VARIATIONS IN THE CALCIUM METABOLISM OF PRESCHOOL CHILDREN

THESIS FOR THE DEGREE OF M. S.

Veda E. Hiller

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OF

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to the Faculty of
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Veda E. Hiller

Department of Foods and Nutrition
Division of Home Economics
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VARIATIONS IN THE CALCIUM METABOLISM OF PRESCHOOL CHILDREN

Introduction

Little is known concerning the calcium requirement of children. Investigators first estimated this requirement by proportionally reducing suggested standards for adults, which had been obtained through dietary and balance studies. The values thus obtained did not indicate the increased need for calcium during the growth period and were therefore inadequate. Later, through dietary studies on children, investigators determined the calcium needs for the growing child more accurately. This type of research measured the amount of calcium which children consumed but did not determine the amount actually needed for optimum storage. To obtain this information, it was necessary to conduct quantitative metabolism studies. Although several of such studies have been made they do not give a complete picture of the child's metabolic processes.

This investigation is an attempt to give additional information on this problem through making a study of the daily variations in the calcium metabolism of two normal preschool children. It includes a study of their reactions on medium and high protein diets.

Review of Literature

Sherman and Hawley (8) conducted a series of calcium metabolism studies, in order to determine the rate of calcium storage in normal children of different ages; the quantity of calcium required for optimum storage; and the most valuable sources of the calcium. Twelve children from three to thirteen years of age served as subjects for periods consisting of one preliminary day plus two or three collection periods of three days each.

In order to measure the rate of storage, the investigators gave the children a fairly constant diet containing 750 grams of milk. The food consumed contained about one gram of calcium per day. The retentions varied from 0.15 to 0.62 gram of calcium, the amount increasing as the age and the size of the child increased. Table I shows that the children of preschool age, on the basis of body weight, received 0.53 gram and stored 0.010 gram of calcium per kilogram per day.

To find the quantity of calcium required for optimum storage, the calcium intake of three of the children was varied by adding 250, 500, 750, 1000 and 1500 grams of milk to a fixed diet. The data for the two preschool children are shown in Table I. The amount retained varied from 0.008 to 0.014 gram of calcium per kilogram of body weight. The differences between the amounts stored on the

500, 750 or 1000 grams of milk were negligible.

To determine whether children could utilize the calcium in the form of vegetables as efficiently as the calcium of milk, the daily allowance of 750 grams of milk was reduced to 375 grams and enough vegetables were added to yield an equivalent amount of calcium. The retention on this diet was 0.006 gram per kilogram, as compared with 0.011 gram per kilogram on the former diet. The authors state that this difference in the retentions indicated that the calcium of milk is somewhat better utilized than that of vegetables. On the basis of all these retention figures they conclude that one quart of milk per day should be given to each child.

Wang and her associates (10) conducted calcium metabolism studies on ten normal children and fifty undernourished children between four and thirteen years of age. The children received a weighed diet during a three day preliminary and a three day experimental period. On the basis of body weight, the calcium intakes of the preschool children (Table I) ranged from 0.061 to 0.082 gram per kilogram and the retentions varied from 0.006 to 0.019 gram. On the whole, the intakes and retentions were higher than those observed by Sherman and Hawley (8). The calcium absorption and retention, however, showed wide individual variations among both normal and undernourished children.

In 1930, Wang and her associates (11) compiled the data on 18 children obtained during their study of undernourished children, in order to show the minimum calcium requirement. The calcium intakes varied from 0.009 to 0.103 gram of calcium per kilogram of body weight per day, and on the same basis the retentions ranged from 0.003 to 0.018 gram. In every case a calcium intake above 0.0278 gram per kilo of body weight resulted in a positive balance and below that figure the balance was negative. This difference may have been due to the difference in the source of calcium, since the milk consumed varied from 130 to 1400 grams per day. The authors concluded from their results that the minimum and not the optimum calcium requirements for a growing child weighing 20 kilograms and living on a mixed diet would be 0.46 gram of calcium per day.

, Willard and Blunt (12) made a study on the comparative influence of evaporated and commercially pasteurized milk on the calcium metabolism of three normal children, three, four and eight years of age. The method of procedure was the same as that used by Wang and her associates (10) (11). The children received a simple, adequate diet which was varied by the addition of 810 grams of pasteurized milk or an equivalent amount of irradiated milk. As shown in Table I, the calcium intakes of the children of preschool age ranged from 0.064 to 0.070 gram per kilogram of body weight and they retained from 0.009 to 0.013 gram per kilogram. The amounts retained varied with the calcium

TABLE I

SUMMARY OF STUDIES ON CALCIUM METABOLISM OF PRESCHOOL CHILDREN

Investigator	Children		24 hours											Remarks
			Intake		Output				Retention			Ca : P		
	Age	No.	Total	Per Kilo	Feces	Urine	Total	Total Per Kilo	Total	Per Kilo	Per Cent of Intake	Intake	Retention	
	(yrs)		(gm)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)	
Sherman-Hawley (8) (1922)	3-5	3	0.883	0.053	0.69	0.02	0.71	0.042	0.17	0.010	19.3	0.8:1	1.2:1	Mixed diet, 750 gm milk
Series II	4-5	2	0.386	0.020	0.23	0.02	0.25	0.013	0.14	0.008	36.3	0.6:1	0.8:1	Fixed diet, 250 gm milk
	4-5	2	0.677	0.035	0.42	0.02	0.44	0.023	0.24	0.013	35.5	0.7:1	0.9:1	Fixed diet, 500 gm milk
	4-5	2	0.932	0.048	0.66	0.03	0.69	0.036	0.24	0.013	25.8	0.8:1	0.8:1	Fixed diet, 750 gm milk
	4-5	2	1.207	0.063	0.90	0.04	0.94	0.049	0.28	0.014	23.2	1.1:1	1.1:1	Fixed diet, 1000 gm milk
	4-5	2	1.722	0.089	1.43	0.03	1.46	0.076	0.27	0.009	15.7	1.1:1	1.1:1	Fixed diet, 1500 gm milk
Series III	4-5	2	0.666	0.033	0.51	0.02	0.53	0.026	0.14	0.007	21.0			Fixed diet, 500 gm milk, potato
	4-5	2	1.030	0.051	0.92	0.02	0.94	0.046	0.09	0.004	8.7			Fixed diet, 500 gm milk, veg.
Series IV	6	1	0.951	0.054	0.73	0.02	0.75	0.043	0.20	0.011	21.0	0.9:1	1:1	Fixed diet, 750 gm milk
	6	1	0.894	0.051	0.76	0.03	0.80	0.046	0.11	0.006	12.3	1.1:1	3.7:1	Fixed diet, 375 gm milk, veg.
Wang (11)(1930)	5	1	0.318	0.019	0.25	0.09	0.34	0.020	0.02					Normal child, low intake
	5	1	1.453	0.082	1.14	0.15	1.29	0.072	0.16	0.009	11.3	1:1	0.7:1	Same child, higher intake
	4	1	0.523	0.041	0.28	0.02	0.30	0.024	0.22	0.017	42.2	0.8:1	0.4:1	Normal child
	4	1	0.666	0.049	0.40	0.02	0.42	0.031	0.25	0.018	37.3	0.7:1	0.4:1	Same child, higher intake
Wang (10)(1928)	5	1	1.450	0.082	1.14	0.21	1.35	0.075	0.11	0.006	7.4			Vigorous normal
	5	2	0.944	0.067	0.74	0.07	0.81	0.057	0.14	0.010	14.8			Normal in weight only
	5	1	0.970	0.061	0.72	0.06	0.78	0.049	0.18	0.012	19.0			5-10 per cent underweight
	4-5	2	0.775	0.061	0.51	0.02	0.53	0.042	0.24	0.019	31.0			10-15 per cent underweight
Burton (2) (1930)	3-5	4	1.49	0.084	0.60	0.06	0.66	0.041	0.82	0.048	55.0	1:1	1.2:1	Wheat cereal, ultraviolet hight
	3-5	4	1.36	0.080	0.69	0.04	0.73	0.045	0.63	0.039	46.0	0.8:1	1:1	Oatmeal, ultraviolet light
Willard-Blunt (12) (1927)	3-4	2	1.12	0.070	0.90	0.02	0.92	0.58	0.21	0.013	18.5	1:1	0.9:1	Fixed diet, evaporated milk
	3-4	2	1.04	0.064	0.88	0.02	0.90	0.55	0.14	0.009	13.0	1:1	1:1	Fixed diet, 810 gm milk

intake and were higher than those given by Sherman and Hawley (8).

Burton (2) conducted a study to determine the influence of cereals upon the calcium retention in four normal boys from three to five years of age. The children received a fixed diet to which was added either refined wheat or oatmeal. The procedure was the same as that used by Wang and her co-workers (10) (11), and only one day was allowed between the change in diets. Since the children had been receiving sun lamp treatments these were continued during the experimental period. The calcium intakes varied from 0.080 to 0.084 gram per kilogram of body weight and the retention ranged from 0.039 to 0.048 gram. These retentions were considerably higher than those reported by other investigators possibly due, either to the high intakes or to the influence of the ultraviolet light. Wang and her associates (11) found that the calcium retention varied directly with the calcium intake. A number of other investigators have shown that vitamin D may be a controlling factor in mineral metabolism whether administered as ultraviolet light, cod-liver oil or irradiated food or ergosterol. In Burton's study the intakes and retentions of calcium were less in the oatmeal period than in the wheat period. Calculated figures showed that the residues of the two diets were practically neutral. According to Blunt and Cowan (1), present day investigators believe that a neutral diet is the most satisfactory for the storage of calcium and that diets with an alkaline residue give better retention than those with an acid residue.

Recently Stearns (9) reported a study on the significance of the retention ratio of calcium and phosphorus in children. She stated that a ratio of two parts of calcium to one of phosphorus would indicate a more rapid growth of bone than of the soft tissues and that ratios above this would signify a previous starvation of calcium. A ratio of less than 1 to 1 would indicate that the major growth was in the soft tissues and if long continued would result in a deficiency in the bone growth. She confirmed the statement made by Sherman and Hawley (8) that more calcium is stored by very young children than by children of school age.

Sherman (7), however, stated in his latest edition of "The Chemistry of Food and Nutrition," that if optimum amounts are retained the ratios will care for themselves. Therefore, with the normal development of the child the optimum ratio will be constantly changing.

These studies show the tendencies in calcium absorption and retention but are not conclusive because of the small number of children studied. The results of different investigators are not entirely comparable on account of the great number of variables. Some of the children studied were not physically normal, nor were their previous environmental conditions comparable in all respects. The diets used also varied to a great extent and some of them were planned to study the comparative values of different foods. The preliminary periods were not more than three days in length and the experimental periods varied from three to nine days. In some studies the time between different diets was not more than one day. Investigators assumed that the body reached equilibrium during the short preliminary periods and that the experimental periods which followed were indicative of the metabolic process occurring in the child at all times. With these facts in mind, the plan of this experiment was to continue a test period for a long period of time and note the ordinary fluctuations which might occur in calcium metabolism.

Plan of Study

The general purpose of this entire investigation was to study the daily variations in the metabolism of two normal, preschool boys, who were fed a fixed diet for an extended period of time. The experiment consisted of three parts, a 15 day preliminary period, a 30 day period when the children received a constant diet containing three grams of protein per kilogram of body weight and a 15 day period when the diet contained four grams of protein per kilogram. This paper is only a portion of the entire investigation and reports the data on the calcium metabolism of the children during the last six days of the medium protein level and the first nine days of the high protein level. There were several reasons for conducting this portion of the investigation; first, to determine the daily variations in the calcium absorption and retention; second, to find the difference in the metabolism on the two levels of protein; third, to discover whether each child reached equilibrium in the nine days following the change in diet; fourth, to study the retention ratio of calcium to phosphorus; and fifth, to compare the variations in the reactions of the two children.

The children D.A. and W.W. came from an institution for orphan children where they had received exceptionally good care. They had followed a regular daily routine of eating, sleep and play. Their diet consisted of practically three-fourths quart of milk, one egg, two or three vegetables, two fruits, meat, cereal, bread and enough of other foods to make a total of 1600 to 1700 calories per day. According to estimations, this diet contained 55 to 65 grams of protein and approximately 1 gram each of calcium and phosphorus. Physical and medical examination of the children in December, 1931, showed that they were, apparently, in good physical condition. Table II shows that D.A., aged four years and nine months, varied from the Woodbury, Height-Weight-Age tables—2.81 per cent according to weight, and +2.08 per cent according to height. W.W., who was four years and seven months old varied -0.60 from the same table according to weight, and +0.56 per cent according to height.

TABLE II
A COMPARISON OF THE HEIGHT AND
WEIGHT OF THE CHILDREN WITH THE WOODBURY TABLES

Subject	Age (Mo)	Height: Observed (In)	Height: Woodbury's Standard: Normal (In)	Height: Woodbury's Standard: Variation (%)	Weight: Observed (Lbs)	Weight: Woodbury's Standard: Normal (Lbs)	Weight: Woodbury's Standard: Variation (%)
D.A.	57	42.88	42.0	+2.08	38.38	39.5	-2.81
W.W.	55	43.75	44.0	-0.56	41.75	41.5	+0.60

During the experiment the boys lived in the apartment in the Home Economics building where they were under constant supervision. The children observed good health habits; such as regular meals, sufficient sleep, both inside and outdoor exercise, fresh air and regular toilet habits. They had their morning play period out of doors with the nursery school children. Immediately after lunch they slept from one to two hours and when possible played out of doors for the remainder of the afternoon.

Both of the diets which the children received were adequate in all dietary essentials as shown by Table III. The amounts in the table are for D.A., since W.W. weighed one and one-tenth times as much, he received one-tenth more food than is recorded. The food each day was identical but the preparation of it varied in order that the diet would not seem so monotonous. The caloric values were the same on the two diets 1494 for D.A. and 1643 for W.W. The vitamin content was adequate as shown by the amount of fruits, vegetables, milk and cod liver oil given daily. As recommended by Sherman and Hawley (8), the calcium as well as the phosphorus content of the diet approximated one gram. Everyday the children received weighed portions of distilled water for drinking.

The subjects ate all of the weighed food. Such precautions as wiping the plate with a small piece of bread, rinsing a cup of milk or orange juice with distilled water insured complete intake of the food. Weighing, cooking and

TABLE III

COMPOSITION OF MEDIUM PROTEIN DIET*†

Food	Weight	Cal-ories	Pro-tein	Calcium	Phos-phorus	Ex-cess Acid	Ex-cess Base
	(gm)		(gm)	(gm)	(gm)	(cc)	(cc)
Milk(whole)	720	496.8	23.8	0.8640	0.6696		13.0
(uncooked) Farina	18	64.8	2.0	0.0038	0.0225	1.7	
Orange juice	180	64.8		0.0522	0.0288		11.1
Beef (raw)	45	52.2	10.1	0.0059	0.1084	5.2	
Egg (raw)	45	66.6	6.0	0.0297	0.0810	5.0	
(strained) Prunes	90	113.4	0.5	0.0144	0.0279	14.6	
(strained) Apple sauce	90	88.2	0.3	0.0054	0.0099		3.1
Lettuce	14	2.2	0.2	0.0060	0.0059		1.0
(strained) Carrots	72	29.5	0.8	0.0403	0.0331		7.8
(strained) Tomatoes	90	18.9	1.1	0.0099	0.0234		5.1
Butter	18	138.4	0.2	0.0027	0.0031		
(cooked) Potato	63	71.8	1.4	0.0145	0.0372		4.0
Sugar	18	72.0					
Bread	72	173.5	7.0	0.0360	0.1260	5.3	
Cod Liver Oil	4.5	40.5					
Total	1539.5	1493.6	53.4	1.0848	1.0968	31.8	45.1

* Allowance for D.A. W.W. received 1.1 times this amount.

† Figures from tables, Sherman, Chemistry of Food and Nutrition. (7)

TABLE IV

COMPOSITION OF HIGH PROTEIN DIET*†

Food	Weight	Cal ories	Pro- tein	Calcium	Phos- phorus	Ex- cess Acid	Ex- cess Base
	(gm)		(gm)	(gm)	(gm)	(cc)	(cc)
Milk (whole)	425	293.3	14.0	0.5100	0.3953		7.7
Milk (skim)	425	157.3	14.5	0.5185	0.4080		7.7
(uncooked) Farina	18	64.8	2.0	0.0038	0.0225	1.7	
Orange juice	200	72.0		0.0580	0.0320		12.4
Beef (raw)	90	104.4	20.2	0.0117	0.2169	10.4	
(uncooked) Egg	90	133.2	12.0	0.0594	0.1620	9.9	
(strained) Prunes	90	113.4	0.5	0.0144	0.0279	14.6	
(strained) Apple Sauce	90	88.2	0.3	0.0054	0.0099		3.1
Lettuce	14	2.2	0.2	0.0060	0.0059		1.0
(strained) Carrots	70	28.7	0.8	0.0392	0.0322		7.6
(strained) Tomatoes	100	21.0	1.2	0.0110	0.0260		5.5
Butter	20	153.8	0.2	0.0030	0.0040		
(cooked) Potato	50	57.0	1.2	0.0115	0.0295		3.2
Sugar	10	40.0					
Bread	60	144.6	5.8	0.0300	0.1040		
Cod Liver Oil	4.5	40.5					
Total	1756.5	1514.3	72.9	1.2819	1.4761	41.0	48.2

* Allowance for D.A. W.W. received 1.1 times this amount.

† Figures from table, Sherman, Chemistry of Food and Nutrition. (7)

serving the food in the same container prevented any loss which would occur if food was transferred. The food was either mixed, ground or seived before it was served to be sure that the children received a fair sample.

To simplify the procedure the food for three days plus that necessary for analysis was prepared at one time and the allowance for each day weighed and stored in the ice box. Exact samples of each of these foods were combined into a diet and two such diets were saved for analysis. These samples were dried on a steam bath and then placed in an electric oven at 60 degrees centigrade until the weights were constant. After that they were ground and stored in glass bottles.

The feces were collected in one day periods which were marked off alternately with carmine and charcoal. The feces for each day were dried and stored in the same manner as the food.

The urine was collected in 24 hour samples. The creatinine values varied between the two periods, but were fairly constant from day to day. These results indicated that there had been little loss in the collection of the samples. For the mineral analysis 200 cubic centimeters of the fresh urine were measured into beakers and dried on a steam bath.

Calcium was determined by the Kramer and Howland method (6). A preliminary testing showed that the method was satisfactory.(Table V). Results on a known solution of

TABLE V

TESTS OF THE ACCURACY OF THE METHOD ON KNOWN SOLUTIONS AND PREVIOUSLY ANALYZED FOOD SAMPLES

Series	Sample	Number of Determinations	Ca	Ca Recovered	Ca Recovered
			(gms)	(gms)	(%)
1	CaO	10	0.0765	0.0758	99.1
	$CaCO_3$	5	0.2830	0.2780	98.2
2	Food	6	0.0508	0.0502	98.8
		7	0.0538	0.0531	98.7
3	Food and CaO	3	0.1405	0.1406	99.8
		2	0.1434	0.1423	99.2

calcium shows averages of 99.1 and 98.2 per cent recovery. Determinations of the calcium in a food previously analyzed checked within two per cent. When a known solution of calcium was added to the food sample, the recovery of calcium was 99.8 and 99.2 per cent.

Weighed amounts of the food and the feces were dryed ashed in a muffle furnace at a dull red heat. The ash was taken up with 1:4 Hcl, evaporated to dryness in order to dehydrate any silica present, again taken up with 1:4 Hcl and made up to volume.

The dried samples of urine were ashed by adding five to ten cubic centimeters of concentrated nitric acid, digested on a steam bath and then ashing in a muffle furnace at a dull red heat. Owing to the small amounts of calcium found in the urine, N/100 $KMnO_4$ was used for titrating instead of the N/20 $KMnO_4$ used in the analysis of the food and feces.

Discussion of Results

Table VI gives the results of the calcium analysis of the food on the two protein levels and the values as calculated from Sherman's tables (7). It will be noted that the calculated values are from 6.1 to 10.3 per cent higher than those found by analysis, possibly due to the method of preparation of the foods or to the variations in the composition of foods. There was, however, only a slight

TABLE VI

COMPARISON OF CALCULATED
WITH ANALYZED VALUES FOR CALCIUM CONTENT OF FOOD*

Diet	Period (3 days)	Calcium Intake Analyzed (gm)	Calculated (gm)	Variation (Per cent)
Medium Protein	1	0.984	1.085	10.3
	2	0.990	1.085	9.5
Average		0.987	1.085	9.9
High Protein	1	1.168	1.282	9.7
	2	1.209	1.282	6.1
	3	1.175	1.282	9.1
Average		1.184	1.282	8.3

* Intakes for D.A. W.W. received 1.1 times these amounts.

variation between the values found during the different periods. The calcium ingested on the high protein diet was 1.184 grams, 0.2 gram higher than that on the medium protein level, due without doubt to the higher amount of milk in the former diet.

The actual quantities of calcium consumed and utilized daily by the children on the medium protein diet, as determined by analysis are given in Table VII. The variations in the intakes throughout the experiment were due to impurities of the carmine and charcoal which were used in marking off the feces. On the respective days when they were used for marking, the children received 0.007 gram of calcium from carmine and 0.289 gram from charcoal in addition to the calcium in their food. Therefore, both children received varied intakes.

It will also be noted that there were large daily variations in the total calcium output. These variations were not due to fluctuations in the calcium of the urine since the calcium thus excreted was quite constant. The values for D.A. varied within 0.008 gram and those for W.W. within 0.015 gram. The total amount excreted in the feces was greater than that in the urine and varied from 0.726 to 1.193 grams for D.A. and from 1.034 to 1.276 grams for W.W. These variations in total output might be due to the varied intake or to the unavoidable experimental errors in the collection of the daily feces samples.

TABLE VII

DAILY CALCIUM BALANCES ON THE MEDIUM PROTEIN DIET

Subject	Day	Intake	Output			Retention		Absorption	
			Feces	Urine	Total	Total	Percentage of Intake	Total	Percentage of Intake
		(gm)	(gm)	(gm)	(gm)	(gm)			
D.A.	1	0.991	1.038	0.085	1.123	-0.132		-0.047	
	2	1.272	0.849	0.083	0.931	0.341	26.8	0.424	33.3
	3	0.991	0.834	0.082	0.917	0.074	7.5	0.153	15.4
	4	1.279	1.108	0.077	1.185	0.094	7.4	0.172	13.4
	5	0.998	0.726	0.081	0.807	0.191	19.1	0.271	27.2
	6	1.279	1.193	0.082	1.275	0.004	0.3	0.086	6.7
Average		1.135	0.958	0.082	1.040	0.095	8.4	0.177	15.6
W.W.	1	1.089	1.162	0.032	1.194	-0.105		-0.073	
	2	1.371	1.034	0.032	1.066	0.304	22.2	0.337	24.6
	3	1.089	1.259	0.028	1.287	-0.198		-0.170	
	4	1.378	1.276	0.027	1.303	0.075	5.4	0.102	7.4
	5	1.097	1.035	0.042	1.077	0.020	1.8	0.062	5.7
	6	1.378	1.103	0.028	1.130	0.248	18.0	0.276	20.0
Average		1.234	1.145	0.032	1.176	0.057	4.6	0.089	7.2

These fluctuations would of course give wide variations in the daily amount absorbed and retained. On some days the children apparently absorbed no calcium and on other days as much as one-third of their intake. The differences between the high and low values for absorption were as much as 0.5 gram of calcium. The retention values were slightly lower than the absorption values, those for D.A. ranging from a negative result of 0.132 to a positive result of 0.341 or 26.8 per cent of the calcium intake. The retention for W.W. ranged from -0.198 gram to +0.304 gram, the latter being 22 per cent of his intake.

When average figures were considered on the basis of grams of calcium per day, D.A. had an intake of 1.135 grams and absorbed 0.177 gram of which he retained 0.095 gram, a percentage absorption of 15.6 and a percentage retention of 8.4. The values for D.A. were quite similiar to those for a five year old vigorous, normal child studied by Wang and her associates (10). W.W. who received 1.234 grams of calcium per day absorbed only 0.089 gram of which he retained 0.057 gram a percentage absorption and retention of 7.2 and 4.6, respectively.

The tow children showed slight variation in the amount of calcium utilized. W.W. absorbed and retained only about half as much calcium as D.A. These differences were probably individual differences which might have due to the differences in the acid-base condition of the intestine.

According to Hawk and Bergeim (4), the acidity of the intestinal contents is important for the absorption of the relatively insoluble salts of calcium. Nevertheless their might have been other differences in their metabolic processes, such as parathyroid activity, or differences in the physical condition. Since D.A. was taller for his weight than W.W. (Table IV), his bony structure might have been developing, at that time, more rapidly than that of W.W. Perhaps both children would have stored more calcium if the experiment had been conducted in the season of the year when there was more sunshine.

Table VIII gives the daily calcium balances of the children on the high protein diet. The intake of D.A. averaged 1.347 gram of calcium on this diet in comparison with 1.135 gram on the medium protein diet. That of W.W. was 1.466 gram in place of 1.234 on the first diet. The daily intake varied again due to the carmine and charcoal used to mark the feces.

There was as much day by day variation on this diet in all the calcium figures as there was on the medium protein diet. The percentage of the intake excreted, as shown in Table IX, was greater on the high than on the lower protein diet. The excretion was from 96.5 to 97.3 percent of the intake on the high protein diet, while it was from 91.6 to 95.3 per cent on the medium protein diet.

The calcium retention and absorption on the high pro-

TABLE VIII

DAILY CALCIUM BALANCES ON THE HIGH PROTEIN DIET

Subject	Day	Intake	Output Feces	Output Urine	Output Total	Retention Total	Retention Percentage of Intake	Absorption Total	Absorption Percentage of Intake
D.A.	1	1.175	1.072	0.105	1.177	-0.002		0.103	8.8
	2	1.457	1.362	0.101	1.463	-0.007		0.095	6.5
	3	1.175	1.128	0.095	1.223	-0.048		0.047	4.0
	4	1.497	1.022	0.101	1.123	0.374	25.0	0.475	31.7
	5	1.216	1.531	0.089	1.621	-0.405		-0.316	
	6	1.497	1.150	0.102	1.252	0.246	16.4	0.348	23.2
	7	1.463	1.150	0.100	1.250	0.214	14.6	0.314	21.5
	8	1.182	1.138	0.102	1.240	-0.058		0.044	3.7
	9	1.463	1.241	0.112	1.353	0.111	7.6	0.223	7.6
Average		1.347	1.199	0.101	1.300	0.047	3.5	0.148	11.0
	1	1.292	1.733	0.039	1.773	-0.480		-0.441	
	2	1.574	0.924	0.035	0.959	0.615	39.1	0.650	41.3
	3	1.292	1.535	0.033	1.568	-0.276		-0.243	
	4	1.618	1.457	0.036	1.492	0.126	7.8	0.161	9.9
	5	1.337	1.244	0.032	1.276	0.061	4.6	0.093	7.0
	6	1.618	1.329	0.042	1.371	0.247	15.3	0.290	17.9
	7	1.581	1.329	0.031	1.360	0.221	14.0	0.252	15.9
	8	1.299	1.564	0.034	1.598	-0.298		-0.265	
	9	1.581	1.406	0.039	1.444	0.137	8.7	0.175	11.1
Average		14 66	1.391	0.036	1.427	0.039	2.7	0.075	5.1

TABLE IX

PERCENTAGE OF THE CALCIUM INTAKE EXCRETED

Subject	Medium Protein			High Protein		
	Feces (%)	Urine (%)	Total (%)	Feces (%)	Urine (%)	Total (%)
D.A.	84.4	7.2	91.6	89.0	7.5	96.5
K.K.	92.8	2.6	95.3	94.9	2.4	97.3

tein diet (Table VIII) again shows irregular daily variations. On only one day did D.A. appear to absorb no calcium, on the other days he absorbed from 3.7 to 31.7 per cent of his intake. On five days he apparently re-excreted more calcium than he absorbed, since the retention figures were negative on those days. Three of these days immediately followed the change in diet. This might have been due to the fact that on the medium protein diet the body had obtained equilibrium and on the changed diet the body was attempting to adjust its processes. However, the fourth day was near the end of the period. There were four days when D.A. did retain calcium, the amount varying from 7.6 to 25.0 per cent of his intake.

The variations for W.W. were similiar, since on three days he apparently absorbed and retained no calcium . Two of these three days were near the beginning of the high protein period. The percentage absorbed varied from 7 to 41.3 per cent and the amount retained from 4.6 to 39.1 per cent of his intake. These daily fluctuations were somewhat greater than those on the medium protein diet.

When all of the data for the high protein diet were considered both children were storing calcium. D.A. absorbed 11 per cent of his intake and of this amount retained 3.5 per cnt. While W.W. absorbed only 5.1 per cent and retained 2.7 per cent. This amounts to a daily retention of 0.047 and 0.039 gram of calcium, respectively. As in the previous diet W.W. did not store quite as much calcium as did D.A. Both children, however, failed to store as much calcium on the high protein as they did on the medium protein diet. Analysis of this data statistically showed that this difference was not significant.

From the results of Wang and her co-workers (11), one would expect better retention on the high protein diet because the calcium intake was increased. Nevertheless, the 380 single balances of children reviewed by Craig indicated, that the quantity of calcium did not consistently follow the intake, results similar to those found in this experiment.

Calculated values for the acid-base residues of the two diets (Tables III and IV) show that the medium protein diet left a slightly more alkaline residue than did the high protein diet. This might have influenced the retentions on the diets as Blunt and Cowan (1) state that most investigators find better assimilation of minerals on diets leaving an alkaline residue than on those leaving an acid residue. The difference, however, of 6.2 cubic centimeters of N/10 excess base on the medium protein diet was probably not enough to influence the retentions greatly.

Table X records the data on the calcium balances of the two children on the medium protein diet per kilogram of body weight. As was previously noted in the data per 24 hours (Table VI), there were daily variations in both calcium intake and output. The intake for D.A. per kilogram, varied

TABLE X
DAILY CALCIUM BALANCES ON THE
MEDIUM PROTEIN DIET CALCULATED PER KILOGRAM OF BODY WEIGHT

Subject	Day	Weight	Intake	Output Feces	Output Urine	Output Total	Retention	Absorption
		(kg)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)
D.A.	1	17.4	0.057	0.060	0.005	0.065	-0.008	-0.003
	2		0.073	0.049	0.005	0.054	0.019	0.024
	3		0.057	0.048	0.005	0.053	0.004	0.009
	4		0.074	0.064	0.004	0.068	0.006	0.010
	5		0.057	0.042	0.005	0.047	0.010	0.015
	6		0.074	0.069	0.005	0.074	0.000	0.005
Average			0.065	0.055	0.005	0.060	0.005	0.010
W.W.	1	19.0	0.057	0.061	0.002	0.063	-0.006	-0.004
	2		0.072	0.054	0.002	0.056	0.016	0.018
	3		0.057	0.066	0.002	0.068	-0.011	-0.009
	4		0.072	0.067	0.001	0.068	0.004	0.005
	5		0.058	0.054	0.002	0.056	0.002	0.004
	6		0.072	0.058	0.002	0.060	0.012	0.014
Average			0.065	0.060	0.002	0.062	0.003	0.005

from 0.057 to 0.074 gram and that for W.W. from 0.057 to 0.072 gram. The urinary excretion was practically constant 0.005 gram for D.A. and 0.002 gram for W.W. The grams excreted through the feces varied greatly. When both children were considered, however, in 7 out of the 12 days the feces as well as the total output followed the variation in the intake. Since the daily output was not constant the absorption and retention varied greatly in both children. On the basis of kilograms of body weight the absorption values varied for D.A. from a negative value of 0.003 to a positive figure of 0.015 gram and the retention values ranged from -0.008 to 0.019. On the other hand W.W. had two days in which the absorption was negative but on the other four days he absorbed values for calcium up to 0.018 gram and retained up to 0.016 gram per kilogram of body weight.

Considering the average values on the basis of kilograms of body weight D.A. shows absorption and retention of 0.010 and 0.005 gram, respectively. The results for D.A. are again comparable to those of the same normal child, who, as reported by Wang and her co-workers (10), retained 0.006 gram of calcium per kilogram of body weight. On this basis D.A., also had a slightly better storage and absorption of calcium than W.W., who absorbed only 0.005 gram and retained 0.003 gram. In her survey of the calcium and phosphorus of children, Craig (3) found that 43 per cent of all cases stored from -0.001 to 0.009 gram per kilogram of body weight. The average figures for both children were within this range.

On this same basis the data in Table XI shows the daily balances of the children on the high protein diet. The same daily variations were again prevalent. The intakes for both children varied from 0.068 to 0.086 gram per kilogram of body weight. The urinary output was again constant , 0.006 gram for D.A. and 0.002 gram for W.W. The excretion of the calcium in the feces did not increase and decrease with the variation in the intake as consistently as they did on the medium protein diet. The total output varied for D.A. from 0.065 to 0.093 and for W.W. from 0.051 to 0.093 gram per kilogram. The variations between the highest and lowest absorption and retention values were 0.045 gram for D.A. and 0.057 gram for W.W.

The averages of the daily calcium balances on the high protein diet showed that even though D.A. and W.W. absorbed, respectively, 0.008 and 0.004 grams per kilogram, they both retained 0.002 grams of this amount. For D.A. the difference in the retention on the two diets was 0.003 grams, while for W.W. the difference was not significant being only 0.001 grams. On the medium protein diet the per cent of absorbed calcium which was retained was 50 for D.A. and 60 for W.W. On the high protein diet, however, these same percentages were reduced to 25 for D.A. and 50 for W.W. This indicates that possibly D.A. was slightly more effective by the change in the diet. The difference in the reactions of

TABLE XI

DAILY CALCIUM BALANCES ON THE HIGH PROTEIN DIET CALCULATED PER KILOGRAM OF BODY WEIGHT

Subject	Day	Weight	Intake	Output			Retention	Absorption
				Feces	Urine	Total		
		(kg)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)
D.A.	1	17.4	0.068	0.062	0.006	0.068	0.000	0.006
	2		0.084	0.078	0.006	0.084	0.000	0.006
	3		0.068	0.065	0.005	0.070	-0.002	0.003
	4		0.086	0.059	0.006	0.065	0.021	0.027
	5		0.070	0.088	0.005	0.093	-0.023	-0.018
	6		0.086	0.066	0.006	0.072	0.014	0.020
	7		0.084	0.066	0.006	0.072	0.012	0.012
	8		0.068	0.065	0.006	0.071	-0.003	0.003
	9		0.084	0.071	0.006	0.077	0.007	0.013
Average			0.077	0.069	0.006	0.075	0.002	0.008
W.W.	1	19.0	0.068	0.091	0.002	0.093	-0.024	-0.023
	2		0.083	0.049	0.002	0.051	0.032	0.034
	3		0.068	0.081	0.002	0.083	-0.015	-0.013
	4		0.085	0.077	0.002	0.079	0.006	0.008
	5		0.070	0.065	0.002	0.067	0.003	0.005
	6		0.085	0.070	0.002	0.072	0.013	0.015
	7		0.083	0.070	0.002	0.072	0.011	0.013
	8		0.068	0.082	0.002	0.084	-0.016	-0.014
	9		0.083	0.074	0.002	0.076	0.007	0.009
Average			0.077	0.073	0.002	0.075	0.002	0.004

the two children might be due to the fact that they were storing their optimum amount of calcium on the medium protein diet and that adjustment to the new diet occurred at different rates.

Since calculations for caloric values are often made on the basis of the body's surface area, the present data calculated on that basis would show if there was any correlation between utilization of calcium and surface area. (Tables XII and XIII). The calculations indicated the same daily variations in all figures as were found for total calcium and calcium per kilogram.

Tables XIV and XV give the daily calcium to phosphorus (5) intake and retention ratios on the medium and high protein diets. There were wide variations in these ratios. For both children the intake ratios vary from 0.8:1 to 1:1. Since there were many days on which there was no retention, and when there was a retention the ratios varied so widely, only the average ratios could be considered. The Ca:P retention ratios of D.A. were 1:1 on the medium protein and 0.7:1 on the high protein diet. According to Stearns (9), the 1:1 ratio would be indicative of very good growth both in bone and soft tissue. While the ratio of 0.7:1 would signify that the major growth was in the soft tissues. This might explain why D.A. did not store as much calcium during the high protein period.

The average retention ratios for W.W. on the medium and high protein diets were 0.5:1 and 0.4:1. Stearns (9)

TABLE XII

DAILY CALCIUM BALANCES ON THE MEDIUM PROTEIN DIET CALCULATED PER SQUARE METER OF BODY SURFACE

Subject	Day	Surface Area	Intake	Output: Feces	Output: Urine	Output: Total	Retention	Absorption
		(Sq. M.)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)
D.A.	1	0.72	1.376	1.441	0.118	1.559	-0.183	-0.065
	2		1.767	1.179	0.115	1.294	0.473	0.588
	3		1.376	1.159	0.114	1.273	0.103	0.217
	4		1.776	1.538	0.107	1.645	0.131	0.238
	5		1.386	1.009	0.112	1.121	0.265	0.377
	6		1.776	1.657	0.114	1.771	0.005	0.119
Average			1.576	1.331	0.113	1.444	0.132	0.245
W.W.	1	0.76	1.433	1.529	0.042	1.571	-0.138	-0.096
	2		1.803	1.361	0.042	1.403	0.400	0.442
	3		1.433	1.657	0.037	1.694	-0.261	-0.224
	4		1.813	1.679	0.036	1.715	0.098	0.134
	5		1.443	1.362	0.055	1.417	0.026	0.081
	6		1.813	1.451	0.036	1.487	0.326	0.362
Average			1.623	1.507	0.041	1.548	0.075	0.116

TABLE XIII

DAILY CALCIUM BALANCES ON THE HIGH PROTEIN DIET CALCULATED PER SQUARE METER OF BODY SURFACE

Subject	Day	Surface Area	Intake	Output: Feces	Output: Urine	Output: Total	Retention	Absorption
		(Sq. M.)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)
D.A.	1	0.72	1.633	1.489	0.145	1.634	-0.001	0.144
	2		2.023	1.892	0.141	2.033	-0.010	0.131
	3		1.633	1.567	0.132	1.699	-0.066	0.066
	4		2.079	1.420	0.140	1.560	0.519	0.659
	5		1.689	2.127	0.124	2.251	-0.562	-0.438
	6		2.079	1.597	0.142	1.739	0.340	0.482
	7		2.032	1.597	0.139	1.736	0.296	0.435
	8		1.642	1.581	0.142	1.723	-0.081	0.061
	9		2.032	1.723	0.155	1.878	0.154	0.309
Average			1.871	1.666	0.140	1.806	0.065	0.205
W.W.	1	0.76	1.700	2.281	0.052	2.333	-0.633	-0.581
	2		2.071	1.216	0.046	1.262	0.809	0.855
	3		1.700	2.020	0.044	2.064	-0.364	-0.320
	4		2.129	1.917	0.047	1.964	0.165	0.212
	5		1.759	1.637	0.042	1.679	0.080	0.122
	6		2.129	1.748	0.056	1.804	0.325	0.381
	7		2.080	1.748	0.041	1.789	0.291	0.332
	8		1.710	2.058	0.044	2.102	-0.392	-0.348
	9		2.080	1.850	0.051	1.901	0.179	0.230
Average			1.929	1.831	0.047	1.878	0.051	0.098

TABLE XIV

DAILY INTAKE AND RETENTION RATIOS
OF CALCIUM TO PHOSPHORUS ON THE MEDIUM PROTEIN DIET

Subject	Day	Intake Ca (gm)	Intake P (gm)	Intake Ca:P	Retention Ca (gm)	Retention P (gm)	Retention Ca:P
D.A.	1	0.991	1.249	0.8:1	-0.132	0.029	
	2	1.272	1.380	0.9:1	0.341	0.191	0.8:1
	3	0.991	1.250	0.8:1	0.074	0.127	0.6:1
	4	1.279			0.094		
	5	0.998	1.178	0.8:1	0.191	0.117	1.6:1
	6	1.279	1.309	1:1	0.004	0.009	0.4:1
Average		1.135	1.273	0.9:1	0.095	0.089	1:1
W.W.	1	1.089	1.374	0.8:1	-0.105	0.098	
	2	1.371	1.505	0.9:1	0.304	0.220	1.4:1
	3	1.089	1.374	0.8:1	-0.198	0.017	
	4	1.378	1.426	1:1	0.075	0.092	0.8:1
	5	1.097	1.296	0.8:1	0.020	0.043	0.5:1
	6	1.378	1.426	1:1	0.248	0.234	1:1
Average		1.234	1.406	0.9:1	0.057	0.117	0.5:1

TABLE XV

DAILY INTAKE AND RETENTION RATIOS
OF CALCIUM TO PHOSPHORUS ON THE HIGH PROTEIN DIET

Subject	Day	Intake Ca (gm)	Intake P (gm)	Intake Ca:P	Retention Ca (gm)	Retention P (gm)	Retention Ca:P
D.A.	1	1.175	1.470	0.8:1	-0.002	0.130	
	2	1.457	1.601	0.9:1	-0.007	0.058	
	3	1.175	1.470	0.8:1	-0.048	0.032	
	4	1.497	1.709	0.9:1	0.374	0.156	2.4:1
	5	1.216	1.579	0.8:1	-0.405	-0.069	
	6	1.497	1.709	0.9:1	0.246	0.142	1.7:1
	7	1.463	1.610	0.9:1	0.214	0.127	1.7:1
	8	1.182	1.480	0.8:1	-0.058	-0.001	
	9	1.463	1.610	0.9:1	0.111	0.063	1.8:1
Average		1.347	1.582	0.9:1	0.047	0.071	0.7:1
W.W.	1	1.292	1.617	0.8:1	-0.480	-0.039	
	2	1.574	1.748	0.9:1	0.615	0.375	1.6:1
	3	1.292	1.617	0.8:1	-0.276	-0.137	
	4	1.618	1.867	0.9:1	0.126	0.158	0.8:1
	5	1.337	1.737	0.8:1	0.061	0.187	0.3:1
	6	1.618	1.867	0.9:1	0.247	0.153	1.6:1
	7	1.581	1.758	0.9:1	0.221	0.161	1.4:1
	8	1.299	1.628	0.8:1	-0.298	-0.046	
	9	1.581	1.758	0.9:1	0.137	0.131	1:1
Average		1.466	1.733	0.8:1	0.039	0.103	0.4:1

states that if long continued such ratios would result in a deficiency of bone growth. As was previously stated Sherman (7) says that with the normal development of the child the optimal ratios will be constantly changing. Since W.W. appeared to be developing normally, this low retention ratio might have been optimum for him. At this particular time his soft tissues might have been developing faster than his bony structure. If this was the case, he needed to retain more phosphorus than calcium.

The difference in the retention ratios of the two children again might indicate that a change in diet slightly effected the metabolic processes of D.A. more than that of W.W.

The following table, number XVI, summarizes the average calcium retention and absorption values for 24 hours and per kilogram of body weight of both children on each level of protein.

There is a difference between the amount of calcium retained on the two levels of protein in twenty four hours of 0.033 gram, but calculated on the basis of body weight the difference is only 0.002 gram - certainly not a significant figure. The results on the absorption of calcium are practically the same 0.022 and 0.002 gram. The experiments indicate that the calcium utilization of these two boys did not vary when the amount of protein in the diet was changed from 3 to 4 grams per kilogram of body weight per day.

TABLE XVI

COMPARISON OF BALANCES OF THE TWO CHILDREN

	Calcium per 24 hours			
	Medium Protein		High Protein	
Subject	Average Retention	Average Absorption	Average Retention	Average Absorption
	(gm)	(gm)	(gm)	(gm)
D.A.	0.095	0.177	0.047	0.148
W.W.	0.057	0.089	0.039	0.075
Average	0.076	0.133	0.043	0.111

	Calcium per kilogram of Body Weight			
	Medium Protein		High Protein	
	Average Retention	Average Absorption	Average Retention	Average Absorption
	(gm)	(gm)	(gm)	(gm)
D.A.	0.005	0.010	0.002	0.008
W.W.	0.003	0.005	0.002	0.004
Average	0.004	0.008	0.002	0.006

Summary

The general purpose of this investigation was to study the actual daily variations which might occur in the calcium metabolism of two normal preschool children on different diets.

1. The daily amounts of calcium absorbed and retained by the children varied greatly, possibly due to the daily variations in the intakes or to the difficulty of collecting daily fecal samples.

2. On the medium protein diet D.A. and W.W. stored daily 0.095 and 0.057 gram of calcium, respectively. On the basis of per kilogram of body weight these same values were 0.005 gram for D.A. and 0.003 gram for W.W.

3. Both children failed to store as much calcium on the high protein diet as they did on the medium protein diet. Statistical treatment of this data, however, showed that this difference was not significant. On the higher protein level D.A. and W.W. retained 0.047 gram and 0.039 gram, respectively. In grams per kilogram of body weight, both children retained 0.002 gram.

4. There was a slight difference in the reactions of the two children, since D.A. stored more calcium than W.W. on the medium protein diet. The difference in the amounts retained on the high protein diet was practically negligible.

5. The average retention ratios of Ca:P for D.A. and W.W.

on the medium protein diet were 1:1 and 0.5:1, respectively. On the high protein diet these ratios were slightly lower and were 0.7:1 for D.A. and 0.4:1 for W.W.

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