%).

DISCUSSION

The present paper investigates a large number of unselected subjects while many previous studies used hospital patients. In this population males outnumbered females, although the difference dwindles in the 4–5 year group because more males die than females. The data on child mortality are taken from the report of Miltényi [23] which covers the same period as our study. The mortality of children is higher than in developed countries but lower than rural areas of Africa where malaria is holoendemic [24].

The decrease of *AA* genotypes in the age stratified population and the increase of *AS* and *AC* shows a differential survival of the heterozygotes. This is the first study which displays the growing percentage of Hb AC carriers in an age stratified population. The differential survival of the latter, however, is less pronounced than that seen in *AS* genotypes. It is noteworthy that the decrease of *AA* genotypes and the increase of *AS* and *AC* take place during the first 3 years of life.

The question may arise whether figures for heterogeneity of the Accra population are distorted because of older children migrating from Northern Ghana where the frequency of *AC* is higher [16, 25], however, the rate of Hb AC carriers among migrants was not higher. The distribution of AS and AC individuals in the three domains is not uniform. In Accra Central, where the southern Ga tribe is indigenous, the number of children with Hb S is higher; those with Hb C, lower. Nonetheless, the survival is not influenced by this difference.

Infection rate is lower than any found by other investigators in holoendemic areas [3, 18]. One would naturally assume that this is due to a lower transmission rate because the population protects itself from mosquitoes. Trent [26], in connection with the malariometric survey, carried out a field study on the causation and treatment of malaria among the population of Accra. The individuals investigated lived in the same three domains which were the subject of our study. In 74 of 93 homes nothing was done to protect against mosquitoes. Only one respondent reported that he gave his children an antimalaria drug regularly [26].

P. falciparum was the parasite most frequently observed in the Accra population. Infection with other parasites is rare. Children under 1 year had the lowest infection rate suggesting transplacental immunity. This supposition is supported by an

immunoglobulin study in Accra of women and their children under 20 weeks of age. The mean IgG level of children less than 10 days old was 680 mg/100 ml; this fell to 510 mg when the children were 20 weeks old. The mothers' IgG level was 1,700 mg/100 ml (B. Ringelhann and S. Pacsa, unpublished). The danger period for a child is between the age of 6 months and 2 years when he has developed his own immunity from infection [8, 27, 28].

More children with Hb AS (22%) were infected than those with AC and AA. Those who are Hb AS [2, 9] and Hb AC [20, 21] have been said to have a lower rate of infection and parasite count than those who are Hb AA. Some literature supports these data [6, 11, 16, 17] while others disagree [5, 8, 29]. It is generally agreed that fewer heterozygotes have severe infection (i.e., parasite counts over 100,000) [4, 5, 11].

We were unable to confirm the report that parasite density is lower in AS and AC than in AA individuals [2, 6, 9, 11, 16, 17]. However, no individual with higher than 50,000 parasites/ml was found with AS and AC. Those with higher parasite counts were all AA individuals. This finding suggests that persistent moderate parasitemia may stimulate the development of active immunity, protecting heterozygotes against severe *P. falciparum* infection.

The immunity acquired after repeated natural infection is directed against the asexual erythrocytic form of parasites but not against gametocytes. The maintained production of gametocytes in the immune person provides more chances for transmission by mosquitoes and thus may be of epidemiologic importance [27]. Indeed *P. falciparum* gametocytes were more frequent in S and C gene carriers in the Accra population.

Females, regardless of their Hb type in all three age groups, had a higher parasite count than males. The apparently better immunological defense of females can explain, in the case of AS girls, why the percentage of carriers rose from 6.25% ($<$ 1 year) to 13.13% (4–5 years). It is known that G6PD deficient heterozygous females are relatively resistant to malaria regardless of Hb type [30], although their resistance is weaker than that of the abnormal Hb gene. A third of the females in the Ghanaian population are estimated to be G6PD heterozygotes. The increasing number of females in this population may suggest a double (Hb type + sex) or triple (Hb type + G6PD + sex) advantage in some individuals. Previous studies have not presented separate data for the two sexes when studying differential survival of the heterozygotes.

The percentage of Hb AC males was not higher in the under 1 year age group. In an earlier study a non-Mendelian segregation ratio was suggested for this Hb type, supported by a 2:3 male to female ratio [31]. This was not seen in our study.

This investigation concludes that there is a higher survival rate for AS than AC individuals and AC than AA individuals, although the effect of AS is more pronounced. This differential survival has been demonstrated in a metropolitan area with relatively low mortality. Presumably higher age differences could be obtained in areas where mortality due to malaria is more frequent. We suggest that pro-

tection is derived from the better immunological defenses of the heterozygotes against malaria. The higher infection rate of AS persons and increased parasite density stimulate the host response and enhances the production of antibodies against *P. falciparum*. A similar but less potent advantage of females was also suspected from the higher parasite rates in females than males. It is conceivable that the protection enjoyed by AS and AC females is further enhanced by heterozygosity for G6PD deficiency.

SUMMARY

A total of 4,097 randomly selected children under 5 years in Accra, Ghana were investigated for Hb type, malarial parasite species, and parasite density. Even though malarial infection rates in this metropolitan population were lower as compared to holoendemic areas, the differential survival of Hb S carriers was confirmed. In addition, similar but less pronounced survival effects were seen in Hb C heterozygotes. Hb S carriers had the highest infection rates. More females than males were infected. Individuals with a moderate parasite count ($<$ 50,000/ml) were seen more commonly among AS and AC individuals as compared to AA controls.

It is postulated that heterozygotes have a better immunological defense against the deleterious effects of *P. falciparum* infection because persistent parasitemia stimulates antibody production.

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