The Effect of Supplements of Animal and Vegetable Protein, Vitamin B_{12}, and Aureomycin on Hematological Values in Central American School Children

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Comparative studies of the effect of animal and vegetable protein-containing supplements on the health and development of school children have been few and of relatively short duration.[1-3] Investigations of the effect of vitamin B_{12} on the development of school children have been generally inconclusive,[4-8] while trials of antibiotics for this purpose have been reported only from this laboratory.[9,10]

The administration of supplements of animal protein, vegetable protein, vegetable protein plus vitamin B_{12}, and vitamin B_{12} alone in school children of El Salvador and Guatemala was begun in 1950. At the same time, vegetable protein plus Aureomycin® and Aureomycin alone were administered in Guatemala. The design of this experiment and the results of successive height and weight measurements are described in the initial report.[9] The children in El Salvador averaged more than one year behind those of the United States in height and weight and those in Guatemala more than two and one-half years behind.[11] Detailed hematologic observations were also considered necessary because of the lack of previous experience with the long continued administration of vitamin B_{12}, or small doses of Aureomycin, to essentially normal children. The present paper presents the hematological findings in this study.

Material and Methods

The subjects were in apparently good health, although heavily infested with parasites. *Ascaris lumbricoides* was found in 79 per cent of the children of El Salvador and in 84 per cent of the Guatemalans. *Trichuris trichiura* occurred in 9 per cent of the former and 36 per cent of the latter. *Necator americanus* was identified in the stools of 13 per cent in El Salvador but was not found in the group studied in Guatemala. All children were treated for intestinal parasites at the beginning of the study in El Salvador and during the first 2–3 months in Guatemala. The opportunities for reinfestation were so great that the antihelminthic did not decrease the incidence of infestation encountered at the beginning of the second year, although it may be presumed to have decreased the number of parasites.

The scheme of experimental treatment in the control groups and those receiving the food supplements are shown in Tables I and II,

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TABLE I

Hematological Findings and Occurrence of Anemia among Treatment Groups in El Salvador

Locality	Treatment	Months Interval	Number 1st Exam.	Number 2nd Exam.	Red blood cells 1st Exam. Mean ± S.D.	Red blood cells 2nd Exam. Mean ± S.D.	Hemoglobin 1st Exam. Mean ± S.D.	Hemoglobin 2nd Exam. Mean ± S.D.	Hematocrit* 2nd Exam. Mean ± S.D.	Distribution of "anemia" and macrocytosis: % MCV >94	children anemic: Total No.	children anemic: No. MCV >94
					Millions per cu mm		*gm per 100 ml*		%			
					Urban							
Colombia	Animal protein lunch	5	50	30	4.45 0.52	4.37 0.39	12.3 1.0	11.5 1.2	39.9 2.5	26	11	4
Roosevelt	Control	5	50	37	4.18 0.60	4.49 0.24	11.6 0.5	11.2 1.0	38.9 2.6	2	16	1
					Rural							
Matazano	Control	3	22	15	4.21 0.45	4.27 0.38	11.6 1.2	11.0 1.6	37.1 3.9	6	6	1
Comecayo	Animal protein lunch	4	26	24	4.17 0.46	4.32 0.30	12.0 1.2	12.3 1.2	37.3 2.1	4	3	0
Portezuelo	Vegetable protein lunch	5	24	23	3.99 0.47	4.19 0.41	10.9 1.1	10.9 1.4	36.9 2.8	34	8	4
Portezuelo	Vegetable protein lunch + vit. B_{12} 20 μg.	5	26	26	3.86 0.35	4.35 0.32	10.9 1.4	11.5 1.1	37.6 2.4	42	7	4

* No hematocrit data available for first examination.

together with the presentation of the results. In one additional village in Guatemala, Xenacoj, some serial observations are mentioned in the text for 3 children receiving 20 μg of vitamin B_{12} and 2 receiving 50 mg of Aureomycin for 18 months. A larger number of children in these two Xenacoj groups are included in the tabulations in which each set of examinations is treated individually.

The initial diets were very low in both animal protein and vitamin A activity. Riboflavin and calcium also fell below the National Research Council (U.S.) recommendations.[12,13] The supplementary feeding improved the situation in regard to protein, riboflavin, and calcium, but still left the diets below the recommended intake of vitamin A.

In the morning as soon as possible after the children arrived at school, blood was drawn and placed in oxalated tubes prepared as suggested by Wintrobe.[14] The blood samples were kept under refrigeration from the time they were drawn and were usually examined the same day. In no case did more than 24 hours elapse before the examinations were completed. The standard instructions of Wintrobe[14] were used for the determinations of hematocrit and red blood cells. New International type B centrifuges, with tachometers, were employed in both Guatemala and the second examinations in El Salvador. Hemoglobin was determined by the method of Sahli, using a visual hemoglobinometer in El Salvador and a Klett photoelectric colorimeter in Guatemala.

Results

All of the mean values for children from El Salvador (Table I) and Guatemala (Table II) fell within the limits for United States children in this age range, and the standard deviations are not remarkable. The results of comparisons of values before and after the treatment administered to the groups in Xenacoj and those in Table I and II do not indicate a consistent advantage for any of the treatments employed.

The children in Xenacoj receiving vitamin B_{12} for 18 months and examined all four times had upon first examination an average red blood cell count of 4.05 millions per cu. mm., hemoglobin of 13.2 gm per 100 ml, and hemato-

TABLE II

Hematological Findings among Treatment Groups in Rural Guatemala

Locality	Treatment	No.	Red blood cells 1st Exam. Mean ± S.D.	Red blood cells 2nd Exam. Mean ± S.D.	Red blood cells 3rd Exam. Mean ± S.D.	Red blood cells 4th Exam. Mean ± S.D.	Hemoglobin 1st Exam. Mean ± S.D.	Hemoglobin 2nd Exam. Mean ± S.D.	Hemoglobin 3rd Exam. Mean ± S.D.	Hemoglobin 4th Exam. Mean ± S.D.	Hematocrit 1st Exam. Mean ± S.D.	Hematocrit 2nd Exam. Mean ± S.D.	Hematocrit 3rd Exam. Mean ± S.D.	Hematocrit 4th Exam. Mean ± S.D.
			Millions per cu mm				*gm per 100 ml*				%			
San Antonio	Control	20	4.38 0.56	4.55 0.32	4.48 0.30	4.32 0.24	12.8 1.2	12.3 0.9	13.5 0.8	13.9 0.8	41 3.0	42 2.3	42 1.9	42 2.1
Santa Maria	Animal protein snack	26	4.47 0.45	4.50 0.33	4.58 0.36	4.50 0.30	13.4 0.9	13.0 0.9	13 0 0.9	14.3 0.9	42 2.7	42 2.2	43 2.2	44 2.2
Magdalena	Vegetable protein snack	11	4.14 0.70	4.48 0.22	4.58 0.56	4.39 0.33	13.1 1.1	11.9 —*	13.1 0.9	14.1 0.9	42 2.6	44 —*	43 1.8	43 2.0
Magdalena	Vegetable protein snack + vit. B_{12} 20 μg	11	4.42 0.42	4.68 0.28	4.36 0 42	4.54 0.24	13.5 1.1	12.0 1.1	13.2 1.0	14.5 0.6	42 2.8	43 3.0	42 2.3	43 1.5
Magdalena	Vegetable protein snack + Aureomycin 50 mg	9	4.57 0.51	4.69 0.41	4.55 0.37	4.85 0.39	13.8 0.7	12.6 —*	13.3 0.8	14.7 1.0	41 1.4	44 —*	44 2.0	44 1.5

All of the same children are represented on first, third, and fourth examinations. Due to local circumstances, fewer were included on the second examination. The second examination means marked with an asterisk (*) represent too few observations to justify a value for standard deviation. The interval between the first and second examinations was 5 months and between the second and third and the third and fourth was 6 months.

TABLE III

Occurrence of Anemia and Macrocytosis among Treatment Groups in Rural Guatemala

Locality	Treatment	1st Examination Total No.	1st Examination % MCV >94 *cu μ*	1st Examination Children "anemic" No.	1st Examination Children "anemic" No. MCV >94 *cu μ*	2nd Examination Total No.	2nd Examination % MCV >94 *cu μ*	2nd Examination Children "anemic" No.	2nd Examination Children "anemic" No. MCV >94 *cu μ*	3rd Examination Total No.	3rd Examination % MCV >94 *cu μ*	3rd Examination Children "anemic" No.	3rd Examination Children "anemic" No. MCV >94 *cu μ*	4th Examination Total No.	4th Examination % MCV >94 *cu μ*	4th Examination Children "anemic" No.	4th Examination Children "anemic" No. MCV >94 *cu μ*
San Antonio	Control	53	49	13	13	43	37	5	4	32	53	2	2	28	64	3	2
Santa Maria	Animal protein snack	61	50	5	5	56	53	4	4	32	47	1	1	30	67	3	2
Magdalena	Vegetable protein snack	30	73	14	13	12	50	0	—	12	50	2	2	14	57	3	3
Magdalena	Vegetable protein snack + vit. B_{12}	22	45	2	2	10	50	1	1	11	64	3	3	18	56	0	—
Magdalena	Vegetable protein snack + Aureomycin	24	66	7	7	9	66	0	—	12	67	2	2	13	23	0	—
Xenacoj	Vitamin B_{12}	18	55	3	2	13	46	4	4	8	25	1	1	6	83	0	—
Xenacoj	Aureomycin	20	40	0	—	18	61	6	5	7	57	0	—	7	71	1	1

The interval between the first and second examinations was 5 months, and between the second and third and the third and fourth was 6 months.

crit of 41 per cent. The two receiving Aureomycin for the same period had an average red blood cell count of 4.26 millions per cu. mm., hemoglobin 12.4 gm per 100 ml, and hematocrit of 41 per cent.

The percentage of children with mean corpuscular volume (M.C.V.) greater than 94 cu μ is given in Table III for Guatemala. The value of 94 cu μ was chosen because Wintrobe[14] has used this value as the lower limit in macrocytic anemia of adults. Since children tend to have smaller mean red cell values this figure should give a conservative estimate of the amount of macrocytosis. Of possibly greater interest in these tables is the distribution of M.C.V. values in children considered to have anemia. For the purpose of this tabulation, children with a red blood cell count of less than 4.1 million per cu mm, a hemoglobin of less than 10.6 gm per 100 ml, or a hematocrit of less than 32.5 per cent were arbitrarily classified as anemic. These figures are an estimated one standard deviation below the normal values for children from 6 to 10 years of age given by Wintrobe.[14] None of these children had a M.C.V. under 86 cu μ. On the other hand, 66 to 100 per cent had a M.C.V. over 94 cu μ.

Unfortunately, comparable values cannot be presented from El Salvador, since a suitable centrifuge was not available when the initial surveys were done. Upon the second examination, the percentage of cases with M.C.V. greater than 94 cu μ varied from less than 10 per cent in Comecayo, Matazano, and Roosevelt schools to 26 and 42 per cent, respectively, in the schools Colombia and Portezuelo. Although the relative number of children with anemia was greater in El Salvador than in Guatemala on this second examination, the incidence of macrocytosis was not as great. The incidence of anemia in the first examination in El Salvador varied from 28 to 91 per cent, a far greater percentage than in Guatemala.

Discussion

Initial Hematological Values

As stated above, the incidence of anemia in El Salvador, as based on the criteria cited, was relatively high. Low hemoglobin values were the most common basis for classifying these children as anemic. The average red blood cell counts were only slightly below those for the United States. Since the average hematocrit values were considerably higher, nearly all the anemia cases were macrocytic in type. No case of microcytosis was observed, even though hookworm infestation was common among the groups studied in El Salvador.

The poor quality of the protein in the diets and perhaps the deficiency of one or more vitamins of hematopoietic importance are believed to be responsible for the tendency to macrocytosis. It is of interest that, in general, the entire distribution about the mean of values for M.C.V. was shifted toward the macrocytic side.

Higher hemoglobin values were observed in Guatemala than in El Salvador. As a result of the higher hemoglobin values, the incidence of anemia tended to be less in Guatemala and to be due largely to low red cell values. There is no reason to believe that the altitude (6,000 feet) at which the Guatemalan children lived was sufficient to explain the increase.[15]

Effect of Treatment

The figures given in Table I show that the treatment had no striking effect on hematological values. Although statistically significant at the 5 per cent level, the slight improvement in red blood cell count in the urban control group in El Salvador and one of the vegetable protein groups in Guatemala is of little practical importance and was probably a reflection of seasonal factors. The seasonal drop observed in hemoglobin in the urban schools in El Salvador was unrelated to treatment.

Treatment differences in hematocrit could be tested only for the data obtained in Guatemala, but no effects on hematocrit values were observed. In Guatemala, where it was possible to evaluate the effect of the various treatments on the incidence of macrocytosis in children with anemia, no consistent change in the high percentage of macrocytosis was observed. It is of interest to note that the vegetable protein snacks offered in Magdalena

were apparently effective in reducing the percentage of children with anemia.

The group receiving vitamin B_{12} in addition to the vegetable protein containing lunch in El Salvador had a slightly lower initial mean red blood cell value. Although this had increased significantly to the values observed for the other rural groups upon re-examination five months later, no special significance is attached to this isolated finding.

Summary

Hematological studies were carried out in two urban and three rural schools in El Salvador and four rural schools in Guatemala. Initial values for 100 urban children in El Salvador were: red blood cells 4.31 (S.D. 0.57) millions per cu mm, hemoglobin 12.0 (S.D. 0.8) gm per 100 ml, and for 98 children from the rural schools, red blood cells 4.05 (S.D. 0.45) millions per cu mm, hemoglobin 11.4 (S.D. 1.3) gm per 100 ml. Corresponding values for 228 rural children in Guatemala were red blood cells 4.40 (S.D. 0.42) millions per cu mm, hemoglobin 13.3 (S.D. 1.0) gm per 100 ml, and hematocrit 42.0 (S.D. 2.5) per cent. In Guatemala, 49 per cent of the children had mean corpuscular volumes greater than 94 cu μ. Only 19 per cent had hemoglobin values less than 10.6 per 100 ml or red blood cell counts less than 4.1 millions per cu mm.

The children in an urban school in El Salvador were given an excellent lunch rich in animal protein without producing any change in five months compared with those in an urban control school. Vegetable and animal protein lunches, respectively, and a vegetable protein lunch plus 20 μg of oral vitamin B_{12} daily had no effect on red blood cell count or hemoglobin values during five months in El Salvador or two years in Guatemala, compared with rural control schools in each country. The daily oral administration of 20 μg of vitamin B_{12} or 50 mg of Aureomycin to rural Guatemalan school children in addition to a vegetable protein lunch, as well as the provision of 50 mg of Aureomycin or 20 μg of vitamin B_{12} alone, had no significant effect on blood values during a two-year period.

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