Studies of Serum Carotenoids and Vitamin A in Iowa School Children

CHARLOTTE E. RODERUCK, PH.D.,* VIRGINIA D. SIDWELL, PH.D.,† EMIL H. JEBE, PH.D.‡ AND ERCEL S. EPPRIGHT, PH.D.§

STUDIES designed to measure the concentrations of carotenoids and vitamin A in blood serum and their relationship to dietary vitamin A and its precursors have been conducted on children and young people from Maine in the northeast region[1,2] to Oregon,[3] and from Michigan[4] to Louisiana.[5,6] Between these points, subjects in similar age groups have been studied in Vermont,[7] New York,[8–10] Pennsylvania[11] and Colorado.[12] In general, serum carotenoids reflect the total vitamin A intake and have been shown to reflect, even more clearly, vitamin A value¶ from plant sources.

A survey of the nutritional status of Iowa school children, six to eighteen years of age, was made from 1949 to 1951 as part of a North Central Regional project. From records of food intake for seven consecutive days, vitamin A value was estimated. Since the serum carotenoid concentration in many Iowa school children was low, a longitudinal study was conducted to examine the serum of about 200 girls at intervals over two and a half years. During two periods, three months during one school year and six months during the following school year, half of the girls received supplementary foods rich in nutrients; the others, a placebo supplement. The nutrient-rich supplement consisted of concentrated and flavored milk plus fruits and vegetables rich in vitamin C and carotenoids. Acceptance of the supplements and their contribution to intake of nutrients have been described.[13] The effects of the total intake of carotenoids on serum concentrations have been considered; changes with age, sex and season as well as variation among replicate values obtained within six months of each girl's birthday in the longitudinal study are reported.

This manuscript is published as Journal Paper No. J-4421 of the Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa. Project No. 1428.

* Professor, Food and Nutrition Department, College of Home Economics, Iowa State University, Ames, Iowa; † Nutrition Specialist, Technological Laboratory, Bureau of Commercial Fisheries, Ann Arbor, Michigan; ‡ Research Mathematician, Institute of Science and Technology, University of Michigan, Ann Arbor, Michigan; § Professor and Assistant Dean, College of Home Economics, Iowa State University; and Assistant Director, Iowa Agricultural and Home Economics Experiment Station, Ames, Iowa.

This study was supported in part by a grant and other assistance from General Mills, Inc., as part of its nutrition education program.

¶ Throughout this paper, the term "vitamin A value" has been used to refer to vitamin A plus its equivalents from precursors.

EXPERIMENTAL PROCEDURES

For the survey, schools were chosen at random from two population groups in Iowa: (1) eleven elementary schools in cities with a population over 50,000 and (2) twenty-six elementary schools and seven junior and senior high schools in cities and towns with populations under 50,000. Within each school, the children were sampled by sex and by age. Permission to participate in the study was obtained from the parents of each child and, when refusals were received (less than 10 per cent), replacements were selected at random.

The entire sample in the state-wide survey consisted of 705 children, for whom 692 seven-day dietary records, 649 observations on the concentration of serum carotenoids and 324 observations on the concentration of serum vitamin A were obtained.

For the longitudinal study, the subjects were 225 girls, aged eight to fourteen years, from four schools in Story County, Iowa. Of these girls, 184 participated for three years. Samples of blood serum were collected at seven different times. Food intake was recorded for two consecutive days at intervals and as many as twenty-six daily records were available

TABLE I
Serum Concentrations of Vitamin A in Iowa School Children

Age (yr.)	1949–1951 (Survey Data)				1954–1956 (Mixed Longitudinal Data)		
	No. of Boys	μg. %*	No. of Girls	μg. %*	Age (yr.)	No. of Girls	μg. %*
6, 7, 8	28	32 ± 7.3	29	33 ± 9.4	8	31	39 ± 9.8
					9	59	41 ± 11.4
9, 10, 11	32	36 ± 8.6	40	34 ± 9.6	10	100	44 ± 11.0
					11	119	46 ± 9.2
					12	110	47 ± 8.9
12, 13, 14	64	40 ± 8.7	52	38 ± 9.3	13	106	48 ± 9.8
					14	84	48 ± 10.1
					15	54	50 ± 7.6
15, 16, 17	42	45 ± 6.6	46	41 ± 7.8	16	21	49 ± 8.0

* Standard deviation.

from the subjects. Supplementary foods, designed to achieve an increase in the intake of vitamin A and its precursors, were given to half the girls and affected the concentration of serum carotenoids at three of seven collection periods. Data for each age group were obtained by using values for all girls who were within six months of a given birthday. When several values were available in this twelve-month period, they were averaged. Hence, each girl contributed values to more than one age group, but only one value to each age group.

The microchemical procedure of Bessey, Lowry, Brock and Lopez[14] was used for serum analyses. Finger-tip blood was collected within one and a half to two hours after the children arrived at school. Since they had been asked to come to school without eating breakfast or eating bread and jelly only, breakfast was served to them after the blood sample was

TABLE II
Serum Concentrations of Carotenoids in Iowa School Children

Age (yr.)	1949–1951 (Survey Data)				1954–1956 (Mixed Longitudinal Data)					
					Supplement Not Given		Supplement Given†		All Subjects	
	No. of Boys	μg. %*	No. of Girls	μg. %*	No. of Girls	μg. %*	No. of Girls	μg. %*	No. of Girls	μg. %*
6	21	106 ± 35.9	24	139 ± 54.6	...	...	...	...	...	...
7	25	125 ± 47.3	31	122 ± 40.3	...	...	...	...	...	...
8	34	116 ± 45.0	25	116 ± 47.8	32	115 ± 37.9	...	...	32	115 ± 37.9
9	31	110 ± 37.3	38	113 ± 34.4	46	117 ± 36.3	18	150 ± 48.0	64	126 ± 42.2
10	34	124 ± 47.4	28	128 ± 59.7	66	111 ± 36.9	36	147 ± 38.9	102	125 ± 40.9
11	26	116 ± 42.2	35	105 ± 39.2	86	119 ± 47.5	34	141 ± 35.0	120	125 ± 45.4
12	65	106 ± 38.8	62	99 ± 31.8	71	117 ± 44.1	39	140 ± 45.5	110	125 ± 45.7
13	26	104 ± 47.3	25	86 ± 38.3	81	112 ± 41.3	26	131 ± 50.9	107	117 ± 44.3
14	17	89 ± 31.4	13	95 ± 33.2	59	118 ± 41.3	25	121 ± 38.7	84	119 ± 40.1
15	13	72 ± 30.1	15	99 ± 26.8	35	109 ± 36.8	19	122 ± 31.4	54	114 ± 35.2
16	13	82 ± 46.8	14	92 ± 29.9	16	119 ± 44.0	5	140 ± 33.8	21	124 ± 42.0
17	7	106 ± 37.1	10	120 ± 38.8	...	...	...	...	...	...
18	10	96 ± 37.6	7	136 ± 34.6	...	...	...	...	...	...

* Standard deviation.
† Supplementary foods were given during part of the time to girls whose serum concentration of carotenoids contributed to these means.

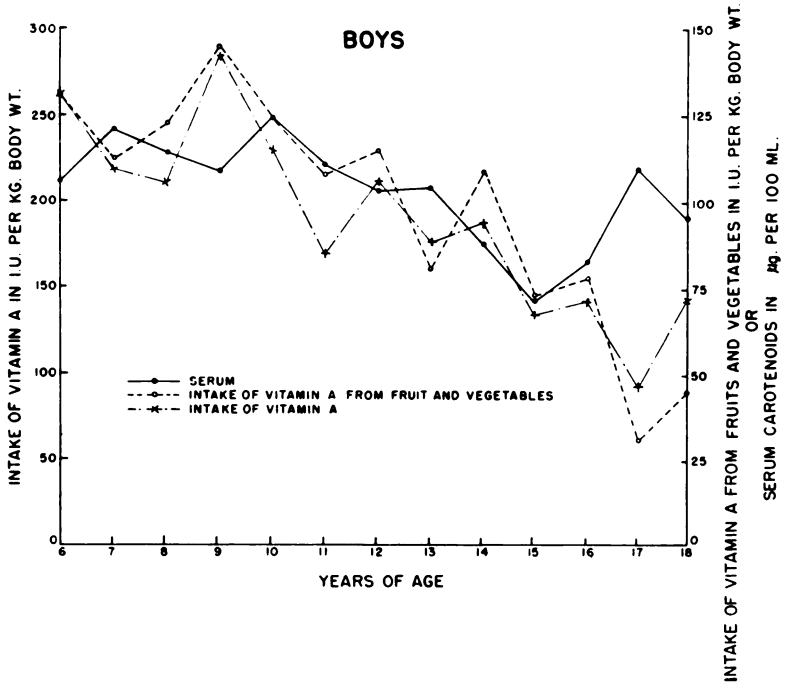

FIG. 1. Mean serum concentrations of carotenoids, mean intakes of total vitamin A per kilogram body weight and mean intakes of vitamin A from fruits and vegetables per kilogram body weight for boys participating in a state-wide survey.

obtained. Samples were refrigerated and transported to the Nutrition Laboratory at Iowa State University for analysis.

RESULTS AND COMMENTS

Tables I and II are summaries of the mean concentrations of serum carotenoids and serum vitamin A in Iowa school children. The concentrations of serum vitamin A increased with age in both populations observed; no difference between sexes was apparent in the concentrations of serum vitamin A included in the survey data.

When the mean total vitamin A value or the mean vitamin A value from fruits and vegetables was considered per kilogram of body weight, these two values decreased with age in much the same way as the serum concentration of carotenoids (Fig. 1 and 2). Minimal values of serum carotenoids occurred at age thirteen for girls and at age fifteen for boys. Age itself, or rate of growth as suggested by Szymanski and Longwell,[12] may be a factor in determining the concentration of serum carotenoids; on the other hand, data from girls in the mixed longitudinal study did not confirm changes in serum carotenoids with age. In girls who consumed their usual diets or their diets with a placebo supplement, the concentrations of serum carotenoids were similar at all ages from eight to sixteen. However, increased intake of foods rich in vitamin A and its precursors during periods of supplementation, did bring about significant increases in serum carotenoids.[13]

Serum Carotenoids and Dietary Sources of Vitamin A

Wide variation in mean vitamin A value was observed in food records obtained in both the survey and longitudinal studies; standard deviations often amounted to three-fourths of the mean value per day. Standard devia-

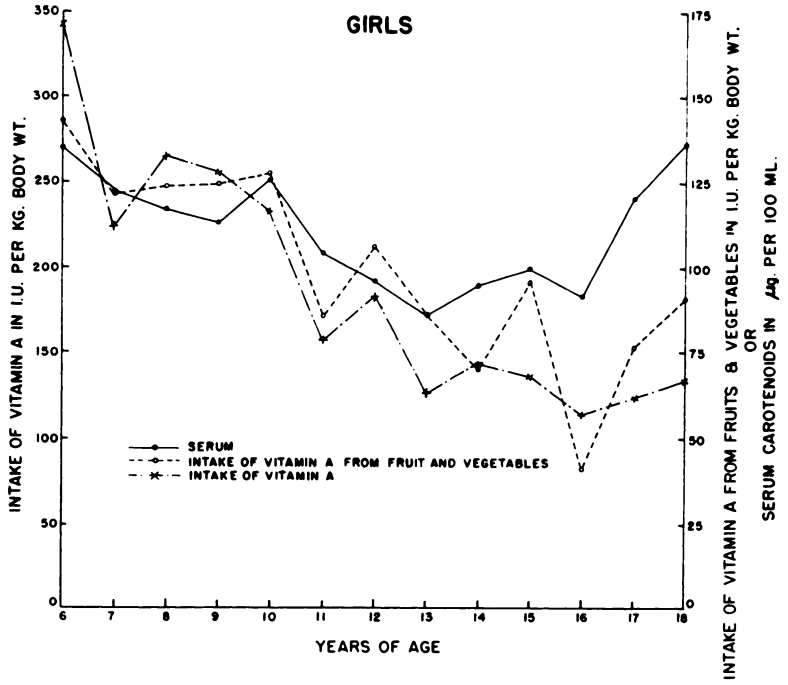

FIG. 2. Mean serum concentrations of carotenoids, mean intakes of total vitamin A per kilogram body weight and mean intakes of vitamin A from fruits and vegetables per kilogram body weight for girls participating in a statewide survey.

tions for serum values were large also, occasionally approaching half the mean value.

The correlation coefficient between concentration of serum carotenoids and daily intake of vitamin A and its precursors was 0.15 for 322 boys in the survey sample. This correlation, although small, was significant. A highly significant correlation, r = 0.30, was obtained between the concentration of serum carotenoids and vitamin A value from fruits and vegetables per kilogram body weight per day. Similar correlations were found in the data for girls aged six to thirteen years in the same survey, but not for girls over thirteen years of age.

The regression line $y = 121.06 + 0.0124X_1 - 2.644X_2$ was calculated for data on boys from six to fifteen years of age; y equalled the concentration of serum carotenoids; X_1, the vitamin A value from fruits and vegetables per kilogram body weight per day; and X_2, age in years. For girls, the regression $y = 139.80 + 0.0118X_1 - 4.280X_2$ applied to the data from girls six through thirteen years of age.

Longitudinal Studies of Serum Carotenoids and Vitamin A

When repeated measurements were made in the same population during different seasons, the mean serum concentration of both vitamin

TABLE III

Seasonal Means for Serum Concentrations of Vitamin A and Carotenoids in Girls Following Their Usual Food Habits

Year	Serum Vitamin A (μg. %)*		Serum Carotenoids (μg. %)*	
	Spring	Fall	Spring	Fall
1954	42 ± 13	51 ± 16	107 ± 39	123 ± 46
1955	44 ± 14	50 ± 10	103 ± 36	119 ± 51
1956	44 ± 8	49 ± 9	101 ± 36	137 ± 52

* Standard deviation (seventy-four to one hundred and one cases involved in each mean).

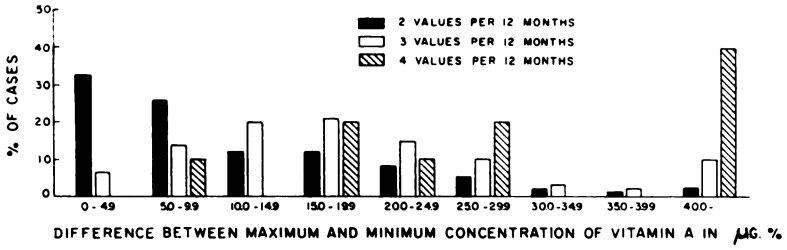

FIG. 3. Distribution of maximal differences among values for serum concentration of vitamin A in samples obtained within six months of each girl's birthday.

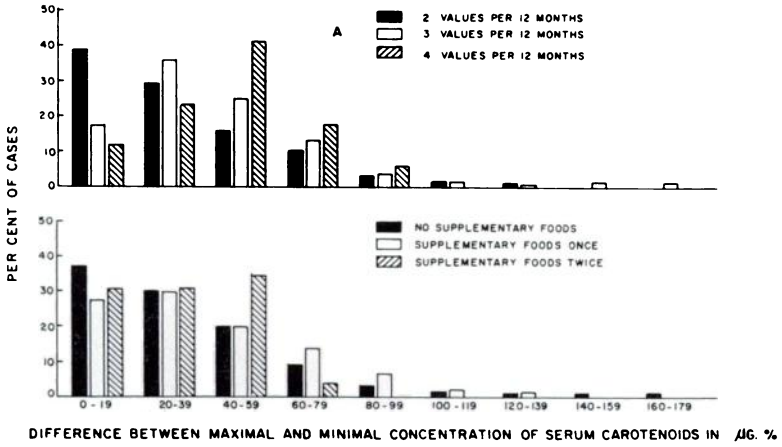

FIG. 4. Distribution of maximal differences among values for serum concentration of carotenoids in samples obtained within six months of each girl's birthday.

A and carotenoids was lower in the spring than in the fall in the girls whose customary food habits were maintained at all times (Table III). In contrast, in the girls who received supplementary foods rich in vitamin A and its precursors during the winter months of two school years, the concentration of serum carotenoids increased in the spring, e.g., 128 µg. per cent in the fall, 145 µg. per cent in the spring; 127 µg. per cent in the fall and 148 µg. per cent in the spring. The serum concentration of vitamin A, however, continued to fluctuate seasonally in a manner similar to that of the girls who maintained their usual food habits at all times. Vitamin A, equivalent to 1,600 I.U. per day, was added to the concentrated milk supplement given during the second year of supplementation. Although the serum concentrations of vitamin A in the specimens collected during the spring of 1956 were not increased significantly, the seasonal variation observed previously was eliminated.

Variability in Concentrations of Vitamin A and Carotenoids in Serum Samples Collected Within Twelve Months

Since values had been obtained for the serum concentrations of vitamin A and carotenoids at seven different times, data from samples collected within six months of each girl's birthday were compiled to study the variability which occurred. Figures 3 and 4 represent the distribution of maximal differences among duplicate, triplicate and quadruplicate observations. Two observations were apt to be more similar than three or four observations. Maximal differences among triplicate observations approached normal distribution. Mean maximal differences are summarized by numbers of samples within twelve months and by

TABLE IV

Variation Observed When Several Observations of Serum Concentration of Vitamin A Were Made Within Six Months of Any Given Birthday

Age (yr.)	Minimal Value		Maximal Difference Between Replicates	
	No. of Girls	μg. %*	No. of Girls	μg. %*
8	7	34.4 ± 5.4 (27.5 - 42.0)	7	18.1 ± 11.8 (2.3 - 33.0)
9	39	32.6 ± 9.6 (16.4 - 59.0)	39	19.8 ± 14.2 (1.7 - 51.8)
10	67	36.4 ± 9.4 (16.8 - 57.5)	67	14.7 ± 13.0 (0.0 - 68.5)
11	68	38.8 ± 9.1 (11.5 - 56.4)	68	13.7 ± 13.8 (0.0 - 64.0)
12	74	41.2 ± 8.5 (23.8 - 64.0)	74	11.6 ± 10.6 (0.0 - 37.8)
13	62	43.3 ± 9.8 (18.3 - 66.0)	62	11.8 ± 10.1 (0.0 - 45.5)
14	53	39.8 ± 9.1 (20.1 - 58.4)	53	15.7 ± 14.0 (0.9 - 75.1)
15	42	44.8 ± 8.3 (28.1 - 59.0)	42	11.5 % 8.6 (0.2 - 28.6)
16	7	44.0 ± 8.7 (29.1 - 54.8)	7	6.1 ± 4.9 (0.4 - 16.2)
All duplicate measurements (within 12 months)			314	11.3 ± 10.7 (0.0 - 68.5)
All triplicate measurements (within 12 months)			95	19.6 ± 12.4 (1.7 - 64.0)
All quadruplicate measurements (within 12 months)			10	33.8 ± 20.7 (7.9 - 75.4)

* Standard deviation. Minimal and maximal values are given in parentheses.

age groups in Table IV and V. The mean of the minimal values involved has been given also. Variability in serum concentrations of vitamin A and carotenoids was not related to the age of the subjects in this study, although the more replications, the more variability was observed.

The magnitude of differences observed between minimal and maximal serum concentrations was very large for both vitamin A and carotenoids. Differences averaged from 30 to 50 per cent of the mean minimal values and, in individual cases, were often considerably greater. Such variability emphasizes the importance of being extremely cautious in interpreting the nutritional status of individual subjects within a population when only single samples have been collected.

In the survey, about 20 per cent of the children under twelve years of age had serum concentrations of carotenoids below 80 μg.; at the same time, 30 to 50 per cent of children aged twelve to eighteen years had serum carotenoid concentrations of this order. When repeated measurements were available for the girls in the longitudinal study, about fifteen per cent of the girls at any one age had serum carotenoid concentrations below 80 μg. per cent; no trend with age was noted. Since data were available for several years for about 180 girls, those who had consistently low concentrations of serum carotenoids were identified. In at least six of seven observations, nine girls had serum concentrations of carotenoids below 80 μg. per cent and an additional ten girls had low values four or more times. Hence about 10 per cent of the girls in the longitudinal study could be considered to have consistently fair to poor concentrations of serum carotenoids, a far smaller number than noted in the survey study.

In the longitudinal study, about 10 per cent of the children under eleven years of age had serum concentrations of vitamin A of less than 30 μg. per cent. Above eleven years of age, less than 2 per cent of the girls had such low

TABLE V

Variation Observed When Several Observations of Serum Concentration of Carotenoids Were Made Within Six Months of Any Given Birthday

Age (yr.)	Minimal Value		Maximal Difference Between Replicates	
	No. of Girls	μg. %*	No. of Girls	μg. %*
8	8	130.5 ± 29.0 (77.9 - 166.9)	8	18.9 ± 11.0 (5.3 - 32.8)
9	44	109.9 ± 44.3 (38.9 - 262.2)	44	43.4 ± 31.3 (4.2 - 143.8)
10	72	108.9 ± 34.4 (55.2 - 206.1)	72	42.4 ± 26.5 (0.2 - 111.0)
11	71	108.4 ± 36.4 (39.4 - 215.6)	71	31.8 ± 24.4 (0.4 - 165.4)
12	78	111.2 ± 44.1 (41.0 - 244.6)	78	37.9 ± 27.8 (0.1 - 174.8)
13	68	105.6 ± 38.5 (41.0 - 228.3)	68	37.3 ± 30.7 (1.4 - 140.7)
14	60	95.2 ± 35.2 (32.7 - 222.8)	60	34.9 ± 27.2 (0.3 - 124.3)
15	43	96.5 ± 32.2 (31.6 - 156.9)	43	30.8 ± 24.0 (2.7 - 84.3)
16	7	111.7 ± 34.8 (50.6 - 155.6)	7	33.3 ± 19.9 (5.0 - 59.9)
All duplicate measurements (within 12 months)			291	32.5 ± 26.1 (0.1 - 129.2)
All triplicate measurements (within 12 months)			144	43.9 ± 29.5 (2.0 - 174.8)
All quadruplicate measurements (within 12 months)			16	46.1 ± 20.6 (15.3 - 88.1)

* Standard deviation. Minimal and maximal values are given in parentheses.

values of vitamin A. None of the nineteen girls classified as having a fair to poor concentration of serum carotenoids could be classified as having a poor concentration of serum vitamin A, since their values for serum vitamin A were usually between 30 and 45 μg. per cent, and occasionally higher. Hence, a poor rating with regard to vitamin A did not accompany a poor rating with regard to carotenoids. Younger girls were more likely to have lower serum concentrations of vitamin A than older girls, but older girls were somewhat more likely to have lower serum concentrations of carotenoids than younger girls. For example, six of nine girls who had the poorest serum concentration of carotenoids were twelve years of age or older at the beginning of the study, whereas seven of ten girls who had fair to poor concentration of vitamin A were below eleven years of age. Over-all, however, little age trend was apparent.

SUMMARY

The serum concentration of carotenoids and vitamin A has been reported for a state-wide survey of about 650 school boys and girls in Iowa and for about 200 girls participating in a longitudinal study. The serum concentration of vitamin A increased with age in both samples. In the survey, the concentration of serum carotenoids appeared to decrease with age; however, the total vitamin A value also decreased with age when it was computed as intake per kilogram body weight. A significant correlation coefficient of 0.30 existed between concentration of serum carotenoids and vitamin A value from fruits and vegetables per kilogram body weight per day. The data from the longitudinal study indicated little fluctuation in serum carotenoids with age for girls whose dietary habits were unchanged. However, serum carotenoids values were lower in the spring compared with those in the fall. When foods rich in vitamin A and its precursors were added as supplements during the winter months to the diets of a similar group of girls, seasonal fluctuation was eliminated.

Considerable variation between serum concentrations of both vitamin A and carotenoids was observed when data collected within six months of each girl's birthday were analyzed. Hence, repeated measurements of serum carotenoids are necessary to assess the true status of any one subject with respect to this nutrient. A poor serum concentration of carotenoids was not accompanied by a poor concentration of vitamin A.

REFERENCES

1. Clayton, M. M., Babcock, M. J., Foster, W. D., Stregevsky, S., Tucker, R. E., Wertz, A. W. and Williams, H. H. Cooperative Nutritional Status Studies in the Northeast Region. v. Blood Findings. Maine Agricultural Experiment Station, Bulletin 516, 1953.
2. Babcock, M. J., Clayton, M. C., Foster, W. D., Lojkin, M. E., Tucker, R. E., VanLandingham, A. H. and Young, C. M. Cooperative Nutritional Status Studies in the Northeast Region. vi. Correlations. West Virginia Agricultural Experiment Station, Bulletin 361T, 1953.
3. Storvick, C. A., Hathaway, M. L. and Nitchals, R. M. Nutritional status of selected population groups in Oregon. ii. Biochemical tests on the blood of native born and reared school children in two regions. *Milbank Mem. Fund Quart.*, 29: 255, 1951.
4. Robinson, A., Lesher, M., Harrison, A. P., Moyer, E. Z., Gresock, M. C. and Saunders, C. Nutritional status of children. vi. Blood serum vitamin A and carotenoids. *J. Am. Dietet. A.*, 24: 410, 1948.
5. Moschette, D., Causey, K., Chealy, E., Dallyn, M., McBryde, L. and Patrick, R. Nutritional Status of Preadolescent Boys and Girls in Selected Areas of Louisiana. Louisiana Technical Bulletin 465, 1952.
6. Haworth, M., Moschette, D. M. and Tucker, C. Hemoglobin values, plasma vitamin A and carotene levels in young women on institutional diets. *J. Am. Dietet. A.*, 27: 960, 1951.
7. Merrow, S. B., Krause, R. F., Browe, J. H., Newhall, C. A. and Pierce, H. B. Relationships between intake and serum levels of ascorbic acid, vitamin A, and carotene of selected groups of children with physical signs of vitamin deficiencies. *J. Nutrition*, 46: 445, 1952.
8. Bessey, O. A. and Lowry, O. H. Meals for Millions, pp. 167–192. Albany, 1947. New York State Joint Legislative Committee of Nutrition.
9. Williams, H. H., Parker, J. S., Pierce, Z. H., Hart, J. C., Fiala, G. and Pilcher, H. L. Nutritional status survey, Groton Township, New York. vi. Chemical findings. *J. Am. Dietet. A.*, 27: 215, 1951.
10. Young, C. M., Day, E. and Williams, H. H.

Nutritional Status Studies of Students at Cooper Union for the Advancement of Science and Art. Ithaca, N. Y., 1954. Cornell University.

11. MACK, P. B. and URBACH, C. A study of institutional children with particular reference to the caloric value as well as other factors of the dietary. Monographs of the Society for Research in Child Development, vol. 13, p. 1, 1948.
12. SZYMANSKI, B. B. and LONGWELL, B. B. Plasma vitamin A and carotene determinations in a group of normal children. *J. Nutrition*, 45: 431, 1951.
13. EPPRIGHT, E. S., RODERUCK, C., GARCIA, P. and WESSELS, K. Effects on girls of greater intake of milk, fruits and vegetables. *J. Am. Dietet. A.*, 42: 299, 1963.
14. BESSEY, O. A., LOWRY, O. H., BROCK, M. J. and LOPEZ, J. A. The determination of vitamin A and carotene in small quantities of blood serum. *J. Biol. Chem.*, 166: 177, 1946.