

HEMOGLOBIN CONCENTRATION AND HYDROXYPROLINE EXCRETION OF HEAD
START CHILDREN IN MONTGOMERY COUNTY, VIRGINIA

by

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CHAPTER I

INTRODUCTION

The Citizens Board of Inquiry in its publication *Hunger USA*, June, 1968, and the one hour CBS television program, *Hunger in America*, 1968, both serious but not scientific efforts, served to stimulate an awareness that malnutrition exists in America as in other countries and to point out the need for epidemiologic data on malnutrition (1).

A standard criterion of health in children is rate of growth; the malnourished child does not grow at a satisfactory rate (2). Differences in the appearance of clinically malnourished and healthy children are easily assessed; perplexities in the detection of impaired growth due to poor nutrition require additional information (3).

Collagen has been demonstrated to comprise about one-third of the total body protein and to be the only body protein containing significant amounts of hydroxyproline. The amino acid is excreted in the urine of man both in the peptide and free forms (4, 5). The free state accounts for 2 to 4% of the total amount excreted. The varieties of peptide bound hydroxyproline, which can be obtained by acid hydrolysis, account for most of the urinary hydroxyproline (4, 5) and about 5% by weight of the total amino acid excretion (6).

As cited by Ziff (6) Kibrick et al. in 1962 and Meilman and co-workers in 1963 reported similar excretion patterns of hydroxyproline and showed that the peptide was comprised of about 60%

prolylhydroxyproline and 15% glycyprolylhydroxyproline. They pointed out that these di- and tripeptides had been demonstrated in the amino acid sequences of normal collagen.

Studies in experimental animals (7, 8) and man (9) show that in malnutrition the total amount of collagen in the body is not reduced and may even be elevated in some tissues. Soluble-collagen turnover has been shown to give rise to about one-third of the urinary hydroxyproline peptides (5, 10).

Failure to grow accompanied by low levels of urinary hydroxyproline reflects a reduced soluble-collagen pool (11). Elevated excretion values may be associated with an increased metabolic rate during recovery from malnutrition (8). Similarly, it has been observed that there is increased excretion of the peptide form in conditions in which there is increased collagen degradation or turnover (6). Thus, the suggestion that low hydroxyproline excretion reflects a reduction in the turnover rate of collagen and this may be a useful criterion of growth failure before the occurrence of significant weight changes.

Children (3, 12) with kwashiorkor and marasmus showed during treatment an increase in the hydroxyproline index

$$\frac{(\text{mM hyp/L} \times \text{kg. body-wt.})}{(\text{mM Creat/L})}$$

and an increase in the hydroxyproline/creatinine ratio. Whitehead (2) has suggested the use of the hydroxyproline index, requiring only random samples of urine, for detection of clinical signs of malnutrition among children. Hydroxyproline excretion has been

shown to decrease in pre-adolescent subjects consuming a low-protein (25 gm) diet (13), whereas, (5) two normal adults on a low-protein diet exhibited no changes in hydroxyproline excretion.

The body hemoglobin present at birth constitutes the bulk of iron in the infant till about six months of age. Thereafter, exogenous iron is necessary to maintain normal hemoglobin levels. Iron deficiency concomitant with a low hemoglobin concentration is known to be widespread in children from a low socio-economic level (14).

Iron, one of the most abundant elements, complexes with a variety of protein molecules. The way it combines with the various proteins confers specific function to that protein. Complexed with globin, the resultant molecule is hemoglobin. Its function is to transport oxygen from the lungs to the tissue and carbon dioxide from the tissues to the lungs. Without adequate iron, blood and tissue hypoxia result. Most of the functional iron is in the red blood cells (15).

It is estimated that there are 6 mg. of iron in every 1,000 calories in the American diet (15). The adequacy of available iron from dietary sources is relative to the requirements of the different age groups. Absorption of iron from foods is variable (16). Ascorbic acid is known to increase iron absorption, whereas phytates and phosphotates decrease absorption (17). The degree of gastric acidity influences solubility and, therefore, availability of the ferric ion (15).

This study is undertaken to evaluate the effect of feeding one adequate meal daily on the nutritional status of children participating in the "Head Start" Program in Montgomery County, Virginia. Comparison of the hemoglobin values at the beginning and at the end of the "Head Start" Program may provide a parameter of the nutritional status in children from low income groups.

CHAPTER II

REVIEW OF LITERATURE

Hemoglobin Concentration and Hydroxyproline Excretion of "Head Start" Children in Montgomery County, Virginia

The majority of body collagen is in the skeletal tissues and comprises about 30% of the total body protein (18). About one-third of the total amino acid content of collagen is hydroxyproline (19). During collagen synthesis proline is incorporated into the peptide chain (20) and then probably enzymatically hydroxylated to form hydroxyproline which is required for collagen formation and stability (21). Free hydroxyproline does not participate in collagen synthesis but it does arise from the breakdown of collagen (20).

Free and peptide hydroxyproline in the urine reflect collagen metabolism (4, 5, 22, 23, 24). Urinary hydroxyproline excretion has been shown to be related directly to collagen metabolism when the intake is held to a minimum by the use of a meat- and gelatin-free diet (5, 6, 11, 25, 26). The daily urinary hydroxyproline excretion is closely correlated also with growth rates (2, 5, 11, 27).

Ziff et al. (4) reported that children excrete three to five times more hydroxyproline peptides than adults even though the total amino acid excretion by children was less. They hypothesized that the increased excretion of hydroxyproline peptides resulted from an increased pool of hydroxyproline peptides "available for collagen synthesis" in the growing child. Higher excretion of hydroxyproline by children than by adults is consistent with the general view that

bone formation and resorption rates are greater in children than adults (4, 28). Similarly, urinary hydroxyproline is increased in young animals as compared with mature animals (23, 29).

The rate of hydroxyproline excretion changes throughout childhood. Whitehead (2) has formulated the hydroxyproline index by multiplying the hydroxyproline to creatinine ratio by body weight and concluded that the index is unaffected by age through six years of age. However, Howells et al. (12) has demonstrated that the hydroxyproline index decreased with age, being high in the fast growing children and lower in the periods of less rapid growth. Kleen et al. (22) reported that both free and peptide hydroxyproline excretions in nine healthy full-term infants rose rapidly during the first two weeks of life (5 to 12 mg/24 hr at birth vs. 50 to 65 mg/24 hr at 10 to 14 days). This phenomena was attributed to changing growth rate and renal function. The period of greatest excretion (102 mg/m²/24 hr) occurs in the first year of life (11). A second period of high hydroxyproline excretion of 80 mg/m²/24 hr in the ten to fourteen year old range (10) as compared to the lower excretion of 66 mg/m²/24 hr average for normal children in the one to ten year old age group has been reported (11). After infancy the highest excretion period for girls is 10 to 12 years of age. Boys were shown to have higher hydroxyproline excretion values in the 11 to 15 year range and to have significantly higher excretion values in this growth period than girls (10).

Other investigations have confirmed the relationship between level of urinary hydroxyproline excretion and rate of growth (11, 12,

25). The rate of hydroxyproline excretion appears to be related to hormones, especially, the growth hormone and thyroxin. The mean excretion of a patient with acromegaly was 101.5 mg/day which on successful irradiation therapy fell to a 58.3 mg/day. Excretion levels of children with dwarfism ranged from 5.6 mg/day to 28.1 mg/day. The hydroxyproline excretion by an 8-year-old pituitary dwarf during growth hormonal therapy rose to average excretion levels for normal 1-to-10-year-old children but fell to pretreatment levels (14.1 mg/day to 18.6 mg/day) on cessation of therapy (25). Concomitant growth and weight gain by dwarfs and cretins receiving hormonal treatment support this evidence (11).

Picou et al. (27) found hydroxyproline excretion in protein-depleted infants (8 to 18 months) below normal (2.6 mg/kg/day). He felt this reflected a reduction in the turnover rate of collagen and could be used to detect early growth failure before objective weight changes occurred. On treatment the average excretion of the ten malnourished infants rose to 6.5 mg/kg/day. Creatinine excretion by the malnourished infants was reduced but not to the same extent as hydroxyproline. When the hydroxyproline index is used with amino acid ratio in blood plasma simultaneously, a differentiation between failure to grow due to a primary deficiency of calories or a failure to grow due to a primary deficiency of protein can be shown (30).

The work of Howells et al. (12) and Rutishauser and Whitehead (3) support the use of random samples of urine and confirm the validity of the Whitehead index as an indication of growth whether during recovery or in normal growth. However, they (12) felt an index based

on height might give a better picture. Anasuya and Rao (2, 3, 27, 31) also reported that urinary excretion of total hydroxyproline (both absolute and per square inch body surface) to be lower in children with kwashiorkor than in normal children and to increase with successful treatment. However, Anasuya and Rao (31) maintain there is no difference in the hyp/creat ratio of normal and kwashiorkor cases because both the hydroxyproline and creatinine excretions are depressed to about the same extent. During treatment of children with kwashiorkor the hyp/creat ratio increases to values higher than in normal children.

The iron balance in infancy is precarious (16). Andelman et al. (14) demonstrated a gain of 166 mg (0.49 mg/day) for a group of infants from birth to 18 months of age fed an iron-containing formula as compared to a gain of 134 mg (0.39 mg/day) for a control group receiving the usual diet. Variations of hemoglobin levels with age is most marked under 3 years of age but is the same for boys and girls (32).

An absorbed iron requirement of 0.4 to 1.0 mg/day and a daily food iron requirement of 4 to 10 mg has been suggested for children 4 to 12 years of age (16). The National Academy of Sciences-Research Council recommends a daily food iron intake of 10 mg for the 6 to 8 year age group (33). The ICNND observed that children under 14 years of age have hemoglobin levels below the average of 16.0 g/100 ml of blood (range from 14 to 18) for normal young men. Darby has shown the mean hemoglobin concentration of 6 year old children is about 12.5 mg/100 ml of blood and continues to increase till about 20 years of age as reported by the ICNND (32).

McCurdy et al. (34) demonstrated that the enhancement of ferrous sulfate absorption by ascorbic acid persists with amounts of iron up to 120 mg (aqueous solution) or 105 mg (tablets). Studies indicate man absorbs 5 to 15 percent of that iron available from dietary intake (15). Finch (15) suggests that animal protein improves iron absorption in other foods, probably through the effects of certain amino acids. The degree of gastric acidity influences the solubility and therefore the availability of iron (35).

"Prevalence figures of iron deficiencies derived from medical literature are of limited value since they deal with iron deficiency anemia and not iron deficiency and since most of the information is derived from patients visiting hospitals rather than from representative populations from different socio-economic classes. Attention generally has been directed first to the detection of anemia and an assumption has then usually been made that most anemic patients have iron deficiencies" (16). The Council on Foods and Nutrition (16) suggests further data are needed to establish the true prevalence of iron deficiency and iron deficiency anemia, and that it is obviously high.

There is a need to evaluate the effect of programs such as "Head Start" on the nutritional status of children. Further study of the validity of using hydroxyproline excretion as a parameter of nutritional status in children beyond infancy would be helpful. Data are limited on hemoglobin values of pre-school children who are not hospitalized. Since malnutrition is frequently seen in the lower

socio-economic groups, I propose to evaluate the effect of the "Head Start" Program on the nutritional status of preschool children.

CHAPTER III

EXPERIMENTAL PROCEDURE

Subjects

The data in this study were collected from 39 pre-school children with an average age of 6 years and 2 months in Montgomery County, Virginia, attending the "Head Start" Program, June 12 through July 25, 1969. The children were recruited from families receiving an average annual income of about \$3,000 and who lived primarily on the less productive farm lands in the area. Permission to collect samples from the children was obtained from the parents of the participating child by the Community Action Center. Height and weight measurements of the children were made without shoes.

The study was designed to compare urinary hydroxyproline excretion values, hemoglobin concentration, and heights and weights at the beginning of the program with the values of these measurements at the end of the program after the children had consumed one nutritionally adequate meal daily for a period of six weeks. This meal consisted of a mid-morning snack and a Grade A lunch. An example of one week's menu and snacks from each of the three participating Montgomery County Schools is shown in Table I.

Urine Collection and Storage

Random samples collected at the first urination after the children arrived at school were used for the hydroxyproline and creatinine assays. The samples were collected by the teachers in

Table I

Examples of Weekly Menus and Snacks Served in the
"Head Start" Program in Montgomery County

Schools	A	B	C
<u>Day 1</u>			
Snack:	Milk	Cornflakes with Bananas	Peanut Butter Crackers, Milk
Lunch:	Pinto Beans Cole Slaw Cornbread, Butter Milk Stewed Prunes	Oven Fried Fish Creamed Potatoes Green Peas Hot Rolls, Butter Milk Apple Pie	Macaroni & Cheese Mixed Greens Yeast Biscuits, Butter Milk Banana Pudding
<u>Day 2</u>			
Snack:	Fresh Orange	Cantalope	Ice Cream Novelty
Lunch:	Broiled Bologna Green Beans Hot Rolls, Butter Milk Cake	Meat Loaf Buttered Corn Mixed Greens Yeast Biscuits, Butter Milk Chocolate Pudding	Fish Sticks Sweet Potatoes Green Beans Pear Salad Rolls, Butter Milk
<u>Day 3</u>			
Snack:	Cereal, Fresh Fruit	Watermelon	Cup Cakes, Orange Juice
Lunch:	Sloppy Joe, Bun Buttered Corn Milk Gingerbread	Baked Beans, Vienna Sausage Cole Slaw Cornbread, Butter Milk Sliced Peaches	Ham Biscuits Potato Salad Green Peas Milk Peach Halves
<u>Day 4</u>			
Snack:	Banana	Fresh Fruit, Milk Crackers	Fruit w/ Whipped Topping & Cookies
Lunch:	Hot Dog, Bun Potato Sticks Milk Jello Peanut Butter Cookies	Vegetable Soup Saltines Peanut Butter Sand. Cheese Sandwich Milk Jello	Meat Loaf Buttered Potatoes Jello w/ Fruit Bread, Butter Milk Cake
<u>Day 5</u>			
Snack:	Vanilla Wafers, Milk	Popsicle	Ham Biscuit, Milk
Lunch:	Fish Squares Mashed Potatoes Buttered Peas Hot Rolls, Butter Milk	Broiled Bologna Creamed Potatoes Green Peas Brown Rolls, Butter Milk Sliced Pineapple	Bologna Cup w/ Potato Salad Buttered Corn Cole Slaw Bread, Butter Milk Rice Pudding

polyethylene collection bottles and frozen immediately at the school. The frozen samples were picked up the day of or within two days following collection and placed in the laboratory freezer.

Individual samples of the initial collection of urine were stored in the original collection bottles to be thawed and refrozen as needed for creatinine assays, and original and duplicate hydroxyproline assays. No preservative was added to the samples. Repeated assays indicated degradation of the hydroxyproline during storage, and therefore were deemed inaccurate and are not included in this study.

Urine samples were collected by the same procedure the final week of the "Head Start" Program. Because of the problem encountered with the initial urine storage and assays, the samples were divided into three parts, placed under toluene and frozen immediately after collection. They were thawed only at the time of the assay. Urinary creatinine content and urinary hydroxyproline assays were determined simultaneously.

Hydroxyproline Assay

The modified Kivirikko, Laitinen and Prockop (36) method was used in determining urinary hydroxyproline values. Newly prepared reagents were used for both the initial and final urinary hydroxyproline analyses and were stored in a refrigerator under toluene until used. Hydroxyproline values are expressed as moles of hydroxyproline per mole of creatinine.

Hemoglobin Determination

The hemoglobin content of the blood was determined by the oxyhemoglobin method described in the Klett-Summerson Clinical Manual (37). The blood for the assay was obtained by finger pricks between 9 and 11 o'clock a.m. and the determinations of oxyhemoglobin in blood using 0.4% dilute ammonia solution were carried out immediately. The standard, Hemotrol, containing 15.7 gm hb/100ml of blood was obtained from the Fisher Scientific Company and manufactured by Clinton Laboratories, Los Angeles, California.

Creatinine Assay

Urinary creatinine was determined by the alkaline picrate method as modified by the Interdepartmental Committee on Nutrition for National Defense, 1964 (32).

Statistical Measurements

The Student one-tailed t test on paired comparisons of the hemoglobin values, heights and weights was used. The values of each subject at the beginning and the end of the program formed the pairs.

CHAPTER IV

RESULTS

The ages of the children attending the "Head Start" Program participating in this study ranged from 4 years 11 months to 7 years and 1 month with an average age of 6 years and 2 months. The initial and final heights and weights of each subject are given in Table II. Heights at the beginning of the study ranged from 106.7 cm to 123.8 cm and at the end of the six weeks ranged from 108.0 to 125.1 cm. The initial and final mean heights were 114.8 cm and 115.7 cm, respectively. The average increase of 0.90 cm was statistically significant at the $P < 0.01$ level.

Cheek (38) observed an average height of 115.0 cm in 5.6 to 6.75 year old normal controls. Subject nos. 2, 8, 11, 19, 23, 24, 33, 60, 65, and 88 (range from 108.0 to 111.0 cm) fell in the lower average height range for this age group when compared to the average height observed by Cheek (38). Subject nos. 25, 26, 27, 71, and 72 ranging in height from 122.0 to 128.0 cm fell within the upper average height range for this age groups.

Subject nos. 25, 44, 72, and 76 showed the largest height increases (2.3 cm, 2.5 cm, 1.5 cm, and 6.1 cm, respectively) during the six weeks of this study. These subjects also had large increases in weight. About 80% of the girls and boys exhibited height increases.

Initial weights of the subjects were from 16.8 kg to 32.7 kg with a mean of 20.7 kg. Final mean weight of the subjects was 22.2 kg with weights ranging from 17.0 kg to 32.0 kg. Fourteen subjects

Table II

Weights, Heights, and Hemoglobin Concentrations of Individual Subjects in the Study^{1/}

Subject No.	Sex	Weight (kg.)		Height (cm.)		Hemoglobin (g/100 ml)	
		Initial	Final	Initial	Final	Initial	Final
2	F	17.93	17.37	109.2	109.9	12.0	12.3
5	M	19.06	18.84	109.2	110.5	11.2	11.3
6	F	23.15	23.04	114.9	115.3	11.5	11.6
8	F	22.13	22.47	123.8	126.0	13.6	13.0
9	F	17.25	17.37	113.7	114.3	11.9	12.3
11	F	18.16	17.93	113.0	111.8	12.2	13.0
12	F	20.20	19.98	114.9	114.9	11.1	12.0
13	F	22.02	21.79	113.7	115.3	12.0	12.5
16	M	^{2/}	19.86	^{2/}	115.9	^{2/}	12.0
18	F	24.51	24.29	118.1	118.1	^{2/}	11.5
19	M	21.45	20.88	110.5	111.8	11.7	12.3
20	F	20.99	20.66	120.7	118.8	12.3	12.3
22	F	19.29	19.29	111.1	111.8	12.0	12.0
23	M	19.29	19.06	111.1	111.1	12.0	12.8
24	F	16.80	17.03	107.3	108.0	12.0	12.3
25	F	21.68	22.93	120.0	122.3	12.8	12.9
26	M	22.47	24.52	121.9	121.9	9.6	13.3
27	F	32.69	32.00	121.9	122.3	11.4	11.5
28	M	20.88	21.11	113.0	113.7	11.9	^{2/}
36	F	19.98	20.43	116.8	118.1	12.3	11.5
37	F	22.93	24.06	116.2	116.8	12.3	12.4
38	F	22.84	24.29	123.8	124.5	10.6	10.3
44	F	18.16	19.98	113.7	116.2	13.1	12.9
50	M	19.75	19.41	113.7	113.7	11.1	11.4
51	M	^{2/}	21.45	^{2/}	127.6	^{3/}	^{2/}
59	M	22.70	22.47	120.0	121.3	13.0	13.0
60	M	17.93	19.06	107.9	109.9	13.6	13.2
62	M	19.06	22.47	113.0	114.3	^{2/}	^{2/}
65	M	18.16	18.27	106.7	108.0	11.8	12.0
66	M	18.61	19.98	114.9	115.9	11.7	11.9
68	M	21.34	21.79	116.2	118.1	^{3/}	^{2/}
69	F	18.61	19.07	114.3	115.6	11.2	11.5
71	M	22.47	22.70	121.3	121.9	13.8	13.5
72	M	22.25	22.47	119.4	121.9	12.3	12.6
76	M	19.52	19.98	108.0	113.0	11.4	10.5
79	F	21.91	21.34	114.3	115.3	13.4	13.5
86	F	21.79	22.25	112.4	114.0	12.6	12.9
88	F	17.25	17.59	111.1	111.8	11.8	12.1
91	F	20.43	21.34	113.7	114.9	11.0	11.7
Mean		20.69	21.05	114.8	115.7	12.0	12.2
6-week Mean		20.88		115.2		12.1	

^{1/}Values used for comparisons only when the initial and final values are available.

^{2/}Absent the day measurements were made.

^{3/}Not analyzed.

showed a decrease, one subject showed no change and 22 showed an increase in weight. As measured by the one-tail t test, the average increase of 0.36 kg was significant ($P < 0.01$).

Twenty-seven children of the study were within the normal average weight-height line established by Sargent (39). By this criterion, nos. 23, 19 and 27 would be classed as stocky, overweight, and obese, respectively. Eight of the children were within the classification of slender (10-20% below the normal base line).

Hemoglobin concentration was determined from blood obtained by the finger prick at the beginning and the end of the six weeks (Table II). The initial and final hemoglobin values with the exception of the value for subject no. 26 were all above 10 g/100 ml of blood. The hemoglobin concentration of subject no. 26 was 9.6 g which increased to 13.3 g/100 ml of blood during the study. The hemoglobin concentration of subject 38, the next lowest hemoglobin value, did not increase during the study, the initial value being 10.6 g and the final value, 10.3 g/100 ml of blood. Four subjects showed no change, 6 showed a decrease, and 24 showed an increase in hemoglobin concentration. Seventy-one percent of the girls as compared to sixty-six percent of the boys showed increases in hemoglobin values. The overall average increase of 0.2 g hemoglobin per 100 ml of blood was significant ($P < 0.05$) as measured by the one-tail t test.

Table III gives the molar hydroxyproline to creatinine ratios and the hydroxyproline indexes observed in the subjects at the end of the study. Molar hydroxyproline to creatinine ratios ranged from 0.02 to 0.31 with the average being 0.16. The average hydroxyproline index was 2.61 with a range of 6.54 to 0.36.

TABLE III

Molar Hydroxyproline to Creatinine Ratios
and Hydroxyproline Indexes

<u>Subject No.</u>	<u>Sex</u>	<u>Molar Hydroxyproline to Creatinine Ratios</u>	<u>Hydroxyproline Indexes</u>
2	F	0.28	4.86
5	M	0.17	3.20
6	F	0.09	2.07
8	F	0.07	1.57
9	F	0.06	1.04
11	F	0.02	0.36
12	F	0.09	1.80
13	F	0.14	3.05
16	M	0.15	2.98
18	F	0.14	3.40
19	M	0.14	2.92
20	F	0.16	3.31
22	F	0.15	2.89
23	M	0.13	2.48
24	F	0.15	2.55
25	F	0.14	3.21
27	F	0.18	5.76
28	M	0.31	6.54
36	F	0.08	1.63
37	F	0.09	2.17
38	F	0.13	3.16
44	F	0.14	2.80
50	M	0.06	1.16
51	M	0.16	3.43
59	M	0.08	1.80
60	M	0.10	1.91
62	M	0.10	2.24
65	M	0.14	2.56
66	M	0.09	1.80
69	F	0.09	1.72
71	M	0.15	3.40
72	M	0.09	2.02
76	M	0.16	3.20
79	F	0.08	1.71
86	F	0.12	2.67
88	F	0.12	2.11
91	F	0.05	1.07
6-week mean		0.12	2.61

CHAPTER V

DISCUSSION

Growth in childhood, expressed by increases in height and weight, proceeds at a rate characteristic of the individual child when adequate quantities of the proper nutrients are consumed. The conventional practice of indicating the nutritional status and interpreting data on the child is to compare his height or weight in relation to his age with some selected height-weight standard (39). Factors such as pre-natal and post-natal nutrition, inherited tendency, activity, and stress conditions lead to wide variations in growth patterns among children. Sargent (39) suggested the use of weight in relation to height as a parameter of nutritional status. In this study 27 subjects were within the normal average weight-height line compared to 8 within the classification of slender as established by Sargent (39). None of the 14 subjects who lost weight showed a decrease in hemoglobin concentration. Subjects within the 10 to 20% below average weight-height base line exhibited less tendency to increase as rapidly in weight and height (averaging 0.34 kg and 0.98 cm, respectively) than those children within the normal average weight-height base line who averaged increases of 0.77 kg and 1.42 cm.

Sargent (39) states that weight increases at a constant rate of 1.8% for each centimeter increase in height and that the relationship between weight and height is about the same for boys and girls before puberty. The tendency toward an increase in height in this

study was about the same for both sexes. The weight difference between the boys and girls of less than 120 cm was very slight (0.8 kg). The average weight for the girls 120 cm and taller was 25.8 kg as compared to 22.7 kg for the boys of the same height range.

Mussen and Heebøll-Nielsen (1956), as cited by Sargent (39) observed that the rate of increase in weight per unit increase in height is slightly higher for girls than for boys. This was not the case with the children in this study as the boys showed an average weight gain of 1.45 kg as compared to 1.16 kg for the girls.

The need for iron in the human diet varies greatly at different ages and under different circumstances. There is a demand for an increased intake during growth when the blood volume is expanding and a more rapid hemoglobin synthesis is required. A function of hemoglobin is its ability to take oxygen to the body tissues and to return carbon dioxide to the lungs. This function depends upon adequate amounts of iron. Because of inadequate intakes of iron, hemoglobin values in children are frequently observed. Although the criterion for classifying anemia varies among authorities, a child with a hemoglobin concentration below 10g /100 ml of blood is considered by most authorities to be anemic (40). By this criterion only subject no. 26 of this study would be considered undesirably low.

Anemias may result from many factors other than inadequate iron intake such as hemolytic anemia when there may be insufficient Vitamin E in the diet. This is rare (especially up to age 10) as the dietary consumption is usually adequate, the main sources being

vegetable fats (41). Other macrocytic anemias may also develop when there is insufficient intakes of folic acid, thiamine or riboflavin. Unglaub (42) in a National Nutrition Survey, found a large number of folic acid, thiamine and riboflavin deficiencies in the lower socio-economic urban areas of New Orleans, Louisiana. Analysis of his Survey Data at this writing was too premature to suggest how these deficiencies and other essential food factors may interfere with biologic alterations and with hemoglobin synthesis (42).

Inadequate Vitamin B₁₂ has been implicated as one causative factor of macrocytic as well as megaloblastic anemia, however, Macdougall and Ross (43) found no evidence of megaloblastic bone marrows and no correlation between the serum Vitamin B₁₂ levels and the degree of anemia in kwashiorkor and marasmic children. The lack of correlation occurred even though the diets of their malnourished children were practically devoid of animal protein and thus, a rich source of Vitamin B₁₂(43).

Shadihi et al. (44) refer to animal studies by Weech and colleagues (1937) that demonstrated hemoglobin synthesis decreased as a result of an inadequate protein intake. The effect of a protein deficiency on the development of anemia has been demonstrated many times.

Most of the iron in foods occurs in the ferric state and many foods containing iron also contain trace amounts of copper (45). Copper is required for iron utilization (17). In an acid medium as the hydrochloric acid of the gastric region, or organic acids in

foods such as ascorbic acid or reducing substances as the SH-groups of cysteine, the ferric compounds of foods are converted to the more soluble and readily absorbable ferrous state (17).

The hemoglobin concentrations of most of the subjects in this study were well within normal ranges as reported for children of this age group (40). It is not clear why 6 of the subjects exhibited a slight decrease in hemoglobin values with the exception of no. 8 who reported illness the evening prior to hemoglobin determination. The illness may have resulted in an increased blood volume. It is known that a decrease in hemoglobin levels is one of the last symptoms to appear in iron deficiency anemias (35). The low hemoglobin status of the subject that exhibited the lowest hemoglobin concentration was most probably due to an inadequate iron intake as the physical examination prior to entry into the "Head Start" Program did not indicate other clinical abnormalities. Subject no. 26, who showed the greatest percentage increase in hemoglobin concentration, was the most economically deprived child in the "Head Start" Program in that particular school. Number 26 also showed a high increase in weight but no change in height. There is usually an increase in weight before height (46) in refeeding following subnutritional states. One explanation for the over-all increase in hemoglobin levels of the "Head Start" children during the six weeks might be that their diets consisted of better nutrient quality, were richer in animal protein and ascorbic acid. Home visitations revealed these particular nutrients to be consumed in smaller quantities. In relation to this, many of the families grew gardens and preserved some of

their food. Dried beans, containing considerable iron (47) were observed to be a staple food in the diets of most of these children. Intakes of large quantities of these foods may account for the low frequency of anemia in these subjects.

Level of creatinine excretion is used frequently as an indicator of lean muscle mass (46) and is almost constant in a given individual (17). When expressed as the creatinine coefficient it is an expression of body size and is nearly constant for different individuals of the same age and sex (17). In protein-depleted children the low level of urinary creatinine reflects a reduction in muscle mass, both absolute and in relation to body weight. Thus, creatinine excretion when compared to body weight can serve as an index of muscular depletion. Lower creatinine excretion level for children of the low socio-economic status as compared to the controls have been reported (46).

Urinary hydroxyproline peptides appear to originate either from a soluble collagen pool (11) or from the degradation of collagen (5, 27, 48). Lindstedt and Prockop (23) have demonstrated that the urinary hydroxyproline peptides are derived from the breakdown of collagen. Urinary hydroxyproline increases with increase in age, height, and weight (6, 49) and the level of excretion seems to be closely associated with growth and a change in weight. The low excretion values in non-growing or slowly growing children and the high excretion levels at puberty are further evidence for the relationship between hydroxyproline and growth (2, 4, 5, 6, 11, 12, 25, 27, 38). Lindstedt and Prockop (23) found less rapidly metabolized

hydroxyproline pools in older experimental animals. Thus, the loss of soluble collagen may explain the decreased excretion of urinary hydroxyproline in older rats (23). A similar difference in the excretion levels of adults and of children has been observed (4, 11).

The hydroxyproline to creatinine ratio can be used as an expression of growth rates, as an index of nutritional status in children (2), and as a measure of metabolic aging of an animal's collagen (23). The hydroxyproline to creatinine ratio has been shown to be higher in younger children and lower in older children (12). Cheek (38) observed the hydroxyproline to creatinine ratio decreased from 3 to 18 months of age, then became constant suggesting the rate of collagen turnover paralleled the increment in muscle mass as growth progresses. The average hydroxyproline to creatinine ratio of 0.12 observed in the children of this study compared with the averages (0.11 to 0.15) in normal children near the same age reported by Cabacungan (12), however, there were more children in the "Head Start" Program with very low hydroxyproline to creatinine ratios. The noticeable difference in the low values in some of the children in this study as compared to the values reported (2, 12, 31) for severely malnourished children may be partially attributed to the older ages of "Head Start" children and the variations from one laboratory to another. The very low values were found in children who excreted very dilute urine.

The hydroxyproline index is a ratio of hydroxyproline to creatinine excretion to which weight is introduced to obtain a normal range independent of age between 1 and 6 years (2). Whitehead

that

(2) reported that the normal range of hydroxyproline index values for children in this age group to be 2.0 to 5.0 with a mean value of 2.9. The mean hydroxyproline index observed in the "Head Start" children closely approximates the mean value of 3.0 observed by Whitehead (2). The children of this study showing indexes ranging from 0.36 to 1.0 originated from homes which have little variety in their diet. There was no correlation between hemoglobin values and the hydroxyproline index.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The study was undertaken to evaluate the effect of the daily consumption of one nutritionally adequate meal on "Head Start" children as indicated by comparing hydroxyproline to creatinine ratios and the hydroxyproline index values at the beginning of the program with those at the end of the program.

Data were collected from 39 pre-school children who participated in the six-week "Head Start" Program. Height and weight measurements of the children were made without shoes.

Urinary hydroxyproline was determined by the procedure as modified by Kivirikko, Laitinen and Prockop and creatinine was determined by using the Picrate Method modified by the Interdepartmental Committee on Nutrition for National Defense. The amount of hydroxyproline in random samples of urine and the molar ratio of hydroxyproline to creatinine were calculated. Hemoglobin values were determined by the oxyhemoglobin method as described in the Klett-Summerson Clinical Manual.

The hydroxyproline to creatinine ratios and the hydroxyproline index values were comparable to those previously reported for normal children within the 6 year old age group.

Subject no. 26 had an undesirably low initial hemoglobin level of 9.6 g and a final value of 13.3 g/100 ml of blood. All other subjects had hemoglobin values above 10 g/100 ml of blood. Four subjects had no change in hemoglobin values, 6 showed a decrease

and 22 subjects had an increase in the hemoglobin level at the end of the study. The over-all increase in hemoglobin averaged 0.29 g/100 ml of blood which was statistically significant ($P < 0.05$).

The average increase in weight was significant at the $P < 0.01$ level. Twenty-seven of the subjects were within the normal average weight-height base line as established by Sargent (39), one was overweight, one was obese. Eight of the children were within the 10 to 20% below normal weight-height base line. Thirty of the children increased in height during the six-week period, 5 had no change in height. This increase was statistically significant. Although no data from children in the "Head Start" Program were obtained for controls, it is likely that the improved hemoglobin status was associated with the nutritionally adequate meal provided by the program.

BIBLIOGRAPHY

1. Latham, M. C. 1968 "The U. S. A. hunger debates". Review, Cornell University, Ithaca, New York.
2. Whitehead, R. G. 1965 Hydroxyproline:creatinine ratio as an index of nutritional status and rate of growth. *Lancet*, 11:567.
3. Rutishauser, I. H. E., and R. G. Whitehead 1969 Field evaluation of two biochemical tests which may reflect nutritional status in three areas of Uganda. *Br. J. Nutr.*, 23:1.
4. Ziff, M., A. Kibrick, E. Dresner, and H. J. Gribetz 1956 Excretion of hydroxyproline in patients with rheumatic and non-rheumatic diseases. *J. Clin. Invest.*, 35:579.
5. Prockop, D. J. and A. Sjoerdsma 1961 Significance of urinary hydroxyproline in man. *J. Clin. Invest.*, 40:843.
6. Smiley, J. D. and M. Ziff 1964 Urinary hydroxyproline excretion and growth. *Physiol. Rev.*, 44:30.
7. Dickerson, J. W. T. and R. A. McCance 1964 The early effects of rehabilitation on the chemical structure of the organs and whole bodies of undernourished pigs and cockerels. *Clin. Sci.*, 27:123.
8. Montgomery, R. D. 1962 Muscle morphology in infantile protein malnutrition. *J. Clin. Path.*, 12:511.
9. Garrow, J. S., K. Fletcher and D. Halliday 1965 Body Composition in severe infantile malnutrition. *J. Clin. Invest.*, 44:417.
10. Anderson, J., D. W. Bannister and R. W. S. Tomlinson 1965 Total urinary hydroxyproline excretion in normal human subjects. *Clin. Sci.*, 29:583.
11. Jasin, J. E., C. W. Fink, W. Wise and M. Ziff 1962 Relationship between urinary hydroxyproline and growth. *J. Clin. Invest.*, 41:1928.
12. Howells, G. R., B. A. Wharton and R. A. McCance 1967 Value of hydroxyproline indices in malnutrition. *Lancet*, I:1082.
13. Gabacungan, N. B. 1968 Use of hydroxyproline excretion for assessing the nutritional status of preadolescent children. Thesis, M. S. VPI, Blacksburg, Virginia.

14. Andelman, M. B. and B. R. Sered 1966 Utilization of dietary iron by term infants. *Am. J. Dis. Child*, III:45.
15. Finch, C. A. 1969 Iron Metabolism. *Nutr. Today*, 4:2.
16. Committee on Iron Deficiency 1968 Iron Deficiency in the United States. *J. Am. Med. Assoc.*, 203:119.
17. Harper, H. A. 1965 Review of Physiological Chemistry, Lange Medical Publications, Los Altos, California.
18. Peterkofsky, B., and S. Udenfriend 1965 Enzymatic hydroxylation of proline in microsomal polypeptide leading to formation of collagen. *Proc. Nat. Acad. Sc.*, 53:335.
19. Pike, R. L., and M. L. Brown 1967 Nutrition: an integrated approach. John Wiley and Sons, Inc., New York 35.
20. Stetten, M. R. 1949 Some aspects of the metabolism of hydroxyproline, studied with the aid of isotopic nitrogen. *J. Biol. Chem.*, 181:31.
21. Boucek, R. J., N. L. Nobel and J. F. Woessner 1959 The effects of tissue age and sex upon connective tissue metabolism. *Ann. N. Y. Acad. Sci.*, 72:1016.
22. Klein, L. and T. M. Terec 1966 Skeletal metabolism in early infancy: urinary hydroxyproline. *J. Ped.*, 69:266.
23. Lindstedt, S. and D. J. Prockop 1961 Isotopic studies on urinary hydroxyproline as evidence for rapidly catabolized forms of collagen in the young rat. *J. Biochem.*, 236:1399.
24. Prockop, D. J., H. R. Keiser, and A. Sjoerdsma 1963 Gastrointestinal absorption and renal excretion of hydroxyproline peptides. *Lancet*, 2:527.
25. Jasin, H. E., C. Fink, J. D. Smiley and M. Ziff 1962 Influence of growth on total hydroxyproline. *J. Clin. Invest.*, 41:1368.
26. Dull, T. A., L. Causing and P. H. Henneman 1962 Urinary total hydroxyproline as an index of connective tissue turnover in bone. *J. Clin. Invest.*, 41:1355.
27. Picou, D., G. A. O. Alleyne, J. C. Waterlow and A. Seakins 1965 Hydroxyproline and creatinine excretion in protein-depleted infants. *Biochem. J.*, 95:18.
28. Dull, T. A. and P. H. Henneman 1963 Urinary hydroxyproline as index of collagen turnover in bone. *N. E. J. Med.*, 268:132.

29. Martin, G. R., S. E. Mergenhagen and D. J. Prockop 1961
Influence of scurvy and lathyrism (odoratism) on hydroxyproline excretion. *Nature*, 191:1008.
30. Whitehead, R. G. and R. F. A. Dean 1964 Serum amino acids in kwashiorkor. *A. J. Clin. Nutr.*, 14:320.
31. Anasuya, A. and B. S. Narasinga Rao 1966 Urinary excretion of hydroxyproline in kwashiorkor. *Lancet*, I:94.
32. Interdepartmental Committee on Nutrition for National Defense 1964 Laboratory Manual for use in Nutrition Surveys. National Institutes of Health, Bethesda, Maryland.
33. Food and Nutrition Board: Recommended Dietary Allowances 1968. Wash., D. C., National Academy of Sciences, Pub. 1964 National Research Council, Wash., D. C.
34. McCurdy, P. R. and R. J. Dern 1968 Some therapeutic implications of ferrous sulfate-ascorbic acid mixtures. *Am. J. Clin. Nutr.*, 4:284.
35. Comar, C. L. and F. Bronner 1962 Mineral Metabolism. Vol II Part B. Academic Press New York.
36. Kivirikko, Kari I., O. Laitinen, and D. J. Prockop 1967 Modifications of a Specific Assay for hydroxyproline in urine. *Anal. Biochem.*, 19:249.
37. Klett-Summerson Photoelectric Colorimetric Manual, Klett Manufacturing Co., New York.
38. Cheek, Donald B. 1968 Human Growth. Lea and Febiger. Philadelphia.
39. Sargent, D. W. 1961 An evaluation of basal metabolic data for children and youth in the United States. Home Economics Research Report No. 14. United States Department of Agriculture, Wash., D. C.
40. Interdepartmental Committee on Nutrition for National Defense Woodruff, C. 1969 Nutritional Anemias in early childhood. *Am. J. Clin. Nutr.*, 22:504.
41. Tulloch, J. A. and N. K. Sood 1967 Vitamin E. Deficiency in Uganda. *Am. J. Clin. Nutr.* 20:884.
42. Unglaub, W. G. Tulane University Dept. of Med. New Orleans, Louisiana. Personal Communication.

43. Maccougall, L. G. and G. I. M. Ross 1960 Serum vitamin B₁₂ concentrations in kwashiorkor and marasmus. *J. Ped.* 57:589.
44. Shadihi, N. T., L. K. Diamond and H. Shwachman 1961 Anemia associated with protein deficiency. *J. Ped.*, 59:533.
45. Zook, E. G. and J. Lehmann 1968 Mineral composition of fruits. *J. A. D. A.*, 52:225.
46. Standard, K. L., V. G. Wills, and J. C. Waterlow 1959. Indirect indicators of muscle mass in malnourished infants. *Am. J. Clin. Nutr.*, 7:271.
47. Watt, B. K. and A. L. Merrill 1963 Composition of foods. Agriculture Handbook No. 8 Consumer and Food Economics Division Agricultural Research Service United States Department of Agriculture Washington, D. C.
48. Prockop, D. J. 1964 Isotopic studies on collagen degradation and the urinary excretion of hydroxyproline. *J. Clin. Invest.*, 43:453.
49. Jones, C. R., M. W. Bergman, P. J. Hettner, and W. W. Pigman 1964 Urinary hydroxyproline excretion in normal children and adolescents. *Proc. Soc. Exp. Biol. Med.*, 115:85.

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HEMOGLOBIN CONCENTRATION AND HYDROXYPROLINE EXCRETION OF HEAD
START CHILDREN IN MONTGOMERY COUNTY, VIRGINIA

Ruth H. Bollman

Abstract

A decrease in hemoglobin concentration is one of the last stages of physiologic alterations in iron-deficiency. To gain more information on the effect of the daily consumption of one nutritionally adequate meal by children from a low socio-economic status, hemoglobin concentration was measured on 5-to-7-year-old children and the ratio of urinary hydroxyproline to creatinine was calculated.

The mean increase in hemoglobin values during the six-week period of the study was 0.24 g hemoglobin/100 ml of blood. Initial and final hemoglobin values were all above 10 g/100 ml of blood with the exception of an initial hemoglobin concentration of 9.6 g/100 ml of blood for one subject. Twenty-four subjects had increases in hemoglobin concentration; the largest percentage of increases were by the girls and by the children weighing above 22 kg.

Twenty-two subjects of the study gained weight. The girls showed a greater tendency to gain. Children within the slender classification of 10-20% below the average normal weight-height base line gained weight less rapidly as compared to those within or above the average normal weight-height base line. The subjects that lost weight either had no changes in hemoglobin concentration or an increase in hemoglobin levels.

A larger percentage of the girls increased in height as compared to the boys. The shortest children averaged a decrease in hemoglobin concentration as compared to an increase by the tallest children.

The average molar hydroxyproline to creatinine ratio at the end of the study was 0.12. The girls losing weight had a higher hydroxyproline to creatinine ratio as compared to the higher hydroxyproline to creatinine ratio for the boys gaining weight. The mean hydroxyproline index of the children in this study approximates the mean value of 3.0 reported for normal children of this age group.