

Serum Protein Concentrations of Healthy Zambians and Europeans Resident in Lusaka

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SUMMARY

A study of the various serum proteins is reported in fifty male and fifty female Zambians. The results are compared with a group of 22 European females and 12 European males. Differences in the albumin levels are expected and are most probably due to diet differences. Similarly the increase in the gamma globulin fraction among Zambians was expected and is most certainly related to prolonged malaria experience.

INTRODUCTION

Substantial differences in plasma protein patterns have been reported for apparently healthy persons living in the tropics (Holmes et al., 1955, Ezeilo 1970, Nantulya and Lindqvist 1973). This seems to be particularly true if values for peoples living in tropical countries are compared with those from temperate regions.

There seems to be little doubt that some of these differences are due to prolonged or repeated exposure to a variety of tropical diseases. Recent investigations of serum protein patterns in subjects with experimentally induced malaria (Collins et al., 1971) showed significantly altered patterns in some individuals many months after a single administration of the parasites. Gilles and McGregor (1962) showed significant alteration towards Western values in serum proteins of Gambian women living in a rural village who received malaria prophylaxis for two years. Untreated women of the same village did not show a similar alteration.

Apart from two previous studies which have been carried out on normal protein patterns in Zambians (Ezeilo 1970; Briggs, Wenlock and Briggs 1972), very little information is presently available on normal serum protein patterns in Zambians. In neither of these studies was a comparison made with a European group living under similar circumstances to the Zambians. It was therefore thought that a study comparing results on normal serum proteins in Zambians with those found in Europeans resident in Zambia would be a useful addition to the already available information.

Various workers have put forward different hypotheses for the albumin and gamma globulin differences found between Negroes and Caucasians. Edozien (1957), puts forward the view that the high globulin fraction of Nigerian subjects is due to a genetic factor. While Ezeilo (1971), studying Zambian and European neonates suggests an environmental factor. In this study an attempt to correlate a possible

increase in gamma globulin with the duration of stay in the Zambian environment, by European subjects has been carried out.

SUBJECTS AND METHODS

Serum from fifty male and fifty female healthy Zambian volunteers, comprising of medical students, student nurses and doctors were used in this study. Their mean weight was (± 1 SEM) 60.2 ± 0.8 kg, while their mean age was 27.1 ± 0.8 years. All Zambian subjects were living on a standard institutional diet comprising, approximately 2,500 calories per day.

The Europeans were a volunteer group, they comprised of 22 females and 12 males. Their mean weight (± 1 SEM) was 58.0 ± 2.6 kg, and their age 31.5 ± 20.0 years. All were healthy and were made up of doctors, nurses, hospital and University staff.

None of the subjects had suffered from malaria, parasitic or other infection for at least four years prior to the study. None of the Zambian subjects had taken anti-malarial drugs for prophylactic purposes for at least three years prior to the study; an occasional course could however have been taken for an acute attack of malaria.

Of the European subjects only 5% of those who had been resident in Lusaka for less than four years were taking anti-malarial drugs regularly. Of the Europeans who had been resident in Lusaka in excess of four years, only 10% had ever taken anti-malarials and then not recently.

Blood for the study was taken from the subjects over a period of three weeks. The serum was separated from the blood clot and divided into two aliquots, one for total protein estimation the other for serum protein electrophoresis. The tubes were stored in a deep freeze at -30°C until required.

Total serum protein was measured using a modified biuret method, Weischselbaum (1946). The coefficient of variation for the method using a standard serum sample (Wellcontrol No. 3, Wellcome Labs. Kent, England, U.K.) with a protein content of $6.2 \text{ g}/100 \text{ cm}^3$ was 1.25%. Protein fractionation was carried out on cellulose acetate media in a Millipore micro cell system, incorporating a barbitone buffer of pH 8.6. The stained and cleared strips were scanned and integrated in a Millipore Phoro-scope (Millipore Corporation Ltd., Boston, Massachusetts, U.S.A.). Statistical analysis was carried out using an Olivetti Programma 101, (Olivetti, Com-

pany Ltd., Italy). Differences between groups were determined by the Student 't' test program.

RESULTS

The mean and standard error of the mean for each protein fraction and total serum protein concentration in each group of subjects are given in Table 1.

Table 1

Serum proteins of Zambian and European subjects resident in Lusaka. Mean values \pm 1 standard error of the mean in grams/100 cm³.

SUBJECTS	No.	SEX	AGE YEARS	WEIGHT kg.	TOTAL PROTEIN	ALBUMIN	ALPHA ONE	ALPHA TWO	BETA GLOBULIN	GAMMA BLOBULIN
ZAMBIANS	50	M	28.5 \pm 1.4	60.0 \pm 0.9	7.2 \pm 0.07	3.6 \pm 0.5	0.13 \pm 0.07	0.77 \pm 0.03	0.90 \pm 0.04	1.9 \pm 0.05
ZAMBIANS	50	F	25.7 \pm 0.8	60.4 \pm 1.4	7.0 \pm 0.07	3.3 \pm 0.06	0.12 \pm 0.02	0.76 \pm 0.03	0.96 \pm 0.03	1.7 \pm 0.04
EUROPEANS	12	M	34.3 \pm 3.8	68.2 \pm 5.2	7.0 \pm 0.22	3.7 \pm 0.12	0.19 \pm 0.02	0.81 \pm 0.06	1.0 \pm 0.05	1.2 \pm 0.06
EUROPEANS	22	F	30.0 \pm 2.3	52.4 \pm 2.2	6.9 \pm 0.09	3.7 \pm 0.06	0.18 \pm 0.01	0.80 \pm 0.04	1.0 \pm 0.05	1.2 \pm 0.05
ZAMBIANS	100	M & F	27.1 \pm 0.8	60.2 \pm 0.8	7.0 \pm 0.30	3.4 \pm 0.04	0.12 \pm 0.01	0.71 \pm 0.02	0.73 \pm 0.02	1.8 \pm 0.03
EUROPEANS	34	M & F	37.0 \pm 2.0	58.0 \pm 2.6	7.1 \pm 0.20	3.7 \pm 0.06	0.18 \pm 0.01	0.81 \pm 0.03	0.95 \pm 0.03	1.3 \pm 0.04

A. Protein Concentrations In Zambians and Europeans.

Total Protein

The mean range and standard error of the mean for the total protein concentrations in the Zambian and European subjects was 7.0 \pm 0.3 (range: 5.9 – 8.2) and 7.1 \pm 0.2 (range: 6.2 – 8.5) g/100 cm³ respectively. No significant difference was seen between the mean of the two groups (P < 0.5).

Albumin

The mean, range and standard error of the mean for the serum albumin of the Zambian and European subjects was 3.4 \pm 0.04 (range: 2.1 – 4.3) and 3.7 \pm 0.06 (range: 2.9 – 4.4) g/100 cm³ respectively.

The serum albumin concentration was significantly higher in the European subjects than in the Zambian subjects, (t = 3.3, P < 0.001, n = 134, df = 132).

Alpha – one globulin.

The mean, range and standard error of the mean for the alpha one globulin in the Zambian and European subjects was 0.12 \pm 0.01 (range: 0.01 – 0.36) and 0.18 \pm 0.01 (range: 0.06 – 0.30) g/100 cm³ respectively. Serum alpha one globulin was significantly higher in the European subjects than in the Zambian subjects (t = 4.5, P > 0.001).

Alpha – two globulin.

The mean, range and standard error of the mean for the alpha two globulin in the Zambian and European subjects was 0.71 \pm 0.02, (range: 0.3 – 1.7) and 0.81 \pm 0.03, (range: 0.49 – 1.3) g/100cm³ respectively.

Serum alpha – two globulin was significantly higher in the European subjects, (t = 3.0, P < 0.001).

Beta globulin.

The mean, range and standard error for the beta globulin fraction in the Zambian and European subjects was 0.73 \pm 0.02 (range: 0.3 – 1.6) and 0.95 \pm 0.03 (range: 0.6 – 1.7) g/100 cm³. The beta globulin fraction was significantly higher in the European subjects (t = 4.6, P < 0.001).

Gamma globulin.

The mean, range and standard error of the mean for the gamma globulin fraction in the Zambian and European subjects was 1.8 \pm 0.03 (range: 0.9 – 2.7) and 1.3 \pm 0.4 (range: 0.7 – 1.9) g/100cm³ respectively. Serum gamma globulin in the Zambian subjects was significantly higher than that seen in the European subjects (t = 8.3, P > 0.001).

B. Sex Differences

Zambian males vs: Zambian females : albumin and gamma – globulin

Zambian males showed a higher mean albumin than the Zambian females 3.6 and 3.3 (range 2.8 – 4.3 and 2.1 – 4.0) g/100 cm³ respectively. The difference between the means of the two groups was significant, (t = 2.8, P < 0.001, n = 100, df = 98).

The Zambian males also showed a higher mean gamma globulin than the female Zambians, 1.9 and 1.7 (range: 1.2 – 3.0 and 0.9 – 2.7) g/100 cm³ respectively. The difference between the two means was significant, (t = 1.7, P < 0.05).

Zambian females vs: Zambian males: alpha and beta globulin.

Zambian female subjects showed a higher mean beta globulin than the male Zambian subjects, 0.96 and 0.90 (range: 0.4 – 1.2 and 0.3 – 1.6) g/100 cm³ respectively. The difference between the means was significant, (t = 1.7, P < 0.05, n = 100 df = 98). No significant differences were observed between the alpha – one and alpha – two fractions in the male and female Zambian subjects, (P < 0.6, P < 0.2), respectively.

European males vs: European females

There were no significant differences seen between the serum protein fractions of the male and

female Europeans, albumin, $P < 0.9$, alpha - one $P < 0.4$, alpha - two $P < 0.8$, beta globulin $P < 0.5$ and gamma globulin $P < 0.6$ ($n = 34$, $df = 32$).

Zambian males vs: European males

(a) Gamma-globulin

Zambian males had a higher mean gamma globulin than the male Europeans, 1.9 and 1.3 (range: 1.2 - 3.0 and 1.1 - 1.6) g/100 cm³ respectively. The difference between the means was significant ($t = 5.2$, $P > 0.001$, $n = 62$, $df = 60$).

(b) Alpha-one and Beta globulin

The European males showed a higher mean alpha-one globulin fraction than the Zambian males, 0.19 and 0.12 (range: 0.11 - 0.3 and 0.01 - 0.3) and 0.01 - 0.3) g/100 cm³. The difference between the two means was significant, ($t = 3.1$, $P < 0.001$, $n = 62$ $df = 60$). The European males also had a higher mean beta - globulin fraction than the Zambian males, 1.0 and 0.90 (range: 0.6 - 1.4 and 0.3 - 1.6) g/100 cm³ respectively. The difference between the means was significant ($t = 4.0$, $P > 0.001$).

Zambian females vs: European females

(a) Gamma-globulin

The mean gamma globulin in the Zambian females was higher than that seen in the European females, 1.7 and 1.2 (range: 1.2 - 3.0 and 0.7 - 1.9) g/100 cm³ respectively. The difference between the means was significant, ($t = 6.1$, $P > 0.001$, $n = 72$, $df = 70$).

(b) Albumin

European females showed a higher mean albumin than the female Zambians, 3.7 and 3.3, (range: 2.9 - 4.2 and 2.1 - 4.0) g/100 cm³ respectively. The difference between the means was significant, ($t = 3.8$, $P > 0.001$).

(c) Alpha-one and Alpha-two globulin

The alpha-one and alpha-two globulin fractions were also higher in the European females, 0.18 and 0.8 (range: 0.06 - 0.3 and 0.49 - 1.3) than the Zambian females 0.12 and 0.7 (range: 0.06 - 0.36 and 0.5 - 1.5) g/100 cm³ respectively. The difference between the means was significant, ($t = 3.2$, $P < 0.001$) and ($t = 3.8$, $P > 0.001$).

(d) Beta-globulin

European females showed a higher mean beta-globulin than the female Zambians, 1.0 and 0.96 (range: 0.4 - 1.2 and 0.6 - 1.4) g/100 cm³ respectively. The difference between the two means was significant, ($t = 2.5$, $P < 0.001$, $n = 72$, $df = 70$).

C. Protein changes due to duration of residence

Figure 1 and Table 2 summarizes the data for the European resident groups. When the European subjects were divided into two groups, those having been resident in Zambia for 1-4 years, mean 1.9 years and those having been resident for 6-46 years, mean 16.2 years. The group that had been resident longest had significantly higher alpha-two globulin ($t = 1.98$, $P < 0.05$, $n = 34$, $df = 32$), and gamma globulin ($t = 3.00$, $P < 0.001$) fractions.

No significant difference due to age, weight or tribe on the serum protein fractions were observed between any of the groups studied. All P values being greater than 0.9.

A comparison of serum protein concentrations of Zambian and European subjects with values obtained elsewhere is shown in Table 3.

DISCUSSION

The results obtained in this study agree with those published by previous workers in Zambia (Ezelio, 1970; Briggs et al; 1972). Similar figures were also reported from East and West Africa (Nantulya and Lindqvist, 1973; Edozien, 1957; Holmes et al., 1951). It is also interesting that studies in the American negro have produced similar figures (Pollak et al., 1961). As in this study, all of these workers used the electrophoretic technique.

The observed increase in the gamma globulin fraction has been largely accounted for by the presence of malaria antibodies (Voller et al., 1962; Voller et al., 1971). This increase has been observed to diminish with residence in a non-malarial environment (Schofield 1957). The increase reported in this study in Europeans after prolonged residence in Zambia without regular protection also suggests a malarial aetiology. The longer the Europeans remained in Zambia the higher their gamma globulin fraction rose.

Albumin changes have always been blamed on the African diet which is deficient in first class proteins (Sandor 1966). This explanation adequately accounts for the findings in this study too.

The finding of significantly higher levels of alpha one and alpha two globulin in European males and females as compared with Zambians of both sexes and the absence of sexual differences also suggests that this may be a dietetic difference as well.

It is surprising that there is no observed sexual difference with reference to beta globulin levels among Europeans. A rise would have been expected in females taking oral contraceptives (Wynn and Doar 1969) but it is possible of course that the female Europeans were not taking oral contraceptives. The sexual difference shown among the Zambian Africans is probably due to this cause and casual enquiries have confirmed this.

Table 2

Comparison of the two European resident groups showing significant differences. Mean values \pm 1 standard error of the mean in grams/100 cm³.

(a) RESIDENT GROUP 1 - 4 YEARS									
	NUMBER OF SUBJECTS	YEARS RESIDENT	AGE YEARS	WEIGHT kg.	ALBUMIN	ALPHA ONE	ALPHA TWO	BETA GLOBULIN	GAMMA GLOBULIN
MEAN	19	1.9	31.6	59.5	3.7	0.14	0.67	0.87	1.16
RANGE	-	1-4	23-48	40-81	3.2-4.3	0.1-0.2	0.4-1.1	0.5-1.7	1.0-1.9
SEM	-	\pm 0.2	\pm 1.7	\pm 2.6	\pm 0.08	\pm 0.01	\pm 0.03	\pm 0.06	\pm 0.05
(b) RESIDENT GROUP 6 - 46 YEARS									
MEAN	15	16.2	31.3	55.9	3.5	0.15	0.80	0.89	1.40
RANGE	-	6-46	8-54	22-96	2.9-4.3	0.1-0.3	0.5-1.2	0.5-1.3	1.2-1.9
SEM	-	\pm 2.7	\pm 4.0	\pm 5.0	\pm 0.1	\pm 0.01	\pm 0.05	\pm 0.04	\pm 0.05
't'	-	-	-	-	1.23	0.58	1.98	0.17	3.00
P	-	-	-	-	<0.2	<0.9	<0.05	<0.8	<0.001

Figure 1

Distribution of serum proteins in the two European resident groups

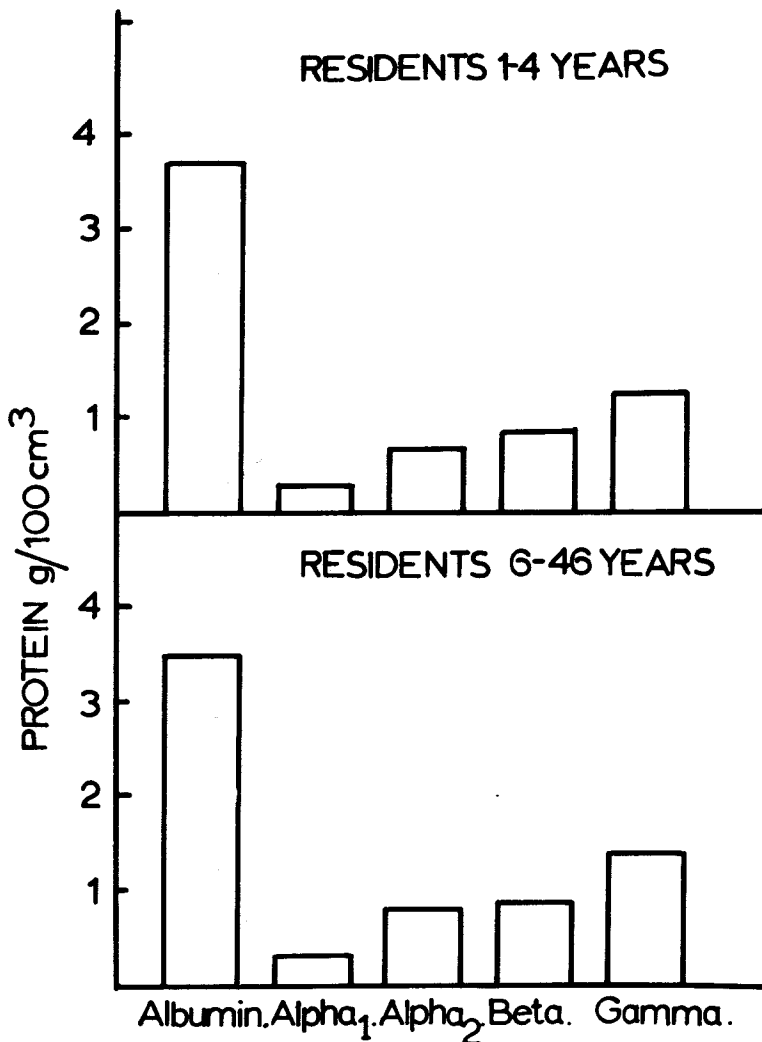


Table 3

Electrophoretic fractionation of serum proteins of Zambian and European subjects resident in Lusaka compared to some values obtained elsewhere. Mean values \pm 1 standard error of the mean in grams/100 cm³.

COUNTRY	SIZE OF SAMPLE	SEX	TOTAL PROTEIN	ALBUMIN	ALPHA ONE	ALPHA TWO	BETA GLOBULIN	GAMMA GLOBULIN	ELECTROPHORETIC METHOD
ZAMBIA (BRIGGS 1972)	104	M+F	7.1	3.5	0.2	0.7	0.9	1.8	MICROZONE
ZAMBIA (EZEILO 1970)	77	M+F	7.1	3.8	0.2	0.7	0.8	1.6	MICROZONE
ZAMBIA (THIS STUDY)	100	M+F	7.1	3.4	0.12	0.71	0.73	1.8	MICROZONE
TANZANIA (NANTULYA 1973)	387	M+F	7.1	4.0	0.2	0.5	0.7	1.6	MICROZONE
UGANDA (LEONARD 1965)	99	M+F	6.6	3.1	0.2	0.5	0.9	1.8	PAPER
CONGO (OYE 1951)	100	M+F	6.5	2.6	0.9		1.1	1.9	PAPER
BRITAIN Caucasians (SMITH 1960)	56	M+F	6.7	4.3	0.2	0.6	0.7	0.9	PAPER
EUROPE Caucasians (SANDOK 1966)	679	M+F	7.4	4.0	0.4	0.6	0.8	1.6	PAPER
ZAMBIA Caucasians (THIS STUDY)	34	M+F	6.9	3.7	0.18	0.81	0.95	1.3	MICROZONE

Apart from albumin and gamma globulin levels there seems to be no other important racial differences revealed in this study.

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