

A STUDY OF THE LITERATURE ON CALCIUM INTAKE AND RETENTION AND THE PARTITION OF OUTPUT WITH CERTAIN OBSERVATIONS ON CALCiUM RETENTIONS OF WOMEN

> Thesis for fhe Degree of M. S. MlCHlGAN STATE COLLEGE Marian Arline Sizelove 1947

## **THESIS**

## This is to certify that the

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A STUDY OF THE LITERATURE ON CALCIUM INTAKE AND RETENTION AND THE PARTITION OF OUTFUT WITH CERTAIN OBSERVATIONS ON CALCIUM RETERTIONS OF WOREN

## presented by

## MARIAN ARLINE SIZELOVE

## has been accepted towards fulfillment of the requirements for

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Margaret G. Ohlson

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## A STUDY OF THE LITERSTURE ON CALCIUM INTAKE AND RETENTION AND THE PARTITION OF OUTPUT WITH CERTAIN OBSERVATIONS ON CALCIUM RETENTIONS

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OF WOMEN

by

## MARIAN ARLINE SIZELOVE

.A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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The author wishes to express her appreciation to Dr. Margaret A. Ohlson for her assistance and guidance during this study and to all of the others who assisted in making this study possible.

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#### INTRODUCTION

An understanding of calcium metabolism of the human involves a study of the intake, absorption, utilization, and excretion of calcium. Although there are several known factors which influence these processes, there are many unknown influences, not clarified by preliminary experiments, which need to be investigated.

Since calcium is not abundantly present in many foods which make up the average American diet and the evidence of calcium depletion in aging women has been demonstrated (Albright, 1929), it is of particular importance that the factors affecting calcium metabolism be clarified so that dietary requirements may be more precisely defined.

Calcium is taken into the body in the form of both inorganic and organic compounds. Hydrolysis of the organic matrix in the digestive process presumably releases inorganic calcium and the process of absorption is thought to be one of simple diffusion from the intestines through the intestinal mucosa into the blood vessels. Thus it would seem to be quite feasible to look at the chemical and physical properties of the element and its compounds as well as the factors which influence these properties and to form certain working hypotheses concerning the flow of calcium in the metabolic stream.

Digestion and absorption of calcium

The chief factors which influence the digestion

and absorption of calcium in addition to the endogenous or endocrine factor are: (1) the total intake of calcium, (2) the reaction of the intestinal contents (Schmidt and Greenberg, 1955), and (5) the normality of the fat metabolism of the body (Everett, 1944). In addition to these major factors, there are many minor influences which may alter the normal metabolism of the element, such as, the ingestion of an acid or of an alkaline forming diet, the quantity of protein ingested, the amount and form of carbohydrate in the diet, the fiber content of the diet, and the presence or absence of vitamin D, the ratio between the calcium and phosphorus ingested, and the intake of other salts (Everett, 1944).

As in the course of digestion the calcium of foodstuffs is set free as inorganic salts, it should follow that the form in wnich the element is ingested is not of great importance. However, for the purpose of absorption, it is essential for the salts to be soluble (Schmidt and Greenberg, 1955). Calcium salts, particularly the phosphates and carbonates, are quite soluble in acid solutions and are relatively insoluble in alkaline solutions (Cantarow and Trumper, 1945). As the saliva is alkaline in character and the calcium salts are relatively insoluble, digestion is not initiated in the mouth. In the stomach under conditions of normal gastric acidity, the compounds of calcium with weak organic acids may be converted into the soluble chlorides and, if retained in the

stomach for a sufficient period, even the less soluble phosphate may go into solution. The rate of the digestion of the organic fraction of the ingested food, especially the cellulose, also may affect the absorption of the calcium. If the calcium is still bound with the indigestible cellulose, there is little chance for absorption to take place in the upper portion of the intestinal tract. The acidity of the duodenum is in all probability quite important as much of the absorption is thought to take place in this area. The normal range of the acidity of the duodenum is from pH 2.3 to 7.0 (Cantarow and Trumper, 1945). This factor determines the state of the solubility of the calcium phosphates, that is, whether they are in the basic or acid form, and since the latter is more soluble, a higher acidity tends to facilitate the absorption of calcium. Therefore, any factor which might influence the pH of the intestinal tract is of importance in the absorption of calcium. .

One of the factors which might influence the reaction of the intestinal contents would be the formation of lactic acid by means of the fermentation of lactose in the intestines. Of course, the increased absorption of calcium when lactose is present in the diet may be due to the formation of calcium lactate (Robinson and Duncan, 1931). There, however, would be such a small amount of the salt formed that it might be questionable as to the extent that it would increase the absorption of calcium.

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It has been suggested that the formation of amino acids from protein digestion will increase the solubility of the dietary calcium and therefore, its absorbability from the intestinal tract (Hall and Lehmann, 1944). If this is the cause of increased calcium absorption, it would seem that an increase in the amount of protein ingested would produce a greater effect upon the amount of calcium absorbed. However, as one of the most common ways of increasing the amount of protein in the diet is the addition of milk which would in it self raise the intake of calcium, one must be sure that the protein increase is not by means of a foodstuff which is high in calcium before any influence of protein is declared.

A diet consisting of large amounts of so called roughage may have a twofold effect upon absorption of calcium. The calcium may be bound in the indigestible portion of the diet and therefore, it would pass through the 'intestinal tract unaffected by the digestive process. The other alternative is that the fiber content of the high roughage diet has a cathartic effect and causes the calcium to be carried through the intestines at such a rapid rate that the absorptive process is limited by the time element. It may be that these two factors operate simultaneously.

The relationship between calcium and phosphorus in the intestines is an important one if the optimal absorption of both elements is obtained. If the calciumphosphorus ratio in the upper intestine is excessively high, calcium absorption is probably diminished due to the production of an unduly large quantity of insoluble tertiary calcium phosphate (Cantarow and Trumper, 1945). This is particularly true if calcium is supplied in insufficient amounts. However, when the calcium was raised by means of <sup>p</sup> the addition of calcium gluconate to the diet, Roberts et a1 (1947) found that although the calcium retentions were increased, the phosphorus retentions were poorer. This would tend to lead one to believe that the ratio itself is important in order to secure adequate retentions of both elements.

The interpretation of the effect of vitamin D upon calcium metabolism has been quite controversial. Vitamin D has been cited as being essential for the absorption of calcium from the intestines. This effect is thought to be brought about by the lowering of the pH of the intestnal contents (Everett, 1944). It appears to be possible, however, that the main activity of the vitamin may be exerted in the intermediary metabolism of phosphorus and calcium in their deposition in the bony structures of the body (Cantarow and Trumper, 1945).

If the hydrolyzed fat of the diet is not absorbed rapidly and quantitatively, the remaining part may combine with the calcium forming insoluble calcium soaps which would not be absorbed. Thus, it would seem that the normality of the fat metabolism would have an influence upon

the absorption of calcium from the intestines. It is thought that bile salts assist in calcium metabolism by accelerating the absorption of fatty acids and thereby, preventing the undue loss of insoluble calcium soaps in the feces (Schmidt and Greenberg, 1935).

## Excretion of calcium

Man excretes, unabsorbed, fully two-thirds of his daily dietary intake of calcium while the rat, under conditions of partial calcium insufficiency, is able to utilize almost all of its intake (Holmes, 1945). Excretion of calcium is by means of two pathways; namely, (a) fecal and (b) urinary output.

(a) The fecal excretion of calcium has at least three possible sources. Some of the calcium taken into the body is not absorbed and is excreted undigested. This was formerly thought to make up the entire fecal excretion of calcium. The older German literature took this viewpoint. However, the viewpoint of intestinal chemistry and physiology has changed somewhat and although it is thought that the feces do contain some residue of materials which the body is incapable of absorbing, it also may contain materials that have been excreted or secreted into the gut (Shohl, 1939).

Another of the sources of fecal calcium may be due to the failure of reabsorption of the calcium of the in testinal secretions which are therefore excreted in the feces. It has been found that these intestinal secretions

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are very similar in their calcium content to the calcium content of the serum. Thus, any such secretion in the feces would probably increase the fecal calcium. Of these secretions, the bile has a relatively high concentration of calcium (Drury, 1924). The use of the radioactive isotopes of calcium and strontium have aided in the study of the fate of calcium in the bile. Greenberg and Troeecher (1942) observed that calcium is excreted with the bile.

Then there is the theory that calcium is excreted through the epithelium of the large intestine (Bauer et a1, 1929). Hamilton and Moriarty (1928) indicate that the calcium in the feces originates chiefly from the calcium excreted into the intestines after having played a role in the acid-base metabolism of the body. They found that the relationship between the calcium excreted in the feces and the fixed base intake showed a correlation of  $0.91 \nleq$ 0.07. However, this theory has been disputed by Telfer (1922) in his study with infants. He based his assumption that there is no re-excretion of calcium into the colon on the parallelism between the calcium intake and the fecal excretion of calcium and also the fact that calcium is excreted chiefly as the insoluble tertiary phosphate. In following this line of thought, McGanoe and Widdowson (1939) followed the fate of the intravenous administration of calcium in a normal person. They found that this provoked no increased excretion of calcium by the intestinal

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tract and supported the view that the intestinal tract does not excrete calcium in amounts which vary with the serum calcium level.

It can be seen that there is much to be learned concerning the source of the fecal calcium.

(b) Although the urinary calcium of individuals. varies considerably, Bauer et a1 (1929) found that about 30 to 50 per cent of the calcium excreted is in the urine. The fact that urinary calcium increases significantly with increase in weight of the individual is based upon the fact that there is a definite relationship between the skeletal weight of the body and the total body weight (Knapp, 1947). This percentage remains amazingly constant with the increase of age and weight. Any increase in body weight caused by the increase in the fat content of the body immediately destroys this relationship. However, for all people of normal body weight the percentage of the total body weight which is made up by the skeleton is fairly constant. The increase in the skeletal weight of children would account for the differences in urinary calcium excreted observed between age groups.

Intake of calcium has been investigated as one of the influences upon urinary calcium excretion. Knapp (1947), eliminating the weight factor by using the excretion per kiIOgram weight, found that there was a definite pattern of excretion, the percentage of intake excreted in the urine decreasing at a decreasing rate as the intake rose.

In addition to these above mentioned influences and the endogenous factor (to be discussed later), there are several dietary factors which have been thought to have an influence upon urinary calcium. These are the increase of metabolites in the body, the calcium-phosphorus ratio, dietary roughage, the Quantity of the protein ingested, vitamin D, and the possible effects of other salt intakes.

Equilibrium of calcium in the body tissues

Absorption of calcium is thought to take place by means of simple diffusion. It is transported in the serum since the membrane surrounding the erythrocytes is impermeable to calcium. The serum calcium for normal adults has been reported to be 10.3  $f$  0.3 milligrams per cent (Everett, 1944). .Approximately 45 per cent of this calcium is ionized and 5 per cent exists as a citrate complex. The remaining 50 per cent consists of non-diffusable calcium-protein complexes whose concentration varies with that of the plasma proteins, especially the serum albumin.

The relative concentrations of the diffusible and non-diffusible forms of the blood calcium are thought to be determined by the parathyroid hormone, vitamin D, and the amount of phosphorus present (Bernheim, 1955). The serum protein concentration constitutes an important regulating factor of the plasma calcium concentration according to the formulas:

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The serum calcium ion concentration decreases as the plasma protein rises and the hydrogen ion concentration falls; tetany developing from hypoparathyroidism is thought to be caused by a decrease in the ionized blood calcium (Everett, 1944).

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Approximately 99 per cent of the calcium in the body is contained in the skeleton; the muscles contain about eight milligrams per 100 grams and the blood calcium con-tent is about 4.5 to 6 milligrams per cent (Best and Taylor, 1945).

Deposition of calcium in the skeletal structure is in the form of tertiary calcium phosphate and of calcium carbonate. Vitamin D is also thought to be necessary for the deposition of calcium. This bony structure is not an inert mass but is in reality a readily available source of calcium when necessary for the maintenance of the calcium requirements of other tissues when the exogenous supplies are deficient (Bauer et a1, 1929). This accounts for the maintenance of a normal serum calcium even when the diet is deficient in calcium. The parathyroid secretion raises the serum calcium by mobilizing these calcium stores (Aub et a1, 1929).

Calcium may be deposited in the soft tissues of the body as well as in the bones. Metastatic calcification usually involves the arteries, the stomach, the lungs, and the kidneys. Urinary calculi are quite common in various bone diseases of a destructive nature. Calculi may be composed of calcium salts quite similar to bone but there are other types of stones as well. This 'depcsition of calcium in the soft tissues has not been considered a normal process but it does occur in the aging as well as in diseases affecting the bone formation. As yet, very little is known about the actual causes for calcium deposition in any soft tissue.

The endocrine balance of the individual has an effect upon the urinary excretion of calcium. Aub et al (1929) believe that the thyroid secretion has a direct stimulating catabolic effect upon the calcium deposits in the bony trabeculae. This causes an increase in the serum calcium which in turn causes an increase in the urinary output of calcium. This would follow the suggestion of Bauer et al (1929) that the calcium liberated in the process of catabolism is not available for anabolisn but must be excreted. Albright et al (1929) found that as the serum calcium rose from 5.2 to 11.2 milligrams per 100 milliliters following an injection of parathormone, there was a critical serum calcium value of about 8.5, at which point the almost negligible urinary calcium excretion suddenly changed to an appreciable one. When the serum calcium was above 8.5 the urinary calcium rose then decreased abruptly as soon as the serum calcium fell below 8.5. This suggests that there is a threshold for urinary calcium and that the threshold is below the normal value for serum calcium (9 to 11.5 milligrams per 100 milliliters, Best and Taylor, 1945). This would mean that at a normal serum calcium there would still be a definite excretion of calcium until the serum calcium fell to the threshold figure of 8.5. This is unique in that most other threshold substances are excreted in quantity only at concentrations above the normal serum value for the substance; i. e., sugar. If calcium is a threshold substance, any increase in the serum calcium above normal ranges should result in an increased urinary excretion of calcium.

A.negative calcium'balance on a zero calcium intake might be thought of as an index of the endogenous calcium metabolism. This would be comparable to the findings of Voit (1881) on nitrogen metabolism wherein the endogenous nitrogen metabolism was obtained by determining the nitrogen excretion during starvation. Although the difficulty in obtaining an otherwise adequate diet with a zero calcium intake has limited experimentation of this type, there have been some such studies (Benedict et al, 1915) (Bauer et a1, 1929).

The National Research Council (U. S. A.) Food and Nutrition Board (1945) have suggested a requirement of 0.8 grams of calcium per day for a 56 kilogram woman. This compares with Sherman's (1946) requirement of 0.45

grams with a recommended 50 per cent safety factor which would mean a calcium standard of 0.68 grams. These values are averages derived from an inspection of the limited metabolism eXperiments recorded in the literature including Work on all adults. More recently, there has been some Work done to investigate the upper limit to the intake of calcium beyond which intake, the calcium is no longer utilized economically. McKay et a1 (1941) studying 109 women predicted that 95 per cent of the subjects would show retention on intakes ranging from 0.758 to 0.895 grams daily.

Steggerda and Mitchell (1941) maintain that Sherman's estimate of the calcium requirement of maintenance in man is too low by reason of the method of derivation, which neglects to consider the close positive correlation between the intake and the excretion of calcium. They in turn suggest that a good average value for the calcium requirement of adults of a nutritional status representative of college students and staff members, subsisting on diets containing dairy products to furnish from one half to twothirds of the calcium content, is 10 milligrams per kilogram of body weight per day (Steggerda and Mitchell, 1946). This would mean an average of 0.6 grams for a woman of 60 kilograms. While the 10 milligrams suggested here is the average intake, Knapp (1947) has suggested that 20 milligrams is the maximum intake beyond which the calcium is not utilized economically.

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per cent. The experiments on the utilization of mil Outhouse et a1 (1941) have also pointed out that Sherman based his requirement of 0.45 grams per day upon the assumption that the milk of the diet was utilized 100 per cent. The experiments on the utilization of milk by the above authors have demonstrated that milk is utilized by the average adult human only to the extent of approximately 52 per cent.

Roberts et a1 (1947) found that the requirements for the aging women whom they studied were higher than the proposed standard for adults. At least one gram of calcium per day was suggested for healthy older women.

The purpose of this study is to analyze the relationship between dietary intake, output and retention of calcium of women over 20 years of age as these relationships influence dietary requirements. Also, the influence of certain factors upon the mode of excretion of the element will be investigated.

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#### SOURCE OF MATERIAL

The data used in this study have been obtained from two sources, namely: (1) the available existing literature and (2) a series of studies from this laboratory.

The data selected from the literature were only. those which were from experiments carried out on normal healthy women over the age of 20 years. Some of the subjects were studied at more than one intake of calcium. Each intake was treated as an individual study. The dietary regimes of the various subjects were quite different in the source of the calcium. Also the length of the balance periods varied with the different experiments. However, as the final comparisons were done on a per day basis, this should not affect the comparative value of the experiments. As the method used in determining the calcium was the McCrudden method or modifications thereof for all of the experiments, no serious error is introduced by the combining of the results of separate studies as long as intake is one of the variables to be considered. The various studies used are cited in the appended list of references. A description of these studies follows:

Adolph and Chen (1952)

This study was done on a Chinese subject to determine the utilization of the calcium of soy beans. Two periods were used, the amount of protein given being different. Milk was the main source of pro-

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tein in the first period. In the second period the protein source was changed from milk to soy beans and the results compared.'

Bauer et a1 (1952)

A 27 year old woman served as the subject for this study to determine the effect of irradiated ergosterol upon the metabolism of calcium, phosphorus and nitrogen of normal individuals. Two different in takes of calcium with three different amounts of irradiated ergosterol were given and the results observed.

Blatherwick and Long (1922)

Two healthy young women served as the subjects for this study of the utilization of the calcium and phosphorus of vegetables. A basal dietary was administered first and then followed by an increased calcium intake provided by added vegetables. The periods observed were four days in length. Bogert and McKitterick (1922)

Four college women were studied on a normal intake of calcium and on high intakes of magnesium and of calcium in order to determine interrelationships between the two elements.

Bogert and Kirkpatrick (1922)

The effects of balanced, basic, and acid forming diets were observed on four college women. The balance periods were divided into four day periods.

### Breiter et a1 (1941)

Four adult women were fed a basal diet and then a diet supplemented by the addition of pasteurized fluid milk until a slightly negative balance was obtained. The purpose of this study was to ascertain the utilization of the calcium of milk. Breiter et a1 (1942)

The subjects for this experiment were the same as for the one cited above. The food studied in this case was carrots.

Leverton and Marsh (1942)

This is a large study involving the average of weekly balances for 100 college girls on their usual dietaries. Calcium, phosphorus, iron and nitrogen analyses were determined.

Mellon et al (1950)

The effect of high and low fat diets upon the utilization of the calcium of a dietary consisting of a constant intake of calcium was observed upon two college age women.' Periods of three days were observed.

## Mellon et a1 (1932)

Two college women served as subjects for this study of the retention of calcium on a diet containing American Cheddar cheese as compared with a milk supplement equivalent to the calcium content of the cheese.

## Mellon et a1 (1955)

The retention of calcium supplied by green leafy vegetables was compared with the retention of the calcium of milk when fed to two college women at approximately equal calcium intakes.

McCance et a1 (1942)

The effect of the addition of a protein supplement to a basic dietary on the absorption of calcium and magnesium of a 26 year old woman was studied. McCance and Widdowson (1942a)

Each of four women was placed on a basic dietary with 40 to 50 per cent of the calories consisting of white bread. The calcium retention on this diet was compared with the calcium retention when sodium phytate was added to this dietary. Then the effect of brown bread was observed and the addition of calciferol to the brown bread dietary also was observed. Calcium salts were added to both the white and the brown bread during a later period.

McCance and Widdowson (1942b)

A study of the effect of phytic acid was carried out on three of the same subjects of the experiment described immediately above. The effects of a brown bread dietary were compared with the effects of dephytinized brown bread.

#### McCance and Widdowson (1942c)

This experiment was a continuation of the one quoted above. A white bread dietary was compared with the others which had been studied.

Pittman (1952)

A series of bean purees and a series of baked bean diets were given to college girls with and without added cystine. The effects upon the matabolism of calcium were observed.

Pittman and Kunerth (1959)

Graduate students on three day balance periods were studied on medium protein intakes. This experiment was part of a long time study of the effect of varying protein intakes on the nitrogen, calcium and phosphorus metabolism.

Roberts et a1 (1947)

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The subjects for this study were over 50 years of age. The calcium intakes of these eight women were observed at their usual intake and then increased by the addition of one and two cups of milk. The effect on calcium retentions were observed. Sherman et al (1918)

Four graduate students served as the subjects for a study of the monthly metabolism of nitrogen, phosphorus and calcium in healthy women.

Sherman et al (1918)

This study was conducted for the purpose of obtaining the nutritive value of maize protein and the phosphorus and calcium requirements of healthy women.

The data used from this laboratory are a part of a series of larger studies on the nutritional status of adult women of varying age groups.

Six normal healthy college girls were the subjects for one of the studies. These girls were fed a standardized basal diet with varying intakes of protein (appendix). In varying the protein intake, the amounts of meat and the quantity of milk consumed were increased or decreased. The change in the milk intake varied the amount of calcium ingested as well as the protein. In this way varying intakes of calcium by the same individuals were observed. The balance periods were of seven days duration and each period was preceded by a preliminary period in order to allow for the adjustment of the subject to the change in the diet.

The subjects for the study on older women were over 50 years of age, had borne and reared one or more children, and were in apparent good health. The fact that these women had families and that they had family responsibilities limited the amount of restriction imposed upon their dietary habits. They were asked to continue their usual

pattern of dietary intake. The experiment was divided into three periods during which the milk intake was varied. Period I contained the usual amount of milk. One cup of milk was added to this intake for Period II. For Period III, another cup of milk was added. An adjustment period consisting of from ten days to two weeks preceded each balance period of ten days.

The samples from both studies were treated in the same way. Food was weighed as eaten and an aliquot of one fifth of the food consumed was collected in hydrochloric acid and saved for the entire experimental period. The urine was collected under toluene, measured daily and saved until the entire collection was completed. Then an aliquot of this collection was digested for analysis. Carmine was used as a feces marker. The entire fecal excretion for the period was saved and used for analysis. Brown digests and wet ashes were prepared according to the method of Stearns (I929). The calcium was precipitated as the oxalate and titrated with potassium permanganate using the McCrudden method modified as described by Stearns (1929).<sup>1</sup> until the entire colle<br>of this collection was<br>used as a feces marker<br>the period was saved a<br>and wet ashes were pre<br>Stearns (1929). The co<br>oxalate and titrated w<br>McCrudden method modif<br>All of the data<br>ing to the amount of c

All of the data were combined and tabulated according to the amount of calcium in grams per day. The intakes and urinary and fecal outputs and retentions were recorded for all of the studies (appendix).

The author is grateful to Norma Jean Rader for her assistance in the mineral analysis.

After the grouping of the data had been done, a statistical analysis of the data was carried out. The regression of the intake of calcium on the urinary output was determined for two ranges of intakes for the age group consisting of women between the ages of 20 to 59 years. The significant difference between these two lines was determined. The methods used were from Snedecor (1946).

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### STATISTICAL ANALYSIS

The data from this laboratory and from the literature were combined and arranged according to the mean intakes of calcium. The data were divided into two age groups. One group consisted of those subjects between the ages of 20 to 59 years and the other group was made up of the subjects over 40 years of age (Table 1).

## Relationship of intake to urinary output

As the subjects of the present study were all over 20 years of age, and were normal individuals, weight may be considered to be relatively unimportant in the interpretation of the urinary excretion (Knapp, 1947).

The calcium intakes of the subjects between the ages of 20 to 59 years ranged from 0.09 to 1.68 grams per day. Upon graphing the mean intakes against the mean urinary outputs (Figure 1), there was a definite break in the direction of the slope of the line at an intake of approximately 0.95 grams. Although there was a steady increase in the mean urinary output as the mean intake rose above 0.95 grams, the mean urinary output increased at a faster rate. Therefore, it was decided to treat the mean intakes below 1.25 grams as a separate group of data and the mean intakes above 0.85 grams as another group alIOWing an overlapping of data around 0.95 grams. The linear regression of the mean intakes upon the mean urinary outputs were determined for each group of

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TABLE 1<br>Eummary of All Data on Intake and Output of Calcium Summary of All Data on Intake and Output of Calcium



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# FIGURE I

# MEAN INTAKE AND MEAN URINARY OUTPUT<br>OF CALCIUM OF ALL SUBJECTS 20 TO 39



data.

These regression lines and their scatter are plotted in Figure 2. for the mean intakes below 1.25 grams of calcium, the predicting equation was  $y - 0.148$  $f$  0.008 x and for the mean intakes above 0.85 grams the predicting equation was  $y = 0.078 \neq 0.100$  x. The error of estimate for each line was determined and the lines were tested for significant differences.<sup>1</sup>

Dietary standards for the intake of calcium have been proposed by varying individuals during the past thirty years. Sherman (1946) set a tentative standard allowance of 0.68 grams per 70 kilograms of adult body weight. The Mational Research Council (1945) recommended an allowance of 0.8 grams of calcium per day for the maintenance of adults. These amounts of calcium represent the median intakes on which calcium equilibrium was maintained using all data on normal adults of both sexes. The figures do not represent the least amount on which many persons are able to maintain a calcium balance nor are they representative r 0.008 x and for the mean 1<br>predicting equation was y =<br>of estimate for each line wa<br>were tested for significant<br>bietary standards for<br>been proposed by varying ind<br>years. Sherman (1946) set a<br>of 0.68 grams per 70 kilogra<br> of maximum intakes apparently required by a few individuals tain a ca<sup>:</sup><br>mum intake<br>ulas used<br>2xy - 2x2y is interest of calcium have<br>the intains the past the<br>intailve standard allowance<br>of adult body weight. The<br>procommended an allowance<br>of the maintenance of<br>the median standard was maintained using<br>the median standard was<br>ex iduals during the past thir<br>
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 $^{\bf 1}$ Formulas used were as follows:

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$$
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t = \frac{(b_1 - b_2)}{b_1 - b_2}
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CALCIUM INTAKE<br>GRANS PER DAY

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FIGURE 2

for equilibrium. They do represent the average requirement of adults.

Leitch (1957) in reviewing the literature, has reported an intake of 0.55 grams daily as the calcium require ment of adults. Outhouse et a1 (1941), using the same techniques as Sherman and the National Research Council, found that an average requirement of 0.75 grams of calcium per 70 kilograms weight for adults of both sexes was needed and Steggerda and Mitchell (1941) have reported an average requirement of 0.67 grams per 70 kilograms per day for men. The average intake of calcium per day for equilibrium of college age women has been reported by McKay et a1 (1942) to be 0.8 grams.

all of these requirements are based upon the idea that a positive retention is not necessary, but rather that equilibrium is all that is to be desired.

As one of the purposes of this study is to define an upper limit of intake beyond which calcium is not utilized efficiently by the body, it is quite interesting to note that the regression lines for the two different intake ranges, namely, 0.11 to  $1.25$  grams and 0.85 to  $1.57$ grams, intercept at an intake of 0.85 grams. The error of estimate of the regression line for the intakes below 1.25 grams of calcium is 0.071 grams and for the intakes above 0.85 grams, the error of estimate is 0.065 grams. The regression lines were tested for significant differences. The "t" value was -1.860 which is just below that

required for significance. Therefore, there is about one chance out of 19 that this result would be repeated if the data were replicated. However, there are many variables which enter into a study such as this which may not be eliminated by means of statistical methods with the infor mation available. Although the weights of the various subjects were within normal limits, there is bound to be variation in height as well as weight of an unsected group of normal women and weight has been shown to be a factor in urinary excretion of calcium. Then too, the age span covered is quite large. The wide variation between individuals quite possibly precludes the demonstration of a greater difference between the regression lines. When factors known to influence variation cannot be excluded. More numerous investigations along these lines might aid in the determination of this difference.

There is a real need for the determination of a maximum intake range for economical utilization of calcium by normal women during periods of reproductive rest as much stress has been placed upon drinking quantities of milk to insure sufficient calcium intake. The danger in this type of instruction is that in order to secure this excessive amount of milk many other foods are omitted from the dietary at the expense of other necessary nutrients. Also the practice of taking calcium salts in order to insure an adequate calcium intake over and above the milk ingested may be of questionable value.

It is interesting to note that the regression lines 'intercept each other at an intake of 0.85 grams of calcium. This falls within the range of calcium intake suggested by the investigators quoted by this study for the maintenance of a state of equilibrium for normal women during periods of reproductive rest.

Below the intake of calcium necessary to maintain equilibrium for the average woman, the percentage of persons retaining calcium or maintaining an equilibrium decreases steadily. McKay et a1 (1942) found that an intake of 0.8 grams of calcium was necessary for equilibrium for over half of the individuals studied. On an intake of calcium of less than 0.8 grams, the retentions steadily decreased until at an intake of 0.4 to 0.5 grams there was no retention by any subject. This was also observable in the combination of the various studies included in this study as shown in Table I. Three fourths of the subjects show a positive retention at an intake of 0.75 to 0.85 grams. Below a mean intake of 0.35 grams there are only five percent of the subjects showing a positive retention.

From this discussion of calcium studies it is possible to postulate a standard for calcium intake which would meet the needs of most normal women as far as equilibrium of calcium is concerned. The lower range of intakes showing retention or equilibrium would be from 0.55 to 0.50 grams per day. The average intake to

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maintain equilibrium would be about 0.8 grams per day. The upper limits of intake are suggested by the fact that out of the 20 subjects from this study who had an intake of calcium over 1.15 grams per day, there were only two negative calcium retentions. Knapp (1947) noted that irrespective of age, very few subjects lost calcium from the body when the calcium intake was greater than 20 milligrams per kilogram of body weight. From this, it would seem that the upper limits of economical utilization of calcium would not be more than from 1.15 to 1.2 grams per day.

In plotting the intakes of the group of subjects who were over 40 years of age against the urinary outputs (Figure 5), the wide variation between individuals made it difficult to see a definite pattern of relationship and there were too few data to justify statistical treatment. However, Roberts et al (1947), by means of covariance, have eliminated the variation between individuals and have reported a slight but definite rise in retention as the intakes rose in women between 50 and 70 years of age. Few women of this age group were in calcium equilibrium on self-selected diets which tended to supply less than a gram of calcium per day. They did find that in a few cases of well nourished older women that it was difficult to persuade them to cut their milk intake. Therefore, more studies involving the lower intakes of calcium would probably add much to the present knowledge of the calcium

### FIGURE 3

# INTAKE AND URINARY OUTPUT OF CALCIUM<br>OF SUBJECTS OVER 40 YEARS OF AGE



metabolism of older women.

In veiw of the fact that calcium is not found abundantly in the natural foodstuffs with the exception of milk, it behooves adults to consider the amount of this element which is necessary for adequate maintenance but factors of economy of food intake preclude an excessive standard. Unless the diet contains liberal quantities of milk and green veretables, it will be low in calcium (Thompson and ohlson, 1942). An average adult dietary containing no milk or cheese provides about 0.2 to 0.25 grams of calcium per 1000 calories of food (Ohlson et a1, 1947). Therefore, the average adult ingesting 2000 calories per day (Pittman et a1, 1942), without milk, would be ingesting about 0.4 to 0.5 grams of calcium per day. The addition of one cup of milk to this dietary would mean the addition of approximately 0.25 grams of calcium which would raise the total calcium intake to 0.65 to 0.75. This would be sufficient to meet the average requirements for equilibrium as defined by most of the investigators quoted earlier. The addition of two cups of milk per day would raise the intake to approximately one gram of calcium which would very nearly meet the maximum intake for efficient utilization as determined by this study of the literature. Therefore, an intake of milk over two cups would seem to be unnecessary for equilibrium for adult women during periods of reproductive rest except in the small proportion of individuals whose

requirements appear to be high or where previous calcium depletion has occurred. The data discussed above assume that equilibrium is a satisfactory state in the adult but it is reasonable to suppose that an increased need would follow a period of depletion. McKay et al  $(1942)$  have shown that late growth in young women is accompanied by a tremendous capacity for calcium retention when intakes are increased.

#### Relationship of intake to fecal output

The theory that the fecal calcium is the calcium which has been taken into the body but not absorbed has been questioned in recent years. Unabsorbed mineral undoubtedly does form part of the excreted fecal calcium, but there may be other sources such as the calcium of the digestive secretions which have not been reabsorbed or a possible secretion by the epithelium of the large intestine.

Data from these studies would seem to support the theory that not all of the fecal calcium is unabsorbed calcium. This is evidenced by the fact that the fecal calcium is equal to or exceeds the calcium intake in 45 out of 951 indifioual studies cited (Table 2). The calcium excreted in the feces exceeded the intake of calcium by amounts varying from 0.0 to 0.403 grams per day. While variations in fecal collections and in the determination of calcium in these materials occur, some of these figures

are higher than the usually accepted errors of measurement when all of the conditions of the original experiments were considered.

It would seem logical to suppose that other sources of fecal calcium were the digestive secretions. Partial reabsorotion of these secretions undoubtedly takes place. However, a percentage of each secretion may not be reabsorbed and thus excreted with the feces. For example, human bile has been found to contain 5.8 milligrams of calcium per 100 milliliters of bile (Ricgcl and Kasinskas, 1941). Zuckerman et a1 (1959) found that a normally functioning liver secreted a total volume of about 480 milliliters of bile per day. Therefore, the calcium content of the bile secreted would be approximately 0.018 grams. As the calcium content of the digestive Juices also reflects the concentration of calcium of the blood, the entire secretion of the digestive juices could be estimated at about 0.109 grams of Calcium per day. In these studies, the mean calcium excreted in the feces in excess of the mean intakes was 0.059 grams with a standard deviation of 0.008 grams and never exceeded 0.109 grams per day. Therefore, it is quite possible that the calcium excreted in the feces in excess of the intake had its source in the digestive juices which had not been reabsorbed by the intestines. This amount remains fairly constant regardless of the intake (Table 2) giving further validity to this conclusion. Apparently, the same

-35-

conditions which limit absorption of food calcium may limit reabsorption of calcium derived from secretions and these data do not justify the assumption that the epithelium of the gut excretes calcium.

Greenberg and Troescher (1942) working with radioactive isotopes of calcium and strontium found that out of a total excretion of about 25 per cent of an injected dose of labeled strontium or calcium, between four and five per cent was eliminated in the bile in bile fistulated rats. The use of radioactive isotopes of calcium and strontium should prove to be helpful in learning more about the mechanism of calcium metabolism in the body.

## TABLE 2

Studies in Which the Fecal Calcium Is Equal to or Exceeds the Calcium Intake TABLE 2<br>Studies in Which the Fecal Calcium<br>Is Equal to or Exceeds the Calcium Intake<br>Calcium in Grams per Day



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Calcium in Grams per Day

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#### **SUMMARY**

Data from the literature and from this laboratory have been combined and analyzed for relationships between the dietary intakes, outputs and retentions of calcium of women over 20 years of age as these relationships influence dietary requirements.

.A total of 251 individual studies were selected from the literature and from this laboratory. The calcium intakes of the subjects between the ages of 20 to 39 years ranged from 0.09 to 1.68 grams per day. For the subjects over 40 years of age, the calcium intake range was 0.23 to 1.77 grams per day.

By means of the calculation of two lines of regression, it was shown that there is a difference between the urinary excretion of calcium of women upon intakes below 1.25 and below 0.85 grams per day. Although the difference in the slope of the regression lines is not significant, it is suggestive of a change in the manner of excretion of calcium as the intake is increased above 0.85 grams per day. An intake of approximately 1.15 to 1.20 grams of calcium is suggested as the upper limit for economical utilisetion of calcium by most normal adult women not previously depleted in this element.

The mean fecal excretion was found to exceed the calcium intake in 43 out of 251 studies investigated. The souree of this fecal calcium was suggested as being derived from the calcium content of the digestive secretions.

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APPENDIX

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CALCIUM STUDIES SELECTED FROM THE LITERATURE

 $\label{eq:2.1} \begin{array}{ll} \mathcal{F}_{\mathcal{A}}(\mathcal{A}) & \mathcal{F}_{\mathcal{A}}(\math$ de de la participat de l<br>Los de los de la participat de la particip  $\mathbf{r}^{(1)}$  .  $\label{eq:2} \begin{split} \mathcal{L}_{\mathcal{A}}(\mathbf{x},\mathbf{y},\mathbf{y}) &= \mathcal{L}_{\mathcal{A}}(\mathbf{x},\mathbf{y},\mathbf{y}) \\ \mathcal{L}_{\mathcal{A}}(\mathbf{x},\mathbf{y},\mathbf{y}) &= \mathcal{L}_{\mathcal{A}}(\mathbf{x},\mathbf{y},\mathbf{y},\mathbf{y}) \\ \mathcal{L}_{\mathcal{A}}(\mathbf{x},\mathbf{y},\mathbf{y}) &= \mathcal{L}_{\mathcal{A}}(\mathbf{x},\mathbf{y},\mathbf{y}) \\ \mathcal{L}_{\mathcal{A}}(\mathbf{x},\mathbf{y$  $\label{eq:2.1} \frac{1}{\sqrt{2\pi}}\sum_{i=1}^n\frac{1}{\sqrt{2\pi}}\sum_{i=1}^n\frac{1}{\sqrt{2\pi}}\sum_{i=1}^n\frac{1}{\sqrt{2\pi}}\sum_{i=1}^n\frac{1}{\sqrt{2\pi}}\sum_{i=1}^n\frac{1}{\sqrt{2\pi}}\sum_{i=1}^n\frac{1}{\sqrt{2\pi}}\sum_{i=1}^n\frac{1}{\sqrt{2\pi}}\sum_{i=1}^n\frac{1}{\sqrt{2\pi}}\sum_{i=1}^n\frac{1}{\sqrt{2\pi}}\sum_{i=1}^n\$ 

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## NITROGEN IITAKES OP GOLLEE GIRLS -61-<br>
NITROGEN INTAKES OF COLLEGE GIRLS<br>
FROM THIS LABORATORY FROM THIS LABORATORY



\*Calculated on the basis of a 60 kgm. person



## COLLEGE GIRL STUDY COLLEGE GIRL STUDY<br>MENUS FOR SEVEN DAY PERIOD MENUS FOR SEVEN DAY PERIOD

 $\Delta \phi = 0.01$ Mar 17 '49

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