THE MAGNESIUM METABOLISM AND ITS RELATION TO CALCIUM RETENTION OF TWO NORMAL PRESCHOOL CHILDREN ON HIGH AND MEDIUM PROTEIN DIETS

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Introduction

While numerous mineral metabolism studies have been conducted on adults and animals, there have been relatively few on preschool children. The majority of the metabolic investigations on humans deal with only two or three of the necessary ash constituents. Very little work has been done on magnesium. Most of the studies on human beings are not comparable because of the variations in age and physical condition of the subjects, and length of the experimental period.

A search of the literature reveals very little information concerning the magnesium requirement and metabolism of normal children. For this reason no standards have been set for the daily amount required to maintain positive magnesium balance as has been done with some of the other minerals.

This study deals with the magnesium metabolism of two normal preschool children on medium and high protein diets, and is a part of an extensive investigation planned and carried out to study the daily variations on the metabolism of these two children.

The objectives of this study are: to determine the daily variations in the magnesium absorption and retention;

to determine if the children reached an equilibrium after being on a constant diet for a considerable period; to compare the individual variations in the metabolism of the two children; to observe whether the different levels of protein intake affects the magnesium metabolism; and to study the relationship of magnesium metabolism to the calcium metabolism.

Review of Literature

Magnesium investigations have been made to study the paths of excretion of this mineral and its relationship to calcium excretion. Nelson and Burns (18) conducted an investigation on the urinary excretion of magnesium and calcium in 25 normal adults and found that 17 of the subjects excreted calcium in larger amounts than magnesium while the output of magnesium was greater in the other 8 subjects. The amount of CaO excreted varied from 0.099 to 0.488 gm. and the MgO from 0.118 to 0.416 gm. They stated that the predominance of one element or the other was practically constant in the individual and appeared to be independent of the character of the food ingested.

Givens (7) also found that, as a rule, more calcium than magnesium was excreted in the urine of 9 healthy men who had been instructed to select their foods with regard to the calcium and magnesium content. On a diet of natural foods containing more magnesium than calcium, the urinary excretion of calcium ranged from 0.05 to 0.24 gm. and the magnesium 0.03 to 0.15 gm. On a diet having more calcium than magnesium, the limits were 0.13 to 0.47 gm. of calcium and 0.05 to 0.23 gm. of magnesium. Contrary to Nelson and Burns (18), he concluded that the variations in the excretion of these elements were influenced by the diet and by the tissue reserves. He found that the administration of milk was more efficacious than a comparable quantity of calcium salts in producing increased urinary excretion of calcium and that magnesium citrate apparently did not affect the excretion of magnesium.

By varying the calcium and magnesium ratio through the addition of magnesium citrate and calcium lactate to the experimental diet, Bogert and McKittrick (1) studied the interrelations between calcium and magnesium metabolism of four normal women. They found that the addition of 6 gm. of magnesium citrate per day increased the urinary and fecal magnesium in all cases. Urinary and fecal calcium was increased in 3 out of 4 cases and the total calcium excretion in all cases. Increased excretion of calcium in the urine and feces in all subjects resulted when 6 gm. of calcium lactate per day was added to the diet and the magnesium output also increased.

Underhill, Honeij and Bogert (25) conducted an experiment on the effect of varying the ratio of the calcium and magnesium intake on the retention of these two minerals in

two normal adults. The calcium intake averaged 0.414 gm. for the low level and 1.416 gm for the high one. The magnesium intake was 0.297 gm. on the low intake diet and 0.549 gm. on the high level. On a low magnesium and low calcium diet for four days, both subjects were in negative calcium and positive magnesium balances. During the following period of high calcium and low magnesium intake, one subject was in negative calcium and positive magnesium balance while the other subject's balances were just the reverse. Both subjects were in negative balance on the third 3 day period when both minerals were low. The high magnesium and low calcium intake produced the greatest magnesium retention in both individuals, while the calcium balance was the least for both subjects of any period.

They also found that, while the food calcium and magnesium may be practically constant, there may be a decided variation in the ratio of these elements in the urine and in the feces and especially in the urine. This was due to the extent of the retention of one or the other of the elements.

Carswell and Winter (3) conducted an experiment on two men, one subject being on the experiment for 20 days and the other for 24 days, to determine the effect of the use of magnesium as a therapeutic measure. During a control period both subjects were in positive balance, while the magnesium balance for one subject was positive and for the other it was slightly negative. The intake of CaO was 1.74 gm. and the P_2O_5 intake was 3.39 gm. daily and remained constant throughout the experiment. To observe the effect of magnesium, 8 gm. of magnesium lactate were added daily. The addition of magnesium caused definite decrease in urinary calcium in one subject, while there was slight retention in the other. The magnesium retention in the first subject was slight while in the latter it was marked. These workers concluded that if the phosphorus intake was adequate, the magnesium appeared to favor calcium storage instead of calcium loss.

Experimental work with animals substantiates the importance of adequate phosphorus in the diet when studying the interrelationship of calcium and magnesium. In a study on the effect of a high magnesium intake on the calcium retention of swine, Hart and Steenbock (10) found that the addition of soluble phosphates decreased the increased calcium excretion brought on by the addition of magnesium salts to the diet.

However, Huffman and his co-workers (14) obtained beneficial results when 3 to 5 per cent magnesium salts, either as phosphates or carbonates, were added to a ration of whole milk and grain and fed to dairy calves. The calcium and phosphorus retentions were unaffected or improved.

In experimental work with rats, Haag and Palmer (9) studied the effect of variations in the proportions of calcium, magnesium and phosphorus contained in the diet. They varied the proportions of these minerals in rations that were adequate in other respects. On a diet low in calcium and high in phosphorus or high calcium and low phosphorus the addition of high magnesium, 0.80 to 0.88 per cent, depressed calcium retention less frequently than when both these elements were low. They concluded that the deleterious effect of high magnesium was conditioned by the intakes of calcium, phosphorus and vitamins. They stated, "The interrelations existing between mineral elements are important factors for consideration in studying the specific role of a mineral element in animal nutrition."

In a study on the growth and nutrition of dairy calves McCandlish (16) found that the addition of grains to a ration consisting solely of milk impaired the growth of calves and eventually brought on death. He concluded that death was caused by the depletion of the body stores of calcium since the grains contain more magnesium than calcium and that calcium was drawn from the bones to counteract the effect of magnesium. The addition of alfalfa hay to the ration of milk and grains brought the animals back to good growing conditions. The beneficial effect was not attributed to any one constituent in hay.

The effect of high and low protein rations on the metabolism of various minerals in dairy cows was studied by Monroe (17). He found that the magnesium balances of the high protein fed animals showed no marked difference from the corresponding balances of those on the low protein ration. The cows receiving the high protein diet stored calcium while those on the low ration were in negative balance. It was suggested that this difference was due to the larger amounts of clover hay contained in the high protein ration.

Studies of factors which may influence mineral metabolism in humans have been made by several workers. A few of these investigations include the metabolism of magnesium. Hobbler (13) varied the fat content of cows' milk fed to an infant and obtained 82 per cent magnesium retention on a 2.5 per cent fat intake, negative balance on a 4 per cent level and 32 per cent retention on a 5.4 per cent intake. The retention of magnesium varied inversely with the calcium retention.

Sawyer, Bauman and Stevens (19) conducted an experiment on two boys, 5 and 8 years of age, to determine the effect of a high fat diet as compared to a normal diet on mineral metabolism. On the normal diet, the average magnesium intake was 0.436 gm. which gave an average retention of 0.064 gm. The high fat diet provided 0.314 gm. of magnesium, but the retention on this diet gave an average negative balance of -0.323 gm.

Shohl and Sato (22) were interested in the effect of acids and alkalis on the salt retention of infants. For subjects, they used three artificially fed infants 7, 9 and 7 months old. A normal diet was fed for a period then hydrochloric acid was added. This was succeeded by a normal diet and then sodium bicarbonate was given. The magnesium intake on the first two periods was 0.123 gm. When only the normal diet was given there was a 37 per cent retention which dropped to 20 per cent when hydrochloric acid was added. The third period which was normal and provided 0.072 gm. magnesium gave a negative balance of -3.0 per cent. The addition of $NaHCO_3$ increased the retention of magnesium to 74 per cent. The acid did not affect the excretion of magnesium in the urine but the fecal excretion increased. The $NaHCO_3$ decreased magnesium excretion in both urine and feces. The authors stated that the explanation for the effect of acids and alkalis on mineral metabolism is often obscure and until facts have been firmly established do not warrant speculation.

Chaney (4) studied the effect of orange juice on the mineral retention of two girls age 10 and 11 years. There were two experiments, the second being a duplicate of the first. During the period when the basal diet alone was given, the average intake of magnesium per day was 0.25 gm. and the output 0.18 gm. with a retention of 0.07 gm. or 28 per cent. The addition of 600 to 700 cc. of unstrained orange juice increased the intake to 0.34 gm. per day and the output to 0.22 gm. with a retention of 0.12 gm. or 35

per cent. The author found that the retention of calcium and phosphorus was more marked than that of magnesium since the body output of these two minerals was actually diminished with the addition of orange juice.

Bogert and Trail (2) made a study of the influence of yeast and butterfat on the assimilation of certain minerals in four adult subjects. The addition of yeast brought the calcium balance from -0.056 gm. to 0.006 gm. and in the case of magnesium the retention was increased from 0.005 to 0.053 gm. After the administration of yeast the subjects were given the basal diet for a period and the balances for both minerals were negative. Then butterfat was substituted for the vegetable fat producing a change in the retention of both minerals. The average magnesium balance increased from -0.007 to 0.037 gm. and the calcium from -0.084 to 0.013 gm.

In a study to determine what the proportion of mineral elements should be in milk modifications for infants, Shohl, Wakeman and Shorr (23) used for their subject an infant with rickets of prematurity. During the first experimental period the infant was fed skimmed cow's milk and cane sugar with salts added so as to make the concentration of each mineral element approximately double that of cow's milk. In the second period the same food was fed without the salt additions. Results from this one study showed that a baby on a skimmed milk diet after the salt constituents were increased from 0.18 to 0.36 gm of MgO retained 0.01 gm as compared to 0.00 gm. when no additional salts were given.

The use of $MgSO_4$ for treatment of the convulsions of tetany in infants led Courtney and Fales (5) to study the rate at which $MgSO_4$ is eliminated after subcutaneous injection. If the doses required more than 24 hours for their excretion then daily repetition of the injection would lead to an accumulation of Mg in body with possible toxic results. Four infants with tetany were treated with subcutaneous injections of 4 to 10 cc. of $MgSO_4$ solution, whose concentration was 8 gm. of anhydrous $MgSO_4$ per 100 cc. They found that the infant organism can incorporate within 2 successive days as much as one or two tenths of a gram of MgO from the $MgSO_4$ and that amounts in excess of this are immediately excreted by the kidneys. There appeared to be no danger of the cumulative effect of magnesium if the dose was not given more frequently than once in 24 hours.

Hamilton, Bengt and Moriarty (8) studied the mineral retention of a premature infant for two months, keeping the subject on a metabolism bed for 31 out of 53 days, each period being 6 days in length. They drew no conclusions in regard to the magnesium metabolism since the mean for all the periods was negative. They stated that on this premature infant study there was a progressive loss of lime salts from the bones, an increase in body fluids and a change in either the composition or the relative mass of cellular tissue.

A portion of the data on mineral metabolism studies on infants in this country and abroad that include the analysis of magnesium have been compiled by Hawks (11) (Table I). The magnesium is calculated as grams of MgO per 24 hours. A comparison of the MgO intake and output of breast fed infants was made with artificially fed infants. The average intake of magnesium per 24 hours of artifically fed infants was higher than in breast fed infants, being 0.160 and 0.057 gm. MgO respectively. The retention of MgO per 24 hours for natural fed infants was 0.022 gm. while the infants on the higher intake retained 0.030 gm. The percentage retention was 38 per cent on the low intake as compared to 12.5 per cent on the high level. This summarization of a number of studies supports the work of Shohl, Wakeman and Shorr (23) that by increasing the intake the amount of the salt retained is increased.

From the above studies on magnesium metabolism it appears that the retention of this mineral increases with increased intake. Factors such as alkali, yeast, butter-fat and orange juice influence magnesium retention favorably, while acids and high fat diets decrease it. There is a close relationship between magnesium, calcium and phosphorus, and the conclusion reached from present data is that adequate amounts of calcium and phosphorus are necessary to counteract the depressing effect of high magnesium intake.

TABLE I

SUMMARY OF MAGNESIUM BALANCES IN INFANTS*

Grams MgO per 24 hours in breast fed infants

Author	Intake	Output: Urine	Output: Feces	Retention	Remarks
Blauberg	0.05	0.01	0.02	0.02	
Klotz	0.04	0.01	0.01	0.02	
Hoobler	0.05	0.01	0.02	0.02	
Lindberg	0.05	0.01	0.02	0.02	
Muhl	0.07 0.07	0.02 0.02	0.03 0.02	0.02 0.03	
Malmberg	0.07	0.02	0.03	0.02	
Average	0.057	0.014	0.022	0.021	

Grams MgO per 24 hours in artificially fed infants

Author	Intake	Urine	Feces	Retention	Remarks
Cronheim	0.15 0.16	0.03 0.03	0.07 0.11	0.05 0.03	Raw milk Boiled milk
Birk	0.18 0.19	0.02 0.02	0.12 0.14	0.04 0.03	 Phosphorated cod liver oil
Bruck	0.13	0.02	0.03	0.08	
Shohl & Sato	0.20 0.20 0.12 0.12	0.03 0.03 0.04 0.01	0.10 0.13 0.08 0.02	0.07 0.04 0.00 0.09	 HCl added $NaHCO_3$ added
Shohl	0.36 0.18		0.35 0.18	0.01 0.00	Minerals doubled
Hoobler	0.11	0.04	0.04	0.03	
Brahm	0.06 0.08	0.02 0.02	0.05 0.03	-0.01 0.03	 Irradiated cod liver oil
Courtney & Fales	0.13	0.02	0.09	0.02	
Average	0.158	0.022	0.102	0.034	

* Compiled by Hawks (11) Unpublished data.

Plan of Study

A comprehensive study of the metabolism of two pre-school boys is being made by the food and nutrition department of the Home Economics Division. There are three parts to the extensive experiment; a 15 day preliminary period, a period of 30 days when the children received a constant diet containing 3 gm. of protein per kilogram body weight, and a 15 day period when the protein intake was 4 gm. per kilogram. This paper is a report of a part of the larger study. It deals with the magnesium metabolism during the last 6 days of the medium protein period and the entire 15 days of the period when the high level of protein was used.

Only a brief summary of the method of conducting the experiment will be given since full details have been given in previous studies (12) (15).

The 2 four year old boys, W.W. and D.A. were healthy, normal subjects who had received excellent care and had good health habits. Table II shows that they did not deviate to any great extent from the computed standard for age, weight and height according to Woodbury (26). The experimental diets for the children were planned to be adequate as to calories, protein, calcium, phosphorus and iron, and were thought to be adequate for the other minerals. The calculated composition of the diets may be

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

Subject	Age	Height			Weight		
		Observed	Woodbury's Standard Normal	Woodbury's Standard Variation	Observed	Woodbury's Standard Normal	Woodbury's Standard Variation
	(Mo)	(In)	(In)	(%)	(Lbs)	(Lbs)	(%)
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

found in tables III and IV.

The food was prepared in three day periods and included a sample for analysis. The feces were collected daily, each day being marked off alternately with carmine and charcoal. Urine was collected in 24 hour samples.

Of the three general methods for the determination of magnesium in biological material, namely gravimetric, titrimetric and colorimetric, the titration method of Tisdall and Kramer (24) was selected, because it was rapid and accurate, and required only a small amount of material. Any inaccuracies in this method as compared to the gravimetric are probably not as great as the errors encountered in the collection of biological material.

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

Two hundred cc. of urine were digested on a steam bath with 5 to 10 cc. of concentrated HNO_3 and then ashed in a muffle furnace at dull red heat. This ash was taken up with 5 per cent HNO_3 and made up to volume.

The magnesium analyses were made on the filtrates from the calcium determinations. One cc. of a 10 per cent solution of $(NH_4)_2HPO_4$ and 5 cc. of concentrated NH_4OH were added to the filtrate, mixed thoroughly and allowed

TABLE III

COMPOSITION OF MEDIUM PROTEIN DIET*/

Food	Weight	Cal-ories	Pro-tein	Cal-cium	Phos-phorus	Mag-nesium	Ex-cess Acid	Ex-cess Base
	(gm)		(gm)	(gm)	(gm)		(cc)	(cc)
Milk (Whole)	720	496.8	23.8	0.8640	0.6696	0.0864		13.0
(uncooked) Farina	18	64.8	2.0	0.0038	0.0225	0.0045	1.7	
Orange juice	180	64.8		0.0522	0.0288	0.0198		11.1
Beef (raw)	45	52.2	10.1	0.0059	0.1084	0.0113	5.2	
Egg (raw)	45	66.6	6.0	0.0297	0.0810	0.0050	5.0	
(strained) Prunes	90	113.4	0.5	0.0144	0.0279	0.0495	14.6	
(strained) Apple sauce	90	88.2	0.3	0.0054	0.0099	0.0072		3.1
Lettuce	14	2.2	0.2	0.0060	0.0059	0.0024		1.0
(strained) Carrots	72	29.5	0.8	0.0403	0.0331	0.0151		7.8
(strained) Tomatoes	90	18.9	1.1	0.0099	0.0234	0.0090		5.1
Butter	18	138.4	0.2	0.0027	0.0031	0.0002		
(cooked) Potato	63	71.8	1.4	0.0145	0.0372	0.0176		4.0
Sugar	18	72.0						
Bread	72	173.5	7.0	0.0360	0.1260	0.0360	5.3	
Cod Liver Oil	4.5	40.5						
Total	1539.5	1493.6	53.4	1.0848	1.0968	0.2640	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. (20)

TABLE IV

COMPOSITION OF HIGH PROTEIN DIET*/

Food	Weight	Calories	Protein	Calcium	Phosphorus	Magnesium	Excess Acid	Excess Base
	(gm)		(gm)	(gm)	(gm)		cc.	cc.
Milk (whole)	425	293.3	14.0	0.5100	0.3953	0.0510		7.7
Milk (skim)	425	157.3	14.5	0.5185	0.4080	0.0510		7.7
(uncooked) Farina	18	64.8	2.0	0.0038	0.0225	0.0045	1.7	
Orange juice	200	72.0		0.0580	0.0320	0.0220		12.4
Beef (raw)	90	104.4	20.2	0.0117	0.2169	0.0225	10.4	
(uncooked) Egg	90	133.2	12.0	0.0594	0.1620	0.0099	9.9	
(strained) Prunes	90	113.4	0.5	0.0144	0.0279	0.0495	14.6	
(strained) Apple sauce	90	88.2	0.3	0.0054	0.0099	0.0072		3.1
Lettuce	14	2.2	0.2	0.0060	0.0059	0.0024		1.0
(strained) Carrots	70	28.7	0.8	0.0392	0.0322	0.0147		7.6
(strained) Tomatoes	100	21.0	1.2	0.0110	0.0260	0.0100		5.5
Butter	20	153.8	0.2	0.0030	0.0040	0.0002		
(cooked) Potato	50	57.0	1.2	0.0115	0.0295	0.0140		3.2
Sugar	10	40.0						
Bread	60	144.6	5.8	0.0300	0.1040	0.0300		
Cod Liver Oil	4.5	40.5						
Total	1756.5	1514.3	72.9	1.2819	1.4761	0.2889	41.0	48.2

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

/ Figures from tables, Sherman, Chemistry of Food and Nutrition. (20)

to stand one hour or longer. The solution was then filtered through No.40 Whatman filter paper. The original method was modified somewhat at this stage. Instead of removing all the precipitated $MgNH_4PO_4$ to the filter paper, the beaker and any remaining precipitate were washed three times with 30 volume per cent alcohol. The precipitate and filter paper were washed an additional three times with the alcohol to insure the removal of all of the ammonia and then transferred to the original beaker. 30 cc. of warm water and exactly 5 cc. of N/10 HCl were added and thoroughly mixed. Brom creosol green was used as indicator instead of cochineal. The excess HCl was titrated with N/10 NaOH. Fiske's (6) method was used to insure accuracy in obtaining the end point. A comparison was made with a standard buffer solution at the correct end point, pH 4.6 containing comparable amounts of indicator and filter paper as the unknown solution. The amount of magnesium in the sample was computed on the number of cc. of N/10 HCl neutralized by the magnesium.

A preliminary test showed that the method selected was satisfactory. (Table V). Analysis of known solutions of MgO and $MgSO_4$ gave average recoveries of 98.3 per cent and 98.6 per cent respectively. The recovery of magnesium from a known solution of calcium and magnesium salts averaged 97.7 per cent. When urine, previously analyzed for its magnesium content was added to the known salt solution, 96.2 per cent of the magnesium was recovered. From these

TABLE V

TESTS OF THE ACCURACY OF THE METHOD ON KNOWN SOLUTIONS, WITH AND WITHOUT ANALYZED URINE AND CALCIUM SOLUTIONS

Series	Sample	Number of Determinations	Magnesium Present (Milligrams)	Magnesium Recovered (Milligrams)	(Per cent)
1	MgO	4	3.098	3.072	99.2
	MgO	8	3.059	2.994	97.9
2	$MgSO_4$	4	3.380	3.301	97.7
	$MgSO_4$	7	3.293	3.263	99.1
3	MgO + $CaCl_2$ —	4	2.994	2.896	96.7
4	CaO + $MgSO_4$	2	3.263	3.248	99.6
5	20 cc. Urine	3		0.164	
6	MgO + 10 cc. Urine	2	3.154	3.023	95.9
7	$MgSO_4$ + 10 cc. Urine	3	3.345	3.223	96.3

results it may be assumed that the method used in this study is within the limits of accuracy as given by Tisdall & Kramer. (24). With the limited quantity of biological materials available for analysis, the method gives satisfactory results.

Discussion of Results

In this study of the magnesium metabolism of two normal children, the magnesium content of the food will be discussed first, then the output of this mineral, and following this the retention and absorption of it on the two diets.

A comparison of the magnesium content of the food on the two protein levels as determined by analysis and the values as calculated according to the food composition tables of Sherman (20) show several interesting facts. (Table VI). It is first noted that the analyzed results are lower than those of the calculated values, the average variation for the period during which the medium level was 7.3 per cent and 7.9 per cent for the period with higher intake. However, the food was analyzed in three day periods and therefore represents an aliquot of a fairly large sample, and the magnesium content may be expected to represent the average of this mineral in these foods. Even these three day analysis show slight variation, usually not more than 4 or 5 mg. The greatest variation

TABLE VI

COMPARISON OF CALCULATED
WITH ANALYZED VALUES OF MAGNESIUM CONTENT OF FOOD*⁄

Diet	Period (3 days)	Magnesium Intake: Analyzed (gm)	Calculated (gm)	Variation (Per cent)
Medium Protein	1	0.2428	0.2640	8.0
	2	0.2464	0.2640	6.7
Average		0.2446	0.2640	7.3
High Protein	1	0.2662	0.2889	7.9
	2	0.2625	0.2889	9.1
	3	0.2676	0.2889	7.4
	4	0.2631	0.2889	8.9
	5	0.2711	0.2889	6.1
Average		0.2661	0.2889	7.9

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

⁄ Figures from table, Sherman, Chemistry of Food and Nutrition.(20)

was found between the 4th and 5th periods of the high protein diet, a difference of 8 mg. or an increase of 3.4 per cent. Possible explanations for this difference in variations in the composition and method of preparation of the food. However, these differences are slight and probably represent an uncontrollable variation.

The magnesium content of the high protein diet for subject D.A. averaged 0.266 gm. for the entire period which was 0.022 gm. or 9 per cent higher than the intake on the medium level. The amounts for W.W. are 1.1 higher than for D.A. since he weighed 1.1 times as much. The increase in the amount of magnesium was to be expected as the calcium intake also increased on the high level. Givens (7) stated that it was practically impossible to increase the dietary calcium without also increasing the magnesium.

Table VII gives the daily amounts of magnesium consumed and excreted by the two subjects on the medium protein diet. While the diet was fairly constant in magnesium content, both subjects showed considerable variation in the total amount of magnesium excreted. The urinary excretion for D.A. was practically constant while W.W. showed slightly more daily variation. The range of variation in the urinary excretion of magnesium for D.A. was from 0.081 to 0.089 gm. and for W.W. it was 0.090 to 0.119 gm. The fecal magnesium for D.A. varied from 0.115 to

TABLE VII

DAILY MAGNESIUM 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)		(gm)	
D.A.	1	0.243	0.180	0.085	0.265	-0.022		0.063	25.9
	2	0.243	0.115	0.085	0.200	0.043	17.7	0.128	52.7
	3	0.243	0.145	0.087	0.232	0.011	4.5	0.098	40.3
	4	0.247	0.158	0.089	0.247	0.000		0.089	36.0
	5	0.247	0.148	0.087	0.235	0.012	4.8	0.099	40.0
	6	0.247	0.160	0.081	0.241	0.006	2.4	0.087	35.2
Average		0.245	0.151	0.086	0.237	0.008	3.3	0.094	38.4
W.W.	1	0.265	0.162	0.112	0.274	-0.009		0.103	38.9
	2	0.265	0.116	0.108	0.224	0.041	15.5	0.149	56.2
	3	0.265	0.170	0.104	0.274	-0.009		0.095	35.8
	4	0.271	0.151	0.113	0.264	0.007	2.6	0.120	44.3
	5	0.271	0.162	0.119	0.281	-0.010		0.109	40.2
	6	0.271	0.138	0.090	0.228	0.043	15.9	0.133	49.1
Average		0.268	0.150	0.108	0.258	0.010	3.7	0.118	44.0

0.180 gm. This extreme variation occurred at the beginning of the period. No explanation of this high output is available since both subjects had been receiving this <u>constant diet for 24 days</u> prior to the start of this study. Following these two high days, the fecal magnesium varied less, the range being within 0.015 gm. W.W., also, showed a day-to-day variation in the fecal magnesium, the output ranging from 0.116 to 0.170 gm. per day. Both subjects showed a decided tendency towards alternating high and low daily fecal excretions of this mineral. The total output varied from day to day which was to be expected since the urinary excretion was practically constant while the amount of magnesium in the feces changed from day to day.

The daily fluctuations in the total output of magnesium would, of course, cause variations from day to day in the amount of magnesium absorbed--that is the total daily intake less the amount excreted in the feces that day. The absorption values for D.A. ranged from 0.063 to 0.128 gm. and, since the amount absorbed varies according to the fecal output of the mineral, this extreme variation followed the fecal variation and occurred at the beginning of the medium protein period. While this occurred at the beginning of the period, it cannot be due to a change in diet, for the child had been on the same one for the 24 preceeding days. After these first two days it became practically constant. W.W. did not show as extreme variation, but the day-to-day variations were greater and indicate an individual variation in the magnesium absorbed on a constant diet. The smallest amount of magnesium absorbed by W.W. was 0.095 gm. and the largest amount was 0.149 gm. The average amount of magnesium absorbed for the entire medium protein period for D.A. was 0.094 gm. or 38.4 per cent of his average intake of 0.245 gm. W.W. absorbed 0.118 gm. or 44.0 per cent of his average intake of 0.268 gm.

A daily variation in the retention of magnesium would, of course, follow daily variations in the absorption values since the amount of magnesium retained is the amount absorbed less the amount excreted in the urine. Because D.A.'s absorption on the first day was lower than the amount excreted in the urine he had a negative balance of -0.022 gm. that day, showing that he lost magnesium from his tissues. Following this first day he was either in equilibrium or in positive balance. The largest amount retained was 0.043 gm. W.W. was less constant than D.A. in his retention of magnesium. He alternated negative and positive retention days, while D.A. had only one negative day. W.W.'s retention values ranged from -0.010 to 0.043 gm.

When the averages for the medium protein period are considered, D.A. received 0.245 gm. of magnesium, and of this amount he absorbed 0.094 gm. or 38.4 per cent. Of the amount absorbed he retained 0.008 gm which is 3.3 per cent of the intake or 8.5 per cent of the amount absorbed.

Of the 0.268 gm. received daily, W.W. absorbed 0.118 gm. or 44.7 per cent and retained 0.010 gm. or 3.7 per cent, which is 8.5 per cent of the absorbed magnesium. Calculated on the percentage basis, W.W. absorbed more magnesium than D.A. but the two subjects retained the same proportion of the absorbed magnesium.

The magnesium balances on the high protein diet are given in Table VIII. D.A.'s intake was 0.266 gm. during this period as compared to 0.245 on the preceeding diet. W.W. received 0.293 gm. of magnesium in place of 0.268 on the medium protein diet.

The day by day variations in the excretion of magnesium through the urine and feces are similar to those on the last 6 days of the medium protein diet, constant for 30 days. It is to be expected that variations would occur in the change from the medium to the high protein diet while the body was becoming adjusted, and by referring to Tables VII & VIII and Charts I & II this is readily seen. W. W. again showed a greater fluctuation in the excretion of magnesium through the urine; his excretion ranged from 0.086 to 0.134 gm. per day as compared with 0.085 to 0.111 gm. per day for D.A. The amount excreted does not be become more constant at the end of 15 days than at the begenning.

The fecal excretion varied considerably from day to day on this diet. When the amount of magnesium excreted

TABLE VIII

DAILY MAGNESIUM BALANCES ON THE HIGH PROTEIN DIET

	13	0.299	0.234	0.113	0.347	-0.048		0.065	21.7
	14	0.299	0.186	0.112	0.298	0.001	0.3	0.113	37.8
	15	0.299	0.142	0.134	0.276	0.023	7.7	0.157	52.5
Average		0.293	0.172	0.106	0.278	0.015	5.1	0.121	41.3

by the way of the intestinal tract is compared with the amount of feces excreted for that day, it is observed that if the total amount of wet feces was high the magnesium excreted was also larger in amount. (Chart II) In spite of the fact that the children were on a constant diet, there was considerable variation in the amount of feces excreted daily, and generally the amount of fecal magnesium increased or decreased with the increased or decreased amount of feces. These extreme daily variations may be due to insufficient information as to what constitutes a daily metabolic cycle.

That both subjects had to adjust themselves to the higher protein intake is shown by the absorption figures for the first three days of this period. The percentage of the intake absorbed by D.A. was considerably lower for these three days than for the preceeding 5 days of the medium protein diet as well as for the succeeding days of the high protein level. There were four days on this diet when D.A. apparently excreted more magnesium than he absorbed. These days were all at the beginning of the new diet and are due, no doubt, to the adjustment to the higher protein intake since he was in equilibrium or storing magnesium previous to the change.

The absorption figures for W.W. on the first 3 days of this diet shows extreme fluctuation from one day to the next. This was also true for the 6 days of the medium protein diet, which had been constant for 24 days previously. However, after the third day, he evidently became adjusted to the dietary change since the absorption figures were higher, although showing daily variations.

Both subjects absorbed slightly more magnesium on the high protein diet. D.A.'s average absorption increased from 0.094 to 0.119 gm. and W.W. from 0.118 to 0.121 gm. Calculated on the percentage basis, W.W. absorbed less of the intake on the high level which was 41.3 per cent as compared with 44.0 per cent on the first diet, while D. A. increased his percentage of absorption from 38.4 to 44.7 per cent.

With an increase in absorption on the second diet, an increase in retention would be expected and this was found to be the case with both subjects. During the first 5 days, D. A. apparently excreted more magnesium than he absorbed since 4 out of the 5 retention figures were negative. However, after this period of adjustment, D.A. retained magnesium throughout the remainder of the period, the amount varying from 0.011 to 0.127 gm.

W.W. showed somewhat similar reaction to the change in diet, although his period of adjustment was shorter in length than for D.A. He had only two negative retention days at the beginning of the period, as compared to 4 negative days for D.A. Although W.W.'s retention on the medium protein diet was less consistent than for D.A., he apparently became more quickly adjusted to the change in diet. After the initial adjustment period both subjects stored magnesium every day until the end of the experiment, with the exception of the 13th day for W.W. which was negative. W.W.'s retention on this diet ranged from 0.001 to 0.067 gm.

The average retention figures for the high protein level are greater for both subjects than on the medium protein diet. D.A.'s average retention increased from 0.008 to 0.024 gm. and there was an increase from 0.010 to 0.015 gm. for W.W. Of the amount absorbed, D.A. retained 20.1 per cent as compared to 8.5 per cent on the medium level. The percentage retained of the amount absorbed for W.W. increased from 8.5 per cent to 12.4 per cent when the diet was changed. The higher magnesium intake appeared to favor increased magnesium retention.

When the magnesium metabolism data are calculated on the 3 day period basis, as given in Tables IX & X, there is practically no difference between the two periods on the medium protein diet in the excretion of the mineral in the urine and feces for both subjects. There is, also, only a very slight variation in the absorption values for the 3 day periods for the subjects, but there is a significant difference in the retention values for the two 3 day periods. The first 3 day period is the higher for D.A. while W.W. has the higher retention the last three days.

There is more variation in the output for the different 3 day periods on the high protein level for both subjects and, in general, the trend is similar to the daily variations.

TABLE IX

AVERAGE DAILY MAGNESIUM BALANCES BY 3 DAY PERIODS ON MEDIUM PROTEIN DIET

As the subjects are maintained on the higher magnesium and protein intake, there is less magnesium excreted for each succeeding period with the single exception of W.W.'s last 3 day period. With a progressive decrease in the excretion there is a correspondingly progressive increase in the amount of magnesium absorbed and retained. Computing the balances on the 3 day period basis, the negative and positive days offset each other and there is only one negative period for each subject throughout the entire 21 days. The two subjects have different negative periods, D.A.'s being the first period on the high protein intake while W.W.'s is the last 3 day period.

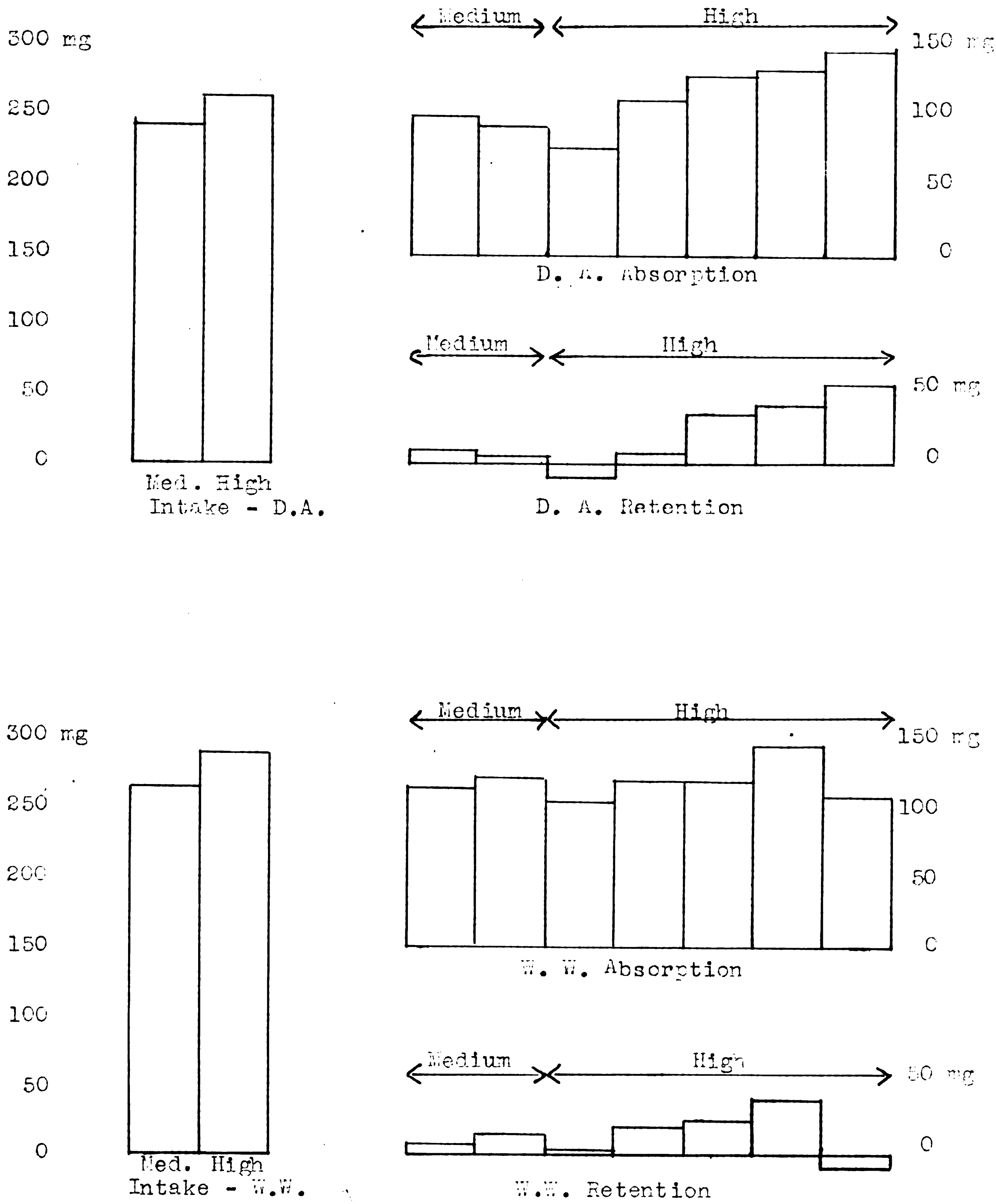
When the averages for the two levels are compared, it will be seen that both subjects absorbed more magnesium on the high protein diet. However, computed on the percentage basis, W.W. absorbed less of the magnesium on the higher intake while D.A. increased his absorption.

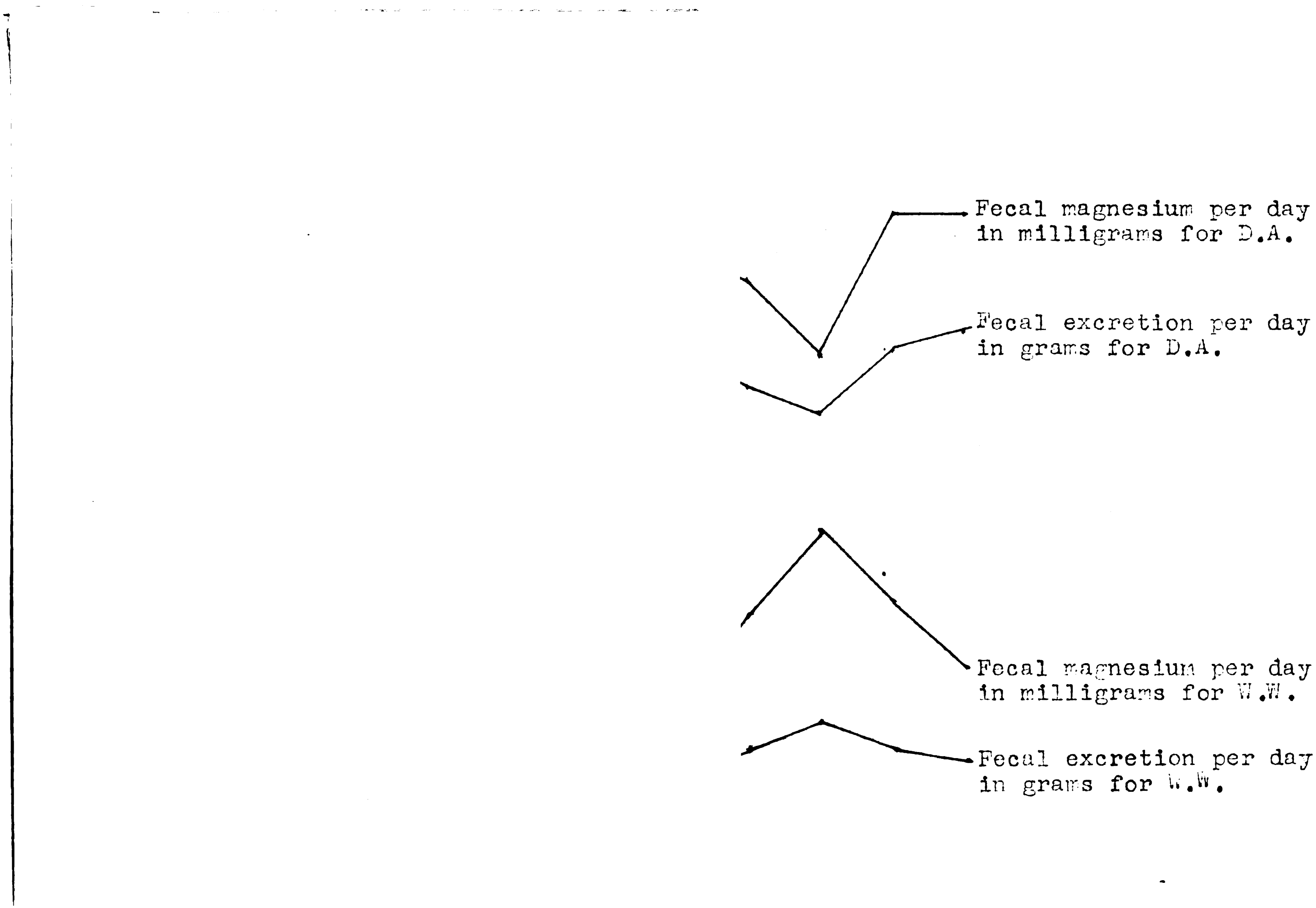
The retention was considerably higher for the high level, D.A.'s markedly so. His retention increased from 3.3 per cent of the intake to 9.0 per cent when on the higher protein and magnesium diet. W.W.'s retention increased from 3.7 to 5.1 per cent. Of the amount of magnesium absorbed that was retained, D. A. retained 8.5 per cent on the low level and 20.1 per cent on the high level, while W.W.'s retention increased to 12.5 per cent on the high intake as compared to 8.4 per cent on the medium protein diet.

A graphic representation of the intake, absorption and retention of magnesium on the 3 day period basis is given in Chart I. This shows that the first 3 day period following the change in diet was a period of adjustment, since the amount absorbed decreased and the retention dropped to almost equilibrium for W.W. and became negative for D.A. Following this period of adjustment there is increasing absorption and retention for each period with the exception of the last period for W.W. when the amount absorbed decreased to approximately 3/4 of the preceeding period and the retention became negative. A reference to all the available data recorded during the study brings forth no information that might explain W.W.'s reaction during this last period.

The acid-base values of the two diets, calculated in cubic centimeters of N/10 acid or base, are given in Tables III & IV. It will be seen that the high protein diet was slightly less basic than the medium protein diet, having 7.2 cc. of N/10 excess base as compared to 13.3 cc. on the medium protein level. The fact that the high protein diet was less basic may have been a factor in favoring magnesium retention on this level. This result is not in agreement with that of Shohl and Sato (22) who obtained greater magnesium retention when $NaHCO_3$ was added to the diet of infants than when HCl was added. Monroe (17) did

Chart I
INTAKE, ABSORPTION AND RETENTION OF MAGNESIUM BY 3 DAY PERIODS





AGNESIUM WITH AMOUNT OF FECES EXCRETED

not obtain any marked difference in the effect of high and low protein diets on the magnesium balances in his animal experiments.

The paths of excretion of the mineral in the two subjects are given in Table XI. The urinary excretion for D.A. on the two diets remained the same, while the fecal magnesium decreased from 61.6 per cent to 55.3 per cent. The total excretion was lowered from 96.7 to 91.0 per cent. The paths of excretion for W.W. were somewhat different. There was a decrease in the urinary excretion from 40.3 per cent on the first diet to 36.2 per cent on the second. The fecal magnesium increased from 56.0 to 58.7 per cent on the higher intake. The total excretion decreased from 96.3 to 94.9 per cent which was less marked than in the case of D.A.

Since caloric and protein requirements are based on body weight, there may be a similar relationship between body weight and mineral requirement. The magnesium balances for the two periods calculated per kilogram of body weight, are recorded in Tables XII and XIII. The intake per kilogram was constant for the two periods on the medium protein diet for both subjects. It was constant, also, for D.A. on the high level. Because W.W.'s weight fluctuated from period to period on the high level, the amount of magnesium per kilogram varied from 0.015 to 0.016 gm. There was very little variation in the output and absorption of magnesium on the medium level while the high

TABLE XI

PERCENTAGE OF THE MAGNESIUM INTAKE EXCRETED

Subject	Medium Protein			High Protein		
	Feces	Urine	Total	Feces	Urine	Total
D.A.	61.6	35.1	96.7	55.3	35.7	91.0
W.W.	56.0	40.3	96.3	58.7	36.2	94.9

TABLE XII

MAGNESIUM BALANCES ON THE
MEDIUM PROTEIN DIET CALCULATED PER KILOGRAM OF BODY WEIGHT

Subject	Period	Weight	Intake	Output			Retention	Absorption
				Feces	Urine	Total		
		(kg.)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)
D.A.	1	17.4	0.014	0.008	0.005	0.013	0.001	0.006
	2	17.4	0.014	0.009	0.005	0.014	0.000	0.005
Average		17.4	0.014	0.0085	0.005	0.0135	0.0005	0.0055
W.W.	1	18.8	0.014	0.008	0.006	0.014	0.000	0.006
	2	19.0	0.014	0.008	0.006	0.014	0.001	0.006
Average		18.9	0.014	0.008	0.006	0.014	0.0005	0.006

TABLE XIII

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

Subject	Period	Weight	Intake	Output			Retention	Absorption
				Feces	Urine	Total		
		(kg)	(gm)	(gm)	(gm)	(gm)	(gm)	(gm)
D.A.	1	17.5	0.015	0.011	0.005	0.016	-0.001	0.004
	2	17.4	0.015	0.009	0.006	0.015	0.000	0.006
	3	17.5	0.015	0.008	0.005	0.013	0.002	0.007
	4	17.5	0.015	0.007	0.005	0.012	0.003	0.007
	5	17.5	0.015	0.007	0.005	0.012	0.003	0.008
Average		17.5	0.015	0.008	0.005	0.013	0.002	0.007
W.W.	1	18.8	0.016	0.010	0.005	0.015	0.001	0.006
	2	18.8	0.015	0.009	0.005	0.014	0.001	0.006
	3	19.0	0.016	0.009	0.005	0.014	0.002	0.007
	4	19.1	0.015	0.007	0.006	0.013	0.002	0.008
	5	19.1	0.016	0.010	0.006	0.016	0.000	0.006
Average		19.0	0.015	0.009	0.005	0.014	0.001	0.006

level shows greater fluctuations for these values throughout the five periods for both subjects. The absorption and retention figures for D.A. are higher on the high protein diet, while for W.W. the absorption figure remains the same with the dietary change, but the retention per kilogram is greater on the higher intake. The per cent of absorbed magnesium for D.A. on the medium protein diet was 39.3 and 42.9 for W.W. On the high protein diet this figure increased to 46.6 for D.A. and was reduced to 40.0 for W.W.

Because of the interrelationship of magnesium and calcium, the ratios of these two minerals, on the two protein levels, are tabulated in Tables XIV and XV. The importance of this interrelationship between these two elements has been studied by a number of workers (1) (3) (7) (9) (10) (14). According to the standards recommended by Sherman and Hawley (21), the amounts of calcium provided in these constant diets were adequate. These workers recommended not less than 1 gm. of calcium per child per day to support optimal rate of storage. The calcium intake on the medium protein diet was 0.991 gm. for D.A. and 1.090 gm. for W.W. The high level furnished D.A. with 1.191 gm. and 1.310 gm. for W.W.

Recent investigations (3) (9) (10) (14) indicate that the amount of phosphorus in the diet is an important factor in the metabolic relationship of calcium and magnesium.

TABLE XIV
DAILY INTAKE AND RETENTION RATIOS OF MAGNESIUM TO CALCIUM* ON THE MEDIUM PROTEIN DIET

Subject	Day	Intake Mg	Intake Ca	Intake Mg:Ca	Retention Mg	Retention Ca	Retention Mg:Ca
		(gm)	(gm)		(gm)	(gm)	
D.A.	1	0.243	0.987	1:4.1	-0.022	-0.147	
	2	0.243	0.987	1:4.1	0.043	0.298	1:6.9
	3	0.243	0.987	1:4.1	0.011	0.088	1:8.0
	4	0.247	0.995	1:4.0	0.000	0.101	
	5	0.247	0.995	1:4.0	0.012	0.171	1:14.3
	6	0.247	0.995	1:4.0	0.006	-0.009	
Average		0.245	0.991	1:4.0	0.008	0.084	1:10.5
W.W.	1	0.265	1.086	1:4.1	-0.009	-0.109	
	2	0.265	1.086	1:4.1	0.041	0.296	1:7.2
	3	0.265	1.086	1:4.1	-0.009	-0.221	
	4	0.271	1.094	1:4.0	0.007	0.077	1:11.0
	5	0.271	1.094	1:4.0	-0.010	0.001	
	6	0.271	1.094	1:4.0	0.043	0.252	1:5.9
Average		0.268	1.090	1:4.1	0.010	0.049	1:4.9

* Figures from Tables, Hiller, Unpublished Thesis (12).

TABLE XV

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

		Intake			Retention		
	14	0.299	1.345	1:4.5	0.001	-0.189	
	15	0.299	1.345	1:4.5	0.023	0.337	1:14.6
Average		0.293	1.310	1:4.5	0.015	0.012	1: 0.8

* Figures for first 9 days from Tables, Hiller, Unpublished Thesis (12). Figures for last 6 days from Unpublished Data, Foods & Nutrition Dept., Michigan State College.

Sherman and Hawley (21) recommend 1.16 to 1.46 gm. of phosphorus per child per day as adequate amounts for optimum retention in a growing child. Subject D.A. received 1.273 and 1.582 gm. of phosphorus on the medium and high protein diets respectively (15). The amounts for W.W. were 1.1 times as much.

The Mg:Ca ratio of the diet was practically constant, although showing slight period variations. The Mg:Ca ratio on the medium protein diet was 1:4 and on the high level it was 1:4.5. Reference to table XIV shows, that on the days of the medium protein level when magnesium and calcium were stored, the retention ratios were higher than the intake ratios for both subjects. The retention ratio of Mg:Ca for D.A. varied from 1:6.9 to 1:14.3 while for W.W. the range was 1:5.9 to 1:11.0.

There was considerable difference in the average retention ratios of the two subjects. D.A. had an average Mg:Ca ratio of 1:10.5 as compared to 1:4.9 for W.W. This wide variation is to be expected when it is observed that D.A. stored more calcium and less magnesium than W.W. on the medium protein level.

After the first 4 or 5 days on the high protein diet the Mg:Ca ratio for D.A. became more consistent. There were day to day variations, but with the exception of the 5th day the daily ratios were never as great as his average retention ratio on the medium level.

The retention ratio of the two minerals for W.W. are less consistent on the higher protein and mineral intake than it was for D.A., and he also showed a greater daily fluctuation. His ratio on this diet varied from 1:0.5 to 1:26.0 while D.A.'s variation ranged from 1:3.9 to 1:17.8. The two subjects did not react alike when the amount of protein and minerals was increased. However, their average Mg:Ca ratio was lower on the higher intake than on the medium level. When the diet was changed D.A.'s ratio decreased from 1:10.5 to 1:5.8 and W.W.'s from 1:4.9 to 1:0.8.

The absorption and retention per 24 hours and per kilogram body weight on the two diets are summarized in Table XVI. The average absorption of magnesium in 24 hours on the high protein intake was 0.014 gm. greater than on the medium level. However, when calculated on the kilogram basis this difference is only 0.0005 gm. which may or may not be a significant amount.

The average retention of magnesium per 24 hours is 0.010 gm. higher on the increased protein diet. The average retention per kilogram for the last 15 days of the experiment is 0.0013 gm. greater and, considering the small amount of magnesium found in the average diet, this difference in retention may be significant.

This experiment indicates that there is more storage of magnesium when the intake of this mineral is increased. Whether this storage is due to the increased intake of mag-

TABLE XVI

COMPARISON OF BALANCES OF THE TWO CHILDREN

Subject	Magnesium per 24 Hours			
	Medium Protein		High Protein	
	Average Retention	Average Absorption	Average Retention	Average Absorption
	(gm)	(gm)	(gm)	(gm)
D.A.	0.008	0.094	0.024	0.119
W.W.	0.010	0.118	0.015	0.121
Average	0.009	0.106	0.0195	0.120
	Magnesium per Kilogram of Body Weight			
	Medium Protein		High Protein	
	Average Retention	Average Absorption	Average Retention	Average Absorption
	(gm)	(gm)	(gm)	(gm)
D.A.	0.0005	0.0055	0.002	0.007
W.W.	0.0005	0.0060	0.001	0.006
Average	0.0005	0.006	0.0018	0.0065

nesium or to the higher protein diet, or to the increase in the phosphorus and calcium intake, or to the less basic diet is a question one cannot attempt to answer from the present data.

The intake of magnesium on the medium protein diet is sufficient to maintain positive balances. However, it appears that 0.250 to 0.270 gm. represents about the minimum requirement for these 2 four year old subjects, since there are occasional negative balances on these amounts. While further studies are needed to establish the quantity of magnesium needed by 4 to 5 year children, these results may be useful until further data are available.

Summary

This investigation was made to study the daily variations in the magnesium metabolism of two normal preschool children on a 6 day medium protein diet and a 15 day high protein diet.

1. There was considerable daily variation in the amount of magnesium absorbed and retained, possibly due to the lack of information as to what constitutes a daily metabolic cycle, and to the difficulty in collecting daily fecal samples.

2. On the medium protein diet D.A. and W.W. stored daily 0.008 and 0.010 gm. respectively. On the basis of per kilogram of body weight these same values were 0.0005 gm. for

both subjects.

3. There was greater retention of magnesium on the high protein diet for both subjects, D.A. storing 0.024 gm. and W.W. 0.015 gm. In grams per kilogram body weight D.A. retained 0.002 gm. and W.W. 0.001 gm.

4. The two children did not react the same to the two diets. D.A. was more constant in his retention on the medium protein diet than W.W., but W.W. became adjusted more quickly when the diet was changed.

5. With the increased protein and magnesium intake there was, with the exception of one period for W.W., a gradual increase in the amount of magnesium absorbed and retained for each 3 day period for both subjects after the adjustment period.

6. Several factors may be responsible for the increased retention during the last period of 15 days. It may have been the increased protein intake, the increase in the amount of magnesium, the increased calcium and phosphorus intake, or since the high protein diet was slightly less basic, it may have favored increased magnesium retention.

7. The average retention ratios of Mg:Ca for D.A. and W.W. on the medium protein diet were 1:10.5 and 1:4.9 respectively. On the higher protein diet these ratios were lower, namely 1:5.8 for D.A. and 1:0.8 for W.W.

8. It appears from this experiment that 0.250 to 0.270 gm. magnesium represents about the minimum requirement for 4 to 5 year old children.

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