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Nutritional Anemia Research

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A report on a field project conducted in Guyana

from

June 23, 1980 to August 6, 1980

by

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A study was conducted to further investigate the roles of iron deficiency, folic acid deficiency, and intestinal parasitic infestation in contributing to anemia in Guyana. The sample consisted of 257 anemic and nearly anemic subjects of all age groups and of both sexes, drawn from households in both urban and rural areas. Anthropometric and hematological measurements, and stool investigations for intestinal parasites were carried out on the subjects. Socioeconomic data, data on the frequency of consumption of foods rich in the erythropoietic nutrients, as well as information on iron and vitamin supplementation were also obtained. A limited amount of obstetrical information was obtained from women of childbearing age. Iron and folic acid deficiencies were found to be the major nutrient deficiencies contributing to nutritional anemia in Guyana. Intestinal parasitic infestation was not found to play a major role in the etiology of this anemia. An applied intervention program was recommended involving a combination of nutrition education, distribution of iron and folic acid supplements to pregnant and lactating women, and fortification of a suitable vehicle with iron and folic acid. A 17-item bibliography is included.

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Abstract

A study was conducted to further investigate the roles of iron deficiency, folic acid deficiency, and intestinal parasitic infestation in contributing to anemia in Guyana. The sample consisted of 257 anemic and nearly anemic subjects of all age groups and of both sexes, drawn from households in both urban and rural areas. Anthropometric and hematological measurements, and stool investigations for intestinal parasites were carried out on the subjects. Socio-economic data, data on the frequency of consumption of foods rich in the erythropoietic nutrients, as well as information on iron and vitamin supplementation were also obtained. A limited amount of obstetrical information was obtained from women of childbearing age. Iron and folic acid deficiencies were found to be the major nutrient deficiencies contributing to nutritional anemia in Guyana. Intestinal parasitic infestation was not found to play a major role in the etiology of this anemia. An applied intervention program was recommended involving a combination of nutrition education, distribution of iron and folic acid supplements to pregnant and lactating women, and fortification of a suitable vehicle with iron and folic acid.

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Dr. Daphne Roe for performing the folate analyses.

Dr. James Cook for performing the serum ferritin analyses.

The 257 subjects who willingly consented to participate in the study, many of them under the impression that themselves and other Guyanese would derive some benefit from the results of the study. Should this not be realised then all of the time and effort expended in the performance of this study would have been wasted.

Introduction

Nutritional anemias continue to be a public health problem of considerable importance in Guyana. This has been emphasized in two previous studies conducted in Guyana. The first study was conducted as part of the 1971 National Food and Nutrition Survey (1). In this study anemia, based on WHO standards (2), was found to be a problem in all age groups. The prevalence of anemia ranged from 35% in males over 14 years of age to 55% in pregnant females. Forty-one percent of the subjects in each of the other groups (children under 14 years of age, females over 14 years of age, and lactating females) were anemic. Suggestive evidence was obtained that deficiencies of iron and of folic acid were contributors to the anemia. Between 13 and 34% of the subjects in the various age groups had mean corpuscular hemoglobin concentration levels below 31%. Also, household dietary intake of iron was significantly related to anemia of household members. Well over half the sample (58.9%) had serum folate values below the normal range (6 - 20 ng/ml.).

The second study (3) was conducted in 1976. In this study high prevalences of anemia, though of a relatively mild degree, were found among all age groups. WHO standards (2) were also used for the definition of anemia in this study. The highest prevalence of anemia was found among pregnant females, of whom 73.7% were anemic, while the lowest prevalence was found among males over 14 years of age, 32.1% of whom were anemic. In the other age groups the prevalence of anemia varied from 44.4% in pre-school children; 57% in schoolage children; 54.8% in nonpregnant, nonlactating females of childbearing age; 52% in females over 45 years of age; to

65% in lactating females. Clear indications were obtained that iron deficiency was contributing to the anemia. The prevalence of iron deficiency, as indicated by low serum iron and/or percent transferrin saturation values, was relatively high among all age groups except males over 14 years of age. Also, significant correlation coefficients were obtained between the measurements of iron status and the hematological characteristics. Evidence for folic acid or vitamin B_{12} deficiencies as contributors to the anemia was inconclusive. However, high prevalences of low serum vitamin B_{12} values and extremely high prevalences of low serum folate values were found among all age groups.

The study which is being reported on here was aimed at the further characterisation of the nutritional anemias in Guyana. Specific emphasis was placed on the roles of iron and folic acid deficiencies, and of intestinal parasitic infestation in the pathogenesis of the anemia. Recommendations were also made for appropriate applied intervention programs which could be used for the control of the anemia.

Objectives

1. To further investigate the roles of iron and folic acid deficiencies, and of intestinal parasitic infestation in contributing to nutritional anemias in Guyana.

2. To suggest appropriate applied intervention programs to control nutritional anemias in Guyana.

Methodology

The sample was made up of 257 anemic or nearly anemic persons. It was comprised of the following seven groups: preschool children (aged 1 - 5 years); schoolage children (aged 6 - 14 years); males over 14 years of age; pregnant females; lactating females; nonpregnant, nonlactating females of childbearing age (aged 15 - 44 years); and females past childbearing age.

The subjects were drawn from households in the following seven areas: Charlestown, Georgetown; Campbellville, Greater Gerogetown; Wismar, Upper Demerara River; Leonora, West Coast Demerara; Aurora and Supenaam, Essequibo coast; Mahaica, East Coast Demerara; and #63 and #64 villages, Corentyne.

A Spencer hemogolobinmeter was used to determine the approximate level of hemoglobin in a "fingerprick" sample of blood obtained from each household member one year of age or older. Each household member found to have a hemoglobin level below 10 - 10.5 g./100 ml. was included in the study. Between 11 and 11.5 ml. of blood were obtained from each subject by venipuncture Eight milliliters of this blood were placed in a heparinised tube. One milliliter of the heparinised blood was later used for the determination of red blood cell count ($10^6/mm^3$), hematocrit (%), and hemoglobin (g./100 ml.). Mean corpuscular volume (MCV) (μ^3), mean corpuscular hemoglobin (MCH) ($\mu\mu$g.), and mean corpuscular hemoglobin concentration (MCHC) (%) were calculated from these three measurements by computer at Howard University using the following formulae:

$$\text{MCV} = \frac{\text{hematocrit} \times 10}{\text{red blood cell count}}$$

$$\text{MCH} = \frac{\text{hemoglobin} \times 10}{\text{red blood cell count}}$$

$$\text{MCHC} = \frac{\text{hemoglobin} \times 10}{\text{hematocrit}}$$

The remaining 7 ml. of heparinised blood were processed for later determination of plasma and red cell folates as described in Appendix 1. The plasma and red cell folate determinations were conducted by Dr. Daphne A. Roe, Division of Nutritional Sciences, Cornell University, Ithaca, New York, U.S.A.

One and a half milliliters of blood were placed in a plastic tube. This blood was centrifuged, and the serum decanted off and stored frozen to be used for the determination of serum ferritin. The serum ferritin determinations were conducted by Dr. James D. Cook, University of Kansas Medical Center, Kansas City, Kansas, U.S.A.

The remaining 1.5 - 2 ml. of blood were placed in a plastic tube. This blood was centrifuged, and the serum decanted off and stored frozen to be used for any further investigations considered necessary.

A stool sample was obtained from each subject and refrigerated until analysed for the presence of occult blood and ova from intestinal parasites (hookworm, roundworm, and whipworm). Ova counts were performed on the

stool samples found to contain ova from these parasites.

Height (cm.), weight (kg.), and percent of standard weight/height were determined as described by Jelliffee (4).

Precoded questionnaires (see Appendix 2) were used to obtain the following information on each subject: age, sex, ethnic group, occupation, educational level, household size, attendance of a health or hospital clinic within the past two months, and the consumption of iron and/or vitamin supplements. Dietary intake was obtained by asking the subject to recall from a list of foods (consisting of meat and dairy products, peas and beans, vegetables, and baked products) the frequency with which these foods were consumed over the past week (Sunday to Saturday).

For women of childbearing age information was obtained on the number of pregnancies and the number of live births within the past five years, and the use of oral contraceptives or intra-uterine devices. The number of months pregnant and the number of months of breastfeeding were obtained for pregnant and lactating women respectively.

The time of day and the environmental temperature at blood collecting were also recorded.

The data were analysed by computer at Howard University using the Statistical Package for the Social Sciences (SPSS) (5). Statistical procedures used included analysis of variance followed by the Scheffe Multiple Range Test, t-tests, Pearson correlations, partial correlations, chi-square associations (6), Spearman correlations, and Wilcoxon Rank Sum tests (7).

Results and Discussion

A total of 1,349 persons was screened with the Spencer hemoglobinometer. Of the persons found by the Spencer hemoglobinometer to have hemoglobin levels below 10 - 10.5 g./100 ml., 257 persons consented to participate in the study and yielded sufficient blood to be included in the study.

Considerable difficulty was encountered in the use of the Spencer hemoglobinometer because of the inaccurate hemoglobin values given by the instrument. The hemoglobin values given by the instrument tended to be lower than those obtained by the cyanmethemoglobin method. Because of this a number of subjects were obtained who were later found to have hemoglobin levels above the WHO standards for anemia (2) (see table 8).. For this reason the author does not recommend that the Spencer hemoglobinometer be used for screening hemoglobin levels. It is also the author's feeling that many of the persons found by the Spencer hemoglobinometer to have hemoglobin levels above 10.5 g./100 ml. and therefore not included in the study may in fact have had hemoglobin levels below the WHO standards (2).

Sample characteristics

The 257 subjects were subdivided into seven groups as shown in table 1.

Table 1. Composition of groups.

Group	Age	Sex	No.	%
Preschool children	1 - 5 years	Males and females	16	6.2
Schoolage children	6 - 14 "	Males and females	63	24.5
Adult males	Over 14 "	Males	16	6.2
Pregnant females	Over 14 "	Females	56	21.8
Lactating females	Over 14 "	Females	21	8.2
Women of childbearing age, Not pregnant, Not lactating	15 - 44 "	Females	64	24.9
Women past childearing age	Over 44 "	Females	21	8.2
Total			257	100.00

The aim at the beginning of the project was to have 42 subjects in each of the seven groups. However, as can be seen by examination of table 1 a number of the groups fell short of this goal. The number of preschool children fell short of the goal for a number of reasons. First of all, it was difficult to obtain sufficient blood from many preschool children because of collapse of the veins, and because it was difficult to keep many of the children sufficiently calm and quiet to draw the blood. Also, many of the children experienced considerable trauma from the fingerprick required for screening so that many parents would not allow

the venipuncture.

In the case of adult males, considerable difficulty was experienced in locating adult males with hemoglobin values below 10.0 - 10.5 g./100 ml. as shown by the Spencer hemoglobinmeter. As mentioned before, this may be due to the inaccuracy of the instrument. It is quite possible that many of the males screened and not included in the sample may have had hemoglobin levels below 13 g./100 ml.

Considerable difficulty was experienced in locating pregnant females, lactating females, and women past childbearing age during the house-to-house visits. In the group of pregnant females, this problem was solved by obtaining names and addresses of pregnant females from health centers and by attending prenatal clinics at the health centers. Names and addresses of lactating females were also obtained from the health centers. However, it was still not possible to reach the desired number by this means. There was no easy or convenient mechanism by which the number of women past childbearing age could be increased.

The sample was made up predominantly of females. Of the 257 subjects, 202 (78.6%) were females while 55 (21.4%) were males. Of the preschool children, 10 (62.5%) were female and 6 (37.5%) were male. In the group of schoolage children, 29 (46.0%) were female while 34 (54.0%) were male.

Table 2 shows the racial composition of the sample.

Table 2. Racial composition of sample.

Race	Number	Percent
Black	125	48.6
East Indian	98	38.1
Mixed	28	10.9
Amerindian	5	1.9
Portugese	1	0.4
Total	257	100.0

The sample was made up mainly of Blacks and East Indians, the two main racial groups in Guyana. The reason for the larger number of Blacks than East Indians, contrary to the fact that there are more East Indians than Blacks in the Guyanese population, was that the Black households visited were somewhat larger than East Indian households

Table 3 shows the occupations held by the subjects.

Table 3. Occupations held by subjects

Occupation	Number	Percent
Homemaker	123	47.9
Student	74	28.8
Skilled Labour	14	5.4
Huckster	8	3.1
Unskilled labour	7	2.7
Teacher	6	2.3
Secretary/clerk	2	0.8
Farmer	2	0.8
Policewoman	1	0.4
Retired	1	0.4
Unemployed	8	3.1
None	11	4.3
Total	257	100.0

The majority of the subjects were home-makers and students. Of the students 59 were primary school students, 9 were secondary school students, 5 were nursery school students and one was attending technical school.

Skilled labourers included factory workers, switchboard operators, mechanics, carpenters, and nurse-aides. Unskilled labourers included janitors and charwomen, domestics, and ditch-diggers. The eleven subjects

who had no occupations were all preschool children.

Table 4 shows the levels of education attained by the subjects.

Table 4. Educational levels attained by subjects

Educational level	Number	Percent
Primary	181	70.4
Secondary	52	20.2
Technical/vocational	9	3.5
No schooling	15	5.8
Total	257	100.0

The majority of the subjects had attained primary and secondary levels of education. Of those who had attained a primary level of education, 5 were preschool children, 58 were schoolage children and 181 were adults. Five of those who had attained a secondary level of education were schoolage children and 47 were adults. Of the 15 subjects who had not had any schooling, 11 were preschool children while 4 were adults.

Mean household size was 8.3 persons. Significant correlations were obtained between household size and both hemoglobin (0.16) and hematocrit (0.14) ($p < 0.05$).

Attendance of health and hospital clinics

Of the 257 subjects, 91 (35.4%) had attended a health or hospital clinic within the past two months. Of these 91 subjects, 4 were schoolage children, 3 were adult males, 53 were pregnant females, 15 were lactating females, 9 were women of childbearing age, and 7 were women past childbearing age.

Use of iron and vitamin supplements

Sixty-seven subjects were taking iron or vitamin supplements. Of these 67 subjects, the majority (33) were pregnant females. Eleven were women of childbearing age, 8 were schoolage children, 5 were women past childbearing age, 4 were adult males, 4 were lactating females, and 2 were preschool children.

Iron supplements were being taken by 47 subjects - 1 preschool child, 2 schoolage children, 2 adult males, 28 pregnant females, 4 lactating females, and 10 women of childbearing age. Only 2 subjects, both of them pregnant females, were taking folate supplements. Two subjects, also, were taking vitamin B_{12} supplements, one being a preschool child and the other an adult male. Vitamin C supplements were being taken by 2 subjects, one a woman of childbearing age, and the other a woman past childbearing age. In many cases it was not possible to ascertain what supplements were being taken.

The results do indicate, however, that more women of childbearing age, especially pregnant females, need to be taking iron supplements, and more pregnant women need to take folate supplements as well.

No significant difference in any of the hematological characteristics was found between those taking supplements of iron, folic aicd, vitamin B_{12} or vitamin C and those not taking any of these supplements. These results indicate that in those taking supplements, the supplements had no beneficial effect. This finding suggests that those taking supplements did not need these supplements, or that they were not taking enough of the supplements for them to have any beneficial effect, or that the supplements were not being well absorbed.

Obstetrical data

Women of childbearing age (including the pregnant and lactating women as well as the women who were neither pregnant nor lactating) had been pregnant an average number of 2.3 times within the past 5 years, and had had an average number of 1.9 live births within the past 5 years. Only 5 of these women were on oral contraceptives and 4 were using intra-uterine devices (coils or loops). Blood losses, and therefore losses of iron, tend to be higher in women using intra-uterine devices than in those using oral contraceptives (8). Iron nutritional status would therefore be expected to be lower in women using intra-uterine devices than in those using oral contraceptives. In the present study, however, no significant difference in serum ferritin or in any of the other hematological characteristics was found between those using intra-uterine devices and those using oral contraceptives.

Dietary intake

Table 5 shows the average weekly frequency of consumption of various food items.

Table 5. Average weekly frequency of consumption of food items

Food Item	No. of persons consuming food item	% of subjects consuming food item	Weekly frequency of consumption of food item Mean ± 1 S.D.
Beef	96	37.4	2.1 ± 1.5
Wild cow	1	0.4	1.0
Pork	49	19.1	2.1 ± 1.5
Mutton	3	1.2	1.3 ± 0.6
Chicken	219	85.2	3.3 ± 2.0
Duck	3	1.2	1.7 ± 1.2
Fish	222	86.4	3.5 ± 2.2
Shrimps	91	35.4	2.6 ± 2.2
Crab	1	0.4	2.0
Liver	2	0.8	5.5 ± 2.1
Heart	2	0.8	1.5 ± 0.7
Sausage	2	0.8	2.0 ± 0.0
Eggs	192	74.7	3.3 ± 2.3
Milk	226	87.9	6.4 ± 1.6
Cheese	97	37.7	2.9 ± 1.9
Pigeon peas	21	8.2	1.7 + 0.5

Table 5. cont'd.

Food Items	No. of persons consuming food item	% of subjects consuming food item	Weekly frequency of consumption of food item Mean ± 1 S.D.
Split peas	201	78.2	2.1 ± 1.4
Blackeye peas	44	17.1	1.6 ± 1.3
Kidney beans	4	1.6	1.0 ± 0.0
Chick peas	1	0.4	1.0
Unknown type of peas	5	1.9	2.4 ± 0.9
Bora	197	76.7	1.6 ± 0.9
Seime	21	8.2	1.0 + 0.2
Callaloo/			
Bhagee/Pakchoy	172	66.9	1.7 ± 1.2
Mustard Greens	12	4.7	1.4 ± 0.9
Ochro	138	53.7	1.6 ± 1.0
Lettuce	22	8.6	2.3 ± 2.0
Cabbage	53	20.6	1.3 ± 0.5
Cucumber	72	28.0	2.4 ± 2.1
Boulanger	174	67.7	1.7 ± 1.1
Squash	104	40.5	1.3 ± 0.8
Carilla	1	0.4	1.0
Bread	235	91.4	5.1 ± 2.4
Roti	151	58.8	4.6 ± 2.5
Biscuit	89	34.6	2.5 ± 1.9

The food consumption data show that chicken, fish, eggs, and milk were the major animal foods consumed by the subjects. Beef was not widely consumed probably because of its expense. Pork was not widely consumed both because of its expense and because of religious restrictions against the consumption of pork. Split peas were consumed by most subjects. Relatively few subjects consumed any other type of peas or beans. Green vegetables such as bora, callaloo, bhagee or packchoy, ochro and boulanger were all widely consumed. Bread was consumed by most subjects. Roti was widely consumed particularly by East Indians. Although many of the foods appeared to be consumed quite frequently, the large standard deviations indicate wide variations in the frequency of consumption of these foods. It should be emphasized that these data represent only frequency of consumption of these food items. The data do not indicate how much of these foods were actually consumed. Therefore, no conclusions can or should be made about the adequacy of consumption of these foods or about the adequacy of nutrient intake.

The seven groups were compared with regard to the daily frequency of consumption of animal foods, peas and beans, vegetables, and baked products. Women of childbearing age (neither pregnant nor lactating) consumed animal foods significantly more frequently than women past childbearing age. The significance of this observation is difficult to explain. No significant differences were obtained among the groups with regard to the frequency of consumption of any of the other food groups.

Hematological characteristics

Table 6 shows the hematological characteristics of the subjects. As was expected, the mean hematological values were low in each of the groups. Pregnant females had significantly lower mean hemoglobin and hematocrit values than schoolage children and adult males. Adult males has a significantly higher mean hematocrit value than preschool children. No significant differences in hemoglobin and hematocrit values were found among the other groups.

Pregnant females also has significantly lower mean MCV and MCH values than schoolage children. No significant differences in MCV and MCH values were found among the other groups. No significant differences in red blood cell count and MCHC values were found among the groups.

Table 7 shows the mean serum folate, plasma, and red cell folate values. These values were low in each of the groups. Both pregnant females and women of childbearing age (neither pregnant nor lactating) had significantly lower mean serum ferritin values than adult males and women past childbearing age.

Schoolage children had a significantly lower mean serum ferritin value than adult males. No significant differences in mean serum ferritin values were found among the other groups.

There were no significant differences in plasma and red cell folate values among the groups.

The large standard deviations indicate considerable variation in each of the measurements within the groups.

Table 6. Hematological characteristics

	Red blood cell count ($10^6/mm^3$) mean ± 1 S.D.	Hematocrit (%) mean ± 1 S.D.	Hemoglobin (g./100 ml.) mean ± 1 S.D.	MCV (u^3) mean ± 1 S.D.	MCH (uug) mean ± 1 S.D.	MCHC (%) mean ± 1 S.D.
Preschool children n = 16	4.9 ± 0.9[a]	33.4 ± 1.8[a,b]	11.0 ± 1.1[a,b]	70.9 ± 12.5[a,b]	23.6 ± 5.7[a,b]	33.0 ± 2.8[a]
Schoolage children n = 63	4.6 ± 0.5[a]	35.8 ± 2.6[a,c]	11.6 ± 1.1[a]	79.2 ± 8.2[b]	25.8 + 3.5[b]	32.5 ± 2.6[a]
Adult males n = 16	4.8 ± 0.8[a]	38.2 ± 6.3[c]	12.2 ± 2.9[a]	79.4 ± 11.4[a,b]	25.2 ± 5.1[a,b]	31.7 ± 3.9[a]
Pregnant females n = 56	4.7 ± 0.6[a]	33.1 ± 3.9[b]	10.6 ± 1.5[b]	71.3 ± 9.3[a]	23.0 ± 3.8[a]	32.1 ± 2.3[a]
Lactating females n = 21	4.7 ± 0.5[a]	35.2 ± 4.0[a,b,c]	11.2 ± 1.5[a,b]	75.7 ± 9.7[a,b]	24.4 ± 3.9[a,b]	31.8 ± 2.5[a]
Women of childbearing age - Not pregnant, Not lactating n = 64	4.8 ± 0.7[a]	35.0 ± 3.1[a,b,c]	11.1 ± 1.4[a,b,]	74.6 ± 12.2[a,b]	23.7 ± 4.6[a,b]	31.7 ± 2.7[a]
Women past child-bearing age n = 21	4.9 ± 0.5[a]	35.4 ± 2.8[a,b,c]	11.5 ± 1.2[a,b]	73.6 ± 8.1[a,b]	23.8 ± 3.6[a,b]	32.3 ± 2.6[a]
Total N = 257	4.7 ± 0.6	35.0 ± 3.7	11.2 ± 1.5	75.1 ± 10.5	24.2 ± 4.2	32.1 ± 2.7

[a,b,c] Means in the same column with at least one common letter are not significantly different ($p > 0.05$).

Table 7. Serum ferritin plasma and red cell folate values (number of subjects in parentheses)

	Serum ferritin (ng/ml.) mean ± 1 S.D.	Plasma folate (ng/ml.) mean ± 1 S.D.	Red cell folate (ng/ml.) mean ± 1 S.D.
Preschool children	25.9 ± 32.7[a,b,c] (16)	9.0 ± 7.5[a] (10)	72.1 ± 50.0[a] (10)
Schoolage children	23.9 ± 21.4[c,d] (62)	10.3 ± 7.1[a] (43)	104.4 ± 44.9[a] (41)
Adult males	67.8 ± 120.7[b] (16)	10.8 ± 8.4[a] (12)	105.3 ± 55.2[a] (10)
Pregnant females	11.4 ± 14.4[a] (56)	7.7 ± 5.0[a] (38)	108.3 ± 57.2[a] (35)
Lactating females	24.0 ± 19.7[a,b,c] (21)	9.2 ± 5.8[a] (15)	93.2 ± 52.0[a] (12)
Women of childbearing age - Not pregnant, Not lactating	14.1 ± 13.8[c] (64)	7.1 ± 4.5[a] (45)	106.9 ± 60.6[a] (38)
Women past childbearing age	58.6 ± 60.9[b,d] (21)	7.0 ± 3.9[a] (14)	104.3 ± 37.0[a] (13)
Total	24.4 ± 41.7 (253)	8.5 ± 6.0 (177)	103.0 ± 52.6 (159)

[a,b,c,d] Means in the same column with at least one common letter are not are not significantly different ($p > 0.05$)

Anemia, iron and folic acid deficiencies

Table 8 shows the prevalences of anemia and of iron and folic acid deficiencies among the groups.

The prevalence of anemia varied between 43.8 and 81.0%. These results demonstrate very well the unreliability of hemoglobin values given by the Spencer hemoglobinometer. All of the subjects included into the study had hemoglobin values below 10.0 - 10.5 g./100 ml. as given by the Spencer hemoglobinometer. Yet when their hemoglobin values were determined by the cyanmethemoglobin method, many of the subjects were found to have appreciably larger hemoglobin values.

Table 8 also shows that an appreciable number of subjects in each group were deficient in iron and in folic acid. These results indicate that deficiencies of these two nutrients do contribute to anemia in Guyana.

Relatively few subjects in each group had low plasma folate values, much fewer than those with low red cell folate values.

The low prevalence of low serum folate values indicates that most of the subjects had consumed folate-conatining foods fairly recently. These results indicate, therefore, that while most of the subjects had consumed folate-conatining foods recently, many were still deficient in folic acid (as indicated by the high prevalence of low red cell folate values).

Table 8. Prevalences of anemia, iron, and folic acid deficiencies, and low serum folate values. (number of subjects in parentheses)

	% anemic	% iron deficient[d]	% folic acid deficient[e]	% with low plasma folate values[f]
Preschool children	43.8[a] (16)	31.3 (16)	80.0 (10)	15.4 (13)
Schoolage children	55.6[b] (63)	22.0 (59)	53.7 (41)	3.2 (62)
Adult males	56.3[c] (16)	50.0 (16)	60.0 (10)	6.3 (16)
Pregnant females	55.4[a] (56)	73.2 (56)	57.1 (35)	7.1 (56)
Lactating females	81.0[b] (21)	33.3 (21)	58.3 (12)	4.8 (21)
Women of childbearing age - Not pregnant, not lactating	78.1[b] (64)	57.8 (64)	52.6 (38)	14.5 (62)
Women past childbearing age	66.7[b] (21)	19.0 (21)	61.5 (13)	4.8 (21)
Total	63.4 (257)	45.5 (253)	57.2 (159)	8.0 (251)

[a] Hemoglobin < 11.0 g./100 ml.

[b] Hemoglobin < 12.0 g./100 ml.

[c] Hemoglobin < 13.0 g./100 ml.

[d] Serum ferritin < 12.0 ng./ml.

[e] Red cell folate < 100.0 ng./ml.

[f] Plasma folate < 3.0 ng./ml.

Morphological classification of the anemias

Table 9 shows the morphological classification of the anemias. Of the anemic subjects 46.2% had a hypochromic, microcytic anemia suggestive of iron deficiency while the remainder, 53.8%, had anemias suggestive of combined deficiencies of iron, folic acid and/or vitamin B_{12} (normochromic, microcytic; normochromic, normocytic; hypochromic, normocytic; and hypochromic, macrocytic). A similar pattern was observed in each of the seven groups. In the 1976 survey (3), similar results were obtained.

Intestinal parasitic infestation

Stool samples were obtained from only 138 of the 257 subjects. As in the 1976 survey, complete collection of stool samples has proven to be a problem. Table 10 shows the percentages of the various groups infested with parasites and the mean ova counts of hookworm, roundworm, and whipworm among the subjects infested with these parasites. Relatively few subjects were infested with hookworm, roundworm, or whipworm, suggesting that intestinal parasitic infestation is not a major contributor to anemia. However, the subjects infested with these parasites had relatively heavy ova counts.

None of the stool samples tested for the presence of occult blood was found to contain any sign of occult blood. This finding was surprising in view of the fact that both hookworms and whipworms cause intestinal bleeding.

Table 9. Morphological classification of the anemias

	% hypochromic microcytic[a]	% normochromic microcytic[b]	% normochromic normocytic[c]	% hypochromic normocytic[d]	% hypochromic macrocytic[e]
Preschool children n = 7	57.1	42.9	0.0	0.0	0.0
Schoolage children n = 35	31.4	34.3	20.0	11.4	2.9
Adult males n = 9	55.6	11.1	11.1	22.2	0.0
Pregnant females n = 31	46.4	42.9	7.1	3.6	0.0
Lactating females n = 17	46.7	40.0	13.3	0.0	0.0
Women of childbearing age - Not pregnant, not lactating n = 50	48.0	32.0	6.0	14.0	0.0
Women past childbearing age n = 14	64.3	35.7	0.0	0.0	0.0
Total n = 163	46.2	34.8	9.5	8.9	0.6

[a] MCHC $<$ 32% and MCV $<$ 82 μ^3

[b] 31% $<$ MCHC $<$ 37% and MCV $<$ 82 μ^3

[c] 31% $<$ MCHC $<$ 37% and 81 μ^3 $<$ MCV $<$ 98 μ^3

[d] MCHC $<$ 32% and 81 μ^3 $<$ MCV $<$ 98 μ^3

[e] MCHC $<$ 32% and MCV $>$ 97 μ^3

Table 10. Prevalence of intestinal parasitic infestation and mean ova counts in subjects infested with parasites

(number of subjects in parentheses)

	% with parasites	Hookworm (no. of ova/g. feces) mean ± 1 S.D.	Roundworm (no. of ova/g. feces) mean ± 1 S.D.	Whipworm (no. of ova/g. feces) mean ± 1 S.D.
Preschool children	9.1 (11)	0	800 (1)	0
Schoolage children	13.3 (30)	1200 (1)	6000.0 ± 1587.5 (3)	2500.0 ± 989.9 (2)
Adult males	22.2 (9)	8850 (1)	2600 (1)	0
Pregnant females	19.2 (26)	5100.0 ± 3598.1 (4)	4400.0 ± 3959.8 (2)	2300.0 ± 2404.2 (2)
Lactating females	27.3 (11)	1733.3 ± 2227.9 (3)	1700.0 ± 989.9 (2)	8000 (1)
Women of childbearing age - Not pregnant, not lactating	10.8 (37)	7200.0 ± 565.7 (2)	5700.0 ± 2121.3 (2)	0
Women past childbearing age	35.7 (14)	6275.0 ± 1320.0 (4)	2233.3 ± 425.6 (3)	0
Total	17.4 (138)	5010.00 ± 3068.4 (15)	3692.9 ± 2421.2 (14)	3520.0 ± 2823.5 (5)

Etiology of the anemias

The primary objective of this study was to further characterise the nutritional anemias with respect to the roles of iron deficiency, folic acid deficiency, and intestinal parasitic infestation with hookworm, roundworm and whipworm. Therefore, the role of each of these factors in contributing to the anemias is discussed in depth.

Role of iron deficiency

In the 1971 National Food and Nutrition Survey (1) and in the 1976 survey (3) evidence was obtained that iron deficiency was contributing to anemia. In the present study, a high proportion of the subjects in each of the groups was deficient in iron (see table 8). Significant correlations were obtained between serum ferritin and both hemoglobin (0.14), and hematocrit (0.16) ($p < 0.05$). A significant chi-square association was obtained between anemia and iron status in the entire sample and in pregnant females ($p < 0.05$). Anemic pregnant females had a significantly lower mean serum ferritin than non-anemic pregnant females ($p < 0.05$).

These results further confirm the findings in the two previous surveys (1, 3) that iron deficiency is a major factor contributing to anemia in Guyana.

A significant negative correlation was obtained between the number of pregnancies in the past five years and serum ferritin (-0.21) ($p < 0.05$) indicating that repeated pregnancies was one factor contributing to iron deficiency in women of childbearing age. A similar finding was obtained in

the 1976 survey (3).

A rather interesting finding was the significant correlation obtained between percent weight/height and serum ferritin (0.20) ($p < 0.05$).

Role of folic acid deficiency

In both of the two previous surveys (1, 3), high prevalences of low serum folate values were found. In the present study, however, relatively few subjects had low plasma folate values (see table 8). No significant correlation was obtained between plasma folate and hemoglobin, and no significant chi-square association was obtained between anemia and plasma folate status. Anemic schoolage children, however, had a significantly lower mean plasma folate than non-anemic schoolage children ($p < 0.05$).

Plasma folate is not a very good index of folic acid status. It is low both in folic acid deficiency and when folate containing foods are not consumed for a long time. Therefore no strong conclusions can be made about folic acid status from plasma folate values. Certainly in this study, no conclusions can be made about the role of folic acid deficiency in contributing to anemia from the plasma folate results.

A better index of folic acid status is red cell folate. In the present study high proportions of subjects in each of the groups had low red cell folate values and could therefore be regarded as deficient in folic acid (see table 8). Significant negative correlations were obtained between red cell folate and both hemoglobin (-0.18), and hematocrit (-0.16) ($p < 0.05$). Significant negative correlations were also obtained between red cell folate and each of the following - MCV (-0.27), MCH (-0.27), and MCHC (-0.14) ($p < 0.05$). These negative correlations were unexpected to say the least.

One explanation for these findings is that in those subjects who were deficient in iron and therefore had decreased production of hemoglobin full use could not be made of the folic acid stores. Of the 74 subjects who were deficient in iron and for whom red cell folate was obtained, 35 (43.7%) had red cell folate values above 100 ng./ml. Another explanation is that the folic acid deficiency was of such short duration that the hemoglobin values had not yet been affected. Of the subjects who were not anemic 45% were deficient in folic acid.

No significant chi-square association was obtained between anemia and red cell folate status. Also, no significant difference in red cell folate was found between anemic and non-anemic subjects.

In spite of these results, the author feels that because of the high prevalence of low red cell folate values, a conclusion can be made that folic acid deficiency contributes to anemia in Guyana.

Role of intestinal parasitic infestation

In the 1976 survey (3) intestinal parasitic infestation was not found to be a major contributor to anemia. A similar conclusion can be made in the present study. Relatively few subjects were infested with parasites. No significant chi-square association was obtained between anemia and intestinal parasitic infestation. Persons infested with parasites had a significantly lower mean MCHC value than persons not infested with parasites ($p < 0.05$). Very little, however, can be said about this finding. No significant difference in any of the other hematological characteristics was obtained between persons infested with parasites and those not infested with parasites. Significant negative correlations were obtained between hookworm ova count and both MCV (-0.54) and MCH (-0.49) ($p < 0.05$). Here again, very little

can be said about these findings. A significant positive correlation was also obtained between whipworm ova count and hemoglobin (0.86) ($p < 0.05$). This sign of this correlation is opposite to what was expected. However, it should be pointed out that, as in the correlations of hookworm ova count with MCV and MCH, it is based on relatively few subjects - 5 in the whipworm correlation, and 15 in the hookworm correlations.

Dietary intake

Because of the nature of the dietary data obtained it was not possible to investigate the role of insufficient dietary intake in contributing to anemia. However, a number of significant correlations were obtained between the frequency of consumption of some foods and some of the hematological characteristics. These are shown in table 11. If one were willing to believe that the more frequently a food is consumed, the greater the intake than all of these correlations would have been expected to be positive. However, a number of the correlations were negative. This is probably a reflection of the fact that increasing frequency of consumption does not necessarily indicate increasing intake.

Table 11. Significant correlation coefficients

	Hemoglobin	Hematocrit	Serum ferritin	Plasma folate	Red cell folate
Beef				0.20	
Pork	- 0.47	- 0.35			
Fish	- 0.17	- 0.13			0.22
Shrimp				0.18	
Egg					0.23
Pigeon peas			- 0.60		
Split peas					0.15
Calalloo				- 0.13	
Ochro	0.19				
Cucumber	0.27				0.25
Squash				- 0.23	
Bread			0.12		- 0.15
Roti			- 0.26		0.25
Biscuit				0.30	

$p < 0.05$

Recommendations for intervention

Based on the results of the present study and of the two previous studies (1, 3) the author feels that the control of the nutritional anemias can be brought about by an applied intervention program incorporating the following three approaches:

(1) Nutrition education

(2) Distribution of iron and folic acid supplements to pregnant and lactating women.

(3) Fortification of a suitable vehicle with iron and folic acid.

Such a program involving a combination of these three approaches is likely to be much more successful than one in which just one approach is applied by itself.

Nutrition education

Although nutrition education has a poor record of changing food habits, the author feels strongly that nutrition education should be an integral part of any applied nutrition program. The nutrition education would focus mainly on the nature, symptoms and causes of nutritional anemia, and how it could be prevented by the consumption of a proper diet incorporating local foods. Some information could also be provided on treatment. Use could be made of the media such as radios and newspapers. Posters could be placed in markets, groceries, shops, hospital or clinic waiting rooms and on billboards. Pamphlets could be prepared and distributed in hospital

and clinic waiting rooms. Demonstrations could be given on the use and proper preparation of local foods that are good sources of iron and folic acid. Cinema advertising could also be used.

The important benefits of nutrition education would be an increased awareness by the general populace of the nutritional anemia problem, the need to do something about it, and how it could be prevented.

Distribution of iron and folic aicd supplements to pregnant and lactating women

The stated policy in Guyana is that iron and folic acid supplements are distributed to pregnant women attending prenatal clinics. However, considering the high prevalence of iron and folic acid deficiency in pregnant women, the program is ineffective. In the present study only 28 of the 56 pregnant females were taking iron supplements and only two were taking folic acid supplements. In the 1976 survey, one finding was that of the 112 women who had attended prenatal clinics during their most recent pregnancies more than one-third (37.5%) never received iron supplements and none received folic acid.

The author feels that there needs to be an evaluation of the present system for distributing iron and folic acid supplements. Depending on the results of this evaluation the present system could be strengthened, or a new one developed and rigidly implemented.

The program should also be extended to lactating women who also have increased needs of iron and folic acid and who also have a high prevalence of deficiencies of these two nutrients. These supplements would not only help to provide for lactation but would also rebuild their stores for

future pregnancies.

Nutrition education should also be previded to these women so as to create or to reinforce the desire to take the supplements.

Fortification of a suitable vehicle with iron and folic acid

The author feels that the most effective and far-reaching approach would be fortification of a suitable vehicle with iron and folic acid. At present all wheat is imported into Guyana where it is milled into flour by the National Milling Company. The wheat flour is then enriched with "Repco Enrichment Concentrate Type R" which contains 4800 mg. reduced iron (metallic iron powder) per ounce as well as niacin and riboflavin. It is believed that 13.0 mg. iron is added per pound of flour (9). Considering the high prevalence of iron deficiency, and the fact that wheat flour is widely used by all segments of the Guyanese population, this enrichment program has been ineffective.

The extent of absorption of iron from the enriched flour is not known. However, it is known that the absorbability of metallic iron powders varies widely, depending on particle size or on surface area per unit weight (8). Consideration may be given to either increasing the level of fortification with metallic iron powder or the use of a more available iron compound.

Several countries in addition to Guyana, (such as Sweden, the United kingdom, and the U.S.A.) already have programs in operation for the fortification of wheat flour with reduced iron and compounds such as iron carbonyl and ferrous sulphate. There is however, no published evidence

concerning the nutritional value of these measures (8). Wheat flour contains an excess of absorption-inhibiting substances which affect in the same proportion the absorption of both native food iron and iron added as fortification (10). Layrisse (13) reported that the mean absorption of iron from wheat was 5% in both normal and iron-deficient subjects.

Another vehicle may be considered for use in place of flour. Careful attention needs to be paid to this vehicle. It should be one that is consumed in adequate amounts by the people in need; it should be available for fortification in relatively few centers so that quality can be adequately controlled and monitored; it should be suitable for fortification on a large scale; and it should result in a product which is stable under extreme conditions of storage (8). A number of vehicles besides flour, that are available in Guyana could satisfy these requirements. These are sugar, rice, and salt. These vehicles would have to be compared as to their suitability for fortification, the absorbability and potential nutritional effect of the compound used for fortification, and its stability under extreme conditions of storage, before one is selected.

Layrisse et al. (10) showed that sugar presented several advantages over other vehicles used for iron fortification. Its interference with the absorption of iron used for fortification was negligible. Iron absorption from fortified sugar was increased more than 50% over that absorbed from vegetable foods when it was administered as a drink during the ingestion of a meal. A further increase in iron absorption was found when fortified sugar was administered with beverages such as orange juice, Coca-Cola, and Pepsi-Cola. Fortified sugar has been successfully used by Viteri and Garcia-Ibanez (11) for the prevention of iron deficiency in Central America. The only adverse effect detected so for occurs when sugar fortified with

ferrous sulphate is added to beverages containing high concentrations of tannin such as tea; the color of the tea changes rapidly to black and the iron is precipitated and poorly absorbed (12). The use of ferric orthophosphate instead of ferrous sulphate overcomes this difficulty but renders the iron much less absorbable (8). Layrisse et al. (12) have reported that iron (III)-EDTA used as an iron fortification compound shows a slow reaction with tea; the color of the tea infusion exhibits a slight change in the first two hours and iron is not precipitated for at least twenty-four hours. The above studies, however, have involved the use of refined sugar. In Guyana mainly "raw" sugar is consumed. Before this "raw" sugar can be considered as a vehicle for fortification, studies would have to be carried out to identify any potential technological problems resulting from fortification. Another problem with the use of sugar is that it is produced in eleven factories in Guyana. This could create difficulties as far as quality control is concerned.

Rice, the main cereal consumed in Guyana, also inhibits the absorption of native iron and of iron added to it (14). Layrisse (13), reported that one percent of iron in rice was absorbed by normal and iron-deficient subjects. Another potential problem with the use of rice as a vehicle for fortification is that Guyanese tend to wash their rice and then discard the wash-water. This could result in the leaching of the supplements used in the fortification of the rice.

Sayers et al. (15) have recommended the use of fortified salt in countries where rice or maize forms the staple food. However, the use of salt as a vehicle for fortification involves considerable technical difficulties. Ferrous salts and reduced iron both discolor salt,

particularly the cruder cooking varieties, the discoloration process being accelerated in hot, humid environments (8). Deterioration in bioavailability has been noted with the passage of time. Ferric orthophosphate has been reported to have reasonable absorbability provided ascorbic acid or sodium hydrogen sulphate is also present (8). The addition of ascorbic acid and starch along with the ferric orthophosphate prevents the discoloration of the salt (15). Another problem associated with the use of salt as a vehicle for fortification is that since relatively small quantities of salt are normally consumed in the adult diet the concentration of fortifying compounds must be high.

The compound used for fortification should be one whose absorption is adequate even in the presence of natural dietary inhibitors; it should not cause undesirable changes in the vehicle or foods consumed along with it; it should be stable under locally prevailing storage conditions; and it should withstand local cooking methods.

At the present time, metallic iron powders, ferrous sulphate, ferrous orthophosphate, and iron sodium pyrophospahte are the most commonly used forms of iron for food fortification (18). Ferrous sulphate, the most absorbable of these salts and also the least expensive is, however, the most chemically reactive. This makes it difficult for use in many foods as it produces a variety of undesirable changes in foods to which it is added. The iron powders are relatively insoluble, and, as mentioned before, their absorbability varies widely depending on particle size. Decreasing the particle size improves absorbability but also tends to increase chemical reactivity. The iron phosphates are poorly absorbed unless given with an

additive such as ascorbic acid or sodium dihydrogen phosphate.

Another iron compound, iron (III)-EDTA, shows great promise based on the work of Viteri and Garcia-Ibanez (11), and of Layrisse et al. (12, 14). Layrisse and Martinez-Torres (12) showed that iron (III)-EDTA as iron fortification presented several advantages over other iron salts previously used, including ferrous sulphate. This iron compound exchanged completely with vegetable food iron in the lumen of the gut but with the characeristic that the absorption from both native iron and iron fortification was higher than that expected from other iron salts. The comparison between the iron absorption from iron (III)-EDTA and ferrous sulphate as iron fortification indicated that the absorption from iron (III)-EDTA was about twice as high as that absorbed from ferrous sulphate. Viteri and Garcia-Ibanez (11) reported that sodium iron (III)-EDTA was absorbed better than ferrous sulphate when given with the diet and particularly in iron deficient sbujects. Furthermore, the addition of sodium iron (III)-EDTA to the diet improved the absorption of the iron contained in the diet. Iron (III)-EDTA would therefore appear to be a leading candidate for use as an iron fortification compound in Guyana.

Ascorbic acid promotes the absorption of iron from foodstuffs. Consideration should therefore be given to the addition of ascorbic acid together with the iron supplement to the chosen vehicle. An additional advantage which may be derived from the addition of ascorbic acid is the preservation of both native folic acid and folic acid added to the vehicle.

Because of the high prevalence of folic acid deficiency, it is recommended that folic acid be added to the vehicle selected along with the

iron. Food fortification with folic acid has not received as much attention as fortification with iron. In one study, the absorption of folic acid from maize (corn), rice and bread fortified with pteroylglutamic acid was compared with that of an aqueous solution of pterylglutamic acid in the same subjects (16). Feeding fortified maize and rice produced similar increments in folic acid concentration, which were approximately half those observed with the pteroylglutamic acid solution. Fortified bread produced a lower increment. Pteroylglutamic acid resisted destruction by boiling and baking temperatures. However, baking for longer periods resulted in a loss of folic acid activity. It was concluded that fortification of staple foods with folic acid may offer a practical solution to widespread folic acid deficiency. It has been recommended that folic acid be added to the vehicle at such a level to provide 0.4 mg. folic acid per day (17). It was unlikely that this level of folic acid fortification would have any deleterious effects in persons with undiagnosed vitamin B_{12} deficiency.

Conclusions

The results of the present study have shown that deficiencies of iron and folic acid are the major nutrient deficiencies contributing to anemia in Guyana. Intestinal parasitic infestation is not a major contributor to this anemia. Because intestinal parasitic infestation does not play a major role in the etiology of the nutritional anemias, it can be concluded that inadequate dietary intake of iron and folic acid is a major contributor to these nutrient deficiencies. Another factor contributing to these nutrient deficiencies is increased requirements of the nutrients particularly in women of childbearing age (including pregnant and lactating women).

It is recommended that control of the nutritional anemias be attempted by a combination of nutrition education, distribution of iron and folic acid supplements to pregnant and lactating women, and fortification of a suitable vehicle with iron and folic acid.

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Appendix I

Protocol for Collecting and Processing of Blood Samples

1. Make up 1% ascorbic acid solution: (prepare fresh daily)

$$1 \text{ g of ascorbate} \xrightarrow[H_2O]{\text{dist.}} 100 \text{ ml.}$$

2. Collect 6.0 ml of more blood by venipuncture into heparinized tube labeled with patients' initials and code number.
3. Put the date of collection, patient's name, code number, age, sex, collecting temperature or other information on the data sheet.

<u>Whole Blood Direct Method Sample</u>

4. a. Label 2 test tube (for sample aliquot with the date, patient's code number and initials.
   b. Invert heparinized tube several times gently.
   c. Pipet 0.2 ml of whole blood into one test tube.
   d. Add 1.8 ml of 1% ascorbate solution (from Step 1) into the same tube. Pipetting in Steps 4c and d <u>has to be accurate</u>.
   e. Cover the tube and invert 5-10 times.
   f. Pipet 1.0 ml of the diluted blood solution into each of the other 2 labeled tubes.
   g. Freeze the samples for assay later at Cornell
   h. Freeze separately 1 ml whole blood (no ascorbate, no dilution).

Plasma Samples

5. Prepare one plain test tube, and also prepare two test tubes with 1.25 mg of ascorbic acid powder.
6. Pipet off 4 ml whole blood and centrifuge for 15 minutes at 2,000 rpm on clinical centrifuge. The speed of the centrifuge should not be too high to hemolyze the blood.
7. Pipet off 0.5 ml of plasma into two test tubes with 1.25 mg ascorbic acid, respectively.
8. Pipet the rest of the plasms into the plain test tube (without ascorbic acid).

Appendix 2

1980 NUTRITIONAL ANEMIA SURVEY QUESTIONNAIRE

SUBJECT'S NAME: _____

SUBJECT'S ADDRESS: _____

_____

DATE: _____

PART I - PERSONAL HISTORY

Column Nos.

1. Card Number [ ][ ][ ] 1 - 3

2. Study Group [ ] 4

   1 - Preschool (1 - 5 Years)
   2 - Schoolage (6 - 14 Years)
   3 - Males (15 Years+)
   4 - Pregnant Females
   5 - Lactating Females
   6 - Non-pregnant/non-lactating Females (15 - 44 Years)
   7 - Females (45 Years+)

3. Case Number [ ][ ][ ] 5 - 6

4. Location [ ] 7

   1 - Campbellville
   2 - Leonora
   3 - Mahaica
   4 - Linden
   5 - Essequibo
   6 - New Amsterdam
   7 - #63
   8 - Charlestown

5. Date of Birth (day/mo/yr) _____ [ ][ ] 8 - 9

   If Not Available, Age Last Birthday in Years for Adult and in Months for Preschool Child

6. Sex 1 - Male 2 - Female [ ] 10

Column Nos.

7. Ethnic Group [ ] 11

1 - Indo-Guyanese
2 - Afro-Guyanese
3 - Amerindian
4 - Portuguese
5 - Chinese
6 - Mixed
7 - Other (specify) _____

8. Occupation (to be coded later) _____ [ ][ ] 12 - 13

_____

9. Highest Level of Education Attained [ ] 14

1 - Primary
2 - Secondary
3 - Technical or Vocational
4 - University
5 - Other (specify) _____
6 - No Schooling
7 - Unknown

10. Total Number of Persons Living in Household [ ][ ] 15 - 16

11. Have You Attended a Health or Hospital Clinic Within The Last Two Months? [ ] 17

1 - Yes 2 - No

12. Are You Taking Any Iron or Vitamin Tablets or Tonics? [ ] 18

1 - Yes 2 - No

13. If Yes, What Type?

1 - _____
2 - _____
3 - _____
4 - _____
5 - _____

(a) Is The Person Receiving an Iron Supplement? [ ] 19

1 - Yes 2 - No

(b) Is The Person Receiving a Folate Supplement? [ ] 20

1 - Yes 2 - No

Column Nos.

(c) Is The Person Receiving a Vitamin B_{12} Supplement? ☐ 21

1 - Yes 2 - No

(d) Is The Person Receiving a Vitamin C Supplement? ☐ 22

1 - Yes 2 - No

FOR FEMALES OVER 14 YEARS ONLY:

14. How Many Times Have You Been Pregnant Within the Last 5 Years? ☐ 23

15. How Many Live Births Have You Had Within the Last 5 Years? ☐ 24

16. Are You on Oral Contraceptives? ☐ 25

1 - Yes 2 - No

17. Are You Using a Coil or Loop? ☐ 26

1 - Yes 2 - No

18. If Pregnant, How Many Months? ☐ 27

19. If Breastfeeding, For How Many Months? ☐ 28

PART II - DIETARY INTAKE

20. During Last Week (From Sunday to Saturday), On How Many Days Did You Eat the Following Foods:

Column Nos.

a. Beef ☐ 29

b. Chicken ☐ 30

c. Pork ☐ 31

d. Fish ☐ 32

		Column Nos.
e. Other Meats (specify) _____	☐	33
_____	☐	34
_____	☐	35
f. Eggs	☐	36
g. Milk	☐	37
h. Cheese	☐	38
i. Pigeon Peas (Green Peas)	☐	39
j. Split Peas	☐	40
k. Black-eyed Peas (Cowpeas)	☐	41
l. Other Peas/Beans (specify) _____	☐	42
_____	☐	43
_____	☐	44
m. Bora Beans	☐	45
n. Seime	☐	46
o. Calalu/Bhagee/Pakchoy	☐	47
p. Mustard Greens	☐	48
q. Okra	☐	49
r. Lettuce	☐	50

		Column Nos.
s. Cabbage	☐	51
t. Other Green Vegetables (specify)_____	☐	52
_____	☐	53
_____	☐	54
u. Bread	☐	55
v. Roti	☐	56
w. Biscuits	☐	57

PART III - ANTHROPOMETRIC AND LABORATORY DATA

		Column Nos.
21.	Card Number	1 - 3
22.	Time of Day	4 - 7
23.	Environmental Temperature (°C)	8 - 9
24.	Height (cm)	10 - 12
25.	Weight (to nearest kg)	13 - 15
26.	Percent of Standard Weight/Height	16 - 18
27.	Hemoglobin (g/dℓ)	19 - 22
28.	Hematocrit (%)	23 - 24
29.	Red Blood Cell Count ($10^6/mm^3$)	25 - 27
30.	MCV (μ^3)	28 - 32
31.	MCH (μμg)	33 - 36
32.	MCHC (%)	37 - 40
33.	Serum Ferritin (μg/ℓ)	41 - 45
34.	Plasma Folate (μg/ℓ)	46 - 49
35.	Red Cell Folate (μg/ℓ)	50 - 54
36.	Occult Blood 1 - Not Done 2 - Absent 3 - Present	55
37.	Ova/Parasites 1 - Not Done 2 - Absent 3 - Present	56
38.	Hookworm (number of ova/g feces)	57 - 61
39.	Roundworm (number of ova/g feces)	62 - 66
40.	Whipworm (number of ova/g feces)	67 - 71