A CALCIUM RETENTION STUDY OF AN ELEVEN AND ONE-HALF YEAR OLD BOY ON A SELF SELECTED DIET

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A CALCIUM RETENTION STUDY OF AN ELEVEN AND ONE-HALF YEAR OLD BOY ON A SELF SELECTED DIET

By

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INTRODUCTION

Many studies have reported calcium retentions of subjects in early childhood and of adult age, but few workers have studied the early adolescent. Due to the lack of information concerning the retention, and therefore the requirement, an interpolated value for the daily requirement for calcium for the early adolescent has been recommended (Recommended Dietary Allowances, 1948).

The daily requirement of calcium may be determined by the use of two types of information. As pointed out in a review by Holmes (1945), this information involves metabolism studies to show the quantity of calcium used by individuals, and records of measurement of growth and body composition to show where the calcium has been used.

It is desirable that more data be provided from which the dietary requirement of calcium for early adolescent children, may be determined.

The object of this study, then, is to supply additional information concerning the calcium retention and increment in body size of an early adolescent boy.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

There are two principal determinants of the requirement for dietary calcium during the growth period (Holmes, 1945). These determinants are the rate of growth and change in composition of the skeleton and the percent of intestinal utilization of the mineral. Various means for obtaining data concerning these two determinants will be mentioned. Each has its disadvantages.

The chemical analysis of the skeleton at various ages provides pertinent information, but data from such studies are limited. X-ray techniques showing the new centers of ossification (Todd, 1938), and determining the bone density (Mack, et al., 1939), have demonstrated the value of that medium in determining skeletal growth and composition. However, a question arises as to the physiological advisability of periodically exposing children to the x-ray. Anthropometric measurements of the skeleton at regular intervals also provide data from which calcium accretion may be estimated (Vernar and Todd, 1933, quoted by Holmes, 1945).

The method for determining intestinal absorption is the balance or retention study. By using data from several balance periods, the percent of utilization may be calculated. Holmes' (1945) formula for the calculation of percent utilization of calcium follows:

$$\frac{\text{Ca. balance during period A} - \text{Ca. balance during period B}}{\text{Ca. intake during period A} - \text{Ca. intake during period B}}$$

times 100 equals percent of utilization.

This formula, requires two levels of intake within the range of positive balance. Period A represents a high calcium intake and

period B, a low calcium intake.

Studies conducted by the use of the methods mentioned provide data from which the daily requirement of calcium may be estimated. Since this paper is concerned with only one of the methods, the retention study, the literature reviewed will be confined to factors affecting the retention of calcium.

First, the length of the experimental period was observed to affect the variability of the results (Porter-Levin, 1933-1934). The retentions for two healthy pre-school children on a balanced diet were observed for a period of sixty days, and from fifteen to twenty-one consecutive days were required before the maximum range of retention was reached. Balanced studies are extremely time consuming and expensive so periods made as short as possible for reliable results are desired.

The "degree of saturation" of the body tissues has been observed by several authors to affect the retention of calcium. In an early study by Daniels (1934), a difference in retention of calcium was observed between children who had been well nourished and those who had been poorly nourished. The poorly nourished group retained more calcium.

Fairbanks and Mitchell (1936), using rats as experimental animals concluded that the "retention of calcium by growing animals under conditions of adequate nutrition measured the requirement of calcium only when the calcium stores had been saturated by appropriate pre-feeding".

Nicolaysen (1943), also observed higher retentions for "unsaturated" subjects. He studied the calcium absorption in relation to the body saturation of that element in the rat. Two groups of rats were fed diets differing only in calcium content for four weeks, then both groups

were given the larger amount of calcium per day for two weeks. The average retentions for these two weeks were higher for the group that had received less calcium previously.

Although the majority of the studies seem to indicate that unsaturated tissues tend to induce an increase in calcium retention when the intake is more plentiful, Stearns, et al., (1941), did not find this true. These authors used malnourished children and observed that the calcium retentions were below the retentions for normal children, but gradually increased as intake increased. They concluded that undernutrition following illness descreased the efficiency of the intestinal mucosa for absorbing calcium. Daniels (1941) studied the calcium retentions for three skeletally retarded boys and expected reduced retentions when the tissues were saturated. However, the retentions remained high throughout the study.

Never-the-less, as a result of these studies and others, many investigators have provided preliminary periods in their work to enable the subjects to adapt themselves to the experimental regimen, and to saturate the tissues if possible.

For example, if the subject has been placed on a controlled diet and the immediate response to the diet is not a variable in the study, sufficient time should be allowed for complete adaptation of the subject to the new regimen. If the effect of change in the diet is the variable to be studied, then a preliminary period for adaptation is not necessary.

Hunscher, et al., (1936), provided one quart of milk per day for three months to ten children in order to avoid any unfilled stores in

the bodies, before a comparison of skeletal maturity of the children to the calcium storage was made. Also to avoid the effects of possible previous deprivation of calcium, Stearns (1934), provided each child on her study with the experimental diet until the calcium retention remained approximately the same for two successive periods.

Another factor influencing the calcium retention is the calcium intake. The quantity of calcium intake has been observed to affect the retention of that element within a certain range which varies for each individual. Johnston (1944), observed a threshhold below which no calcium retention occurred and a physiological ceiling, above which no increased retention was observed. In this study, the calcium retention in relation to calcium intake, increased as did the intake until a given level for each subject was reached. When the "physiological ceiling" was reached the retention dropped. No ill effects were reported as a result of the extra intake of calcium.

Kinsman, et al., (1937), also observed an apparent physiological ceiling. With intakes of 370, 610 and 880 mg. of calcium per day, five pre-school girls showed a retention which indicated that the 610 mg. intake was more nearly optimum. The retentions for 370 and 880 mg./day intake were lower than the retention for 610 mg./day.intake.

However, no physiological ceiling was reported by Stearns (1934). She reported that for each child the amount of calcium retention increased with increased intake. The series of studies included 347 balance periods using 66 infants and children from 7 weeks to 12 years of age. The range in gm./day of calcium intake was not reported in this paper.

McKay, et al., (1942), observed the affect of intake on retention using young women as subjects. One hundred twenty five women were used, and the intake was found to be significantly related to retention at all intakes studied. The range of intake for this group of women was from 0.322 to 2.323 gm./day, and the mean calcium intake was 0.941 gm.

Hunscher, et al., (1933), observed children differing in their respective retentions on each calcium intake. The studies included twenty days, fourteen days, thirteen days and eleven days consecutive observation for each of four children, the first three of them ten years old. When the diet contained one gm. calcium per day the average retentions were 0.26, 0.31, 0.30 and 0.48 gm./day, but when the intakes of the first three children were increased to 1.9 gm./day, the average retentions were increased to 0.55, 0.64, and 0.67 respectively. Although the retentions varied, each increased with increased intake.

To illustrate further the individual variations of retention a study by Hunscher (1936) is cited. She observed healthy children who were five to eight years old and showed no deficiency of calcium stores as determined by the x-ray. The subjects received equal intakes of calcium. The retentions per day over periods of from twenty-five to sixty-five days varied from 0.27 to 0.48 gm./child.

The relationships of the intakes of other substances to the calcium retentions have been reported in various studies. The calcium and phosphorus retention ratios,. the quantity of nitrogen ingested, and the addition of vitamins D or C seem to be the most important and will be discussed here.

The effect of different intakes of protein has been studied by McCance and others. McCance (1942), suggested that "very little calcium would be absorbed if no protein or amino acids were in the diet", and reported results from metabolic studies on five healthy adults. The results showed that increasing protein intake increased the calcium and magnesium absorption. McCance in his review mentioned that Mellanby, in 1921, observed improvement in canine rickets by feeding lean meat. In 1940, Kempster, et al., in determining the relation between the utilization of calcium in milk and the calcium in dicalcium phosphate, observed that dicalcium phosphate was not superior to milk as a source of calcium. McCance suggested as a reason, that "the better utilization of the calcium in the milk was due to the presence of protein".

Stuart (1945), observed a definite correlation between the amount of protein in a mother's diet and the length of the baby at birth.

However, the results from a study by Hawks, et al., (1942), on the interrelationship of calcium, phosphorous and nitrogen in metabolism of the pre-school child, indicated that increased protein had no effect on calcium absorption or retention.

Calcium and phosphorous are both found in the bony tissues and musculature, or soft tissues, but the ratios are different. During growth, when a subject retains more phosphorous than calcium, it is usually suggested that soft tissue has been formed, and, similarly when more calcium than phosphorous has been retained, bony tissue has been formed.

A dietary ratio of one part of calcium to one part of phosphorus has been found to give desirable retentions during periods of rapid growth (Hunscher, 1933). The subjects varied in age from three to ten years. The retentions of calcium and phosphorous respectively for three of the subjects were 0.26, 0.31, and 0.30 gm. and 0.36, 0.40, and 0.35 gm. When the intakes of calcium and phosphorous were increased but the intake ratio was kept about the same, the retentions of both elements were increased.

It is known that vitamin D is required for the efficient use of calcium. Vitamin D induces an increased "net absorption" of calcium from the intestines, and so alters the body fluids that bone salts may be incorporated into growing bone.

Johnston (1944), observed the calcium retention of adolescent girls with a range of daily doses of vitamin D from 0 to 5000 I.U. When calcium intake was given in quantities to produce no further increase in calcium retention, the addition of 650 I.U. of vitamin D resulted in the highest calcium retention of the series. Johnston found that the addition of vitamin D would reduce the negativity of calcium balance and promote a positive balance, only if the intake of calcium were marginal; however he suggests that supplementary vitamin D during the accelerated growth in height during early adolescence would be comparable to that period of growth during infancy.

In areas where an abundance of sunlight is available and the habits of living are such that the body is regularly exposed the value of the ingestion of vitamin D after the period of infancy may be questioned.

The effect of tropical sunlight on the development of bones of children in Puerto Rico was studied by (Eliot, 1933). The incidence of

rickets was very low as determined by calcium and phosphorous blood tests and x-rays. The few cases of rickets found were in invalids or persons who had reasons for not spending time in the sunlight.

The studies made by McKay (1943), and Greenberg (1939), used young women as subjects and reported no effect of vitamin D on calcium retention. But these subjects were past the rapid growth period and probably received enough vitamin D from the sunlight for proper utilization of the calcium intake.

Vitamin C as a factor influencing calcium retention was studied by Mallon and Rosenblatt (1946). Two groups of rats in positive calcium balance were each fed a basic diet. Grapefruit juice was added for thirty-six days to the diet of one group of animals. When the carcasses were ashed, and the calcium determined, the result showed that the group fed grapefruit had a much higher calcium content.

As reported in Brenneman's Practice in Pediatrics, a vitamin C deficiency may cause a cessation of growth of the long bones, and the ends of the bones may mushroom out and acquire a grotesque shape similar to the condition in rickets. Scurvy interferes with the mechanism for removal of calcified cartilage matrix and suppresses the formation of new trabeculae.

Thyroid medication in small doses resulted in increased retention of calcium for children on a constant intake of food (Johnston, 1941). When the doses of thyroid were large the retention was decreased. The doses of thyroid when given to subjects whose basals were lower than average, tended to raise the metabolism, and during growth, if the metabolism were not stimulated beyond normal, calcium retention was

increased (Johnston, 1939). Higher retentions were observed when the basals approached normal; above or below normal, retentions were decreased.

It is believed that the pH of the intestine is the factor that influences the solubility of calcium and therefore the intestinal absorption of that element. The solubility is greater in an acid solution and less in alkali; therefore most of the absorption should take place in the upper segment of the small intestine. Some of the calcium, however, will continue through the alimentary tract and be excreted. Christiansen (1936), discussed the conditions governing calcium absorption and reduced them to one main controlling agent, namely the intestinal pH. She observed a small amount of calcium excretion from the intestine and no active excretion into the intestine. Adolph (1940), also studied the calcium excretion into the intestine. He observed the effect of calcium in the form of diborgluconate injected intravenously into rats on the calcium content of the intestinal tract, and no significant increase was noted.

Jones (1942), investigated the effect of different substances on the pH of six portions of the intestine of the rat and the simultaneous calcification of the bone. A greater acidity was found in the lower ileium and a definitely more antirachitic action was observed when oleic acid was ingested than when sodium oleate was given. Vitamin D did not lower the pH beyond that of oleic acid, but a greater antirachritic action was noted.

The effect of the sex hormone estrogen, administered in varying amounts between 12,000 and 36,000 units to apparently normal girls

during adolescence (Johnston, 1941) was a decrease in calcium retention by five out of six subjects. The decrease was due to increased losses in both urine and feces. The administration of stilbestrol to one normal girl at puberty also resulted in a depressed calcium balance.

Additional factors influencing calcium retention as pointed out by McCance (1943), Handler (1947), Silberberg (1948), and others are depletion of the subject in one or more factors of the vitamin B complex, caloric restrictions, and seasonal and annual changes. McCance reported the retentions were higher for a group of children in the summar months than for other seasons, probably because more sunshine was available.

Handler demonstrated in young rats that caloric restriction to one-half of ad libitum feeding, other nutrients being adequate, resulted in a complete cessation of both skeletal and generalized body growth. The plasma concentration of calcium was normal in all rats.

For B complex depleted rats (Silberberg, 1948), there was a retardation and subsequent cessation of growth of cartilage and bone. With the return to a complete diet the skeletal growth was resumed and normal conditions were restored.

The calcium retention is affected by the rate of excretion of that mineral from the body, mainly by way of the intestines and kidneys. By far the larger quantity is excreted in the feces, but the excretion in the urine is more significant (McCance, 1942, and Knapp, 1947). McCance (1942) after observing that urinary excretions of normal persons rise and fall with intestinal absorptions, concluded that specific excretions "might be used as an index of change in amount of calcium absorption".

Knapp (1947) did not observe any relationship between the retention and urinary excretion. She states that the quantity of urine calcium is dependent on an endogenous factor, presumably endocrine, and also on calcium intake per unit of weight. The urinary calcium expressed as percent of intake varies inversely with intake per kg. of weight, and is an exponential function of the latter.

Among the few investigators who have made contributions to the calcium retention as affected by the preadolescent spurt of growth are Wang, Johnston, Stearns and Jeans, and Sherman and Hawley.

Wang (1928 - 1936), in a series of studies, used twenty-three adolescent girls ranging in ages from eleven to fifteen years, but investigation revealed that these subjects were deficient in calcium stores. The average intake of calcium was 1604 mg./day, and the average retention was 11 mg./kg. of body weight per day, or 417 mg. with a range of 79 to 823 mg./day. About eight percent of the calcium intake was eliminated through the kidneys and sixty-five percent was fecal loss.

Johnston (1939 - 1950), in a number of investigations, studied the factors influencing calcium retention during periods of growth. The subjects were girls, and the retentions were observed under conditions of the reinfection type of tuberculosis. A variation of some intake factors was also studied. Some of the results of Johnston's work and other authors are recorded in Table 1.

The range of retentions found by the workers indicated in Table 1 is from 0.070 to 0.825 gm./day. The greatest retention observed was for a normal subject on a mixed diet, and the least retention was for a malnourished child. Other data include retentions of subjects under various conditions such as added estrogen and thyroid medication.

Table 1

CALCIUM RETENTION DATA FROM THE LITERATURE CONCERNING SUBJECTS OF PRE-ADOLESCENT AND ADOLESCENT AGES

Investigator	Age of subjects	Sex of subjects	Calcium intake	Calcium retention	Calcium retention per kg. weight	Conditions of study
			gm./day	gm./day	gm./day	
Sherman and Hawley (1922)	11 yr.	F	0.748	0.456	0.013	Normal subject on "mixed diet"
			0.994	0.729	0.021	
			1.273	0.782	0.022	
			1.794	0.825	0.023	
			1.262	0.636	0.018	
			1.015	0.544	0.015	
			0.741	0.403	0.011	
	12 yr. 9 mo.	F	1.027	0.406		Normal subject on "mixed diet"
			1.047	0.473		
			1.057	0.436		
			1.030	0.226		
			1.076	0.326		
			1.091	0.229		
			1.002	0.163		
			1.056	0.090		
			1.053	0.333		
			1.068	0.371		
Wang (1936)	12 yr.	F	1.364	0.529		Probably had calcium deficient stores
			1.799	0.591		
			1.799	0.466		
Johnston (1941)	13 yr.	F		0.570		No estrogen
				0.299		Estrogen
				0.433		No estrogen
Johnston (1941)	13 yr.	F		0.503		No estrogen
				0.328		Estrogen
				0.458		No estrogen

Table 1
(continued)

Investigator	Age of subjects	Sex of subjects	Calcium intake	Calcium retention	Calcium retention per kg. weight	Conditions of study
			gm./day	gm./day	gm./day	
Johnston (1941)	12 yr.	F		0.247		No thyroid
				0.310		One grain thyroid
				0.359		One grain thyroid
				0.193		Two grains thyroid
	11 yr.	F		0.245		No thyroid
				0.215		One grain thyroid
				0.342		Two grains thyroid
				0.346		One grain thyroid
Stearns, et al., (1941)	11 yr.		1.586	0.235	0.008	Malnourished children were used
			1.952	0.288	0.009	
			1.927	0.169	0.005	
	12 yr.		2.353	0.509	0.016	
			1.955	0.447	0.013	
			2.056	0.369	0.010	
	13 yr.		1.937	0.179	0.006	
			3.252	0.554	0.016	
			3.325	0.700	0.020	
			2.288	0.070	0.002	

EXPERIMENTAL PROCEDURE

EXPERIMENTAL PROCEDURE

This study reports the calcium intake and retention of one male subject, eleven and one half years of age, from November to March, 1950 - 1951. The diet was self selected. The experimental study contained a preliminary period and four sampling periods; the sampling periods were one week in length and approximately one month apart. Period one was the preliminary period and collections were made and analyzed only for practice. The results for period one are not recorded in this paper.

At the beginning of the experimental period, an examination to establish the physical status of the subject was made by a physician[1]. The examination consisted of the evaluation of the weight and subcutaneous tissue in relation to height, and the condition of teeth, gums, and skin. A patch test and chest x-ray for tuberculosis, a throat culture and an x-ray of the hands to determine the osseous development also were made.

The subject weighed 32.9 kilograms and was 140.5 centimeters tall at the beginning of the study. The subject, described as the wiry type possessed very little excess subcutaneous tissue. The subcutaneous tissue present, however, was firm, muscular, and possessed "spring". The subject was very active and seemed to have an abundance of energy.

The skin of the arms and face was clear, smooth, and moist; and the lips were pink, moist, and full. The condition of the oral cavity was

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[1] Dr. Edith H. Kent, Lansing, Michigan

good at the time of the study. The teeth were clean, seemingly well formed, and all cavities were filled. The gums were pink, firm, and filled between the teeth well. However the six year molars contained very large fillings. Most of the temporary teeth had been badly decayed before they were extracted. No temporary teeth remained.

The subject had received ample quantities of milk throughout infancy and early childhood. The child was a breast fed baby, and had consumed between one and two quarts of milk per day for the last several years. Scars were present indicating a history of rickets. Protruding scapulas and the presence of slight chest beadings were observed, however the x-ray of the wrists showed normal ossification to date. The radiographic report of the subject indicated that the development of the carpal bones was well within the normal limits, as measured by the Todd Table or any other standard of bone development. A picture of the x-ray is found in Figure 1. The subject lived in the south until one year before this study was made. Additional vitamin D had not been given since infancy, but extra doses of vitamin D were again started approximately two months before the experimental period.

Results from the patch test and chest x-ray for tuberculosis were negative. Negative results also were reported for the throat culture.

As a summarization, the physical condition of the subject may be considered as good. He appeared to be a healthy, well nourished, and contented child.

Basal metabolism rates[2], hemoglobin, and plasma ascorbic acid determinations were made three times during the course of study. Blood

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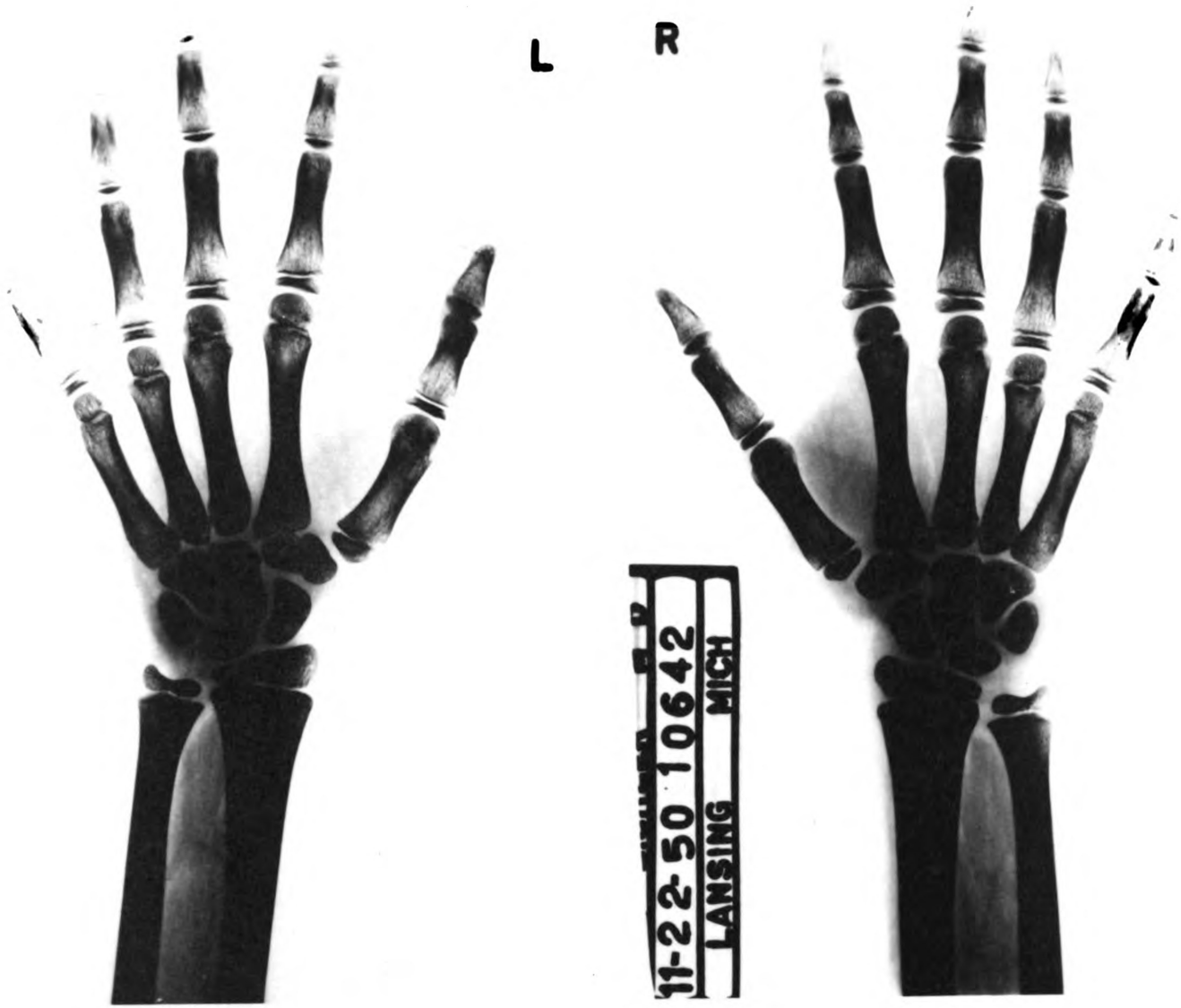
[2] A Benedict - Roth metabolism apparatus was used.

Figure I. An x-ray of the wrists and hands of the subject showing the ossification of the carpal bones.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samples were taken from a pricked finger, and hemoglobin was determined colorimetrically using a photolometer[3] and the method suggested by Waddell, Steinbock, Elvehjem, and Hart (1938). The micro-method of Farmer and Abt (1936) was used for the ascorbic acid analysis of blood.

Anthropometric measurements, taken once a month, were similar in technique to those described by Meredith (1935), Davenport (1927) and Hrdlicka (1939). The measurements[4] taken are listed below:

Standing height

Sitting height

Maximum head length

Maximum head breadth

Morphological face height

Physiological face height

Nose height

Nose breadth

Maximum length of pinna (ear)

Breadth of pinna

Hand length

Hand breadth

Foot length

Foot breadth

Girth of lower leg

Chest breadth

Chest depth

Arm span

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[3] A Cenco-Sheard photolometer was used.

[4] The Baldwin Paper Measuring Scales and Square, a metal tape, and metal spreading and sliding calipers were the instruments used. When possible the left side of the subject was measured.

Weight[5] was taken with shoes removed, but underwear and pants were kept on by the subject. Similar articles of clothing were worn at each weighing.

Grip strength[6] was determined using a hand grip dynomometer. The subject was instructed to take a firm hold and exert the maximum strength possible without allowing the arm to come in contact with the trunk or lower extremity. One trial for each hand was made and the record was taken in kilograms.

The blood pressure[7] and the pulse rate[8] was taken each time the subject was measured.

A number of the measurements were discontinued for the last periods. It was felt that the growth changes by these measurements were not significant for an experimental period of only four months.

The diet was well balanced and checked by reference to the "Basic 7". The menus were made each week in advance, and foods were chosen that were seasonal and easily prepared. A typical dietary record for one day is given in Table 2. A calculated analysis of that days dietary is found in Table 3. The subject ate from the family table, and the method of

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[5] A Fairbanks Scales was used.

[6] A Narragansett dynomometer was used.

[7] A Mercury monometer and cuff was used.

[8] The pulse rate was determined as the sum of two consecutive half minutes.

Table 2

FOOD INTAKE RECORD FOR THE SUBJECT

FOR JANUARY 15, PERIOD III

Meal	Foods Eaten	Weight in Grams
Breakfast	Orange halves	70
	Cooked rolled oats	150
	Oleomargarine	10
	Sugar	4
	Scrambled eggs	35
	Enriched bread	25
	Milk	300
Lunch	Vegetable soup	185
	Roast pork	50
	Bread	55
	Salad dressing	10
	Baked custard	110
	Milk	450
	Cookie	10
	Orange	230
Dinner	Grilled hamburger	85
	Bread	100
	Celery	25
	Lettuce	25
	Corn	70
	Milk	375
	Oleomargarine	10
After Dinner	Banana	88
	Coca Cola	340

Additional vitamin D equivalent to 312 I.U. was taken each day in tablet form. The tablets were manufactured by White Laboratories, Inc.

Table 3

CALCULATED FOOD VALUES* OF DAILY INTAKE FOR THE SUBJECT
FOR JANUARY 15, PERIOD III

FOOD	Cal.	Prot.	Ca.	P	Fe	Vit. A	Thia.	Ribo.	Niacin	Ascorbic Acid
		gm.	mg.	mg.	mg.	I.U.	mg.	mg.	mg.	mg.
Milk	765.9	39.2	1324.8	1044.6	0.84	1860.0	0.42	2.02	1.3	15.6
Orange Halves	98.0	2.0	72.0	50.0	0.8	400.0	0.16	0.06	0.6	106.0
Cooked Rolled Oats	93.9	3.43	13.34	100.3	1.09	-	0.14	0.03	0.26	-
Oleomargarine	142.5	0.14	4.2	2.8	-	655.0	-	-	-	-
Sugar	16.0	-	-	-	-	-	-	-	-	-
Scrambled Eggs	49.9	3.95	16.8	65.4	0.84	356.4	0.03	0.09	-	-
Enriched Bread	491.4	15.6	164.2	179.8	3.13	-	0.05	0.31	3.9	-
Vegetable Soup	60.7	3.11	23.7	37.0	0.59	-	0.04	0.06	0.74	5.9
Pork (roast)	119.5	8.22	3.95	84.3	1.08	-	0.30	0.09	1.81	-
Salad Dressing	38.9	0.13	0.67	2.6	0.06	13.4	-	-	-	-
Baked Custard	125.4	10.68	125.4	130.7	0.53	372.1	0.05	0.22	0.09	0.44
Cookie	43.6	0.60	2.0	6.0	0.1	-	0.01	0.01	0.1	-
Grilled Hamburger	298.3	17.9	7.55	126.5	2.27	-	0.07	0.15	3.9	-

Table 3
(continued)

CALCULATED FOOD VALUES* OF DAILY INTAKE FOR THE SUBJECT
FOR JANUARY 15, PERIOD III

FOOD	Cal.	Prot.	Ca.	P	Fe	Vit. A	Thia.	Ribo.	Niacin	Ascorbic Acid
		gm.	mg.	mg.	mg.	I.U.	mg.	mg.	mg.	mg.
Celery	4.4	0.32	12.6	10.1	0.13	-	0.02	0.01	0.13	2.0
Lettuce	3.62	0.3	5.4	6.22	0.12	135.6	0.01	0.02	0.05	2.0
Corn	45.9	1.38	2.7	35.4	0.35	140.4	0.06	0.05	0.65	3.8
Banana	51.7	0.68	4.7	16.44	0.35	252.4	0.02	0.03	0.41	5.87
Coca Cola	163.0	-	-	-	-	-	-	-	-	-
TOTALS	2359.62	107.64	1784.01	1898.16	12.27	4185.30	1.38	3.15	13.40	141.61

* Composition of Foods -- Raw, Processed, Prepared, 1950 --
U.S. Department of Agriculture, Agriculture Handbook Number 8

service was unchanged from previous eating procedure. The plates were filled in the kitchen then brought to the table. The subject was served a portion of each food on the menu and was expected to consume all of it; additional portions were available if desired.

Each of the four sampling periods began on Monday morning before breakfast. During these four balance periods, all food and liquids consumed by the subject were weighed[9] and these weights recorded. Samples of food and excreta were collected during the balance periods. A description of the methods used in the collection and preparation of urine, feces, and food for sampling is given below.

URINE

The urine was collected in one liter, screw top brown bottles that contained five ml. of concentrated acetic acid as a preservative. The first day's collection excluded the first voiding of the bladder upon rising, but included the morning voiding twenty four hours later. The succeeding days collections were separated similarly. Each twenty four hours collection was measured in a graduated mixing column, and with distilled water, made to volumne easily divisible by five. The urine was mixed fifty times; samples were taken for creatine and creatinine (Folin, 1914); and an aliquot of one fifth of the total volume was saved for the composite. The composite was kept refrigerated in a brown bottle until the end of each balance period.

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[9] A Hansen scale was used.

After the last day of each balance period the urine composite was allowed to reach room temperature, then mixed thoroughly by inverting and rotating. The composite ready for sampling was equal to one fifth of the urine excreted in seven days. Samples were wet ashed as described later.

FOOD

Samples of food similar to that served and equal to one fifth the weight of that consumed by the subject were collected after each meal. The food was weighed on wax paper or in small beakers, and washed with distilled water into large containers. The solid and liquid foods were kept separate for ease in later handling. Both containers were stored in a deep freeze until time for sampling.

The food was removed from the freezer the night before sampling to allow for thawing. Thawed food was transferred to a blender[10] cup, and enough thawed liquid was added to fill the cup slightly over one half full. Care was taken not to fill the cup too full since the food was apt to foam over. The cup was covered and allowed to blend for five minutes. After all food was blended and transferred quantitatively to as many two liter volumetric flasks as were needed, the foam was allowed to subside. The flasks were made to volume, mixed thoroughly, and were ready for sampling. The total contents of all the flasks represented one-fifth of a seven day food intake. Each flask was sampled separately for ashing. The sum of calcium content of the flask was the sample total.

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[10] A Waring blender was used.

The fecal samples were collected in quart-sized paraffined lined cardboard containers and carmine was used as a marker. A carmine capsule was taken before breakfast of the first day and another before breakfast on the day following the last twenty four hours of the balance period. The fecal samples containing the first dose of carmine were separated and that portion of the stool excreted before the color showed was discarded. The colored portion and the portion that followed were saved, as well as the succeeding samples until the second dosage of carmine appeared. Any part of the stool containing color from the second capsule was discarded. The samples were dated and frozen until the composite was to be made.

The thawed feces were placed in a blender cup, covered with distilled water, and blended for five minutes. The blended material was transferred quantitatively to a two liter volumetric flask, and after the foam had subsided, the flask was made to volume, and finally mixed. After being thoroughly mixed, this solution was ready for sampling and represented the total fecal excretion for seven days. Samples were taken for ashing.

Triplicate samples were taken from each composite (food, feces, urine) for ashing, and duplicate portions were frozen in twelve ounce pharmaceutical bottles for later sampling if necessary.

An additional experimental problem in ashing techniques was performed. Both wet and the dry ashing of certain food and fecal composites were done. The methods of ashing as used by this laboratory at the present time are described below.

WET ASHING

Triplicate samples of the blended composites were pipetted into Erlenmeyer flasks and placed on a hot plate. Five ml. each of concentrated sulfuric acid, nitric acid, and hydrogen peroxide were slowly added, in that order, to each sample. Additional quantities of one to two ml. of nitric acid were added upon each appearance of charring. The samples were allowed to digest until no further charring was observed then heated for about twenty minutes. The contents of the flasks were dissolved with a few ml. of a 1:4 hydrochloric acid solution and transferred quantitatively to a volumetric flask. Each flask was made to a volume of one hundred ml., mixed, and poured into a paraffined stoppered six ounce bottle and kept for chemical analyses.

DRY ASHING

Triplicate samples of the composites were poured into separate weighed porcelain evaporating dishes and dried. The fecal samples were dried partially over a steam bath under a hood, then to constant weight under infra red lamps. The food samples were dried in an oven at 40° C. All samples were stirred often.

After being sufficiently dried, the samples were weighed, scraped, ground, and stored in labeled bottles in a desiccator.

Duplicate one gram samples of the food and duplicate one-half gram samples of the feces were ignited in silica dishes in a muffle furnace at approximately 500° F. When the ashes were white or an even gray in color, they were dissolved with a few ml. of a 1:4 hydrochloric acid solution and transferred quantitatively to a one-hundred ml. volumetric flask. The flask was made to volume, mixed, and the contents were poured

into a bottle for chemical analyses.

The calcium content of all the ashed samples was determined using McCrudden's method (1911). The analysis consisted of precipitating the calcium as calcium oxalate and titrating with standard potassium permanganate at 90° - 100° C. Reagent blanks were prepared for each period.

The results in mg. calcium /day as determined from both types of ashes are compared in Table 4.

Higher values were found from the dry ash. This indicated that part of the calcium was not recovered from the wet ash, probably for one of two reasons. First, the organic matter might not have been completely destroyed; or, secondly, some of the hydrogen peroxide might have been left which interfered with the titration with permanganate. The higher values obtained from the dry ashing method were used in calculating the retentions of calcium.

Table 4

CALCIUM IN FOOD INTAKES AND FECAL EXCRETIONS

AS DETERMINED BY WET AND DRY ASHING METHODS

Periods	Calcium Intake			Fecal Excretion of Calcium		
	Dry Ash	Wet Ash	Deviation fr. Dry Ash	Dry Ash	Wet Ash	Deviation fr. Dry Ash
	mg./day	mg./day	%	mg./day	mg./day	%
II	2242.47	1918.05	11.4	1350.45	1403.85	3.7
III	2020.92	1712.32	15.3	1327.25	1123.54	15.3
IV	2043.38	1768.93	13.4	1733.37	1351.16	12.1

RESULTS AND DISCUSSION

EXPERIMENTAL RESULTS

During the four balance periods the subject ingested 2.043, 2.021, 2.043 and 1.873 gm./day of calcium, and retained 0.519, 0.520, 0.194, and 0.371 gm./day respectively. These data are found in Table 5. The calcium intake averaged 1.995 gm./day, and the retention ranged from 0.194 to 0.520 gm./day.

Certain of the anthropometric measurements are recorded in Table 6. Only the measurements that seemed to indicate a significant change within the experimental time limit were related to the calcium retention in that table. A complete record of the measurements may be found in the appendix Table 7. A total increase in height of two cm. for the three month period, and a total gain in weight of 1.7 kg. were observed. The increment for each period is given in Table 6.

A record of the basal metabolic rates, hemoglobin and ascorbic acid determinations are found in Table 8. The results of all tests were within the ranges considered normal.

The intake of certain food nutrients for each period of study is given in Table 9. Total calories, protein, and ascorbic acid were calculated. An average of 2369 calories, 97.4 gms. of protein, and 115 mg. of ascorbic acid were taken each day.

DISCUSSION OF RESULTS

The range of calcium retention of this subject was within the range found in the literature. Sherman (1922) reported retentions for two early adolescent girls that ranged from 0.090 to 0.825 gm./day. The

Table 5

THE INTAKE, OUTGO, AND RETENTION OF CALCIUM OF THE SUBJECT ON A SELF SELECTED DIET

Period	Intake	Outgo		Retention	
		Urine	Feces		
	mg./day	mg./day	mg./day	mg./day	%
II	2043.47	173.66	1350.45	519.36	25.4
III	2020.92	173.61	1307.25	539.06	25.7
IV	2043.38	116.36	1733.37	193.63	9.4
V	1873.15	141.85	1360.10	371.20	19.8

Table 6

INCREASE IN CERTAIN ANTHROPOMETRIC MEASUREMENTS AS RELATED TO CALCIUM RETENTION OF SUBJECT ON A SELF SELECTED DIET

Periods	II		III		IV		V	
Age	11 yr. 7 mo.		11 yr. 8 mo.		11 yr. 9 mo.		11 yr. 10 mo.	
Calcium Retention (mg./day)	519.36		520.06		193.63		371.21	
	Measurement		Measurement	Increment	Measurement	Increment	Measurement	Increment
Stature (cm.)	140.5		141.5	+1.0	142.0	+0.5	142.5	+0.5
Weight (kg.)	32.94		32.84	-0.10	33.9	+1.06	34.64	+0.74
Leg Length (cm.)	67.3		68.0	+0.7	68.5	+0.5	68.8	+0.3
Chest Breadth (cm.)	14.0		14.4	+0.4	14.8	+0.4	15.8	+1.0
Chest Depth (cm.)	22.0		22.2	+0.2	22.2	0.0	22.6	+0.4
Length Left Hand (cm.)	14.3		15.0	+0.7	15.3	+0.3	15.9	+0.6
Breadth Left Hand (cm.)	6.5		6.7	+0.2	7.0	+0.3	6.7	-0.3
Length Left Foot (cm.)	22.1		23.7		22.3		22.7	
Breadth Left Foot (cm.)	8.1		7.9		7.9		8.1	
Girth Left Leg (cm.)	28.2		28.0	-0.2	28.0	0.0	28.6	+0.6

Table 8

BASAL METABOLIC RATES, HEMOGLOBIN, AND ASCORBIC ACID DETERMINATIONS OF SUBJECT ON A SELF SELECTED DIET

Age	Basal Metabolic Rates	Hemoglobin	Plasma Ascorbic Acid
	percent	gm./100 ml.	gm./100 ml.
11 yr. 6 mo.	+8.04	13.63	1.5
11 yr. 8 mo.	-11.0	15.63	1.6
11 yr. 10 mo.	-2.6	14.40	1.4

Table 9

THE AVERAGE INTAKE OF CALORIES, PROTEIN, AND ASCORBIC ACID OF SUBJECT ON SELF SELECTED DIET

Period	Calories Number/day	Protein gm./day	Ascorbic Acid mg./day
II	2074.86	88.57	105.91
III	2469.53	98.13	115.89
IV	2635.87	98.23	111.30
V	2294.42	104.67	126.72
Average	2368.67	97.40	114.96

conditions under which Sherman observed that range of retentions, were very similar to the conditions of this study. Wang (1936) observed that a twelve year old subject retained from 0.466 to 0.591 gm./day. The retentions as reported by Johnston (1941) and Stearns (1941) ranged from 0.070 to 0.700 gm./day, and incidently, both extreme values were recorded for the same subject. The subjects used by these authors were not considered in normal physical condition. They were either malnourished or had the reinfection type of tuberculosis. Other variables such as the addition of estrogen or thyroid also were included with one group of the subjects.

The retention pattern of the subject of this study varied as did the intake pattern except for one period. For two periods when the intakes were practically the same, the retentions were the same, and for the last period as the intake was decreased, the retention also decreased. This variation with the intake was in accordance with the results observed by Stearns, Sherman, Johnston, and others.

It is not known why the lowered retention occurred in the fourth period. The subject had a slight cold during that period but this was not deemed sufficient to terminate the study at that time. The retention for three of the periods ranged from 19 to 25 percent of the intake.

An interesting relationship was observed between the growth of the subject and the calcium retention. It was observed that the skeleton of the subject made several changes between periods two and three (Table 6). The stature increased one cm.; the leg length increased 0.7 cm.; the hand changed; and the chest depth and breadth increased.

The retention for period three was 0.520 gm./day of calcium.

For period four a retention of 0.194 gms. of calcium per day was recorded. Less change in the skeleton was observed, although the subject gained 1.06 kg. of weight during this period. The stature increased only 0.5 cm. The subject probably increased in soft tissues. Again for period five, there was an indication of an increase of soft tissue with a continuation of the slowed rate of skeletal change. The increase in leg girth of 0.6 cm. and the gain in weight of 0.74 kg. indicated growth in soft tissues. The skeletal changes were about the same as for period four.

This rhythm of growth from the skeleton to the soft tissue and back to the skeleton, are similar to the changes as recorded by Meredith (1935).

The excretion of calcium through the kidneys varied with the calcium retention. This relationship was similar to that reported in the literature by McCance, although McCance did not use children as subjects.

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

The calcium intake and excretion of a healthy adolescent boy were studied for an experimental period of approximately four months. Certain anthropometric measurements also were made periodically. The rate of growth was compared with the retention of calcium.

The calcium intakes for the four balance periods were 2.043, 2.021, 2.043 and 1.873 gm./day, and the retentions were 0.519, 0.520, 0.194 and 0.371 gm./day respectively.

The total increase in stature for the subject was two cm.; the leg length was 1.5 cm.; chest breadth was 1.8 cm., and the increase in weight was 1.7 kg.

The calcium retentions seemed to correlate with the rhythm of growth.

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APPENDIX

Table 7

ANTHROPOMETRIC MEASUREMENTS DURING THE GROWTH OF AN EARLY ADOLESCENT BOY

Age	11 yr. 7 mo.	11 yr. 8 mo.	11 yr. 9 mo.	11 yr. 10 mo.
Stature (cm.)	140.5	141.5	142.0	142.5
Weight (kg.)	32.94	32.84	33.9	34.64
Maximum Arm Span (cm.)	144.0	144.2	142.4	144.3
Sitting Height (cm.)	73.2	73.5	73.5	73.7
Head Length (cm.)	18.2	18.6	18.2	18.5
Head Breadth (cm.)	13.6	13.5	13.8	13.9
Morphological Face Length (cm.)	11.8	11.8	11.7	
Physiological Face Length (cm.)	17.3	17.6	17.5	
Length of Ear (cm.)	5.7	5.5	5.6	
Breadth of Ear (cm.)	3.4	3.4	3.6	
Nose Height (cm.)	4.9	4.6	4.6	
Nose Breadth (cm.)	3.0	2.9	2.9	
Hand Length (cm.)	14.3	15.0	15.3	15.9
Hand Breadth (cm.)	6.5	6.7	7.0	6.7
Foot Length (cm.)	22.1	23.7	22.3	22.7
Foot Breadth (cm.)	8.1	7.9	7.9	8.1
Girth of Lower Leg (cm.)	28.2	28.0	28.0	28.6
Chest Breadth (cm.)	22.0	22.2	22.2	22.6
Chest Depth (cm.)	14.0	14.4	14.8	15.8

Table 7
(continued)

ANTHROPOMETRIC MEASUREMENTS DURING THE GROWTH OF AN

EARLY ADOLESCENT BOY

Age	11 yr. 7 mo.	11 yr. 8 mo.	11 yr. 9 mo.	11 yr. 10 mo.
Grip Strength (kg.) R	25.0	25.0	20.0	25.0
L	20.0	22.0	15.0	20.0
Blood Pressure	96/66	94/54	98/48	104/70
Pulse Rate	80	95	84	95
Time of Measurements	4 pm	4 pm	4 pm	4 pm