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## The Calcium Needs of Pre-School Children

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<u>Alma E. Wyatt</u>

<u>1945</u>

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I wish to express my grateful appreciation to Dr. Marie Dye, whose guidance and help was a source of inspiration for me; and also to Miss Mary Lewis who gave me valuable help in organizing the material for this paper.

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Alma E. Wyatt

The purpose of this paper is to set forth what is known about the calcium metabolism of pre-school children, and to summarize what is the trend of evidence as to the calcium needs for this age group.

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The first part of the paper will concern itself with a brief discussion of methods used for the study of calcium needs. This will be followed by a discussion of normal utilization of calcium and phosphorus in growing children, including distribution of calcium and phosphorus in the body, the picture of bone growth, and the sequence of calcium and phosphorus metabolism with its problems of digestion and absorption and deposition in the bone, losses of these minerals from the body through urine and feces, and the variation of metabolic response to a given intake of calcium.

From this point the discussion will proceed to a consideration of the factors affecting calcium and phosphorus metabolism such as age, previous nutritional history, availability of calcium and phosphorus in the diet, the levels of calcium and phosphorus in the diet, the effect of vitamins C and D on calcium and phosphorus utilization, the interrelationship of calcium, phosphorus and nitrogen in the metabolism of calcium, the effect of the parathyroids, and the effects of such factors as sugar and oxalic acid. The paper deals

principally with calcium needs of pre-school children, but the interrelationship of calcium, phosphorus and nitrogen as well as other factors mentioned above brings them into the discussion briefly, where relationships indicate the necessity.

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The various studies which have been conducted upon children to determine their calcium needs are similar in technique. In general, children are selected who have no known organic trouble. These subjects are fed an experimental diet of known calcium level for definite periods of time. There is usually a preliminary period during which time the body is assumed to adjust itself to the new experimental diet. Samples of urine and feces are collected at specified intervals, and analyzed for amount of calcium excreted. Knowing the intake, and the amount excreted, it is possible to determine the amount retained. The amount retained is taken as evidence of the amount needed. This method of study is known as a balance study.

Another method of studying calcium needs of children which is probably not as common as the balance study, is to make roentgenograms of bone to determine the degree of ossification. This method is used and reported by McNair and Roberts (13) as follows: "Ossification in wrist and forearm has been widely used by anatomists, educators and other investigatros as an index of anatomic age. As such, it has been correlated with mental age, the intelligence quotient and other factors, and important conclusions have been drawn from the findings. Only

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rarely has the suggestion been made that this index might be modified by the state of nutrition, although it seems almost self evident that any prolonged deficiency or marked improvement in diet would have an appreciable effect on development of bone."

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In McNair's and Roberts' study the area chosen for study was the wrist because it had been shown by other workers to <sup>be</sup><sub>4</sub> representative of development in other centers. This does not mean that development will be precisely the same throughout the body, but merely if ossification is accelerated or retarded in the wrists, it will be correspondingly retarded or accelerated elsewhere. Two additional reasons for using the hand were the greater practical ease of taking roentgenograms of this area and especially the fact that the norms for skeletal development available for comparison at the time of the experiment (1938) were based on measurement of these centers of ossification. Daniels (5) also reports the use of this method.

Let us consider the distribution of calcium and phosphorus in the body. <sup>M</sup>inerals are the chief elements in skeletal structures. Approximately 99 per cent of the calcium found in the body is contained in the bones. (3) 70 per cent of the phosphorus of the body is found in the bones, and 30 per cent of the phosphorus is combined with nitrogen.(12) Calcium and phosphorus are essential substances in living muscle and nerve. Phosphorus is

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essential in the structure of all cells. It exists in organic combinations in nucleo-proteins, and in phospholipins; and in inorganic form the phosphorus of the soft\_tissues is combined largely with potassium. Muscle contractility is dependent upon balance of calcium, sodium and potassium. Calcium and phosphorus are essentials in the blood. In health, calcium and phosphorus of the blood are constant within narrow limits, (parathyroids and vitamin C and vitamin D help regulate blood calcium and phosphorus); phosphates and carbonates of calcium, sodium and potassium act as buffers to prevent changes in reaction of blood; both minerals regulate osmotic pressure of blood and other tissues; and calcium is essential in the clotting of blood. (3).

In normal bone development (23) the region of the junction between the epiphysis and shaft is divided into three zones; (a) The distal zone of epiphysis is composed of resting cartilage the cells of which are arranged in a somewhat irregular fashion, but with a tendency to a transverse pattern. (b) Toward the shaft the second zone is that of a proliferating cartilage which is the regions of active growth and ossification. It is this area which by its growth and ossification adds to the length of the long bones. These rows of cartilage cells are separated from one another by a jelly-like collagenous material containing cells known as osteoblasts. These rows of cartilage cells lying in their collagenous material end where the shaft

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or third zone begins. (c) At the junction of these rows of cartilage cells and the shaft there is a lane or zone of capillary loops which form a smooth even surface at the end of the shaft from which they originate. (d) During the process of bone growth and ossification these capillaries invade the columns of cartilage cells destroying them as the capillaries advance. (e) A calcification of the collagenous framework or wall between the rows of cartilage cells slightly precedes the advance of the capillaries. The calcification appears to be due to the action of the osteoblasts. This forms the organic framework of the bone and, as the advancing process continues. the lime salts of true bone are deposited in these osteoid Thus we can see that in normal bone development there tissues. are four stages; the capillaries advance, the cartilage cells disappear the osteoid cells appear, and finally, the osteoid cells are calcified.

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Since rickets occurs in most cases some months after birth, a time when ossification of a large part of the diaphysis (shaft) has occurred, the area affected is principally the region of the junction of the epiphysis and diaphysis where most active growth and ossification are occurring. (23).

The factors affecting digestion and absorption of calcium and phosphorus are quantity intake, conditions in the blood stream and conditions in the alimentary tract. These are summarized by Chaney and Ahlborn (3) as follows:

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Normally the ratio of calcium to phosphorus in the blood stream of a child is 2 to 1. If this altered, rickets This has been demonstrated experimentally in develops. animals by disturbing the proportions of these minerals in the diet. Few such studies have been made on children with intentional variations in calcium-phosphorus ratio. Chaney and Ahlborn (3) report one by Orr and Holt who used two subjects, one healthy, one with incipient rickets. They determined calcium and phosphorus output on three diets, one with the correct proportion of calcium, one with high calcium added as calcium chloride, and one with high phosphorus, added as sodium phosphate. In both children the large amounts of calcium favored the retention of that element, and the loss of phosphorus, and with high phosphorus intake the reverse was true. Macy (12) states that we need to keep calcium and phosphorus in ratio in the diet, because phosphorus fed in excess causes corresponding increase in the excretion and of calcium vice versa. Chaney and Ahlborn (3) continue their summary as follows: Conditions in the alimentary tract thought to affect the absorption of calcium and phosphorus, but for which conclusive proof is lacking, are the fat and fiber content of the diet. Excess fat is thought by some to cause increased elimination since soaps formed in the intestine carry calcium with them into the feces; on the other hand some believe increase in dietary fat causes greater mineral retention. Holt and Fales

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in a balance study on 7 normal children, 2-6 years of age, secured better calcium retention when the daily diet contained 30-60 grams of fat than when only 5-8 grams were included; they recommend 3 grams per kilogram of body weight daily and 0.021 to 0.036 grams calcium per gram of fat. Their study indicates the importance of the use of whole rather than skimmed milk as a means of preventing rickets in infants.

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Excess fiber in the digestive tract may, by increasing fecal excretion, decrease the amount of calcium and phosphorus absorbed. This was shown by Ascham to occur in dogs when large amounts of agar or cellulose flour were fed. If this is likewise true with the human species, it may account for the lessened utilization of calcium observed by Sherman and Hawley in a balance study conducted on children in which the source of the mineral was vegetable rather than milk. Before conclusions can be drawn in regard to the effect of fiber on mineral retention, more experiments must be performed.

It is believed that the reaction of the intestinal contents influences the solubility of the calcium phosphates which is greater in an acid medium, less in alkali. If this is true, most of the calcium absorption will take place in the upper segment of the small intestine where the reaction is still acid, and the salts are in an absorbable state; it is to be expected that some of the mineral consumed will pass along the tract till it becomes insoluble, and in this form will be excreted unused. Recent

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studies have shown that in rachitic animals the contents of the alimentary tract tend to be alkaline throughout; in such cases normal absorption of calcium and phosphorus is not possible, and the bone fails to secure its quota. It is not as simple to control the reaction of the digestive tract as it is to regulate the fat or fiber content in the In ordinary infant feeding and in rickets an acid such diet. as lactic may be prescribed. Lactose, when fed to rats in amounts equal to 25 per cent of the diet, has been shown by Bergeim to be more effective than other sugars in favoring calcium and phosphorus retention; this is thought to be due to the slower absorption of this sugar and to its fermentation in the intestines with the production of acid. The practical value of this finding may be questioned, since the large proportion of lactose required to improve utilization is never incorporated in any ordinary menu, not even in infant feeding.

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Sherman (18) gives a good description of the various kinds of rickets and their causes:

When the calcium content of blood serum is normal, but phosphorus (phosphate ion) content is subnormal, there results the so-called low phosphorus rickets. There is a tendency to over growth of cartilaginous or osteoid tissue at the ends of the long bones, and at the rib junctions. Because retarded calcification prevents the bones from acquiring normal rigidity, the pressure of the body weight tends further to enlarge the

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ends of the bones. Together these abnormalities make a typical picture recognized in part by the obvious clinical signs of enlarged joints and the "rachitic rosary", and in part by means of the Roentgen ray photograph and the determination of inorganic phosphate in the blood serum; and confirmable at autopsy by histological examination. There has sometimes been a tendency to confine the term rickets or "true rickets" to this low phosphorus type.

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When the phosphorus content of the serum is normal, but its calcium content is subnormal there results gross abnormality, but a somewhat different histology of the bones. This has sometimes been called a "rickets-like condition", or a second type of rickets, but is now commonly known as low calcium rickets. Low calcium rickets is often accompanied by tetany; and it seems not improbable that in such cases the trouble with the nerves as well as with the bones is attributable (at least in part) to the deficiency (subnormal concentration) of calcium in the blood.

When both calcium and phosphorus are reduced below normal concentration in the blood serum, calcification is retarded, but the structural abnormality of the bone differs from that of the two types described above. The condition constitutes a third type. Because of difference in clinical and histological appearance it is sometimes called an osteoporosis.

Unless the mineral supply is very deficient, any of the three types can be prevented by vitamin B which acts to restore to normal the concentration of calcium and phosphate ions in the blood.

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The chief organ of excretion so far as calcium is concerned is the intestine through which 87 per cent of the calcium list is excreted. In the case of phosphorus, 55 per cent of the daily loss is through the kidney, 31 per cent through the intestine, and 14 per cent is retained. (12)

Studies over a period of years show a wide physiological variation in metabolic responses of different children to a given intake of calcium. Consecutive balance periods reveal individualities in response of different subjects to identical conditions and also show variations from period to period. Hunscher, Hummel and Macy (8) showed evidence of this in a study of six children to the same calcium intake (1 gram per day). In this study age did not explain wide individual variations in response. Their data was illustrative of increments and decrements of storage of calcium observed in healthy individuals maintained under strict metabolic conditions provided they are followed continuously over a considerable period of time. Consecutive balance periods in their study revealed individualities in response for acid-base mineral elements and nitrogen of different subjects to identical conditions, and variations from period to period.

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Porter-Levin (16) also showed evidence of a variation in calcium and phosphorus storage on the same level of intake in three normal children. Her study also showed a wide variation from period to period as well as variation in storage for each child. This study indicates it required from five to seven successive balances or from fifteen to twenty-one consecutive days, to cover the entire range of variation in the retentions.

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It is becoming increasingly evident that the amount of calcium stored depends upon several factors: (17) namely, age, previous nutritional history, availability of calcium in the diet, the level of calcium in the diet, the changing demands of growth impulses, the interrelationship of calcium, phosphorus and nitrogen in the metabolism of pre-school children, adequate amounts of vitamin D and vitamin C, and the action of the parathyroid glands. <sup>1</sup>/<sub>2</sub> ach of these will be discussed in detail.

Obviously age is a factor affecting calcium and phosphorus needs. Growing children will need adequate amounts for normal development. This is particularly true during the times when children are going through rapid growing periods. Sherman (18) states that during periods of rapid growth the food as a whole should have a calcium phosphorus ratio of between 1 to 1 and 2 to 1, and that the ratio of calcium to phosphorus in the body as a whole is increasing throughout growth. Blunt and Cowan (1) state that the ratio between calcium and phosphorus retained is optimum if the ratio is 2 to 1, then normal bone development appears to take place.

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That previous nutritional history is a factor affecting calcium storage has been shown by many workers. Pierce and others (15) observe large amounts of calcium being stored at the beginning of an experiment of retention of calcium and phosphorus by pre-school children, and attributed this to previous depletion. Outhouse and others (14) in the study of the calcium requirements of five pre-school girls did not consider period 1 in their study because of the possibility of calcium storage during the first period being the result of depleted stores. The child whose store of calcium has been depleted is going to store more when more becomes available. Wang (21) made a comparison of the calcium metabolism of ten normal and fifty undernourished children, and found a slight tendency toward increased calcium storage among the underweight children.

In 1922 Sherman and Hawley reported that a daily intake of 1 gram of calcium was necessary for optimum storage in children 3 to 13 years of age. <sup>S</sup>ince then, studies have been made on calcium retentions at various levels of intake for many children. From these studies there is some evidence that calcium retentions on daily intakes of 1 gram or more were higher than on intakes of less than 1 gram. However, there is a great variation in data as to the minimum quantity necessary to insure maximum retention. It would not be wise to predict the quantity of calcium which might be retained on a given level of intake.

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In a study of the effect of milk supplement on the physical status of institutional children, Roberts and others (13) wished to determine whether the daily addition of a pint of milk to diets of children already receiving approximately one pint would produce any measurable improvement in physical status in one year, and whether any advantage could be demonstrated for irradiated over nonirradiated milk. The growth of three groups of institutional children consisting of thirty-six children, each, was followed through out a calendar year. One group remained on the usual institutional diet as the control, one received daily an additional pint equivalent of evaporated milk, and one an equal amount of irradiated evaporated milk.

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The measurements used to determine physical growth were monthly measurements of height and weight, Franzen indices of nutrition, Lucas-Pryor-Stalz width-length index, roentgenograms of the wrists as a measure of bone development, dental examinations for determination of the arrest or progress of dental caries, and medical examinations. The study was divided into three parts. The first part deals with growth, the second with osseous development and the last with dental caries, all in relation to level of calcium intake as supplied by milk.

By all means of comparison the growth of the children given a supplement exceeded that of the controls. The average excess in means gain in height was 0.9 centimeters (0.3 inches)

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for both milk groups, and in weight was 1.03 kilograms (2.27 pounds) for the plain milk and 0.62 kilograms (1.4 pounds) for the irradiated milk group. The percentages of expected gains made by the three groups were 106, 117, and 115 for height and 109, 122, and 118 for weight for the control, the plain milk and the irradiated milk groups respectively. The percentage of individual children in these groups who made expected gains or better was 61, 67, and 69 for the height, and 58, 70, and 75 for weight of the groups in the same order. When comparison was made of the gains of the individual children in 24 matched trios, the gains of weight in the control child were best in 12 per cent, of the child given plain milk in 46 per cent and of the child given the vitamin D milk in 42 per cent. The gains in height were in the same order, but were less marked. "Though the differences are not large, and do not always attain statistical significance, they tend consistently in the same direction by all methods of comparison, and probably represent real differences which can be attributed in part, at least, to the dietary supplement," is the conclusion of the authors.

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As a part of this study McNair and Roberts (13) studied the osseous development of the wrists and hands in 108 children. Roentgenograms were taken at the beginning and end of a calendar year, and the progress in osseous development, as indicated by

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changes in the Carter ossification ratio was followed. There was no significant difference between the two groups receiv-Though all groups at the beginning were retarded ing milk. in respect to the Carter norms, and were still retarded at the end of the study, both the groups given a supplement reduced their deficits to a greater degree that did the control The percentage of children in more favorable relation group. to the Carter standard for age at the end than at the beginning of the study was 17 for the control, 50 for the milk, and 46 for the D milk group. The mean gain in Carter points for the three groups was 6 for the control, 8.3 for the milk, and 8.2 for the D milk, an advantage of 2.3 and 2.2 points respectively, in favor of the milk groups. The control groups as a whole gained 79 per cent of the expected quota; each group given a supplement gained 115 per cent of the expected amount. In matched trios, the control child made the greater annual gain in 5 (21 per cent) of the comparisons, the child given plain milk in 10-1/2 (44 per cent) and the child given D milk in 8-1/2 (35 per cent). The amounts of the superiority is shown in a comparison of the partmers in the groups as to differences in variations from expected gain, the most test of The mean advantage of the children in the groups the data. receiving milk over their control partners was 2.7 points. This difference is statistically significant. In terms of growth it amounts to more than a third of a year's quota of expected gain.

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As another part of this study Roberts and others (13) studied the effect of milk supplement with and without vitamin D on the development and spread of dental caries in 90 children in an institution over a calendar year. The children were divided into three groups matched as closely as possible on the basis of age, sex and degree of caries. One remained as controls, the other two received daily per child one pint equivalent of evaporated milk, one having it in irradiated the other in nonirradiated form. All children showed extremely progressive For the entire group there was a 26 per cent advance caries. in number of carious teeth, and 98 per cent in the caries score. Although the difference in advance in caries between the groups was small and statistically insignificant, there was a consistent tendency according to all methods of comparison for the progress of caries to be slightly less for the children given a supplement than for the control children, with no consistent difference between the two groups given different types of milk. The percentage of increase in carious teeth was 28.7 for the control, 26.2 for the milk, and 21.5 for the D milk group. The corresponding percentage of increase in score 107, 92.6 and 94.6 respectively for the three groups. These results indicate that the supplement tended to inhibit the rate of dental decay, but that other factors were involved. In view of striking results by other workers for arrest of caries with dietary improvement, the findings in this study are disappointing, say the authors.

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There are several possible explanations. First, even with the addition of a supplementary pint of milk, diets were still inadequate in a number of important elements, most notable vitamin C. Lack of vitamin C may well have been the limiting factor in the present study. The relatively high percentage of carbohydrate may also have been a limiting factor. Those who believe in harmful effects of carbohydrate on teeth will find support for these theories in this study. Whatever the factor or factors responsible for the extreme degree of caries in this institution, they were modified only slightly by the addition of a pint of milk either with or without vitamin D (either the children were already receiving all the needed D or the 70 units supplied was not enough to produce measurable results under conditions of the experiment.)

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The significant observation regarding the entire investigation as reported in this and two preceding ones, is that the results of all three studies are uniformly consistent. By every method of comparison, the children given milk supplement are found to have an advantage over the controls. They made better growth in height and weight, more progress in bone development as judged by ossification of bone of wrist, and there was a slight tendency toward retardations of dental decay.

Daniels, <sup>H</sup>utton, Knott, Everson and Wright (5), conducted a study to determine the relationship of the ingestion of milk to calcium metabolism in children fed standard weighed diets differing in amounts of milk and vitamin D. All but four were given two teaspoons daily of cod liver oil and ten minute daily exposure to ultraviolet ray. Four were studied during June and July and given no cod liver oil or ultraviolet or viostrol. During certain periods four drops of viostrol were given to all to obtain information on optimum retentions of calcium in one pint and one quart of milk respectively.

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Results showed the amount of calcium retained during two periods when cod liver oil was given when receiving one pint and one quart of milk respectively, suggests one pint of milk used as part of a diet optimum in other respects furnishes sufficient calcium for physiological needs of children three to five years of age, provided the calcium is available.

There was a wide variation in amounts of calcium, phosphorus and nitrogen retained by the different children of the same age under varying conditions of diet and vitamin, but results still indicate one pint of milk would provide sufficient calcium for the normal child three to five years old if the diet has enough protein, phosphorus and vitamins from other sources. The authors comment that the results are not in line with present theories (1934) and that the high retentions in previous studies may be due to the fact that the children were depleted in the substances studied. They

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remark too, that the marked difference in amounts retained by the children in their own study may be due to childrens physiological conditions at the time of study or to the children's potentialities of growth.

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In a study to determine the effect of orange juice on the calcium, phosphorus, magnesium and nitrogen retention and urinary organic acids of growing children Chaney and Blunt (4) showed the following effect on calcium metabolism: The children in the experiment showed a slight postive calcium balance on the basal diet, but this was greatly increased during the orange juice period. The extra calcium retention during the orange juice period was considerably more than the little excess calcium in the diet of that period. The calcium assimilated was decidedly benefitted with oranges, the retention being considerably greater than the calcium of oranges, and than might be expected from stimulus to retention caused by larger calcium intake.

The basal intake of calcium was kept at approximately that the children had previous to the experiment, neither it nor the slightly increased amount in the orange juice period was up to the optimum of 1 gram per day recommended by Sherman and Hawley. It might be thought increased retention was due to increased intake nearer the optimum, but the intake of the basal period in the second experiment was higher than the first (due to increased bread consumption) yet the percentage retention was decidedly less, and the variation irregular.

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(one child greater, one less) Sherman and Hawley also found when they increased milk intake there was an increase in actual amount of calcium retained, but in many cases a lowered percentage retained. This seems to demonstrate that some factor in orange juice beside the additional calcium must have caused the retention of calcium.

The results with phosphorus were similar to those with calcium. During the basal diet there was a slightly negative balance. The addition of orange juice made the balance positive. In all cases but one, the additional amount of phosphorus retained was greater than the amount added by the oranges. It seems probable, here as in the case of calcium, there was some factor that helped the body make economical use of the mineral. The phosphorus retention was even more marked than calcium, or in fact, three times as much both in grams and per cent. Chaney concludes that the increased vitamin C and the possible increase acidty of the intestinal tract may explain the beneficial results obtained.

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Hummel, Hunscher and Macy (10) made a comparison of the effect of irradiated milk upon the storage of nitrogen and acidbase minerals in children, judged by the average daily retention of these factors in the children studied. From the standpoint of the actual average daily retention alone, it does not appear that the additional vitamin D in irradiated milk had any nutritive advantage in the deposition of the minerals. On the other hand

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the children showed a more rapid rate of growth during the periods when vitamin D was the only factor added. The increase in rate of skeletal growth was in general, accompanied by a higher calcium-phosphorus ratio in the retention. This indicates a stinulus to formation of bone and, by the decrease in retention of nitrogen, phosphorus and sulphur a shift from the formation of soft tissue to that of skeletal structure. Moreover, the variations which occurred in the average daily storage of calcium during the five day balance periods were reduced, which shows a regulating effect of the vitamin D during the administration of the irradiated milk. In addition, all children were storing base in excess of acid while ingesting additional vitamin D whereas several were actually storing more acid while taking nonirradiated milk, which again shows the development of skeletal structures. From the cumulative results just listed, it seems justifiable to conclude that vitamin D supplements in the form of irradiated milk included in a dietary known to be nutritionally good, and given to children who have good health and good nutritive histories not only exert a regulating influence on the growth impulses, but stimulate skeletal development. These problems emphasize, however, the need for more precise less laborious methods and for further knowledge of the chemical and physiological processes accompanying growth as well as of the physiologic effect of the administration of vitamin D to human beings.

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In a study by Shepherd and Macy (17) to determine the relative advantages of plain milk, evaporated milk, and irradiated milk, there is evidence that evaported milk gives a more rapid synthesis of soft tissue. Vitamin D (in the form of irradiated milk) gave an increased rate of skeletal over soft tissue formation. Out of this study also comes evidence that the inclusion of 400 grams of milk per day predicts a more favorable state of nutrition for a child, especially if his mineral stores are not filled in abundance, provided adequate supplies of other nutrients are available to the body.

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Shohl (19) writes "many experiments have shown that vitamin D draws calcium from the bones only when given in toxic doses; normally it increases absorption of calcium, but parathyroid always draws it from the bones. Vitamin D cures rickets, whereas parathyroid does not. Rickets can be produced and cured in animals without parathyroid glands. Increase of vitamin D intensifies the action of the hormone in raising blood calcium and causes abnormal calcification. Without vitamin D this parathyroid action is lessened. Although parathyroid deficiency causes diminished serum calcium, absence of vitamin D does not. Both parathyroid and vitamin D raise blood calcium, but the former lowers blood phosphate, whereas the latter raises it. Therefore, in spite of their common relation to calcium and phosphate metabolism, the

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action of the two is essentially dissimilar."

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In a study to establish the relationship of calcium, phosphorus and nitrogen in metabolism of pre-school children, Hawks, Bray Wilde and Dye (7) fed two groups of pre-school children first a diet containing 3 grams, then 4 grams per kilogram of body weight of protein. The metabolic processes were not the same on the two different levels. The increased protein (either from meat, milk and eggs or egg white and gelatin) produced similar changes in growth. The calcium phosphorus retention ratios indicated a difference in the relative growth of bone and soft tissue on the two different levels of protein. All children but one were probably producing normal proportions of bone and muscle tissue on 3 grams of protein, as shown by calcium-phosphorus ratio. The 4 gram level of protein accelerated growth of soft tissue as compared with bone growth, (A low ratio indicates increased muscular development, a high ratio increased bone development).

All the children grew faster on the 4 gram protein level. The composition of weight gains probably was not the same for all children on the same diet, or for each child on two different diets. If increased weight gains had caused acceleration of growth of bone, there should have been increased calcium retention. Two children showed no increased calcium retention. Three showed cnly slight increase in calcium retention. Since increase of bone was small on high proteins,

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soft tissue must have composed a large part of the increased weight gains. Higher nitrogen retentions on 4 grams of protein substantiate this fact. Water storage may have produced some of the extra weight gains because there was an increase in sodium and chlorine retention on the higher protein and these minerals are associated with fluid tissues in the body. Fat deposit may cause increased weight gains; and some potassium may have been deposited with these tissues.

The ratio of bone growth to protein tissue may also be expressed as nitrogen to calcium, assuming all nitrogen is utilized by protein tissue, and all the calcium in bone. The 4 gram level of protein increased nitrogen to calcium retention ratios in all children in the experiment (more nitrogen was stored than calcium), so protein tissue increased more rapidly than bone.

The lower nitrogen to phosphorus ratio on the 4 gram protein level gave evidence that all five children were not producing the same type of body tissue. It showed increased prosphorus retention in relation to nitrogen for three children. The additional phosphorus probably deposited with extra calcium these children stored.

Stearns (20) states that absolute retentions of calcium and phosphorus are important, but that the relationship between the amounts of calcium and phosphorus is equally significant;

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and that calcium retention is an index of the rate of bone growth, while the amount of phosphorus retained is used both by bone and soft tissue, and its distribution is an important indication of the comparative rates of growth of bone and soft tissue.

Daniels (5) also showed a relationship of phosphorus and nitrogen to calcium retention.

Shohl (19) states that the main regulator of calcium in the blood is parathyroid hormone; the level of calcium of the serum rises or falls according to the amount of hormone available. Shohl further states that removal of the parathyroids reduces serum calcium to about half the normal amount, and tetany follows. In hyper-parathyroidism the primary effect is in drawing out calcium phosphate from the bones, the most rapid growing bone is first drawn upon. In absence of vitamin D as in rickets, the parathyroid is stimulated and compensates and maintains the level of calcium of the blood. (19) Parathyroid hormone not only governs, but is in turn governed by calcium and phosphorus metabolism. (19)

Bonner and others (2) experimented to determine the influence of a daily serving of spinach or its equivalent in oxalic acid upon the mineral utilization of children. Since free oxalic acid of the spinach will combine with the calcium of the other foods eaten simultaneously, any untoward effect on calcium retention will depend upon the quantity of spinach

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consumed, and the adequacy of calcium in the diet. Each child was studied with and without spinach or oxalic acid, thus each child acted as his own control. The daily intake of simply prepared common foods was kept constant in quantity and quality for each child through the control and experimental periods except for the addition of 100 grams of canned spinach (pureed to assure complete sampling of plant parts) during the latter periods. Each day for five days immediately following spinach addition, and with the same basal diet, oxalic acid equal to that determined in the spinach fed, was given together with the calcium (calcium acetate) equivalent of the spinach. The basal diet contained the equivalent of 90-100 U. S. P. units of vitamin D and 90-120 milligrams of ascorbic acid daily. The calcium intake of eight of the children approximated 0.8 grams daily, while that of the two eight-year Oxalic acid content of the 100 grams olds was 1.3 grams. spinach fed was 0.7 grams. Calcium contributed by the spinach supplement represented only 5.7 per cent of the total intake.

The control periods of twenty-five days on the ordinary mixed diet of common foods showed the children to be storing nitrogen, calcium and phosphorus. Storage rates of these elements were not significantly altered by the daily consumption of 100 grams of spinach; moreover, they were

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compatible with normal variations observed in growth. No cumulative toxic or untoward effects could be determined either in the average daily retentions, or in the progrossive storage curves of nitrogen, calcium and phosphorus either during the period of consumption of spinach and oxalic acid, or during the control period immediately following. The supplementation of an already adequate diet with a generous serving of spinach daily, even for as long as forty consecutive days, did not change the rate of calcium storage in growing children when their calcium intake was adequate to cover the precipitating effect of the Oxalic acid, and provide for their fluctuating growth needs.

The practical conclusion is that spinach is not harmful, even in servings of 100 grams daily, at least in preadolescent children, but because of its richness in vitamins, minerals, especially iron, and other nutritive essentials, it should retain its customery place along with other green leafy vegetables.

Fairbanks and Mitchell (6) in a study to determine the availability of calcium in spinach, in skim milk powder, and in calcium oxalate concluded that the calcium of spinach, either fresh, cooked or canned is very poorly utilized for the mainténance and growth of rats, in all probability it is quite unavailable under conditions permitting a very high utilization of milk calcium. Calcium oxalate appears to be entirely

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unutilizable by the growing rat. All pairs fed a skim milk supplement to the basal diet exhibited the greater amount of calcium in the body. The average amount of stored calcium for the rats on the milk supplement was 760 milligrams and for those on the spinach supplement was 342 milligrams. The latter is 92 per cent of the calcium consumed in the basal diet, and probably was derived entirely from it. Assuming that the utilization of the calcium in the basal diet was not differently affected by the different calcium supplements fed, the average difference in calcium storage must have resulted from the difference in utilization of milk and spinach calcium.

In an experiment by Hubbel and Koehne (9) to determine the effect of varying sugar intake on the nitrogen, calcium and phosphorus retention of children, there was a tendency toward increased nitrogen and phosphorus retention, while calcium retention decreased in five out of seven cases.

From the evidence presented we have seen that a number of factors affect calcium metabolism, namely, age, previous nutritional history, availability of calcium in the diet, the level of calcium intake, vitamin D and probably vitamin C, action of the parathyroids, changing growth impulses, and conditions in the blood stream and the alimentary canal. Two of these factors lie within our control, namely, the level of calcium intake, and to a certain extent the amount of vitamin intake.

In view of the evidence presented it would seem that the

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calcium needs of pre-school children are best met by including at least one quart of milk per day in the diet. The studies which suggest one pint of milk per day as being adequate were not followed over a period of years, and were conducted under the best possible conditions where there was no deviation in living regime, which would not be possible under family conditions. There is also evidence that vitamin D tends to stimulate an increase in calcium retention and bone formation, but just how much vitamin D should be included for best possible results is still open to question. As to the relationship of vitamin C to calcium metabolism, this phase too needs more study. However, it seems safe to assume that some vitamin D at least in amounts which children may be getting under the best possible dietary conditions in the home is actually beneficial as far as calcium metabolism is concerned, and there is a possibility that more may be better, though to what extent it is as yet impossible to say. The one definite conclusion that can be drawn from evidence here presented is that every child should have at least one quart of milk per day.

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