ABSTRACT OF THESIS

THE EFFECT OF ASCORBIC ACID ON THE RETENTION OF CALCIUM IN NORMAL ADULT WOMEN

Submitted by
Shizuko Higano

In partial fulfillment of the requirements

for the Degree of Master of Science

Colorado State College

of Agriculture and Mechanic Arts

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ABSTRACT

A high intake of calcium is necessary for optimum skeletal development and maintenance, but relatively few foods with the exception of milk and milk products are good sources. In many countries milk is not available owing to transportation difficulties or to geographic and economic conditions. If the utilization of a minimum intake of calcium is improved by ascorbic acid, nutritional disturbances caused by a low intake of calcium may be alleviated. Too, the United States is planning to assume responsibility in feeding the war-torn countries, and if ascorbic acid aids in the retention of calcium, such information may prove helpful.

Numerous experiments reported in the literature indicate a distinct relationship between ascorbic acid and calcium metabolism. In guinea pigs, and to a certain extent in monkeys and in rats, research workers have shown that ascorbic acid aids in the retention of calcium and enhances the calcification of bones and teeth. Since rats synthesize ascorbic acid, results on them cannot be compared to those on man. Experiments on normal human adults have been few and inadequate.

Therefore, the object of this experiment was to study the effect of ascorbic acid on calcium retention in normal adult women.

Five women college students and one faculty member in good health with known age, height, and weight were used as subjects for a 30-day balance experiment. The entire experimental period consisted of a preliminary period of five days, a first experimental

period of 15 days, an interval period of two days, and a second experimental period of 15 days. During the first and the second experimental periods the subjects consumed a balanced, weighed diet of known calcium and ascorbic acid content. Five fixed menus were used in rotation. The ascorbic acid intake in the first period was limited to 11 milligrams, and in the second period it was increased to 111 milligrams in order to study the effect of increased ascorbic acid on the calcium metabolism. A self-chosen diet was permitted during the preliminary period and the interval period. During the preliminary period both calcium and ascorbic acid intakes were limited to approximately 0.4 grams and 20 milligrams respectively. During the interval period only calcium intake was restricted.

Excretions were collected daily. Composite samples of feces and urine representing one-fifth of the daily excretions were made and preserved until there was sufficient time for analysis for calcium. Ascorbic acid determinations on the urine were made daily each morning during the first five days and during the last three days of each experimental period to check upon the saturation of the body with respect to ascorbic acid.

Aliquot samples of food were taken daily and analyzed later for calcium content. Determinations of ascorbic acid were not made on the food because the urinary excretions indicated that the intake was sufficiently low.

The difference between the intake and the output of calcium for each subject was calculated for each of the three five-day periods in the first and the second experimental periods. The differences in mean balances for the two main periods were then

compared.

The comparisons of the means of the balances showed that three subjects had a definitely more negative balance, two subjects had about the same balance when the balance was calculated on a daily basis, and one subject showed a decreased negative balance during the second experimental period when the diet was supplemented with ascorbic acid. The balances were changed in the second period because of changed fecal excretions of calcium for urinary excretions remained fairly constant.

The balances of five subjects indicated that during the second experimental period when the ascorbic acid intake was high, calcium retention was not improved. The one subject who had a marked decreased negative balance during the second period may have shown a favorable effect of ascorbic acid on calcium retention. Mean balances of each subject for the first and second experimental periods were paired; the differences were averaged and analyzed statistically by Student's Pairing Method. The "t" value was found to be 1.454 which was less than that at either of the two levels of significance at $P = 0.01$ and $P = 0.05$. Since the "t" value was low one may conclude that ascorbic acid has no significant effect on the retention of calcium in this experiment.

Possible causes for variations in the balances were the failure of one subject to become saturated with ascorbic acid, the delay in saturation of all subjects with ascorbic acid, the development of a cold in one subject, and the state of previous saturation of the subjects with calcium. However, consideration of these factors did not affect the conclusion that ascorbic acid did not influence significantly the retention of calcium in the subjects in this experiment.

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Shizuko Higano

ENTITLED THE EFFECT OF ASCORBIC ACID ON THE RETENTION OF CALCIUM IN NORMAL ADULT WOMEN

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

MAJORING IN NUTRITION

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Permission to publish this thesis or any part of it must be obtained from the Dean of the Graduate School.

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INTRODUCTION

One of the most common deficiencies in the present-day American diet is calcium (27, p. 253). As much as 99 per cent of the body calcium is contained in the skeletal system, i.e. bones, teeth, cartilages, and tendons, and one per cent circulates in the body fluids and permeates the soft tissues. Calcium is not only necessary in the sound development of teeth and bones, but it is important in the coagulation of blood, irritability of muscles, and regulation of the heart (27, p. 253).

Relatively few foods with the exception of milk and milk products are good sources of calcium. In many countries milk is not available owing to transportation difficulties or to geographic and economic conditions. Moreover, much of the calcium from plant sources is unavailable (16).

One factor which may affect the metabolism of calcium is ascorbic acid. If the utilization of a minimum intake of calcium is improved by ascorbic acid, nutritional disturbances caused by a low intake of calcium may be alleviated. Too, the United States is planning to assume responsibility in feeding the war-torn countries. If ascorbic acid aids in the retention of calcium, such information may prove helpful.

Although many histological studies on the effect of vitamin C on calcium metabolism have been studied on guinea pigs and monkeys, very few quantitative experiments have been conducted on normal human adults. The results on animals show that ascorbic

acid from foods or in pure form affects the calcification of bones and the formation and the structure of teeth.

Therefore, the object of this experiment was to determine the effect of ascorbic acid on calcium retention in normal adults.

REVIEW OF LITERATURE

Numerous experiments have been reported in the literature on the relationship of ascorbic acid to calcium metabolism, but many of them have been indirect results from the study of scurvy on various animals.

Lanford (15), however, used growing rats to determine the effect of orange juice on calcium retention, and reported that the calcium assimilation was enhanced by the addition of orange juice to the basal diet. Calcium retention was increased eight percent and a higher percentage of utilization was noted. On the other hand, Mullick and Ahmad (23) found that the addition of orange juice to the diet caused no significant increase in the retention of calcium in rats. There was a normal utilization of calcium and phosphorus, but they concluded that it was not because of the ascorbic acid in the orange juice. Mallon and Lord (20) did not find any improvement in calcium retention in rats given lemon juice in addition to the basal diet. The citric acid content of lemon juice is six times that of orange juice(20); therefore, any increase in the retention of calcium cannot be attributed to citric acid. Shields and et al (28) reported the addition of ascorbic acid at the rate of one to three milligrams per rat daily did not increase the utilization of calcium.

Experiments reported on guinea pigs seem more applicable, for unlike rats, guinea pigs, and primates do not synthesize ascorbic acid (1, p. 331). Robb, Medes, McClendon, Graham, and Murphy (24), using a balance study on guinea pigs, found that the animals

continuously lost calcium after they had been on a scorbutic diet containing sufficient calcium for two weeks, even though no marked clinical symptoms of scurvy were observed. Results obtained by Salter and Aub (25), on a histological study of bones of guinea pigs, indicated that calcium failed to be deposited in the bones when the diet was adequate in calcium but was low in ascorbic acid. Wolbach and Howe (34) have reported that there was a very poor development of new bones, and Kodicek and Murray (14) reported that there was a complete absence of new bone in scorbutic animals. Bourne (5) observed that the bone healing in scorbutic guinea pigs was proportional to the amount of ascorbic acid given.

Ascorbic acid seems to affect the teeth as well as the bones in guinea pigs, as shown by Fish and Harris (6), Wolbach and Howe (34), Howe (11), and Hanke (9, p. 36). In scorbutic guinea pigs the teeth grew four times as slowly as those in normal animals. The dentine and the odontoblast degeneration was accelerated. Toverud (32) has shown in the teeth of scorbutic guinea pigs that the ash is lower in calcium and higher in magnesium than that from the teeth of a normal control.

Experiments on monkeys, too, have shown that there is some relationship between calcium retention and ascorbic acid intake. Tomlinson (31) found that artificially induced scurvy in monkeys caused lesions of the bones, joints, and adjacent tissues when calcium intake was low, but these lesions were less severe when ascorbic acid was given and only calcium was withheld from the diet. Fraser (8) reported that there was a marked depletion of bone calcium in animals which were chronically deficient in

calcium. Distinct decalcification of the jaw bones of scorbutic monkeys was noted by Tomlinson (31) and Howe (11). Excessive calcification was observed by Howitt (13) when orange juice and cabbage were given to scorbutic animals.

Howe (12) and Hanke (9, p. 143) both reported that a high intake of ascorbic acid decreased dental caries in children. On the other hand, Sandburg and Dagulf (26) in a comparative investigation of 190 tubercular and normal individuals over a period of one year found that although the tubercular patients had a lower ascorbic acid level in the blood, there was no greater frequency of dental caries.

Other acids besides ascorbic acid may affect calcium retention. Outhouse (21) has reported that lactose aids in greater retention of calcium in children, for lactose is converted to lactic acid.

The study which is most nearly comparable to the one reported here is that of Ludwig and Schuck (18). They performed an experiment on five human subjects to study the influence of orange juice on calcium retention. They obtained suggestive results but concluded that the experimental periods (two three-day periods) were too short.

Hence, human experiments, especially on adults, have been scanty and inadequate. The rats synthesize ascorbic acid; therefore, results on them can not be compared to those on man. However, experiments on guinea pigs are numerous and indicate a positive effect of ascorbic acid on calcium retention.

EXPERIMENTAL PROCEDURE

Six normal women were used as subjects on a controlled diet for 30 days. One of the subjects was a member of the faculty, and the remainder were students enrolled in the Division of Home Economics. They were selected upon the basis of good health and willingness to participate in the experiment. All the subjects were given complete physical examinations, and the examinations and the laboratory findings were found to be negative. Basal metabolic rates were taken, and all were within the normal range.

The entire experimental period consisted of a preliminary period of five days, a first experimental period of 15 days, an interval period of two days, and a second experimental period of 15 days. The two experimental periods of 15 days were further subdivided into three five-day periods each.

The menus for the six five-day periods were identical, but the daily menus varied (Table 1). The recommendations of the Committee on Food and Nutrition of the National Research Council (7) were used to check the adequacy of the diet. It was found that niacin, riboflavin, and thiamin were lower than the recommended level. This deficiency was corrected by giving one vitamin B complex tablet every morning at breakfast. Each tablet contained 10.0 milligrams niacin, 1.0 milligram thiamin, and 1.5 milligrams riboflavin. Iron intake for some days was also found to be low, (Table 2) but since the amount was very small, no supplement was given. All foods consumed were prepared under standard conditions, weighed on

TABLE 1

MENUS FOR FIVE-DAY PERIODS

First Day		Second Day		Third Day		Fourth Day		Fifth Day	
Food	Amt. gms.	Food	Amt. gms.	Food	Amt. gms.	Food	Amt. gms.	Food	Amt. gms.
Breakfast		**Breakfast**		**Breakfast**		**Breakfast**		**Breakfast**	
Farina, fortified	150	Ralston's wheat cereal	125	Oatmeal	150	Cornmeal	150	Farina, fortified	150
Sugar	*	Sugar	*	Sugar	*	Sugar	*	Sugar	*
18% cream	60	18% cream	50	18% cream	60	18% cream	60	18% cream	60
Bread, whole wheat	30	Bread, whole wheat	30	Bread, whole wheat	30	Bread, whole wheat	30	Bread, whole wheat	30
Butter	*	Butter	*	Butter	*	Butter	*	Butter	*
Coffee or Postum	*	Coffee or Postum	*	Coffee or Postum	*	Coffee or Postum	*	Coffee or Postum	*
Lunch		**Lunch**		**Lunch**		**Lunch**		**Lunch**	
Plain omelet	50	Beef Boullion	150	Bacon	15	Macaroni Salad		Baked Eggs	
Canned beets	100	Frozen squash	100	Canned corn	100	Macaroni, cooked	100	Eggs	50
Bread, whole wheat	30	Bread, whole wheat	30	Bread, whole wheat	30	Tuna, canned	20	Bacon	15
Canned pear	100	Peanut butter	15	Stewed prunes	100	Mayonnaise	15	Canned corn	100
Canned pear juice	50	Milk	153	Milk	153	Bread, whole wheat	30	Bread, whole wheat	30
Milk	153	Cookies	*	Cookies	*	Canned beets	100	Applesauce	100
Butter	*	Butter	*			Milk	153	Milk	153
Cookies	*					Butter	*	Cookies	*
						Cookies	*		
Dinner		**Dinner**		**Dinner**		**Dinner**		**Dinner**	
Ground beef	100	Ground beef	100	Ground pork	100	Ground beef	100	Ground pork	100
Canned peas	100	Canned string beans	100	Canned peas	100	Canned carrots	100	Canned carrots	100
Rice	*	Rice	*	Rice	*	Rice	*	Rice	*
Gravy	*	Gravy	*	Gravy	*	Gravy	*	Gravy	*
Biscuits	*	Biscuits	*	Biscuits	*	Biscuits	*	Biscuits	*
Butter	*	Butter	*	Butter	*	Butter	*	Butter	*
Canned apricots	100	Canned peach juice	50	Canned pears	100	Canned apricots	100	Canned peaches	100
Canned apricot juice	50	Canned peaches	100	Canned pear juice	50	Canned apricot juice	50	Canned peach juice	50
Cookies	*	Cookies	*	Cookies	*	Cookies	*	Cookies	*
Coffee or Postum	*	Coffee or Postum	*	Coffee or Postum	*	Coffee or Postum	*	Coffee or Postum	*

* ad libitum

TABLE 2

CALCULATED COMPOSITION OF DIET USED FOR CALCIUM BALANCE EXPERIMENT

	Protein gm.	Fat gm.	COH.* gm.	Calcium gm.	A.A.** mgm.	Vit.A I.U.	Thiamin ug.	Niacin mgm.	Riboflavin ug.	Iron mgm.
Menu #1.	64	72	273	.406	11	8199	893	6.77	1122	10.1
Menu #2.	62	78	242	.406	9	7511	799	3.4	623	7.5
Menu #3.	64	73	293	.403	11	4385	804	6.25	829	11.7
Menu #4.	60	82	249	.406	10	9776	558	10.08	843	8.0
Menu #5.	60	111	279	.407	11	6529	1049	7.70	1115	7.2

*Carbohydrate

**Ascorbic Acid

a balance, and served in Guggenheim Hall. To meet individual caloric requirements different weighed amounts of cookies, butter, gravy, crackers, and biscuits were consumed.

The purpose of the preliminary period was to stabilize the calcium intake and to find the level of ascorbic acid nutrition. During this period (five days) the subjects were on a self-chosen diet, but the calcium intake was limited to approximately 0.4 grams per day. This was done by decreasing the intake of milk to a half-cup and omitting all foods with high calcium content. Each subject was required to record the amount and type of food consumed during the period of self-chosen diets.

In order to determine the ascorbic acid status, a saturation test was performed on each individual. During the first two days previous to the day of the saturation test, a normal intake of ascorbic acid was allowed, but from the third day and throughout the remainder of the preliminary period, the intake was restricted to approximately 20 milligrams. On the third day 200 milligrams of ascorbic acid (two cevitamic acid tablets each containing 100 milligrams of ascorbic acid) were given before breakfast. Complete urinary collections were made each day. To the urine collected for ascorbic acid determinations a preservative containing two normal sulfuric acid and two per cent metaphosporic acid was added to the amount of ten per cent of the volume of each collection. Ascorbic acid determination was made on the 24-hour urine sample by using the indophenol titration method (2). The saturation level is considered reached when 50 per cent of the total intake is excreted in the urine in 24 hours (28).

Immediately after the preliminary period, the first experimental period consisting of three five-day periods began. The ascorbic acid and calcium intakes were limited to approximately 11 milligrams and 0.432 gram per day respectively. The National Research Council recommends (7) 70 milligrams of ascorbic acid daily for an adult woman of moderate activity; therefore, 11 milligrams is considered very low. The level of calcium was chosen to approximate the amount yielding a small negative balance (25, p. 252). During the entire period both fecal and urinary collections were made to be analyzed for calcium. These were composited into samples for each five-day period. To check upon the intake of ascorbic acid, the urinary collections were divided into half during the first five days and the last two days of the fifteen-day period. One half of the samples was analyzed each morning for ascorbic acid.

To relieve the monotony of the diet an interval period of two days was given between the two experimental periods. On their self-chosen diets, as in the preliminary period, the subjects were limited to 0.4 gram of calcium but were encouraged to consume much citrus fruit and other foods containing vitamin C. This was done to bring the ascorbic acid level of body up to the saturation point.

Although the length of the period, the collection of excreta, and the calcium intake during the second experimental period were the same as those of the first experimental period, the ascorbic acid intake was increased to 100 milligrams. To make the administration more nearly normal, the 100 milligrams were given

in two portions of 50 milligrams, before breakfast and before lunch.

Aliquot samples of the daily food representing one-fifth the total intake were taken, dried, ground, and kept in tightly covered jars until there was sufficient time to analyze them. Because of different amounts of cookies, butter, gravy, biscuits, graham crackers, and soda crackers consumed by the subjects, these in addition to milk and cream were analyzed separately for calcium. Ascorbic acid analysis of the food was not made because the urinary excretions during the period of minimum intake were sufficiently low.

Fecal samples saved for analysis were marked off by carmine given in gelatin capsules at the first breakfast during the five-day period and again before dinner of the closing day. Collections were made directly into glass-covered pint jars. Daily samples were weighed and transferred into large jars containing the five-day composite sample. All transferring was done with distilled water, and the amount used was recorded. Twenty per cent hydrochloric acid was used to acidify the feces, and an aliquot portion from each period was dried, ground, and stored.

For the calcium determination the urine composite for the five-day period was made by combining one-fifth of half of the day's collection in glass-stoppered storage bottles and acidifying the samples with 20 per cent hydrochloric acid.

Triplicate samples of all biological materials were used for calcium determinations by McCrudden's method (20).

RESULTS

The calcium intakes, excretions, and balances of the six subjects during the experimental periods are recorded in Table 3.

The average calcium intake for five days for each individual for the first experimental period ranged from 2075 to 2264 milligrams, and for the second experimental period it varied from 2054 to 2346 milligrams. Approximately 50 per cent of the calcium was obtained from milk. While the average intake estimated before the experiment was 2025 milligrams, the averages actually provided in the diet during the first and second periods were 2140 and 2177 milligrams respectively. The slightly higher intake of calcium was primarily because of a greater consumption of biscuits, cookies, and butter than expected.

Although, for the purposes of the experiment, the intake of ascorbic acid was limited to 11 milligrams daily in the first period and increased to 111 milligrams during the second period, ascorbic acid determinations on the urine of the subjects were carried out in order to check the saturation of the body with ascorbic acid. The output by the subjects during the two experimental periods was charted (Figures 1, 2, and 3). Figure 2 showed that the intake during the first experimental period was sufficiently low. On the fifth day of the first fifteen-day period, ascorbic acid determinations on 24-hour urine samples read as follows: for subject A, 4.80 milligrams; B, 13.17; C, 7.74; D, 7.16; E, 8.02; and F, 8.93 milligrams. On the fourteenth day determinations were run

TABLE 3

CALCIUM BALANCES DURING
THE SIX FIVE-DAY PERIODS

Subject	Exper. Period	Intake mgm.	Output Feces mgm.	Output Urine mgm.	Total Output mgm.	Balance mgm.
A.	I.	2131	707	546	1253	+ 878
		2163	1191	599	1790	+ 372
		2209	1854	367	2221	- 11
	II.	2147	2178	214	2392	- 145
		2234	2440	506	2046	- 712
		2119	3123	436	3559	- 1440
B.	I.	2128	1431	1053	2484	- 356
		2142	1658	1000	2658	- 516
		2264	2064	717	2781	- 517
	II.	2054	2666	708	3374	- 1320
		2312	2081	1187	3268	- 956
		2156	2062	1004	3066	- 910
C.	I.	2096	1946	1035	2981	- 885
		2125	1514	968	2482	- 357
		2092	1392	848	2240	- 148
	II.	2137	1063	813	1876	+ 251
		2183	1887	901	2788	- 605
		2107	1876	1027	2903	- 796
D.	I.	2081	1849	354	2203	- 122
		2154	1839	535	2374	- 220
		2208	1267	548	1815	- 393
	II.	2202	2206	544	2750	- 548
		2326	4071	524	4595	- 2269
		2216	2248	644	2892	- 686
E.	I.	2075	2032	431	2463	- 378
		2175	1610	558	2168	- 7
		2082	2322	563	2885	- 803
	II.	2085	2510	430	2940	- 855
		2247	1909	553	2463	- 215
		2109	2095	449	2544	- 435
F.	I.	2081	2598	513	3110	- 1029
		2141	2453	575	3028	- 887
		2179	2794	547	3341	-1162
	II.	2142	1973	474	2447	- 304
		2258	1820	475	2295	- 37
		2161	1899	616	2515	- 354

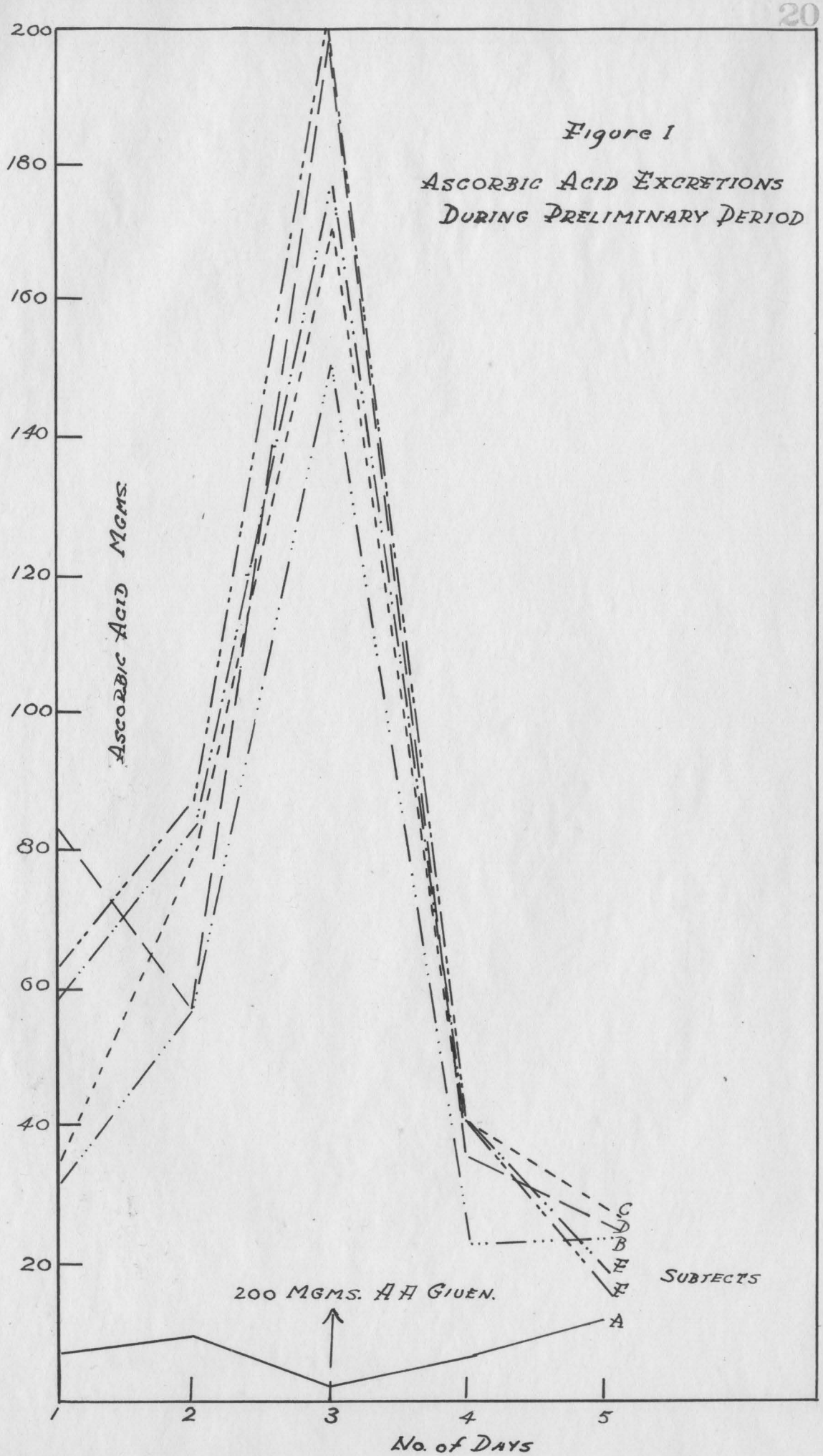
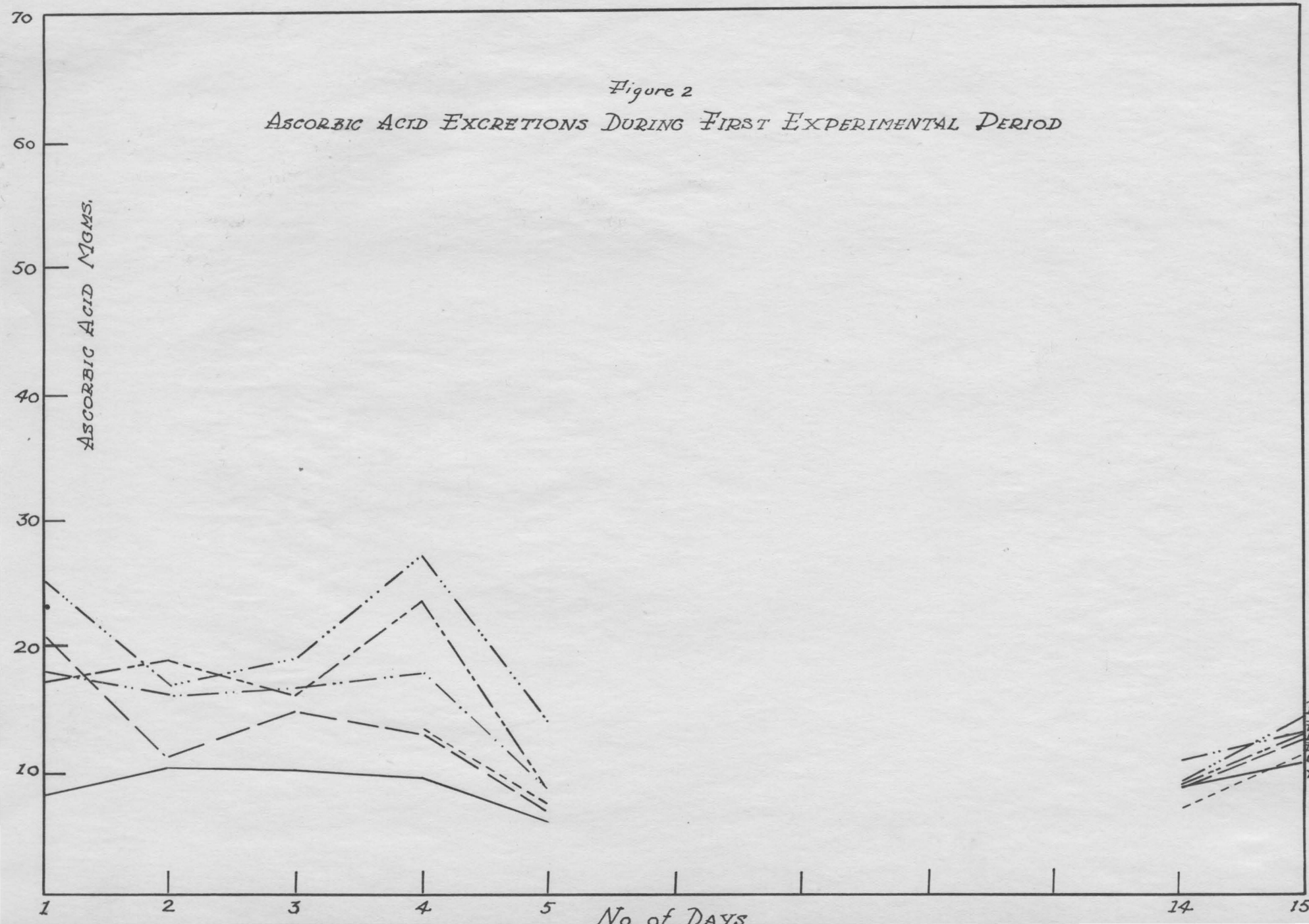


Figure 1
Ascorbic Acid Excretions
During Preliminary Period
Ascorbic Acid Mgms.
200
180
160
140
120
100
80
60
40
20
C
D
B
E
F
Subjects
200 Mgms. A.A Given.
A
1
2
3
4
5
No. of Days

Figure 2

ASCORBIC ACID EXCRETIONS DURING FIRST EXPERIMENTAL PERIOD



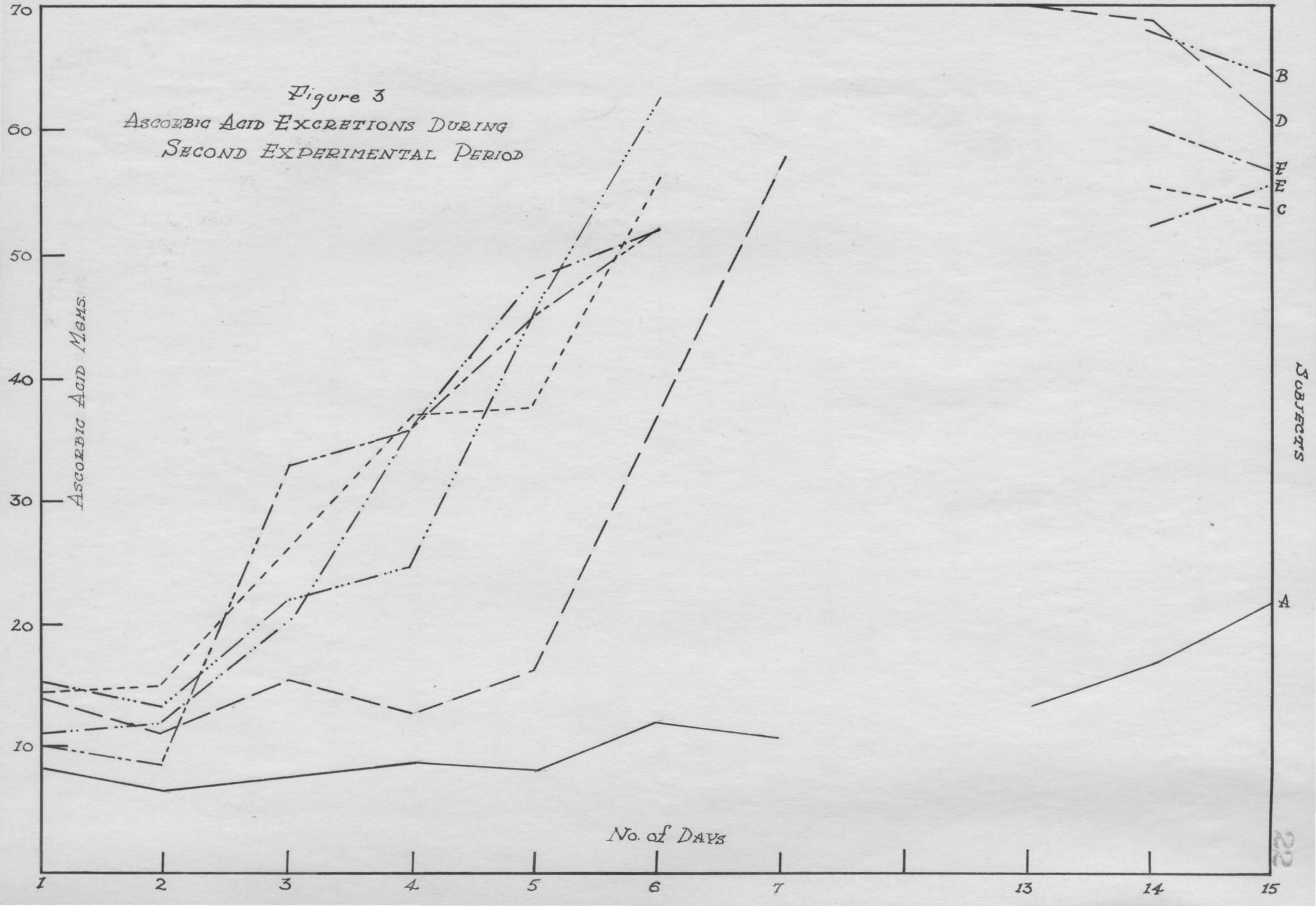


Figure 3
Ascorbic Acid Excretions During
Second Experimental Period
Ascorbic Acid Mgms.
70
60
50
40
30
20
10
No. of Days
1
2
3
4
5
6
7
13
14
15
B
D
E
E
C
A
Subjects

again to recheck on the intake. Milligrams excreted for subjects A, B, C, D, E, and F were 8.50, 8.77, 7.40, 8.54, 10.60, and 9.40 respectively.

During the second experimental period, the ascorbic acid intake was approximately 111 milligrams per day. This included the amount in the diet and in the supplement of crystalline ascorbic acid. By the seventh day all but one subject were excreting 50 per cent of the intake and were maintaining this level throughout the remainder of the period. When 50 per cent of the intake was excreted, the subjects were presumed to be saturated with ascorbic acid (30).

The average calcium balances for five days for each subject are noted in Table 4. During the first and the second experimental periods the balances for subject A were +413 and -765 milligrams respectively. Her excretions for each of the five-day periods were successively +878, +372, -11, -145, -712, and -1440 milligrams. This subject maintained a positive balance during the first period. Since the figures for the latter part of the first experimental period and first part of the second experimental period when calculated on a daily basis showed a very small negative balance, one may assume that she was more or less at an equilibrium. These two balances were much less than her other negative balances and those of other subjects.

Subjects B and D, like subject A, showed a substantial increase in excretion with mean balances of -463, and -1062 milligrams, and -245 and -1134 milligrams respectively in the two periods. Although there was a definite increase in the excretion of calcium by

subjects A, B, and D during the second experimental period when the ascorbic acid intake was high, the amount by which it was increased varied with the individual. The average balances of -386 and -501 milligrams for subject E, too, showed an increased excretion in the second period, but the increase was not as great as in the subjects just mentioned. The remaining two subjects, C and F, followed the opposite trend. The averages for C were -464 and -383 milligrams. The difference between the two means was small; therefore, it probably was not significant. The averages for subject F were -1026 and -232 milligrams, which showed a definite decrease in the negative balance and indicated that more calcium was being retained in the second period. The balances for each five-day period and for each individual, with the exception of subject A, did not show a step-like increase or decrease (Figure 4).

The urinary excretions of calcium for all subjects remained fairly constant. In the first experimental period an average of 25 per cent of the total output appeared in the urine, and in the second period 22 per cent was found. Steggerda and Mitchell (29) reported that 24 per cent of the calcium excreted was found in the urine when the diet provided enough calcium for body equilibrium. Urinary calcium is presumed to indicate that utilized from food or withdrawn from body stores.

The fecal calcium represents unabsorbed calcium and calcium excreted into the intestine (25, p. 252). Fecal excretions of calcium increased in subjects A, B, and D, thereby increasing the total excretion and the negative balances. In subjects C and E fecal excretions did not show much change when the ascorbic acid

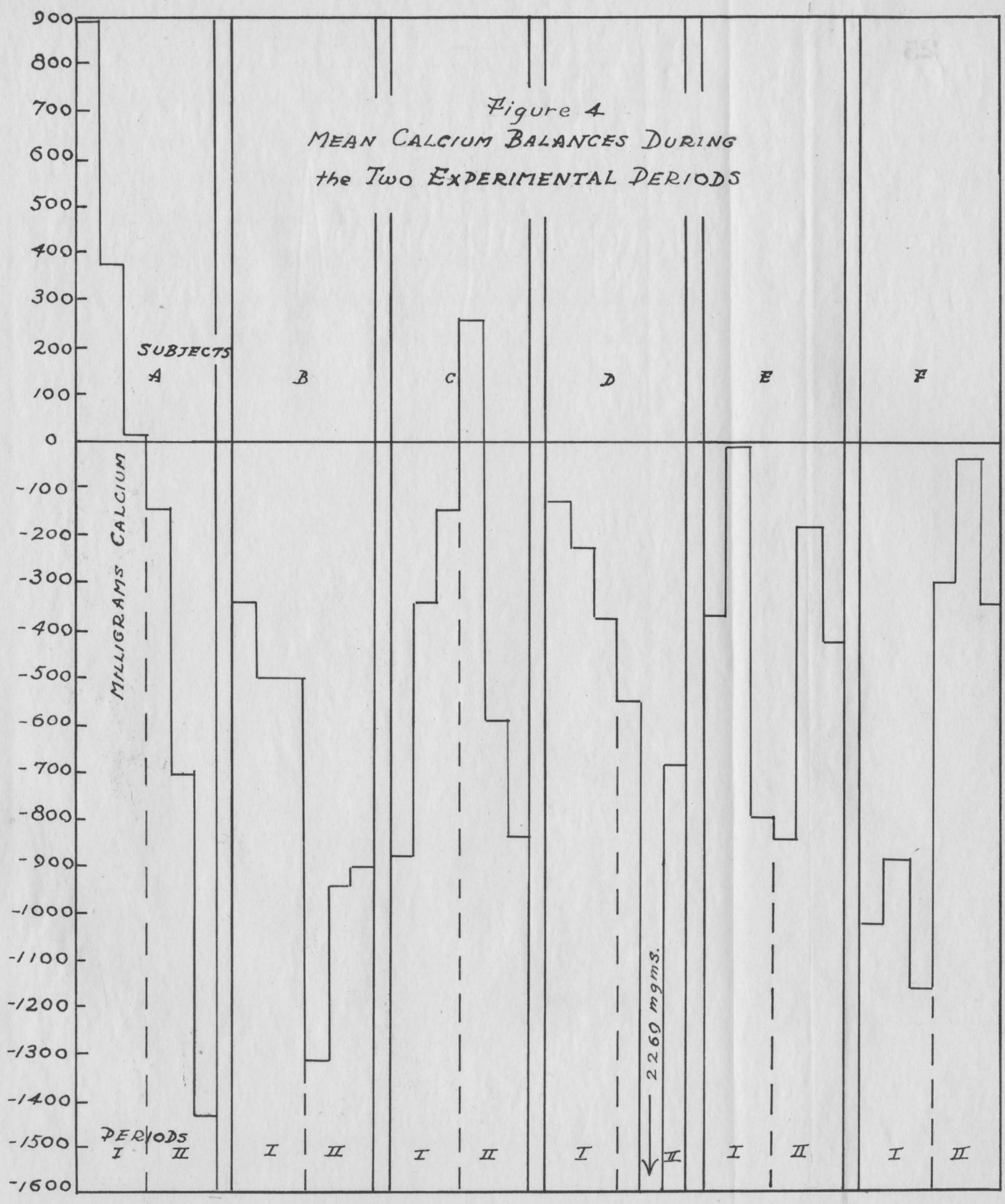


Figure 4
MEAN CALCIUM BALANCES DURING
the TWO EXPERIMENTAL PERIODS

in the diet was increased, and in subject F fecal excretions were definitely decreased.

Since intake and urinary excretions did not fluctuate greatly, changes in the balances were affected mainly by fecal excretions. Three subjects showed definitely greater negative balances in the second period, one showed a definitely smaller negative balance in the second period, and two showed little change when the differences in balances were calculated on a daily basis (-21 and +16 milligrams).

DISCUSSION

The calcium balances found in this experiment were fairly comparable to those reported in the study (5) of calcium requirements of adults in which approximately the same level of calcium was fed.

The weights and heights of the subjects in the present experiment were found to be very much alike (Table 4). McKay et al. (19) reported that age and weight did not make a significant difference in the retention of calcium and phosphorus in the 124 college students they studied. For these reasons in analyzing the data, the results were not calculated according to weight.

The differences between the means of the balances showed that three subjects had a definitely more negative balance, two subjects had about the same when the balances were calculated on a daily basis, and one subject showed a decreased negative balance during the second experimental period when the diet was supplemented with ascorbic acid.

Student's Pairing Method, which has been devised to compare two results on a probability basis, was used to interpret the data statistically (17, p. 66). This method was used because it is applicable to small samples and was recommended for making comparisons between pairs of variates or treatments when the scope of the experiment is limited to a few pairs of observations and where only two or more things are compared.

TABLE 4

MEAN CALCIUM INTAKES AND BALANCES
DURING SIX FIVE-DAY PERIODS

Subjects	Periods	Mean Intake mgm.	Mean Balance mgm.
A.	I.	2134	+ 413
162 cm. 54 kg.	II.	2166	- 746
B.	I.	2178	- 463
164 cm. 57 kg.	II.	2174	- 1062
C.	I.	2104	- 464
166 cm. 56 kg.	II.	2142	- 383
D.	I.	2149	- 245
163 cm. 59 kg.	II.	2248	- 1134
E.	I.	2110	- 396
163 cm. 47 kg.	II.	2147	- 501
F.	I.	2134	- 1026
167 cm. 52 kg.	II.	2187	- 232

The balances for each subject for the first and for the second experimental period were paired. The variance (s^2) and "t" values were calculated by using the following formula:

$$s^2 = \text{variance} = \frac{S^{*}(d^2) - (Sd)^2/N^{**}}{N - 1}$$

$$t = \bar{d}\sqrt{\frac{s^2}{N}}^{***}$$

The value of "t" for this experiment was 1.454. It was compared with the "t" values in Fisher's t-table (17, p. 254) at P = 0.05 and P = 0.01. Since the computed value of "t" is much smaller than that at these two levels of significance, the differences of the means were not considered significant. Hence, according to this analysis additional ascorbic acid did not affect the retention of calcium significantly.

Possible explanations for the variations in the balances of each subject are discussed below. Five of the six subjects followed about the same pattern during the saturation test and throughout the experiment. Subject A did not excrete 50 per cent of her intake of ascorbic acid during either the preliminary period when the saturation test was made or during the second experimental period when the ascorbic acid intake was 111 milligrams. Therefore, this subject never became completely saturated during the experiment. Immediately after the completion of the experiment, another saturation test was performed on the same subject. Her excretion was 65.56 milligrams which was not 50 per cent of the

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*S(d) = sum of the differences between the paired observations.
** N = number of pairs.
***d = mean of the differences.

intake. After being on a normal diet for approximately a month, she was given a third saturation test using 400 milligrams of ascorbic acid. Her excretion, which was 72.93 milligrams, did not reach the saturation level and was only about 18 per cent of the test dose given. The subject's inability to become saturated may be because of the fact that her customary diet was low in ascorbic acid or that her renal threshold was high.

The fact that five days were required for most of the subjects to become saturated during the second experimental period with 111 milligrams of ascorbic acid suggested that a longer interval period seemed necessary or that the intake of ascorbic acid should have been increased during the interval period.

If the omission of the fourth five-day period during which the subjects had not become saturated was assumed in the analysis of the results, there would be no significant changes in the average balance figures with the exception of those of subject E. In subject E, the average balance of the second period would be changed from - 501 to -325 milligrams, and the difference in the balances between the two periods would be reduced from -105 to +71 milligrams. Although the direction of the differences in the balances was changed from negative to positive, the magnitude was still small.

The average intakes of calcium for the individuals during the first and the second experimental periods were 2140 and 2177 milligrams for the five-day periods. The difference of 37 milligrams was very small; therefore, the increase in the intake does not account for the differences in excretion in the two periods.

If there is less efficient utilization of calcium in a well nourished individual and an efficient utilization in persons whose skeletal tissues have been depleted (5), then the latter reason might be the case in subject A who showed a positive balance during the first experimental period. Since she is not accustomed to drinking milk in her normal diet, she may have been able to store calcium at this low level of intake.

In subjects B and D, calcium intake in the ordinary diet was high; therefore, when the intake was decreased to a low level, there may have been a delay in adjusting to this level. However, since the other subjects, with the exception of subject A, drank a considerable amount of milk in their usual diets, the delay in the adjustment does not seem to be an adequate reason for the large negative balances. It is interesting to note that subjects B and D both have had previous history of urticaria. During the second experimental period subject D required an additional day to become saturated with ascorbic acid. The reason may have been because of a cold which began during the third five-day period of the first experimental period and persisted until the end of the fifth five-day period. Although the balances of this subject became more negative as the cold progressed, the effect of a cold on calcium metabolism is unknown.

In subjects C and E there was little difference between the means of the balances in the first and second periods. The individual five-day balances for one subject varied from -177 to +50 milligrams daily and for the other subject from -1 to -171 milligrams daily. No regularity in the balances can be observed.

The results indicated that ascorbic acid during the second experimental period had no effect on the calcium retention of these two subjects.

Subject F showed a marked decrease in the excretion during the second experimental period when additional ascorbic acid was given. Since the mean balance during the first experimental period is very much less than the mean balance of the second experimental period, it appeared that ascorbic acid aided in the utilization of calcium in this subject.

Many of the studies which reported that ascorbic acid aided in the assimilation of calcium have been made on growing animals. In adults growth is not marked, and the need for ascorbic acid in the utilization of calcium in normal individuals might be small.

The subjects participated enthusiastically in the experiment. Their cooperation and interest throughout the entire experiment were excellent. No ill effects were suffered by the subjects, although the intakes of calcium during the entire experimental period and of ascorbic acid during the first experimental period were low.

Although the caloric intakes did not meet the requirement recommended by the Committee on Foods and Nutrition, National Research Council (7), there was no loss in weight except in one subject who lost five pounds. This subject had the cold. The mean caloric intakes for the subjects A, B, C, D, E, and F were 2381, 2419, 2256, 2538, 2277, and 2367 respectively. (Table 5). The diet, on the whole, was palatable and appetizing; however, the low roughage content caused slight constipation in all subjects. The use of

TABLE 5

AVERAGE DAILY CALORIC INTAKES OF SUBJECTS DURING SIX FIVE-DAY PERIODS ON CALCIUM BALANCE EXPERIMENT

	Experimental Period I				Experimental Period II			
	1.	2.	3.	Mean	4.	5.	6.	Mean
Subjects								
A.	2372	2360	2444	2392	2425	2348	2335	2369
B.	2308	2352	2471	2377	2435	2512	2437	2461
C.	2299	2274	2263	2279	2267	2204	2229	2167
D.	2255	2374	2454	2361	2508	2541	2495	2518
E.	2193	2393	2213	2266	2174	2343	2346	2288
F.	2259	2347	2443	2349	2397	2401	2368	2389

frozen squash proved to be unsatisfactory although it was thoroughly heated and cooked for 20 to 30 minutes. It caused an increased excretion of ascorbic acid as shown in Figure 2, and it was unpalatable because of its rawness in flavor and grittiness in texture.

In spite of the demands made upon the subjects and the care and intelligent coooeration required of them in carrying out a study of this kind, the experiment ran smoothly and without difficulties.

SUMMARY

Calcium is important as it is necessary in the development and maintenance of the skeletal system, in coagulation of blood, and in the maintenance of irritability of muscles and nerves. A deficiency of calcium in the present-day American diet is a serious problem in nutrition for rich sources of calcium are limited to milk and milk products, and calcium from plants is not easily available to the human body.

Numerous experiments reported in the literature indicate a distinct relationship between ascorbic acid and calcium metabolism. In guinea pigs, and to a certain extent in monkeys and in rats, research workers have shown that ascorbic acid aids in the retention of calcium and enhances the calcification of bones and teeth. Since rats synthesize ascorbic acid, results on them cannot be compared to those on man. Experiments on normal human adults have been few and inadequate. Hence, the object of this experiment was to study the effect of ascorbic acid on calcium retention in normal adult women.

Six normal women, five college students and one faculty member, were used as subjects on a calcium balance study for thirty days. The entire experimental period consisted of a preliminary period of five days, a first experimental period of fifteen days, an interval period of two days, and a second experimental period of fifteen days. All foods consumed during the first and the second experimental periods were prepared under standard conditions and were weighed for each meal and for each individual. During the two experimental periods, excreta were collected and analyzed for cal-

cium and ascorbic acid.

The intake of ascorbic acid during the first period was limited to approximately 11 milligrams per day. During the second period 111 milligrams of ascorbic acid were taken each day. This included the amount in the diet and in the supplement.

The calcium intake was kept fairly constant during the two periods. The average intakes per five-day periods for the first and the second periods were 2140 and 2177 milligrams respectively.

Three of six subjects showed an increased excretion of calcium during the second experimental period, and two subjects showed about the same balances when they were calculated on a daily basis. The balances of these five subjects indicated that during the second experimental period when the ascorbic acid intake was high, the retention of calcium was not improved by an increase in ascorbic acid. One subject showed a decided decreased negative balance which meant that less calcium was lost. Ascorbic acid may have aided in the retention of calcium in this subject.

The differences in the means of the balances during the first and second periods of all the subjects were averaged and analyzed statistically by Student's Pairing Method. According to this analysis there was no significant difference in the average balances; therefore, one can conclude that ascorbic acid did not significantly affect the retention of calcium under the conditions of this experiment.

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