Serum Ascorbic Acid, Riboflavin, Carotene, Vitamin A, Vitamin E and Alkaline Phosphatase Values in Central American School Children

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THE value of determinations of nutrients in blood serum has not been fully appreciated by workers in areas in which the population is well nourished and the range of variation in nutritional status relatively small. In areas in which there is considerable malnutrition, however, blood serum levels of specific nutrients may be useful indicators of nutritional status.[1]

As part of the nutrition surveys of INCAP, it has been possible to determine several serum constituents in persons (principally school children) living in Costa Rica, El Salvador, Guatemala, Honduras and Panama. Serum ascorbic acid, free riboflavin, carotene vitamin A, vitamin E and alkaline phosphatase were measured in over 800 children attending eighteen public schools in both urban and rural areas. To avoid confusing seasonal trends with age trends, seasonal fluctuations in the blood constituents measured were determined from nearly 1,000 adult blood samples. The findings have been useful for comparison with parallel dietary and clinical data from the same populations.

MATERIAL AND METHODS

Blood was taken by venipuncture from children seven to twelve years of age attending public schools in both rural and urban areas of Central America. Blood samples from adults were obtained over a period of two and a half years while the initial nutritional surveys of INCAP were under way. All samples were allowed to clot and then centrifuged within six hours to obtain the serum. Special care was taken to avoid prolonged exposure to light by keeping the tubes containing the blood samples tightly stoppered in a covered portable refrigerator. Aliquots of the serum were taken and stored at approximately minus 20°c., for all determinations except ascorbic acid. For the latter, a trichloroacetic acid protein free serum filtrate was prepared and aliquots of this were stored frozen until the time of analysis. Blood samples from Guatemala were brought directly to the central laboratory, while those from the other countries were sent via air, at convenient intervals, packed in salted ice. All assays were performed within three months after the samples were obtained.

The micromethods of Lowry et al.[2] for the determination of serum ascorbic acid, Burch et al.[3] for riboflavin, Bessey et al.[4] for vitamin A and Bessey et al.[5] for alkaline phosphatase were used. At the time these determinations were made, the measurements of "free" riboflavin were not corrected for the contribution of flavin-adenine dinucleotide riboflavin. Vitamin E was determined according to Quaife et al.[6] while carotene was estimated inter-

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TABLE I

Blood Serum Values in Central American School Children

No. of Children	Constituent of Blood Serum	Mean	Standard Deviation
850	Free riboflavin (µg./100 ml.)	1.53	0.93
850	Ascorbic acid (mg./100 ml.)	1.55	0.61
849	Vitamin A (µg./100 ml.)	26.4	8.4
849	Carotene (µg./100 ml.)	90.0	44.0
691	Vitamin E (mg./100 ml.)	0.65	0.26
868	Alkaline phosphatase (mM/L./hr.)*	5.25	1.99

* Milimoles of p-nitrophenyl phosphate per liter per hour. Approximately equal to 1.79 Bodansky units.

changeably by the method of Bessey et al.,[4] or the method of Quaife et al.[6] The results obtained from these two procedures proved identical for practical purposes and the simultaneous determination of total carotenoids with either vitamin A or vitamin E simplified the laboratory routine

RESULTS

The average free riboflavin, vitamin C, vitamin A, carotene, vitamin E and alkaline phosphatase serum values for school children in Central America, with their standard deviation, are given in Table I. These values were obtained from the total sample which includes children from both urban and rural lower income populations. Mean values for these serum nutrients, according to country and place of residence, are given in Table II. School children from both urban and rural communities were studied in Guatemala and El Salvador. Children living in rural areas had lower serum values for vitamin A, carotene, vitamin E and alkaline phosphatase, but higher serum values for riboflavin than children living in urban communities. Serum levels of ascorbic acid were high in the urban Guatemalans and much higher in the rural school children from El Salvador.

The seasonal variations in nutrient levels in the adult group sampled are shown in Figure 1. The levels of all the nutrients except riboflavin tended to be highest from November to March, which are dry months, and lowest during the rainy season months, May to September. Riboflavin behaved in the reverse manner with a peak in July. The average levels for ascorbic acid tended to be lower in the adult group than in school children in the same area, but the levels for vitamin E, carotene, vitamin A, and free riboflavin were unaltered or higher. Seasonal trends for alkaline phosphatase were not evaluated because of insufficient determinations in adult blood samples for this purpose.

TABLE II

Blood Serum Values in Central American School Children by Country and Place of Residence

Country	Place of Residence	Maximum No.*	Free Riboflavin (µg./100 ml.)		Ascorbic Acid (mg./100 ml.)		Vitamin A (µg./100 ml.)		Carotene (µg./100 ml.)		Vitamin E (mg./100 ml.)		Alkaline Phosphatase (mM/L./hr.)†	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Costa Rica...	Urban	145	1.45	0.94	1.47	0.42	28.4	10.2	75	37	0.74	0.22	4.65	1.40
	Urban	131	1.65	0.96	1.28	0.45	25.8	8.1	85	35	0.80	0.26	4.96	1.68
El Salvador..	Rural	86	1.44	0.70	2.65	0.87	19.6	7.7	44	32	0.55	0.20	4.88	1.52
	Urban	19	1.34	0.72	1.85	0.24	30.5	10.1	157	60	0.44	0.20	6.98	1.80
Guatemala...	Rural	232	1.66	1.15	1.14	0.42	24.8	7.1	108	50	0.55	0.24	5.22	2.06
Honduras....	Urban	92	1.73	0.99	2.66	1.14	28.6	9.6	57	28	0.67	0.20	5.49	1.44
Panama.....	Urban	183	1.32	0.71	1.37	0.39	25.0	14.3	101	60	...	...	6.19	3.20

* Minor differences in number of determinations occurred for the different constituents because of breakage or loss.
† mM of p-nitrophenyl phosphate per liter per hour. Approximately equal to 1.79 Bodansky units.

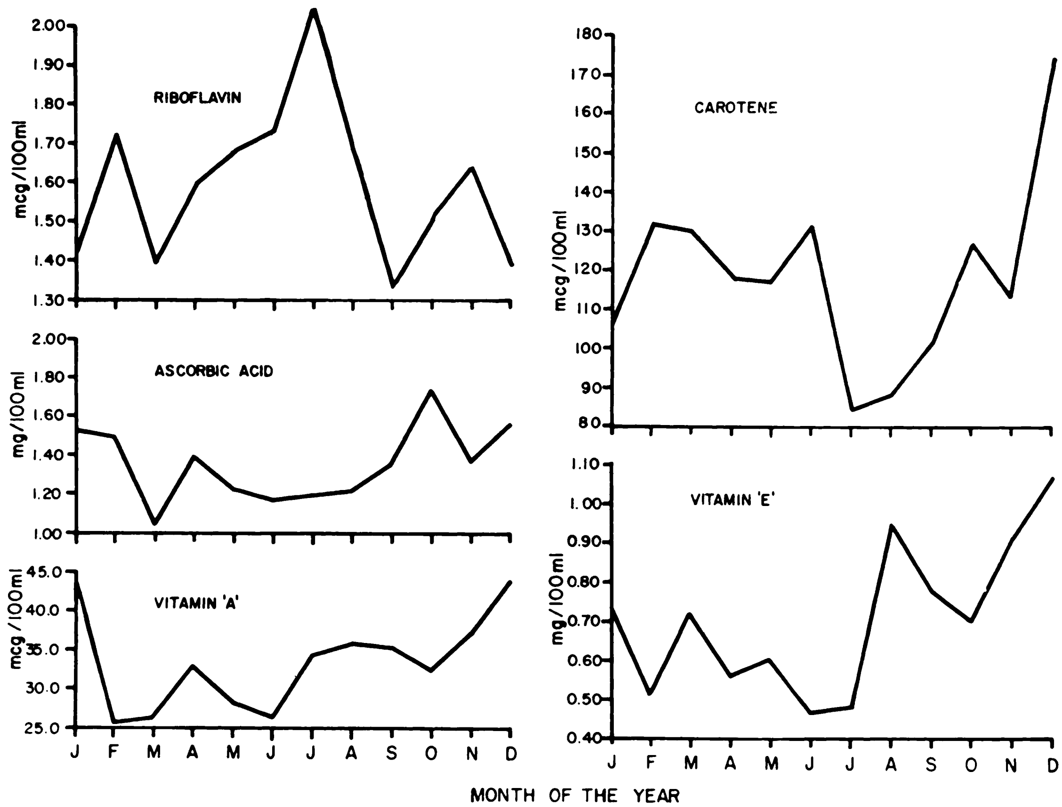

FIG. 1. Seasonal trends in serum values in Central American adults.

COMMENTS

Serum protein levels for the Central American area have been previously described.[7] The data indicate that not only are all of the average values well within normal limits, but also very few individual values fall below the 6 gm. level which has been considered to be critical from a public health point of view.[1,8] Persons consuming diets in which the protein is predominantly of vegetable origin are known to have serum protein values the same or even higher than those whose diets contain abundant animal protein.[7] Thus, serum protein values reveal protein malnutrition only when it is sufficiently severe as to be manifest by clinical signs. No child attending school was found to have reached this degree of protein malnutrition, although cases of kwashiorkor are common in most of the populations studied.[9,10]

The average values reported for serum ascorbic acid, riboflavin, vitamin A, carotene, vitamin E and alkaline phosphatase were well above levels considered to represent deficiency.[1,11-17] Since the standard deviations are large, however, levels suggestive of deficiencies for some of the nutrients were found in many children.

Average values for riboflavin in school children were quite uniform among the groups sampled and also within normal limits[13] although the estimated dietary intake is approximately 50 per cent[18,19] of the recommended INCAP[20] and N.R.C.[21] allowances. In physical examinations among these children, signs suggestive of riboflavin deficiency, such as cheilitis, angular stomatitis and nasolabial seborrhea, were found only rarely, although hypertrophy or atrophy of the papillae of the tongue are regularly found in about 15 per cent of the children.[22]

The levels of ascorbic acid are considerably higher than those reported for school children in the United States[23] where a level of 1 mg. per 100 ml. has been considered an indication of saturation associated with dietary intakes of 75 to 100 mg. per day.[24,25] Even though average values in excess of 2.6 mg. per 100 ml. were encountered in the population of urban Honduras and rural El Salvador, and no group averaged below 1.1 mg. per 100 ml., dietary surveys in the area did not estimate dietary intakes in excess of 50 mg. per day. Since analytical error has been ruled out by repeated analysis of known serum samples, it seems probable that although the diets are exceedingly rich in ascorbic acid, much fruit, which is the major source of ascorbic acid, is consumed between meals and not reported to the nutritionists as part of the food consumption measured in dietary surveys. The same type of difficulty has been encountered in other tropical regions in measuring the relatively large amount of fruit consumed between meals.[26]

According to dietary surveys, vitamin A is probably the most deficient vitamin in the diet in all the countries represented in the study. Vitamin A activity of the diets is estimated to vary from 690 μg. (2,300 I.U.) in localities where green leaves are more abundant and used in the regular diet[18] to 290 μg. (980 I.U.) in communities where the diet is relatively lacking in both animal and vegetable sources of vitamin A activity.*

In the clinical examinations, frank xerophthalmia and Bitot's spots are not found in school children, and such common suggestive signs as mild xerosis and follicular hyperkeratosis are notoriously nonspecific. The mean values for vitamin A and carotene are both considerably lower than those for comparable groups in the United States, and individual values below the 15 to 20 μg. per 100 ml., which Bessey[17] considered to be suggestive of deficiency, were observed in about 15 per cent of the children.

Carotene levels were highest in the population of Guatemala, especially in the highland areas, where green leaves and yellow corn are consumed in larger quantities than in the other countries. It is probable, however, that some of the carotene activity measured represents inactive carotenoid pigments. The carotene levels are relatively low in the other countries and suggest that the intake of biologically active carotenoids is probably inadequate to make up for the relative lack of preformed vitamin A in the diets of children in the lower income groups.

Little is known about either the normal serum level of vitamin E or its significance in human nutrition, although it has been established that the vitamin E level in plasma raises and falls with intake in both experimental animals and man.[17] The large whole grain cereal consumption of populations in the lower income groups in Central America should ensure an abundance of this vitamin in the diet. All the average values in the present study were within the limits of values for population groups considered well nourished.[15,16]

The alkaline phosphatase values for Central American school children are below those reported for well nourished children of the same age in the United States. No elevated alkaline phosphatase values suggestive of rickets were encountered. A possible interpretation of the lowered alkaline phosphatase values is that they reflect a poor nutritional status with respect to protein or other nutrients. This is supported by the finding that alkaline phosphatase values in Guatemalan and foreign children from upper income families attending a private school in Guatemala City, are higher than those in Guatemalan children from low income groups and similar to those found in the United States.[27] In severe protein malnutrition (kwashiorkor), markedly lowered alkaline phosphatase is one of the biochemical characteristics of the syndrome.[28,29]

The apparent seasonal trends in the serum values of all the nutrients studied are of particular interest because they suggest that the levels are sensitive to seasonal variations in nutrient intake. Fruits rich in ascorbic acid are abundant at all times; their price is lower, however, and certain foods which are particularly good sources such as mangoes (Mangifera indica L.), guavas (Psidium guajava L.) and jocotes (Spondias purpurea L.) are more available during the dry season. Many of these same fruits are also sources of biologically

* INCAP unpublished data, kindly supplied by Miss Marina Flores.

active carotene and would be partly responsible for the variations in serum carotene and vitamin A.

SUMMARY

Microchemical analyses of blood samples taken from school children in different surveys conducted in Central America give the averages for serum constituents shown in Table I.

Comparison of the present results with similar studies in the United States indicates that in the Central American children studied, the serum ascorbic acid values were higher, the serum riboflavin and vitamin E values were in the same range, and the serum vitamin A and total carotenoids were lower. No elevated serum alkaline phosphatase values were encountered. In approximately 1,000 adults, ascorbic acid, vitamin A, total carotenoids and vitamin E levels were highest during the dry season, while riboflavin values tended to be highest during the rainy season.

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