FOURTH SEMIANNUAL REPORT

to the

National Aeronautics and Space Administration

on a

FUNDAMENTAL INVESTIGATION OF LOSSES OF SKELETAL MINERAL IN YOUNG ADULT HUMAN MALES AND COLLATERALLY IN YOUNG ADULT MALE PIGTAIL MONKEYS (MACACUS NEMESTRIMA) THROUGH IMMOBILIZATION FOR VARYING PERIODS OF TIME, COUPLED WITH A STUDY OF METHODS OF PREVENTING OR REDUCING MINERAL LOSS

(Grant Number NsG-440)

from the

Nelda Childers Stark Laboratory

for Human Nutrition Research

TEXAS WOMAN'S UNIVERSITY

Denton, Texas

March 31, 1965

FOURTH SEMIANNUAL REPORT

NASA Grant Number NsG-440

PERSONNEL:

Pauline Beery Mack, Ph.D., Director

Betty Bohon Alford, Ph.D.

Ralph E. Pyke, Ph.D.

Elsa Arcienegas Klapper, Ph.D.

Sue Nell Brooks English, Ph.D.

Walter W. Gilchrist, M.S.

John A. Bateman, Jr., B.S.

Bill J. Stover

Grady Dozier, B.A.

PRELIMINARY REPORT ON TWO 14-DAY BED REST STUDIES AND AUXILIARY AMBULATORY PERIODS

INTRODUCTION

This is a preliminary report on two 14-day bed rest units studied during this period, together with related ambulatory tests, as a part of a research program sponsored by the National Aeronautics and Space Administration. The two units include the feeding of 2.0 grams and of 0.5 gram of calcium during the respective bed rest periods.

In the Third Semiannual Report of 30 September, 1964, two men participating in the first unit to be discussed in this Report were included because they were the same men who had taken part in two previous units covered in this earlier report. The full analysis of data on the other two men in this bed rest sequence had not been completed at that time, and hence this unit is reviewed here.

SUBJECTS OF THE STUDIES

The two units covered in this report are based on the participation of seven healthy adult males who had been screened from a large number of applicants on the basis of medical examinations, clinical blood and x-ray tests, and psychological assessments. The subjects are identified by letters as follows:

Unit in which 2.0 grams of Calcium were Provided during Bed Rest and Auxiliary Ambulatory Periods

Subject	Age (years)	Height (inches)	Weight (pounds)	Former Occupation
A	24	69	163	Assistant to father--a farmer and rancher
D	22	72	182	University student
G	40	75	204	Retired Air Force Officer
H	21	72	164	Semi-pro Baseball Flayer
J	26	68	159	Teacher
K	22	70	162	Semi-skilled Laborer
L	34	71	198	Construction worker

FOURTEEN-DAY BED REST UNIT DURING WHICH 2.0 GRAMS OF CALCIUM WERE PROVIDED

Four subjects took part in the 14-day bed rest unit in which 2.0 grams of calcium were provided. Two subjects, Subjects A and D, were included in the previous semiannual report because it was desired to compare the results of the bed rest period of these two subjects on this level of calcium with those of the same men who had engaged in two previous bed rest units which were covered in this earlier report, as

noted. A review of the data for these two subjects for this bed rest period have been combined with the data for the two subjects who joined the investigation at the beginning of this period.

Blood Tests for Nutritional Status of Subjects

The subjects were given certain blood tests initially and throughout this unit of study in order to aid in following their general nutritional status.

Hematological Analyses

Hematological values (hemoglobin, erythrocyte count, and packed cell volume) tended to fall within or close to the normal limits initially and as the study progressed.

Blood Plasma Tests

Plasma vitamin A tended to be high initially and to increase slightly as the study progressed. Plasma carotene increased markedly in all subjects during this unit of the investigation.

Plasma ascorbic acid was extremely low initially for all four subjects. Orange juice has been fed from the beginning of each unit, with daily amounts which provided 125 milligrams of ascorbic acid. With initial plasma levels which ranged from 0.1 to 0.5 milligram per

100 milliliters, the subjects increased rapidly to 1.0 milligram and over, and they maintained this higher level throughout.

Plasma phosphorus was slightly below the normal limits for this plasma component in two subjects initially, with the other two subjects falling within the normal limits at the beginning of the study. Wohl and Goodhart (1) gives 3.0 to 4.0 milligrams per 100 milliliters as the adult phosphorus level in the blood. All four subjects were within these limits shortly after their feeding program began.

Serum Values. For serum total protein values, Wohl and Goodhart suggest a normal range for adults of 6.0 to 8.0 grams per 100 milliliters. These same authors suggest a range of 4.0 to 5.5 grams per 100 milliliters as a normal range for serum albumin. All four of the subjects met or approached these limits initially and throughout the study.

Serum calcium, with a normal range of 9.0 to 11.0 milligrams per 100 milliliters as suggested by Cantarow and Shepartz (2) fell within these limits from the initial through the final tests.

Urinary B-Complex Vitamins

The subjects were normal throughout in urinary levels of thiamine, riboflavin, and N'-Methylnicotinamide, according to the Standards of Normality suggested by Sinclair (2).

Blood Tests for Cholesterol

The normal range for serum cholesterol of 120 to 280 milligrams per 100 milliliters as suggested by Wohl and Goodhart (1) was not exceeded initially or during the study by any of the four subjects.

Summary of Biochemical Tests for Nutritional Status

The biochemical tests discussed above demonstrate the fact that the four subjects were maintained in an optimum state of nutrition insofar as the blood and urine analyses which were made. Therefore it is possible to emphasize the belief that the variables of this section of the study were calcium and phosphorus, with changes not due to take place in other nutrients.

Consumption and Excretion of Calcium

Table I gives the mean daily quantities of calcium consumed by the four subjects of this unit of the investigation during the control period, the bed rest period, and the post-bed rest period.

Table II contains the mean daily excretion values of calcium for the four subjects taking part in the 14-day bed rest and the auxiliary ambulatory periods when 2.0 grams of calcium were provided.

With each subject, the calcium excretion levels increased during bed rest and decreased following the bed rest period.

Figure 1 illustrates the urinary and calcium excretion of Subject H during the last nine days of the ambulatory control period, during the 14-day bed rest period, and during a 15-day post-bed rest period when this group of subjects was given a 2.0 gram daily provision of calcium. This figure serves as an example of the general calcium excretion behavior of the four subjects.

Status of Calcium Balance

The four subjects of this unit of the study were in calcium balance on 2.0 grams of calcium during the control period before they went into the 14-day bed rest phase on the same calcium level. All four subjects went into a negative calcium balance during bed rest, but they returned to a positive calcium balance during the post-bed rest period. See Table III.

TABLE I

Mean Daily Consumption of Calcium by Four Subjects during the Control, Bed Rest, and Post-Bed Rest Periods when 2.0 grams of Calcium were Provided (Calcium in grams)

Subjects	Control Period	Bed Rest Period	Post-Bed Rest Period
A	1.944	2.017	2.128
D	2.051	2.038	2.18[illegible]
G	2.096	2.034	2.071
H	2.159	2.110	2.126

TABLE II

Mean Daily Excretion of Calcium by Four Subjects during the Control, Bed Rest, and Post-Bed Rest Periods when 2.0 grams of Calcium were Provided

Subjects	Control Period	Bed Rest Period	Post-Bed Rest Period
A	1.514	2.274	2.010
D	1.819	2.358	2.00[illegible]
G	1.836	2.377	1.84[illegible]
H	1.977	2.115	1.847

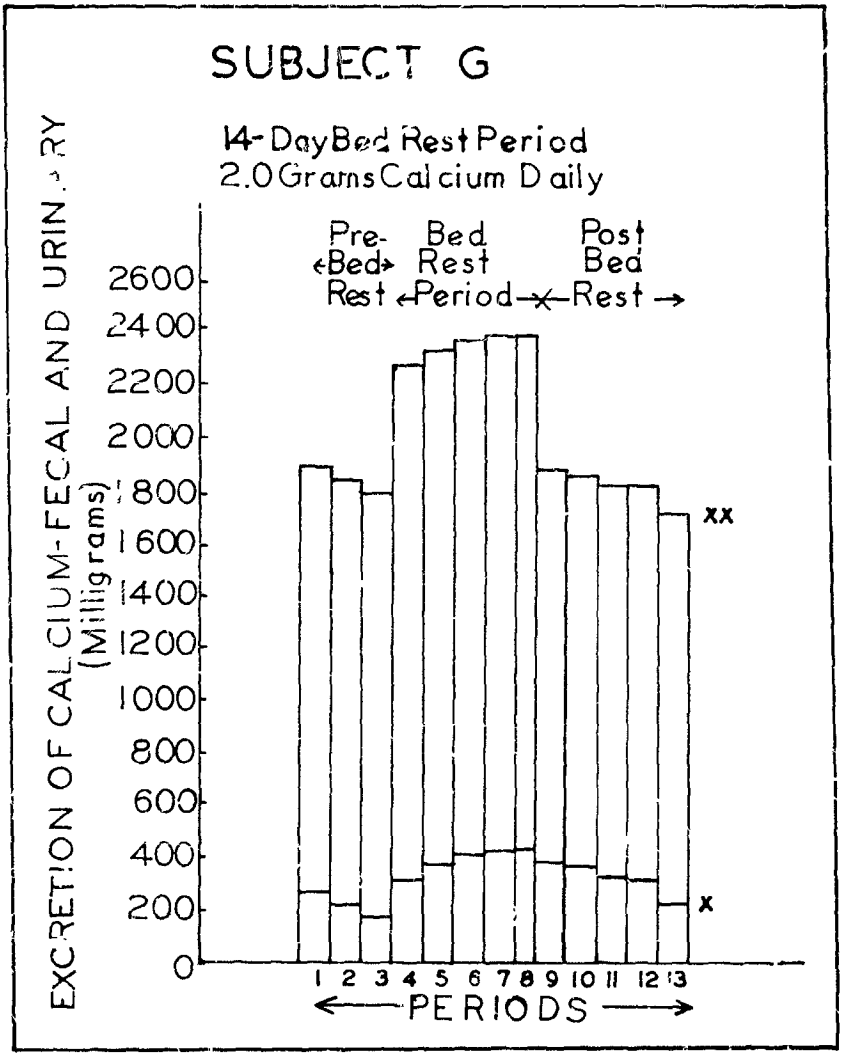


Figure 1. Calcium Excretion by Subject G when 2.0 grams of Calcium were Provided throughout: Urinary (*) and Urinary plus Fecal (**), with Periods in Terms of Average milligrams per Three Days except Period 8, which Represents a Two-day Average. Periods 1 to 3 Cover the Last Nine Days of a Pre-Bed Rest Phase; Periods 4 to 8 Represent the 14-day Bed Rest; and Periods 9 to 13 include the Two-week Post-Bed Rest Phase of the Overall Unit

TABLE III

Calcium Balance during the Control, the Bed Rest, and the Post-Bed Rest Periods of Four Subjects when 2.0 grams of Calcium were Provided Throughout

Subjects	Period	Mean Calcium Consumed Daily (grams)	Mean Daily Calcium Excreted			Mean Daily Calcium Balance
			Urinary	Fecal	Total	
A	Control Period (ambulatory)	1.944	0.480	1.034	1.514	+0.430
D		2.244	0.310	1.509	1.819	+0.425
G		2.096	0.244	1.592	1.836	+0.260
H		2.159	0.277	1.700	1.977	+0.182
A	Bed Rest Period	2.017	+0.460	1.814	2.274	-0.257
D		2.028	+0.380	1.978	2.358	-0.320
G		2.084	+0.406	1.971	2.377	-0.293
H		1.910	+0.325	1.790	2.155	-0.205
A	Post-Bed Rest Period (ambulatory)	2.128	0.364	1.646	2.010	+0.118
D		2.183	0.283	1.725	2.008	+0.175
G		2.071	0.342	1.501	1.843	+0.228
H		2.126	0.232	1.615	1.847	+0.279

Consumption and Excretion of Phosphorus

When the subjects were provided 2.0 grams of calcium daily, a calcium:phosphorus ratio of 1 : 1.31 was maintained. This fell within the satisfactory limits for this ratio suggested by Cantarow and Shepartz (3).

The subjects had mean intakes of phosphorus which were close to the level intended, although their excretion levels varied with the individuals. During the control period and the post-bed rest period, all four subjects were in positive phosphorus balance. During the bed rest period, two subjects were in positive and two were in negative balance.

Bone Mass Changes during Unit in which 2.0 grams of Calcium were Fed Throughout

Bone mass was evaluated by the method of radiographic bone densitometry as developed by Mack and associates (4) (5) (6).

Figures 2 through 5 include the bone mass data points for the four subjects during the unit when 2.0 grams of calcium were fed throughout. Each figure applies to one of the four subjects.

The data on bone mass in this report have been calibrated in terms of calcium hydroxyapatite equivalency. The reporting of bone

mass in such a term is not intended to denote that a bone being reported contains this amount of this calcium complex, but that the total substances in the portion of the x-ray which was evaluated had an x-ray absorption value equivalent to the designated quantity of calcium hydroxyapatite.

The upper section of each of the figures mentioned consists of the data points and a regression line for the bone mass measured during the last nine days of the control period when the subject was ambulatory. The middle graph gives the same data for the 14-day bed rest period; and the lower graph also consists of a regression line and the data points for the bone mass values found during 15 days following the bed rest.

The regression lines have been calculated and plotted by the 1620 IBM Computer in the Texas Woman's University Data Processing Center, based on a program which includes the first order computation of least squares, following this formula:

$$Y = A_0 + A_1(x)$$

where

Y is the dependent variable (bone mass in this case);

x represents time;

A_0 is the Y intercept; and

A_1 is the slope of the regression line.

Simultaneously with plotting the regression line, the data points also were plotted by the Computer, as noted. Also the following data were obtained from the machine in each case:

(a) the variables involved;

(b) the range covered by the plot;

(c) the slope of the curve, which denotes the rate of change of the factor being plotted;

(d) the standard error of estimate.

In each linear plot which has been produced, the standard error of estimate has indicated that the regression line which was plotted is a reasonable approximation of the change which has occurred with time in the factor involved.

In the case of the regression line in each section of each figure, a "t" test was run to test the hypothesis that the regression coefficient was equal to a specific value. The result of this test showed whether or not the line differed significantly from zero.

Table IV summarizes the data of this section of the report. This table shows that all four subjects who received 2.0 grams of calcium daily had negative regression lines during the bed rest period, which varied from zero by highly significant differences

($P < 0.001$ in three cases and $P < 0.01$ in one case), as shown by the application of the "t" test.

During the control period, all four subjects have been shown to be in positive calcium balance before the bed rest period began. Nevertheless, two subjects, Subject G and Subject H, gained slightly in bone mass during this period, causing their regression lines to have a slightly positive slope. The slope of the regression lines during the control periods of Subjects A and D did not differ significantly from zero.

The regression line for the post-bed rest period for Subject A did not have a positive or negative slope, with the "t" test showing that it did not differ from zero. The regression lines for the other three subjects had slightly positive slopes, with the line varying from zero by a difference in all cases which was significant at the 10 per cent level.

Figure 2. Data Points and Regression Lines for Bone Mass of a Central Os Calcis Section of Subject A when 2.0 grams of Dietary Calcium were Provided throughout the Unit of Pre-Bed Rest or Control, Bed Rest, and Post-Bed Rest:

(Left) Control Period
Slope —0.00030, not significantly different from zero

(Middle) Bed Rest Period
Slope —0.00603, significant downward slope

(Right) Post-Bed Rest Period
Slope —0.00281, not significantly different from zero

Bone Mass is given in Terms of Calcium Hydroxyapatite Equivalency

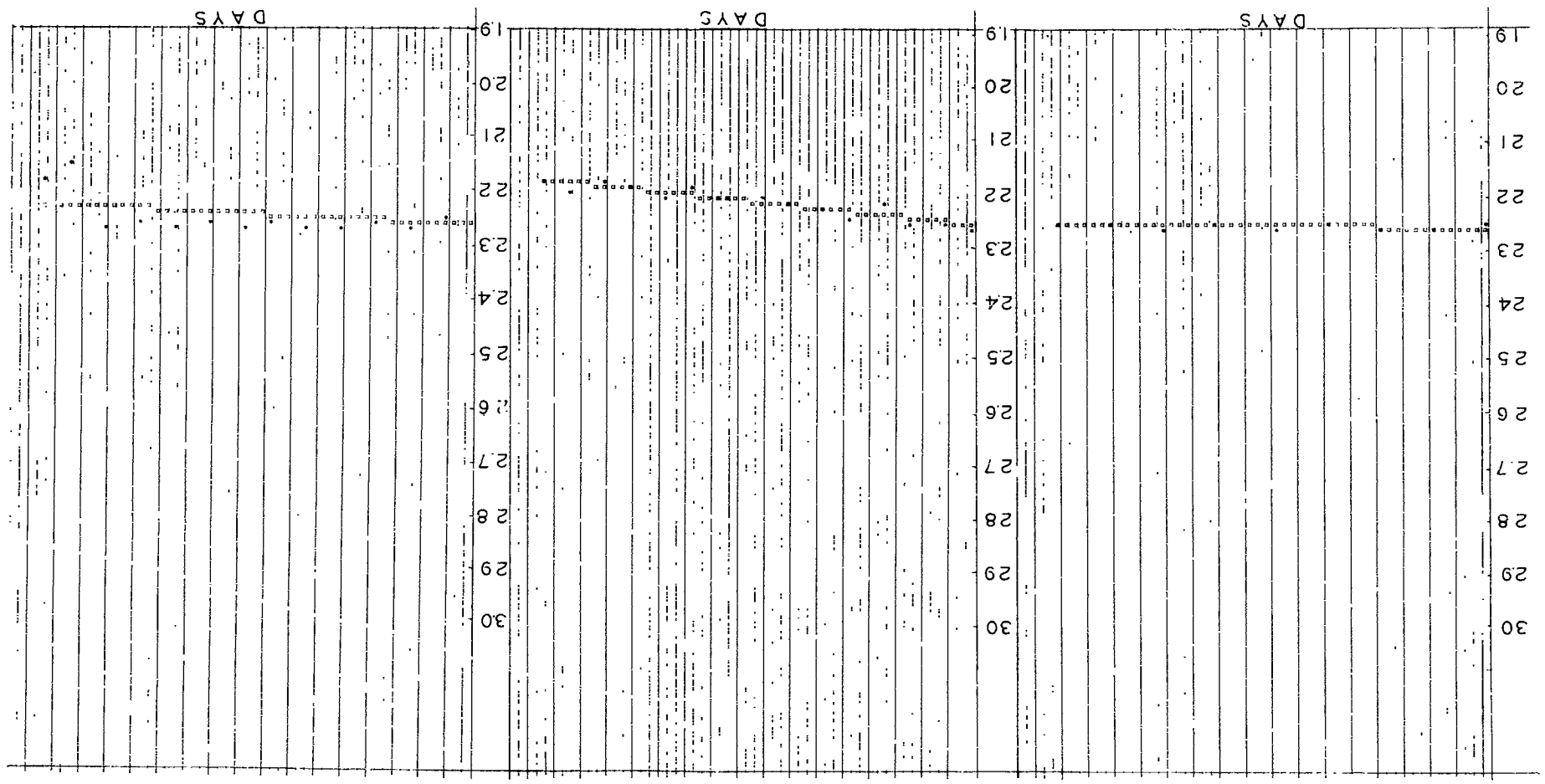

DAYS
19
20
21
22
23
24
25
26
27
28
29
30
DAYS
19
20
21
22
23
24
25
26
27
28
29
30
DAYS
19
20
21
22
23
24
25
26
27
28
29
30

Figure 3. Data Points and Regression Lines for Bone Mass of a Central Os Calcis Section of Subject **D** when 2.0 grams of Dietary Calcium were Provided throughout the Unit of Pre-Bed Rest or Control, Bed Rest, and Post-Bed Rest:

(Left) Control Period
Slope —0.00061, not significantly different from zero

(Middle) Bed Rest Period
Slope —0.00590, significant downward slope

(Right) Post-Bed Rest Period
Slope +0.00402, significant upward slope

Bone Mass is given in Terms of Calcium Hydroxyapatite Equivalency

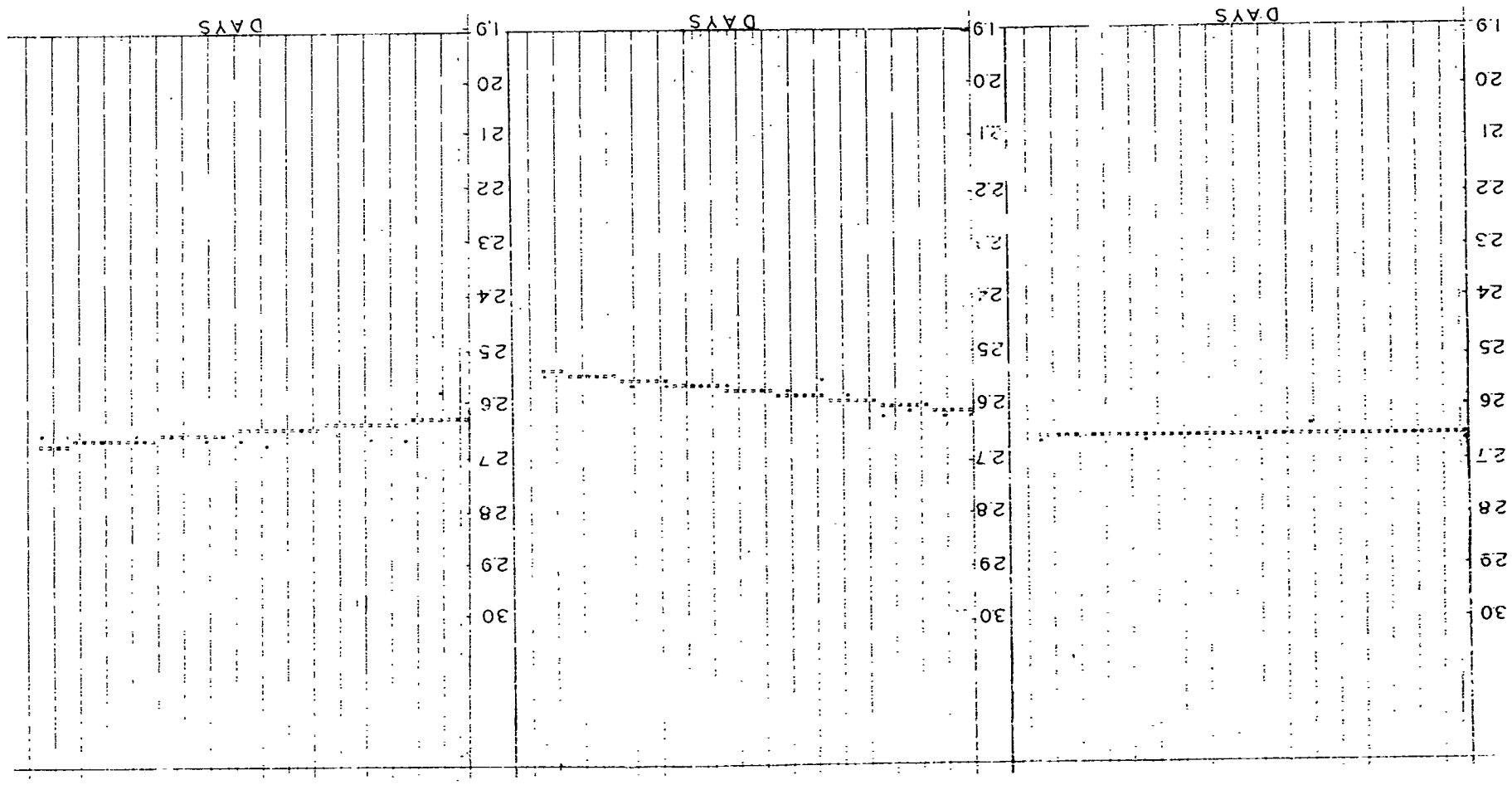

DAYS
1.9
20
21
22
23
24
25
26
27
28
29
30
DAYS
1.9
20
21
22
23
24
25
26
27
28
29
30
DAYS
1.9
20
21
22
23
24
25
26
27
28
29
30

Figure 4. Data Points and Regression Lines for Bone Mass of a Central Os Calcis Section of Subject G when 2.0 grams of Dietary Calcium were Provided throughout the Unit of Pre-Bed Rest or Control, Bed Rest, and Post-Bed Rest:

(Left) Control Period
Slope +0.00114, significant upward slope

(Middle) Bed Rest Period
Slope —0.01291, highly significant downward slope

(Right) Post-Bed Rest Period
Slope —0.00310, slight upward slope

Bone Mass is given in Terms of Calcium Hydroxyapatite Equivalency

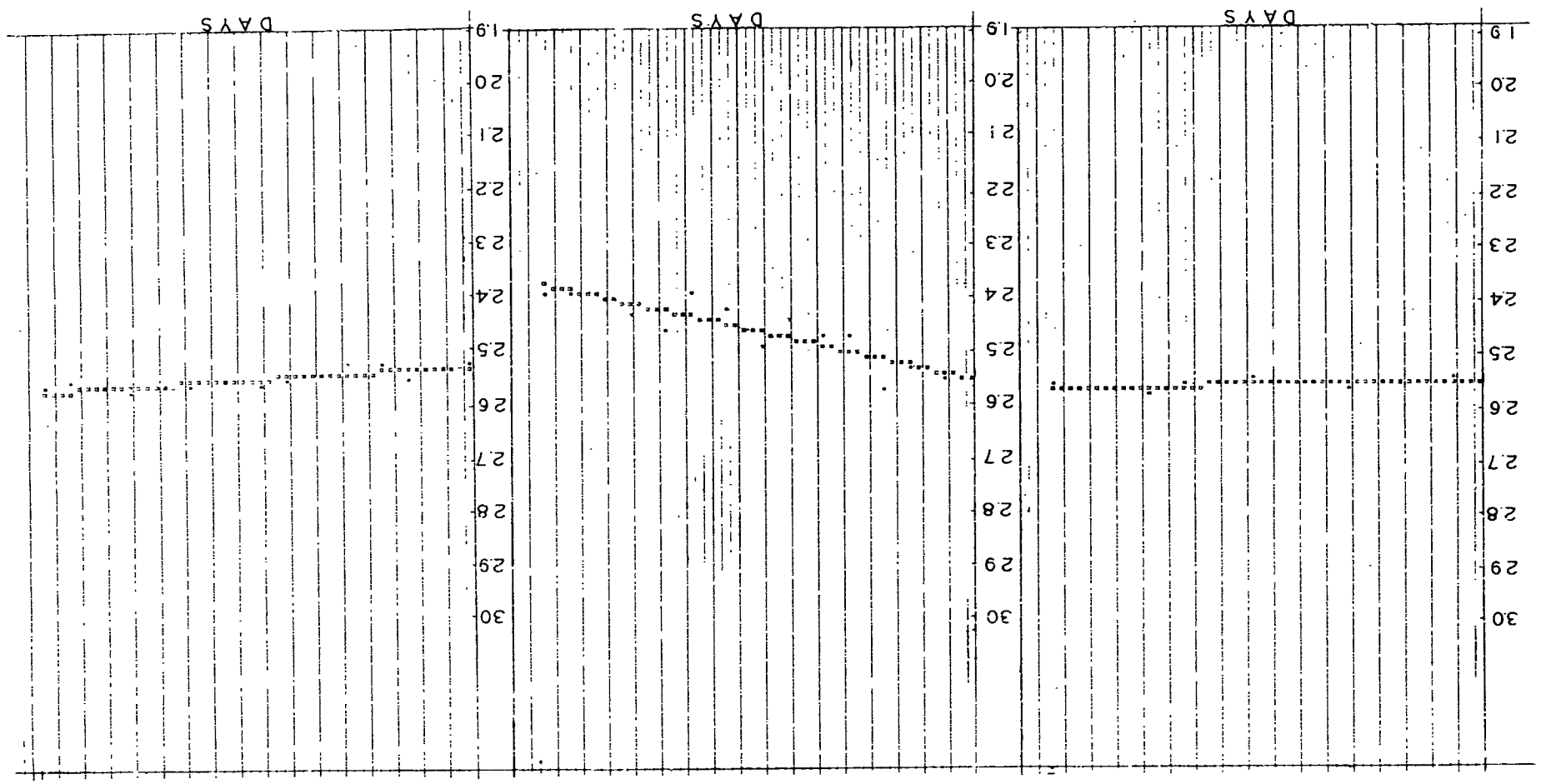

DAYS
1.9
2.0
2.1
2.2
2.3
2.4
2.5
2.6
2.7
2.8
2.9
3.0
DAYS
1.9
2.0
2.1
2.2
2.3
2.4
2.5
2.6
2.7
2.8
2.9
3.0
DAYS
1.9
2.0
2.1
2.2
2.3
2.4
2.5
2.6
2.7
2.8
2.9
3.0

Figure 5. Data Points and Regression Lines for Bone Mass of a Central Os Calcis Section of Subject H when 2.0 grams of Dietary Calcium were Provided throughout the Unit of Pre-Bed Rest or Control, Bed Rest, and Post-Bed Rest:

(Left) Control Period
Slope +0.00344, very slight upward slope

(Middle) Bed Rest Period
Slope —0.01445, highly significant slope

(Right) Post-Bed Rest Period
Slope +0.00344, slight upward slope

Bone Mass is given in Terms of Calcium Hydroxyapatite Equivalency

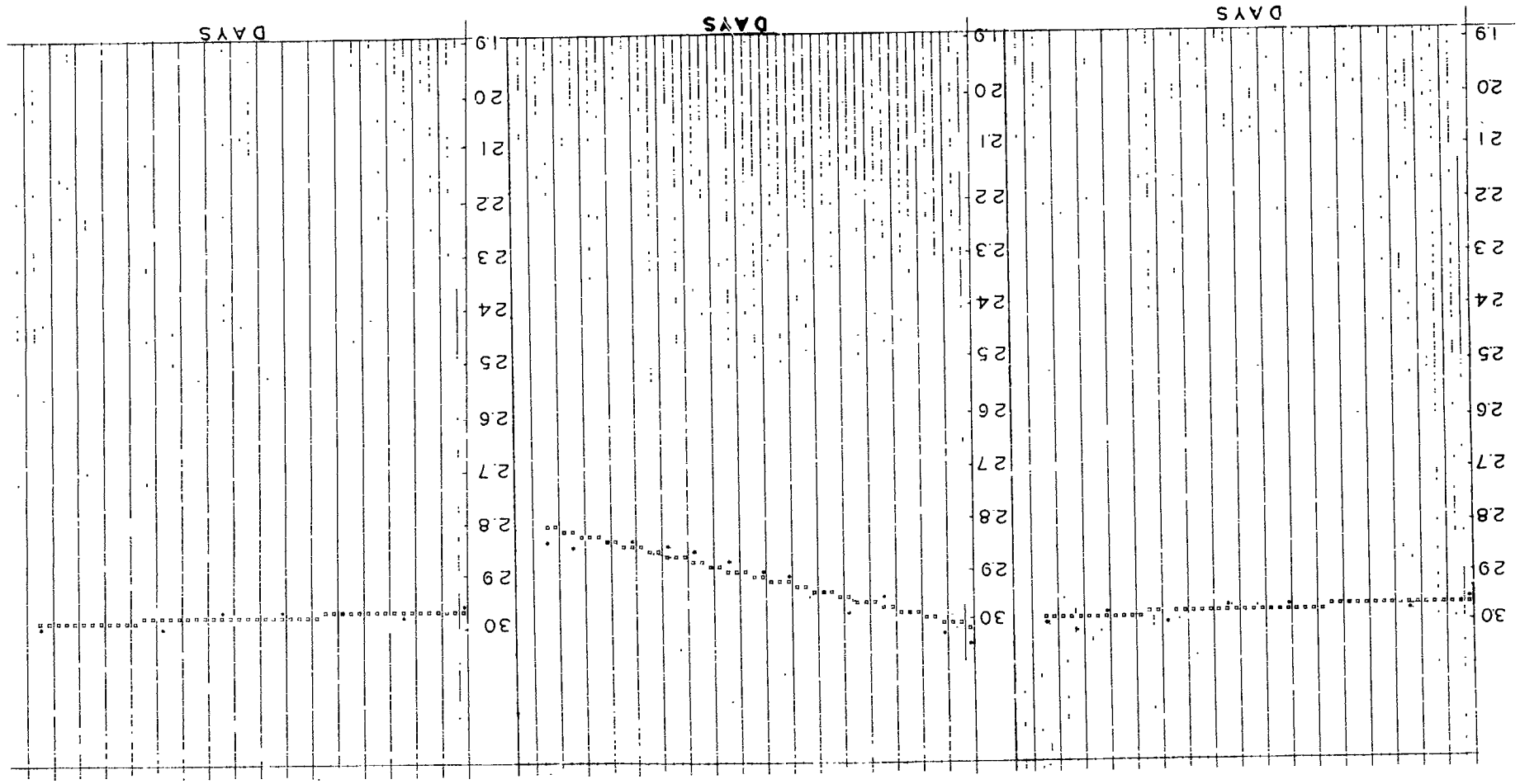

DAYS
19
20
21
22
23
24
25
26
27
28
29
30
DAYS
19
20
21
22
23
24
25
26
27
28
29
30
DAYS
19
20
21
22
23
24
25
26
27
28
29
30

TABLE IV

Slope of Regression Lines for Control, Bed Rest, and Post-Bed Rest Periods for Four Subjects when 2.0 grams of Calcium were Provided Throughout

Subject	Portion of Unit	Slope of Regression Line	Probability that the Slope of the Regression Line Differed from Zero	Per Cent Loss in Bone Mass during Bed Rest
A	Control	-0.00030	Not significantly different from zero	
	Bed Rest	-0.00603	$P < 0.001$	3.86
	Post-Bed Rest	-0.00281	Not significantly different from zero	
D	Control	-0.00061	Not significantly different from zero	
	Bed Rest	-0.00590	$P < 0.01$	3.00
	Post-Bed Rest	+0.00402	$P < 0.05$	
G	Control	+0.00114	$P < 0.05$	
	Bed Rest	-0.01291	$P < 0.001$	6.50
	Post-Bed Rest	+0.00310	$P < 0.10$	
H	Control	+0.00344	$P < 0.10$	
	Bed Rest	-0.01445	$P < 0.001$	6.48
	Post-Bed Rest	+0.00344	$P < 0.10$	

FOURTEEN-DAY BED REST UNIT DURING WHICH 0.5 GRAM OF CALCIUM WAS PROVIDED

The second bed rest covered during this semiannual period also was 14 days in duration with 0.5 gram of calcium provided per day. This was preceded by a control period during which 1.5 grams of calcium were provided, with the subjects brought to a state of calcium balance during this period, while also being equilibrated with respect to the bone mass of the central section of the os calcis. Following the bed rest a post-bed rest period was maintained, again with 1.5 grams of calcium provided.

Five subjects took part in this unit of the overall investigation. They are designated as Subject G, Subject H, Subject J, Subject K, and Subject L. The first two subjects had taken part in the previous 14-day bed rest period when 2.0 grams of calcium were provided.

Tests for Nutritional Status of Subjects

As in the case of the four subjects who participated in the 14-day bed rest unit, these subjects tended to meet or approach throughout the normal limits for the hematological values, for plasma vitamin A, for plasma carotene, for phosphorus, for total serum protein and albumin, for serum calcium, and for urinary B-complex levels. Subjects J, K, and L who had not been in the study before this unit increased in plasma

vitamin A and carotene during the unit, although they were not below the normal range initially.

Initially Subjects G and H, who had been in the previous unit of the study, had plasma ascorbic acid levels of 1.6 and 1.8 milligrams per 100 milliliters, respectively. They maintained values of a similar magnitude through this unit of the study. Subjects J, K, and L, who were new participants, on the other hand, had very low initial values. Subject J had a value of 0.2 milligram of ascorbic acid per milliliter of plasma when he entered the study. The initial values for Subjects K and L were 0.5 and 0.4 milligrams per 100 milliliters, respectively. The value for J increased to 1.5, that for K to 1.5, and that for L to 1.6 milligrams of ascorbic acid per milliliter of plasma shortly after the daily orange juice began to be administered.

Blood Tests for Cholesterol

The cholesterol values initially were low for each subject. Also, they did not increase during this unit of the study, but remained within the suggested normal limits throughout.

Consumption and Excretion of Calcium

Table V gives a summary of the daily amounts of calcium which were consumed by the five subjects in this unit of the study. During

the control period, each subject consumed an average amount of calcium which closely approached the 1.5 grams of calcium planned.

During the bed rest period when 0.5 gram of calcium was offered, each subject consumed a mean quantity of this nutrient which was slightly less than this amount. On this low level of calcium, the refusal even of a small amount of food which contains calcium will result in a notable difference in total intake. The major food which was refused by most of the subjects was bread, except for Subject J who had little appetite and who rejected small amounts of other foods as well.

In the post-bed rest period, the three subjects who remained in the study consumed on the average the desired amount of calcium, with Subject G slightly surpassing the 1.5 grams desired.

Table VI gives the mean daily quantities of calcium excreted both in urine and in feces in this part of the study. The data in this table must be considered in relation to the level of calcium intake, since the bed rest period when 0.5 gram of calcium was provided fell between the control period involving the provision of 1.5 grams and the post-bed rest level when there was a return to this same higher level of calcium.

The excretory losses of calcium during bed rest were considerable. Considering the fact that the mean intake level was slightly

below 0.5 gram daily, each subject lost almost twice as much as he consumed.

Figure 6 shows graphically the mean excretory levels of Subject G during the three periods of this unit of the study.

Status of Calcium Balance

Table VII shows the status of calcium balance between intake and outgo of this nutrient during the three periods of this study. As in the case of the previous unit, all subjects were in positive balance during the control period, in negative balance during the bed rest phase, with a return to a mean positive balance during the post-bed rest periods. The degree of negative balance was more severe than when higher levels of calcium were fed.

TABLE V

Mean Daily Consumption of Calcium by Four Subjects during the Control, Bed Rest, and Post-Bed Rest Periods when 0.5 gram of Calcium was Provided during Bed Rest

(Calcium in grams)

Subjects	Control Period (1.5 grams provided)	Bed Rest Period (0.5 gram provided)	Post-Bed Rest Period (1.5 grams provided)
G	1.531	0.466	1.168
H	1.489	0.431	X
J	1.489	0.395	1.548
K	1.509	0.431	X
L	1.520	0.436	1.524

TABLE VI

Mean Daily Excretion of Calcium by Four Subjects during the Control, Bed Rest, and Post-Bed Rest Periods when 0.5 gram of Calcium was Provided during Bed Rest

Subjects	Control Period (1.5 grams provided)	Bed Rest Period (0.5 gram provided)	Post-Bed Rest Period (1.5 grams provided)
G	1.155	0.982	1.328
H	1.268	1.058	X
J	1.367	0.917	1.178
K	1.260	0.938	X
L	1.375	1.249	1.214

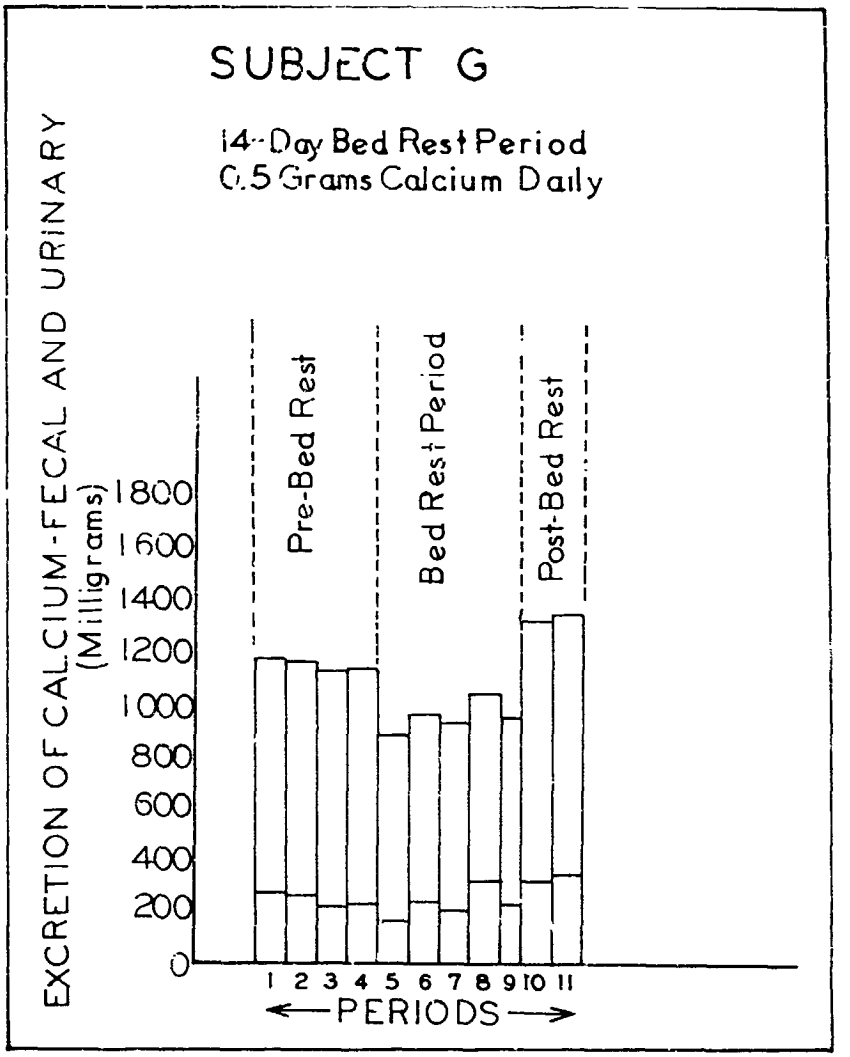


Figure 6. Calcium Excretion by Subject G when 1.5 grams of Calcium were Provided during the Initial Pre-Bed Rest Control and the Final Re-conditioning Period and 0.5 gram was Provided during the Intermediate Bed Rest Phase: Urinary (*) and Urinary plus Fecal (**), in Terms of Average milligrams per Three Days except Period 8 which Represents a Two-day Average. Periods 1 to 4 Cover the Last 12 Days of a Pre-Bed Rest Equilibration Phase; Periods 5 to 9 Represent a 14-day Bed Rest; and Periods 10 and 11 include Six Days of a Post-Bed Rest Phase

TABLE VII

Calcium Balance during Pre-Bed Rest, 14-Day Bed Rest and Post-Bed Rest of Five Subjects when 0.5 gram of Calcium was Provided during the Bed Rest

Subjects	Period	Mean Calcium Consumed Daily (grams)	Mean Daily Calcium Excreted			Mean Daily Calcium Balance
			Urinary	Fecal	Total	
G	Pre-Bed Rest (1.5 grams of Calcium Provided)	1.531	0.251	0.904	1.155	+0.376
H		1.489	0.142	1.126	1.268	+0.221
J		1.489	0.318	1.049	1.367	+0.122
K		1.509	0.179	1.081	1.260	+0.249
L		1.520	0.213	1.162	1.375	+0.145
G	Bed Rest (0.5 gram of Calcium Provided)	0.466	0.260	0.722	0.982	-0.516
H		0.431	0.257	0.801	1.059	-0.627
J		0 395	0.246	0.672	0.917	-0.522
K		0.431	0.212	0.726	0.938	-0.507
L		0.436	0.178	1.071	1.249	-0.813
G	Post-Bed Rest (1.5 grams of Calcium Provided)	1.618	0.332	0.996	1.328	+0.290
H		---	---	---	---	---
J		1.548	0.316	0.862	1.178	+0.370
K		---	---	---	---	---
L		1.524	0.253	0.961	1.214	+0.310

Consumption and Excretion of Phosphorus

During the control and the post-bed rest periods, the subjects in this unit of the study received dietary calcium to the extent of 1.5 grams per day, as has been noted, with a quantity of phosphorus sufficient to yield a calcium:phosphorus ratio of 1 : 1.4. During the bed rest phase, with the extremely low level of calcium, a calcium:phosphorus ratio of 1 : 2.99 was maintained.

Throughout all three parts of this unit, all subjects were in positive phosphorus balance except for Subject H during bed rest, who had only a very slight level of negative balance.

Bone Mass Changes during Unit in which .5 gram of Calcium was Provided during Bed Rest

The results of the radiographic bone densitometric measurements made during this unit of the study are shown: (a) by means of graphs made of the bone mass data points throughout the unit; (b) by regression lines made by the 1620 IBM computer for representative periods of the unit; and (c) by a Table in which the slopes of regression lines for different periods and sub-periods are shown.

Figures 7, 8, 9, 10, and 11 include graphs of the actual data points for the bone mass measurements made on the five subjects in

this study. Figures 12 and 13 show linear regression lines for two subjects of this unit of the investigation which are representative.

Table VIII summarizes the slopes of the designated regression lines and related data for all five subjects.

BONE MASS CHANGES IN SUBJECT G

Subject G consumed a mean of 1.531 grams of calcium during the control period when 1.5 grams were planned, as has been noted. During the bed rest period, this subject ingested a mean of 0.466 gram of this nutrient, when 0.5 gram was planned. During the post-bed rest period, he requested extra helpings, generally of bread, and averaged 1.618 grams of calcium per day, when 1.5 grams again were planned.

The mean calcium excretion during these periods, as well as the status of calcium balance have been summarized in Tables VI and VII. In the latter table it is noted that this subject was in positive balance during the control and post-bed rest period, but in distinctly negative balance during the bed rest phase (-0.516 gram per day on the average).

The bone mass values made throughout this unit of the study on Subject G (Figure 7) reflect these previous findings. This figure shows relatively stable bone mass values through the control or pre-bed rest

period. During bed rest there was an immediate sharp decline through the first few days, followed by an intermediate period when the values tended to level off, and a final second period of decrease in bone mass.

At the beginning of the post-bed rest period there was a sudden increase in bone mass values, with the last one-half of this period approximately equivalent to the control bone mass levels.

Figure 12 shows data points and linear regression lines for three sections of the bed rest period based on Days 0 through 5, 6 through 10, and 11 through 14 for Subject G. The data concerning Subject G summarized in Table VIII show that, when the "t" test was applied to the data of the control period, the regression line did not differ significantly from zero, and hence the line had neither a positive nor a negative slope. In short, there was no statistically significant change in bone mass during this period.

When the bed rest was considered in three sections, the period which encompassed the last day of the control period through the fifth day of bed rest had a distinct negative slope. Bed rest days six through 10 did not have a regression line of which the slope differed from zero, while the last few days of the bed rest period again had a distinct negative slope ($P < 0.001$).

The increase in bone mass during the first one-half of the post-bed rest period gave the entire period a regression coefficient which had a positive slope.

When the initial bone mass value measured just before the bed rest began was compared with the final value, there was an overall loss of 10.8 per cent in this factor throughout the entire bed rest period.

The marked decline in bone mass during the first few days of bed rest for this subject, together with the equally marked increase in bone mass after the bed rest period had ended, has posed a problem as to whether this bone section can experience such sudden changes as are indicated here. Several of the subjects of this bed rest unit followed a similar pattern. On the other hand, when bed rest units have been conducted with 2.0 grams or 1.5 grams of calcium fed both during the equilibration and the bed rest periods, the reduction in bone mass during bed rest is gradual, tending to be more or less continuous while the bed rest phase lasts.

Marked changes in bone mass within a relatively short period of time seem to be a possibility if one considers a description of mineralization of bone by Cantarow and Shepartz (3). According to these authors, the modern concept is that blood plasma is supersaturated with Ca and HPO_4 ions, and that, under the correct circumstances,

crystal growth may continue spontaneously in the unit of bone mineral, visualized as a crystal complex. They state further that, since the ground substance in which these mineral units are embedded is a direct extension of the extracellular fluid, its ionic constituents will be influenced by those of the blood plasma.

BONE MASS CHANGES IN SUBJECT H

Subject H consumed a mean of 1.489 grams of calcium during the control period of this unit of the study, with 1.5 grams provided. He averaged 0.431 gram of this nutrient during the 14-day bed rest period when 0.5 gram was planned. This subject remained only five days after the bed rest ended, before taking a brief leave of absence; during this post-bed rest period he consumed a mean of 1.5 grams of calcium.

In Table VII it is seen that this subject was in positive calcium balance during the control and during the post-bed rest periods. During the bed rest phase, however, he exhibited a mean negative calcium balance of -0.627 gram per day.

These findings are consistent with the bone mass changes found in this subject. Figure 8 shows the data points for individual bone mass measurements. This subject, a semi-professional baseball player who was cooperating with us in his off-season, frequently took

a brief leave of absence between units of the study to engage in some baseball practice. Hence he was not equilibrated with respect to bone mass at the beginning of the control period as was Subject G. After coming up gradually in bone mass during this period, he experienced a relatively gradual decline during bed rest, with an advance after bed rest during the short time he remained with this unit of the study.

The linear regression lines for the bone mass of this subject indicated that he was making positive bone mass gains during the control period, with the regression line as demonstrated by the "t" test having a slope which differed from zero by a highly significant amount ($P < 0.001$). During the first six bed rest measurements including that made earlier in the day when bed rest began through Bed Rest Day 5, the regression line had a slope which was significantly negative ($P < 0.02$). During Days six through 10, the regression line had a slope which was not significantly different from zero. During the last four days, however, the regression line differed from zero by a slightly negative difference ($P < 0.10$). See Table VIII.

The overall total bed rest yielded a regression line with a slope of 0.01457, which was found by the "t" test to be significantly different from zero by a high probability ($P < 0.001$).

From the last bone mass measurement made the morning the

bed rest phase began through the last day of bed rest, Subject H lost 10.5 per cent in bone mass.

BONE MASS CHANGES IN SUBJECT J

Subject J consumed a mean of 1.489 grams of calcium during the control period, 0.395 gram during the total bed rest period, and 1.548 grams during the post bed-rest phase.

The mean calcium excretion data (Table VI) and the calcium balance data (Table VII) show the situation with respect to the negative calcium balance of this subject (-0.522 gram/day).

Figure 9 gives the bone mass data points for this subject throughout the three parts of this unit of the study. During the control period he showed some gain in bone mass during the last few days. During bed rest his bone mass measurements reflected his extremely low calcium intake, with the pattern during this period bearing some similarity to that of Subject G except that, after the initial fall in bone mass there continued to be a more gradual but consistent decrease in bone mass. After the bed rest period the bone mass reached even a higher level than during the control period. It will be noted from the data given above that the calcium intake of this subject was higher during the post-bed rest than during the control period.

Table VIII shows the slopes of the linear regression lines for Subject J, during the various phases of this unit. During the control period the regression line had a slope of 0.00568 which was positive, with a probability significant at the 10 per cent level that the line differed from a zero slope. This is consistent with the data points shown in Figure 9.

The total bed rest period had a negative regression line (slope -0.01323), with a "t" test which showed that the slope differed significantly from zero (P 0.001). When the bed rest period was divided into three parts for statistical analysis, each segment had a negative slope of the regression line, shown to be significantly different from zero. During the post-bed rest period, there was no statistically significant difference between the slope of the regression line and zero, showing that bone mass changes during this period were neither in the positive nor negative direction.

Subject J began the bed rest period with a bone mass level of 2.140 gram-equivalence of calcium hydroxyapatite. This decreased to 1.841 by the close of this period, representing an overall loss of 14.0 per cent. This is consistent with his extremely low calcium intake during this period.

BOI MASS CHANGES IN SUBJECT K

Subject K entered the study at the beginning of the control period, and discontinued his participation after the close of the bed rest phase. During the control phase, he consumed a mean of 1.509 grams of calcium daily. During bed rest he averaged 0.431 gram/day. This subject had a mean negative calcium balance during bed rest of -0.507 gram per day, as shown in Table VII.

Figure 10 gives the data points for bone mass for the two sections of this unit of study in which he took part. His bone mass level showed little change during the control period, with a gradual but consistent decrease throughout the bed rest phase. The overall bone mass loss during bed rest was 11.5 per cent.

Table VIII and Figure 13 show that there was no statistically significant difference between the regression line of the control period and zero, and that the regression line of the total bed rest period was negative with the "t" test showing a highly significant difference between the slope of this line and zero ($P < 0.001$).

When the bed rest period was divided into three segments with respect to time, the slope of the regression line was negative in all three cases, with differences from zero which were statistically significant.

BONE MASS CHANGES IN SUBJECT L

It has been shown in Table V that Subject L had a mean calcium intake of 1.520 grams during the control period, 0.436 gram during bed rest, and 1.524 grams during the post-bed rest phase. Table VII shows that this subject had the lowest negative mean calcium balance of any subject of the group during bed rest (-0.813 gram/day).

Figure 11 shows the bone mass data points for Subject L during all three phases of this unit of the study. This graph shows little change in this factor through the control period, but a definite decrease during bed rest, and a marked increase during the post-bed rest period.

From Table VIII it is seen that the control period gave a regression line the slope of which was not significantly different from zero. When the bed rest period was considered as a whole, it had a negative slope which differed significantly from zero ($P < 0.001$). When the bed rest period was considered in three separate sections, each section had a definite negative slope. During the post-bed rest period the slope was positive, with the difference between this and zero highly significant ($P < 0.001$).

During the overall bed rest period, this subject lost 11.3 per cent in bone mass of the central os calcis section which was being evaluated.

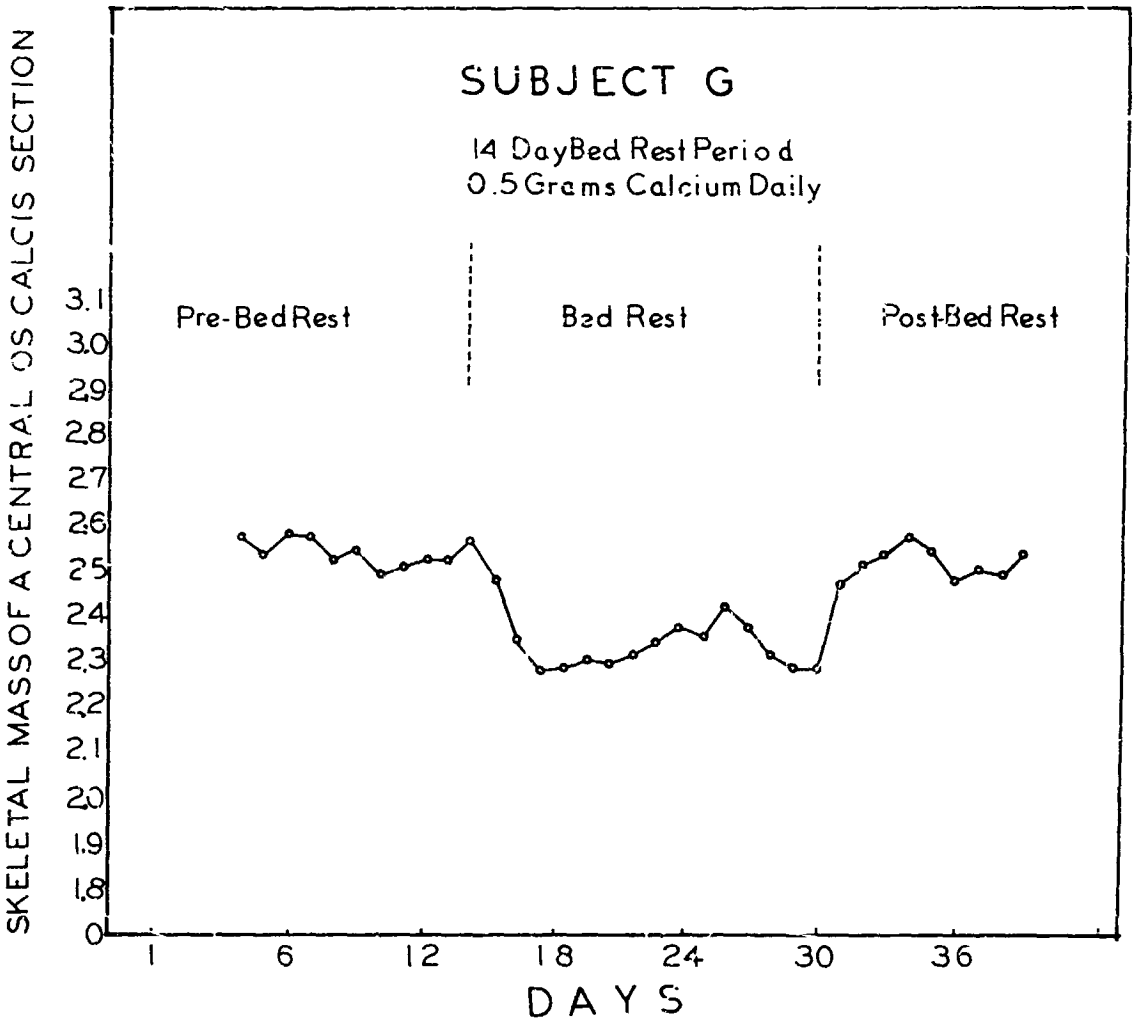


Figure 7. Data Points for Bone Mass Values for Subject G of a Central Os Calcis Section in Terms of grams of Calcium Hydroxyapatite Equivalency through the Pre-Bed Rest, the Bed Rest, and the Post-Bed Rest Periods

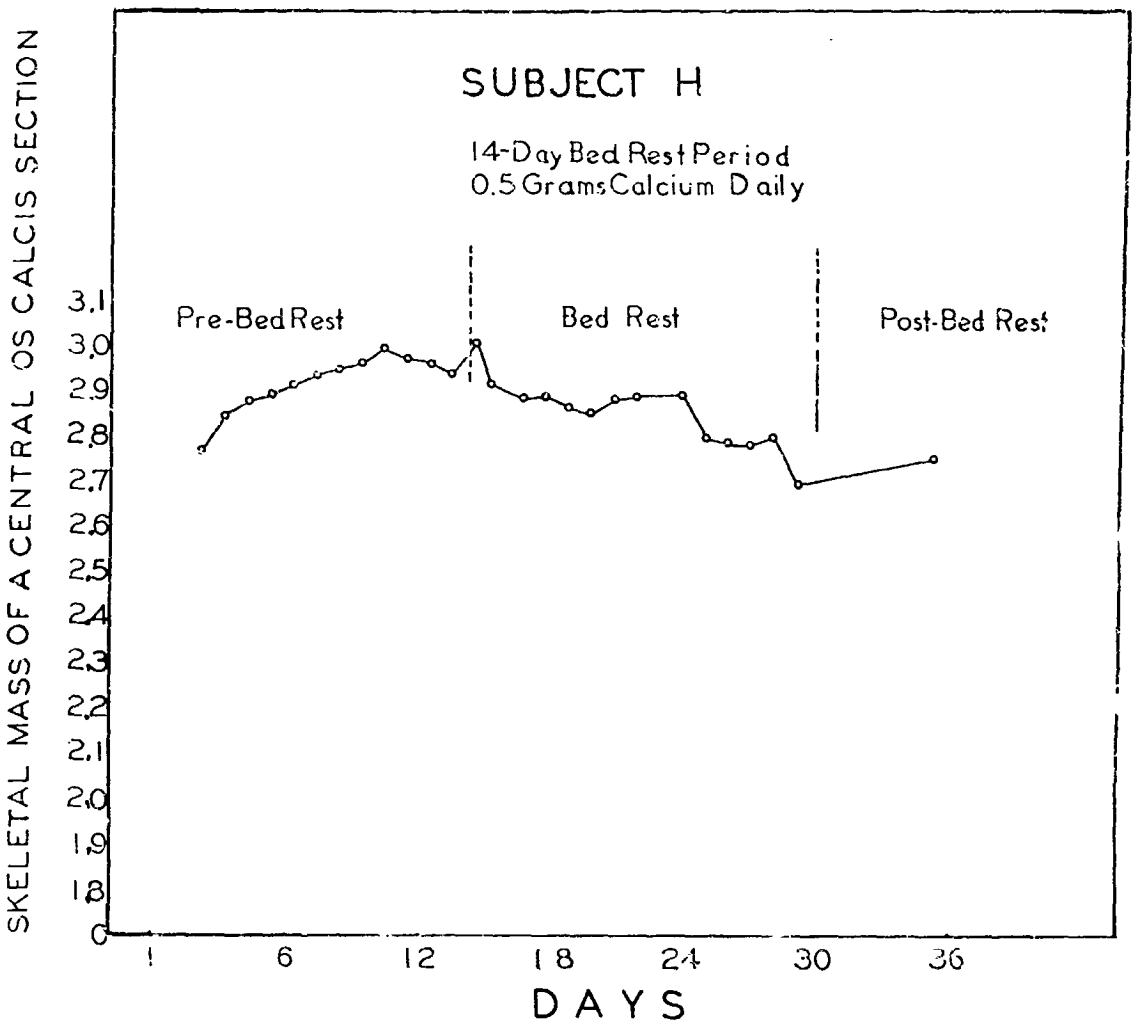


Figure 8. Data Points for Bone Mass Values for Subject H of a Central Os Calcis Section in Terms of grams of Calcium Hydroxyapatite Equivalency through the Pre-Bed Rest, the Bed Rest, and the Post-Bed Rest Periods

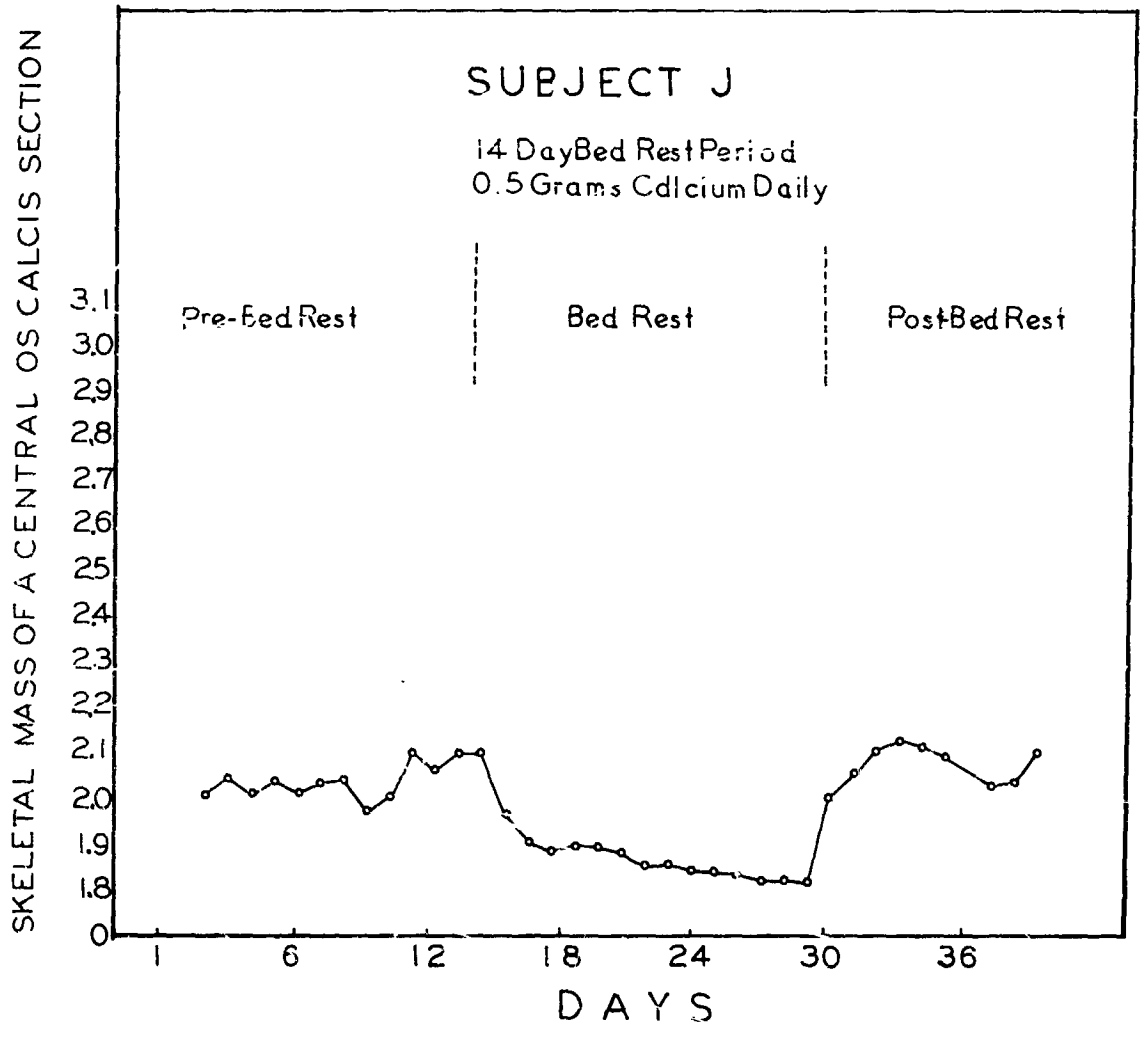


Figure 9. Data Points for Bone Mass Values for Subject J of a Central Os Calcis Section in Terms of grams of Calcium Hydroxyapatite Equivalency through the Pre-Bed Rest, the Bed Rest, and the Post-Bed Rest Periods

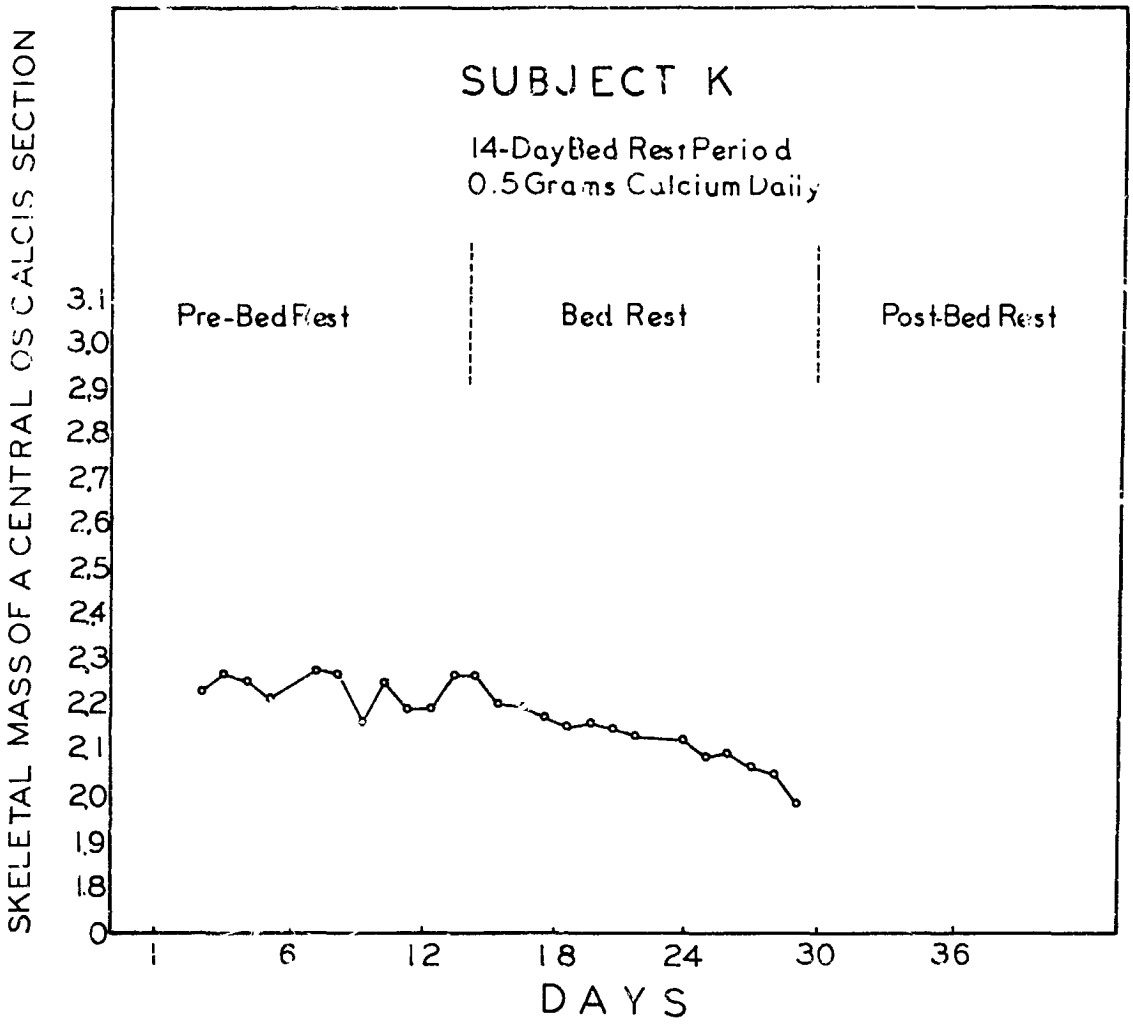


Figure 10. Data Points for Bone Mass Values for Subject K of a Central Os Calcis Section in Terms of grams of Calcium Hydroxy-apatite Equivalency through the Pre-Bed Rest, the Bed Rest, and the Post-Bed Rest Periods

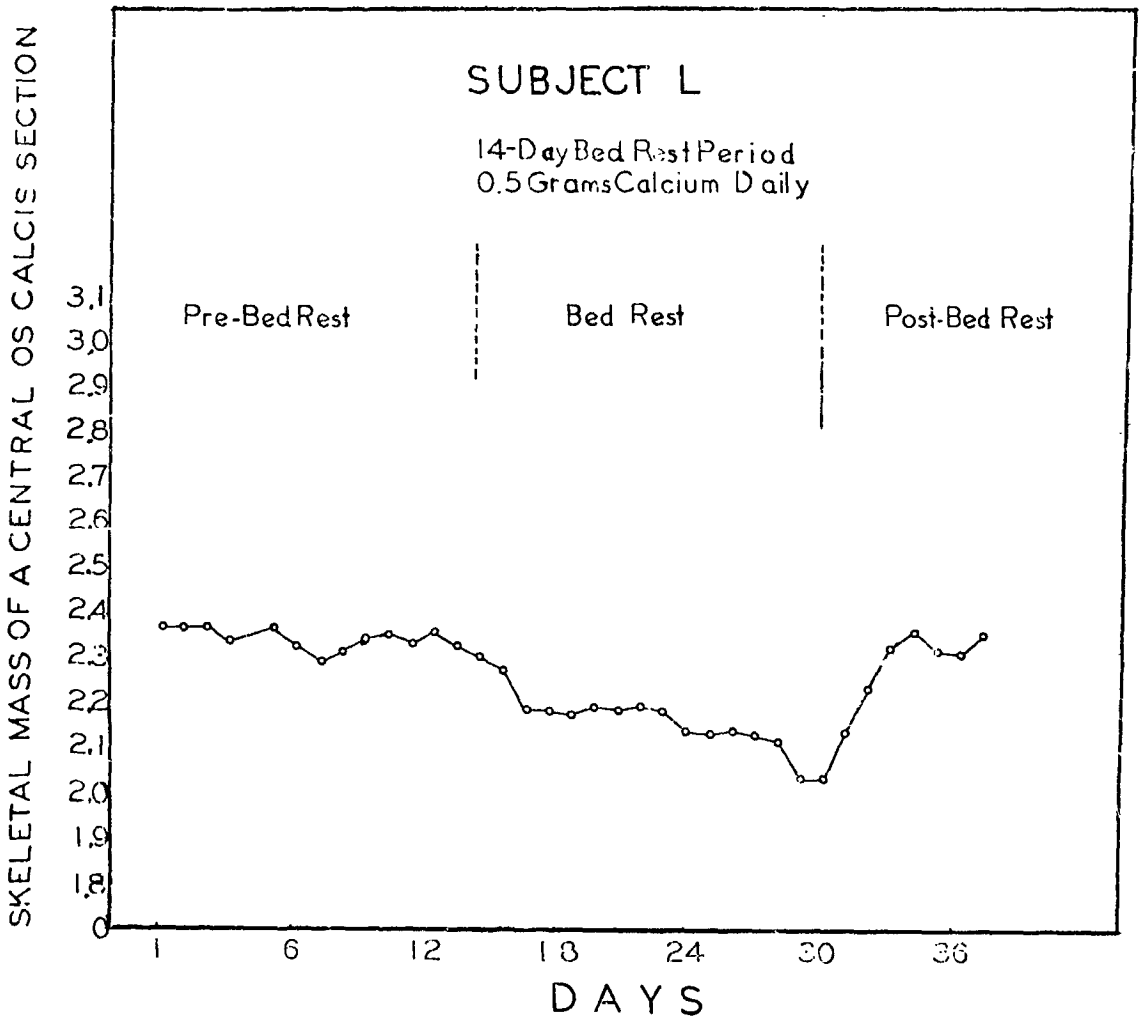


Figure 11. Data Points for Bone Mass Values for Subject L of a Central Os Calcis Section in Terms of grams of Calcium Hydroxyapatite Equivalency through the Pre-Bed Rest, the Bed Rest, and the Post-Bed Rest Periods

Figure 12. Data Points and Regression Lines for

Bone Mass Values of Subject G of a Central Os

Calcis Section for Three Parts of the

Bed Rest Period:

Initial through Five Days, Slope —0.05120, significant downward slope

Days Six through 10. Slope —0.01030, not significantly different from zero

Days 11 through 14, Slope —0.03750, significant downward slope

Bone Mass is given in Terms of Calcium

Hydroxyapatite Equivalency

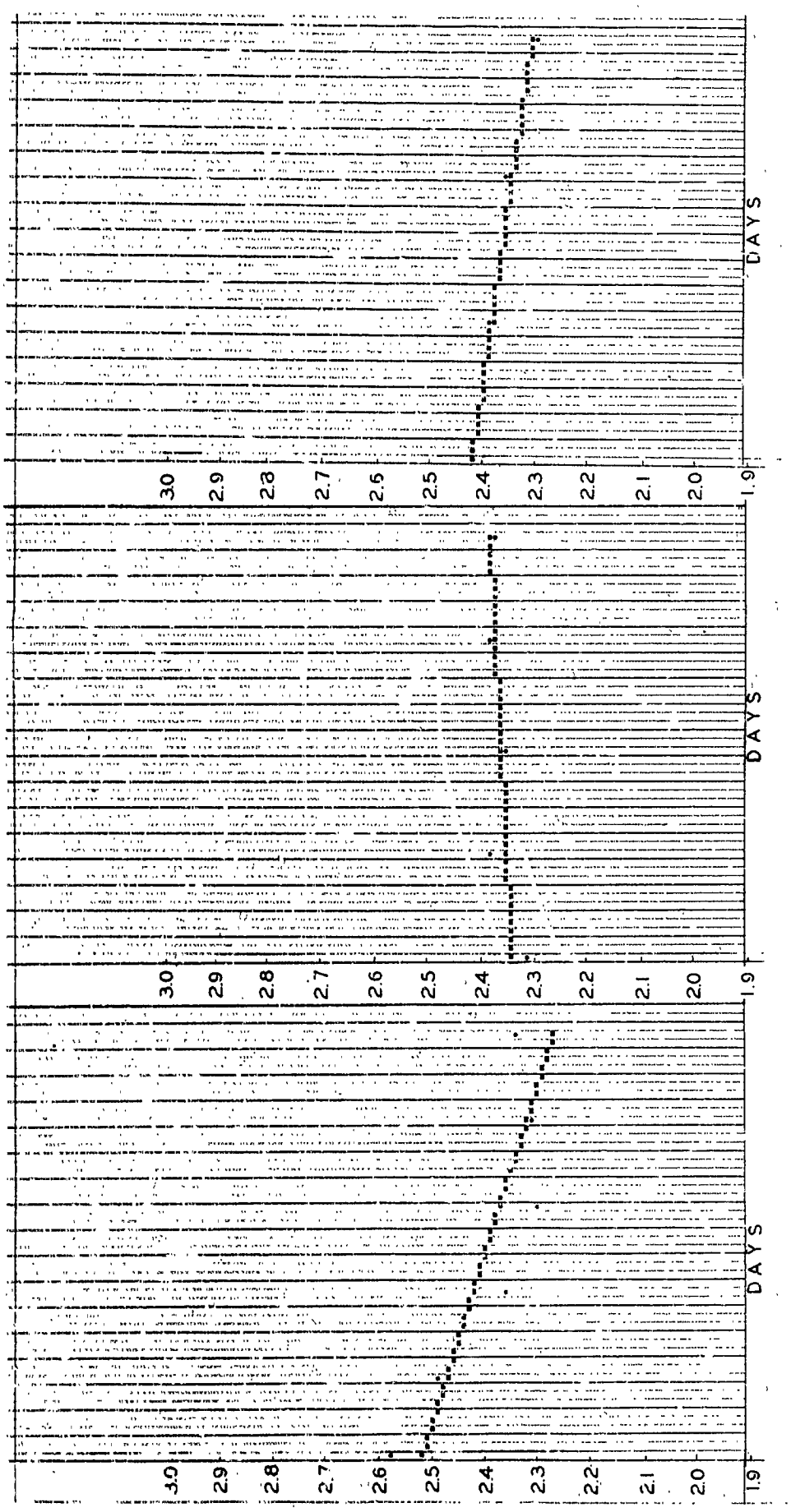

3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
DAYS

Figure 13. Data Points and Regression Lines for

Bone Mass Values of Subject K of a Central Os

Calcis Section for the Control Period

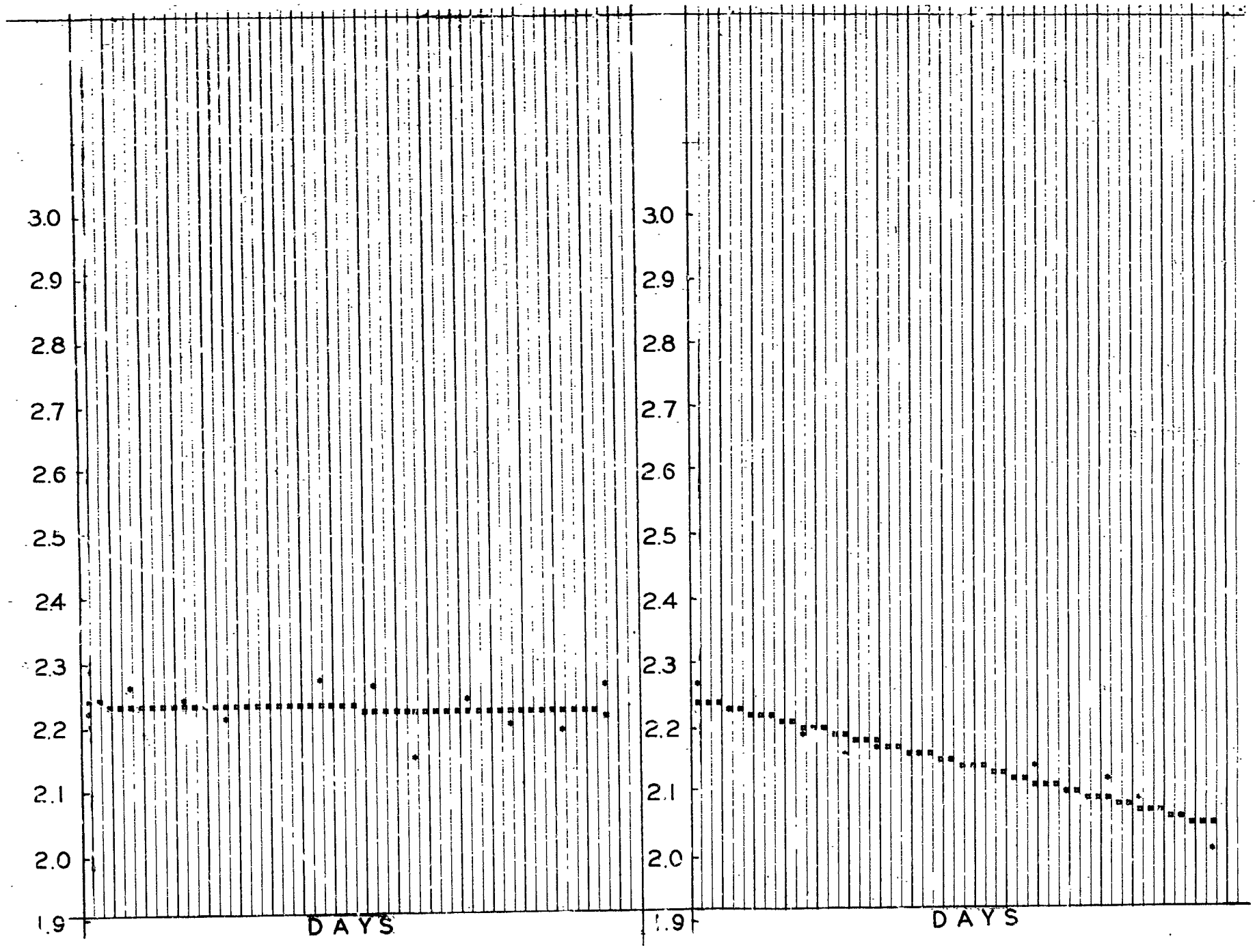
and the Total Bed Rest Period:

Control Period, Slope —0.00194, not significantly different from zero

Total 14-day Bed Rest Period, Slope —0.01437, significant downward slope

Bone Mass is given in Terms of Calcium

Hydroxyapatite Equivalency


3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
DAYS
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
1.9
DAYS

SUMMARY

This fourth semiannual report includes a discussion of two 14-day bed rest units, including collateral ambulatory pre-bed rest and post-bed rest periods. In the first of these units of the overall series of bed rest studies sponsored by the National Aeronautics and Space Administration, four subjects were fed a basic diet which provided 2.0 grams of calcium per day during the pre-bed rest, the bed rest, and the post-bed rest periods.

The four subjects lost, respectively, 3.86, 3.00, 6.50, and 6.48 per cent of bone mass during bed rest. During this period they had a mean overall daily calcium balance of -0.257, -0.320, -0.293, and -0.205 gram, respectively.

In the second of the 14-day bed rest studies conducted during this semiannual period, 0.5 gram of calcium was provided daily during the bed rest, after the five subjects who cooperated in this unit of the investigation had been equilibrated on a diet which provided 1.5 grams of calcium per day. During the bed rest the five subjects did not consume all of the food offered them, rejecting chiefly some of the bread. As a result they consumed a mean ranging from 0.395 to 0.466 gram of calcium daily. This group was in marked negative calcium balance, with mean daily calcium balance values ranging from -0.507 to -0.813 gram.

Following the bed rest, the subjects were placed on a post-bed rest diet again which provided 1.5 grams of calcium.

First order regression curves for the different periods of the two units covered in this report were calculated and plotted by a 1620 IBM computer, which aided in finding the trends in bone mass changes.

In the Fifth Semiannual Report of this series, which will be filed during the last part of August, 1965, the results of two 30-day bed rest units including auxiliary ambulatory periods will be described briefly. The first includes a 30-day bed rest for five men during which 2.0 grams of calcium were provided daily. This was preceded and followed by ambulatory periods with the same calcium level used. In addition to the tests given for the 14-day bed rest units described in this report, this unit also included tests for urinary nitrogen, creatine, and creatinine.

The second of the 30-day bed rest units to be described briefly in the next semiannual report included a 30-day bed rest period during which 1.0 gram of dietary calcium per day was provided, after a 29-day pre-bed rest ambulatory period on the same calcium level. Five men also took part in this unit. The bed rest was followed by a 31-day post-bed rest period with 1.0 gram of calcium in the diet, followed in turn by a post-post bed rest period during which the dietary calcium level was raised to 1.5 grams daily.

The same tests were done as in the previous 30-day bed rest unit, with the addition of cardiovascular measurements.

REFERENCES

1). Wohl, Michael G., and Robert S. Goodhart, Modern Nutrition in Health and Disease, Second Edition, Lea and Febiger, Philadelphia (1960)

2). Sinclair, H. M., The Assessment of Human Nutrition, pages 101-162 in Vitamins and Hormones, Volume VI, by Robert S. Harris and Kenneth V. Thimann, Academic Press, New York (1948)

3). Cantarow, Abraham, and Bernard Shepartz, Biochemistry, Third Edition, W. B. Saunders Company, Philadelphia (1952)

4). Mack, Pauline Beery; Anne T. O'Brien; Janice M. Smith, and Arthur W. Barman, Method for Estimating Degree of Mineralization of Bones from Tracings of Roentgenograms, Science 89: 467 (1939)

5). Mack, Pauline Beery, Walter N. Brown, Jr.; and Hughes Daniel Trapp, The Quantitative Evaluation of Bone Density, American Journal of Roentgenology and Radium Therapy, 61:808 (1949)

6). Mack, Pauline Beery; George P. Vose; and James Donald Nelson, New Development in Equipment for the Roentgenographic Measurement of Bone Density, American Journal of Roentgenology, Radium Therapy, and Nuclear Medicine, 82:303 (1959)

TABLE VIII

Slope of Regression Lines for Control, Bed Rest, and Post-Bed Rest Periods for Five Subjects when 0.5 gram of Calcium was Provided during the Bed Rest, with 1.5 grams Provided during the Control and the Post-Bed Rest Periods

Subject	Period of the Unit	Slope of Regression Line	Probability that the Slope of the Regression Line Differed Significantly from Zero	Per Cent Loss in Bone Mass during Bed Rest
G	Control	0.00511	N.S.	10.8
	Bed Rest			
	0-5 days	-0.05120	$P < 0.02$	
	6-10 days	-0.01030	N.S.	
	11-14 days	-0.03750	$P < 0.001$	
	Post-Bed Rest	0.01309	$P < 0.10$	
H	Control	0.01563	$P < 0.001$	10.6
	Bed Rest			
	0-5 days	-0.03230	$P < 0.05$	
	6-10 days	0.00714	$P < 0.10$	
	11-14 days	-0.01850	$P < 0.10$	
	Post-Bed Rest	X	X *	
J	Control	0.00568	$P < 0.10$	14.0
	Bed Rest			
	0-5 days	-0.05130	$P < 0.05$	
	6-10 days	-0.01520	$P < 0.01$	
	11-14 days	-0.00450	$P < 0.01$	
	Post-Bed Rest	0.00165	N.S.	

*This subject remained for five additional days of the post-bed rest period with bone mass returning to the control level during this period. After this he took a short leave of absence and then later returned.

TABLE VIII. CONTINUED

Slope of Regression Lines for Control, Bed Rest, and Post-Bed Rest Periods for Five Subjects when 0.5 gram of Calcium was Provided during the Bed Rest, with 1.5 grams Provided during the Control and the Post-Bed Rest Periods

Subject	Period of the Unit	Slope of Regression Line	Probability that the Slope of the Regression Line Differed Significantly from Zero	Per Cent Loss in Bone Mass during Bed Rest
K	Control	-0.00194	N.S.	11.5
	Bed Rest			
	0-5 days	-0.02091	$P < 0.001$	
	6-10 days	-0.01060	$P < 0.05$	
	11-14 days	-0.03560	$P < 0.05$	
	Post-Bed Rest	X	X **	
L	Control	-0.00169	N.S	11.3
	Bed Rest			
	0-5 days	-0.03320	$P < 0.01$	
	6-10 days	-0.01190	$P < 0.10$	
	11-14 days	-0.02350	$P < 0.05$	
	Post-Bed Rest	0.04185	$P < 0.001$	

**This subject left the study after the bed rest phase of the unit.