

THE EFFECT OF BEDREST ON VARIOUS PARAMETERS
OF PHYSIOLOGICAL FUNCTION

PART XII. THE EFFECT OF BEDREST ON BONE MASS AND
CALCIUM BALANCE

By F. B. Vogt, P. B. Mack, W. G. Beasley,
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Prepared under Contract No. NAS 9-1461 by
TEXAS INSTITUTE FOR REHABILITATION AND RESEARCH
Houston, Texas

for

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

For sale by the Office of Technical Services, Department of Commerce,
Washington, D.C. 20230 -- Price \$3.00

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ABSTRACT:

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Seven subjects participated in 14-day bedrest studies using approximately 1.0 g. calcium diets. A 14-day bedrest period was followed by a 14-day recovery period, which in turn was followed by a second 14-day bedrest period to which was added an isometric exercise program. The exercise program appeared to prevent the loss of bone density which occurred in the bedrest without exercise period. Calcium balance results are difficult to interpret because of short control periods to attain an equilibrium to the test conditions.

Author's

FOREWORD

This study is a part of a NASA investigation of the effect of bedrest on various parameters of physiological function. It was sponsored by NASA Manned Spacecraft Center under Contract NAS-9-1461 with Dr. Lawrence F. Dietlein, Chief, Space Medicine Branch serving as Technical Monitor.

This study was conducted in the Immobilization Study Unit of the Texas Institute for Rehabilitation and Research, the Texas Medical Center. The authors are affiliated with Baylor University College of Medicine as follows: Dr. Vogt, Department of Rehabilitation; Dr. Beasley, Departments of Rehabilitation and Physical Medicine; Dr. Spencer, Department of Rehabilitation; Dr. Cardus, Departments of Rehabilitation and Physiology; and Dr. Vallbona, Departments of Rehabilitation, Physiology, and Pediatrics. Dr. Mack is affiliated with the Texas Woman's University.

The authors wish to acknowledge the assistance of Mrs. Patsy Dominy and Mrs. Carolyn Caldwell in the typing of this manuscript; of Mary Walters, who was dietitian for the study; of Troy Allen in the analysis of data; of Judy Brock and Carmen Langford for biochemical determinations of the food and feces; of Bill Stover, Travis Driskill, and Grady Dozier for measurement of bone X-ray density; and of Dr. Ralph Pyke, John Bateman, Walter Gilchrist, and Elsa Klapper for urinary and analytical work.

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SUMMARY

An experimental study was performed at the Texas Institute for Rehabilitation and Research in the Summer of 1963 to evaluate (a) the changes in os calcis bone density with bedrest and (b) the effect of isometric exercises in preventing the loss of calcium during prolonged bedrest. Seven healthy young adult males participated in the study which was divided into two 14-day periods of bedrest, preceded and followed by an observation period. The first bedrest period consisted of bedrest without exercise. During the second bedrest period, a program of isometric exercise was added in which the subject developed a thrust force between his shoulders and feet. The subjects ate a controlled diet of 2200 calories with approximately 1.0 grams of calcium content.

Bone densitometry measurements of the os calcis showed a diminished density with the institution of bedrest, the greatest changes occurring in the first 3 or 4 days, with a later return toward normal. These appeared to be a lessening of the loss of bone density during the period in which exercise was added. Calcium balance studies showed a negative calcium balance during bedrest, but interpretation of the meaning is limited because of the short control periods to attain equilibrium to the test conditions.

INTRODUCTION

Changes in the mineral content of bone and in calcium balance on prolonged space flights have been predicted on the basis of clinical and experimental observations over the past several decades. One of the earliest reports of an experimental study on atrophy of bone from disuse was reported by Allison and Brooks,¹ in 1921. Since then, there have been numerous reports confirming this finding.^{2,3,4} Related observations, such as the changes in bone with nerve section, also have been reported.⁵ Cuthbertson, in 1929⁶ reported changes in balance of parameters of bone metabolism with prolonged muscular rest.

There have been other experimental studies made in an attempt to define the musculoskeletal changes that occur with inactivity. The studies of Deitrick, Whedon, and Shorr,^{7,8} performed in 1944 and 1945, have become a classic to describe the effect of prolonged bedrest on four normal adult males. Brannon and associates,⁹ studied thirty subjects for a bedrest period of 60 days. Birkhead et al.,¹⁰ have reported on metabolic effects of prolonged inactivity in four healthy trained men. Information associated with space flights¹¹ indicates that the Soviet Union reported calcium loss in Vostoks 3 and 4. Evaluations of calcium mobilization in astronauts participating in space flights of the United States have been inconclusive because of the lack of controlled dietary conditions prior to the flights and the short duration of the flights.^{12,13}

Bedrest can be used to simulate some of the conditions which may be associated with space flight. The effect of gravity forces acting down the long axis of the body is removed by having the subjects maintain a recumbent position. Muscular activity is likewise reduced, but it is not completely restricted, since cessation of activity would not be found in space flight. The subjects may be fed food of the type used in space flight, kept indoors and not exposed to sunlight. Coincidentally, X-ray measurements, and selected blood, urine, and feces analyses may be made. Therefore, bedrest was selected for the investigation as an appropriate method for obtaining a meaningful experimental design which could be used for more detailed future experiments.

The experimental design of this study is an outgrowth of earlier studies conducted to evaluate "cardiovascular deconditioning" that results from bedrest immobilization. The results reported herein constitute only a part of the multisystem evaluation on the effect of bedrest; and for this reason, the number and frequency of tests have been limited, as was the duration of the control or equilibrium condition prior to immobilization.

METHOD

A. Experimental Design

The experimental design provided for study of the same group of six subjects for two bedrest periods. The purpose of the first bedrest period was to obtain control data on each individual which would be representative of the effect of bedrest. The second bedrest period was identical to the first except for the addition of periodic isometric exercises. Comparison of the data obtained on the subjects during the bedrest plus exercise period with that obtained during the bedrest period provides information concerning any effect that may result from the exercise procedures.

Figure 1 shows a calendar that indicates the various periods of the study. The subjects were admitted to the Texas Institute for Rehabilitation and Research 7 days prior to the start of bedrest. Baseline or equilibrium data were obtained on all of the subjects as they were acclimated to their new environment. The subjects then were kept at bedrest for 14 days, after which they were observed

S	M	T	W	T	F	S
July	15	16	17	18	19	20
1963						
21	22	23	24	25	26	27
28	29	30	31	Aug.	2	3
4	5	6	7	8	9	10
11	12	13	14	15	16	17
18	19	20	21	22	23	24
25	26	27	28	29	30	31
Sept. 1	2	3	4	5	6	

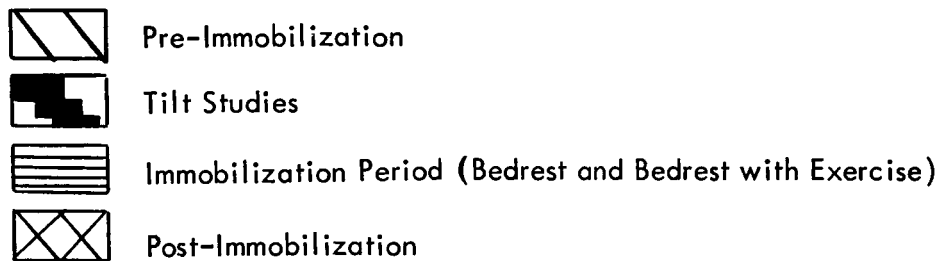


Figure 1. Periods of study.

and tested for 4 days, and then allowed to go home to resume their usual activities before starting the second period of study. Five days prior to the second bedrest period, the subjects were readmitted to the Texas Institute for Rehabilitation and Research for pre-bedrest evaluation as was done in the first period. An experimental procedure identical to the first was carried out except for the addition of controlled isometric exercises.

The subjects were housed in an experimental research ward which had beds arranged side by side. During the bedrest periods, the subjects were allowed to have one pillow under their heads. They were allowed to feed themselves, read in bed, and were provided radio and television entertainment during portions of the day. The subjects were not allowed to sit up in bed, but were allowed to turn over and roll from side to side. They were not allowed out of bed for bathroom privileges. Excreta were collected in bedpans for laboratory analyses. Tilt table tests were performed on each subject immediately before starting bedrest and again at the end of the bedrest period. An orderly was in attendance at all times to provide for the needs of the subjects and to assure that they did not deviate from the restrictions and requirements of the study. A physician supervised the studies and was present, or immediately available 24 hours a day.

B. Subjects

Six healthy adult males whose ages ranged from 21 to 34 years were used in each bedrest period. Five of the subjects participated in both the bedrest and bedrest with exercise periods. Subject T.O. participated in only the bedrest period and was replaced by subject A.I. in the bedrest with exercise period. The subjects were selected and considered for use in the study on the basis of their interest to participate in the experiments and on the basis of their availability during the prolonged periods of study. The details of the experimental procedures were explained to them prior to their selection. The subjects then were given a complete medical history and physical examination which was followed by routine laboratory and X-ray tests. Table I gives the physical characteristics and occupations of the subjects participating in this study.

C. Diet

The subjects were given identical diets and were required to sleep in the TIRR experimental ward. During the pre-bedrest and post-bedrest phases, the subjects were allowed to leave the building for short periods of time when they were not participating in a test. They were instructed not to eat or drink any foods other than those given them in the study.

Dietary needs were provided under the direction of a graduate research dietitian. The subjects ate low residue diets during the study period to provide

TABLE I
PHYSICAL CHARACTERISTICS AND OCCUPATIONS OF SUBJECTS
PARTICIPATING IN FOURTEEN-DAY STUDY

Patient		Age (Years)	Height (Cm.)	Weight (Kg.)	Body Surface Area (m ²)	Usual Occupation
TIRR No.	Name					
70-0-11	A. L.	33	170.3	62.7	1.73	Student (athlete)
70-0-12	T. O.	21	188.0	79.2	2.06	Student
70-0-13	M. O.	24	177.8	79.2	1.97	Student (athlete)
70-0-14	D. C.	24	180.4	75.0	1.94	Student
70-0-16	C. B.	24	185.5	85.7	2.10	Student counselor
70-0-17	C. P.	34	180.4	77.0	1.97	School teacher
70-0-18	A. I.	22	165.0	50.0	1.54	Student (athlete)

information on its effect in reducing the frequency of defecation. Figure 2 shows the dietary program that was used in the 14-day study. Dehydrofrozen foods of the type being evaluated by NASA for use in space flights were used during the times indicated. A low-residue diet prepared at TIRR was used at all other times of the experiment. The daily diet was designed to approximate a 2400 calorie, 1000 mg. calcium, 100 g. protein composition. The actual nitrogen and mineral contents were measured in the laboratory by analysis of a pooled daily sample of meals identical to the three eaten by the subjects. The average calcium intake during the first study period was 1088 mg. calcium daily; the average during the second study period was 1035 mg. calcium daily.

When the subjects were fed NASA dehydrofrozen foods, the diets were determined on the basis of calcium content specified by the manufacturer of the food. Selected samples of the food were analyzed by the analysis technique used at the TIRR, and the results were in good agreement with the calcium values specified by the food manufacturer.

The subjects were required to eat all food served them, but were allowed to drink water ad libitum. Meals were served at 8:30 a.m., 12:30 p.m., and 5:30 p.m.

D. Exercises

During the second immobilization period, the subjects were required to perform controlled isometric exercises at hourly intervals beginning at 9 a.m. and ending at 4:30 p.m. The exercise procedure was designed to provide the following:

1. Musculoskeletal exercise on the os calcis and lower back, since X-ray densitometric studies were used to evaluate the os calcis and third lumbar vertebra. Previous experience has been accumulated on radiographic analysis of these anatomical sites.
2. Exercise to massive muscle groups of the lower extremities, back, and shoulders, since it was thought that lack of exercise may contribute to the cardiovascular deconditioning of bedrest.
3. Relative gravitational constancy (i.e., action of gravity vectors on body does not change direction) as required in the design of the cardiovascular portion of the study.

Apparatus was constructed for use in bed to provide musculoskeletal exercise to the lower extremities and back with the body maintained in a horizontal position. Figure 3 shows a picture of the apparatus used.

The apparatus, referred to as a thrust rack for convenience, was constructed to allow performance of the exercise in a horizontal position on the

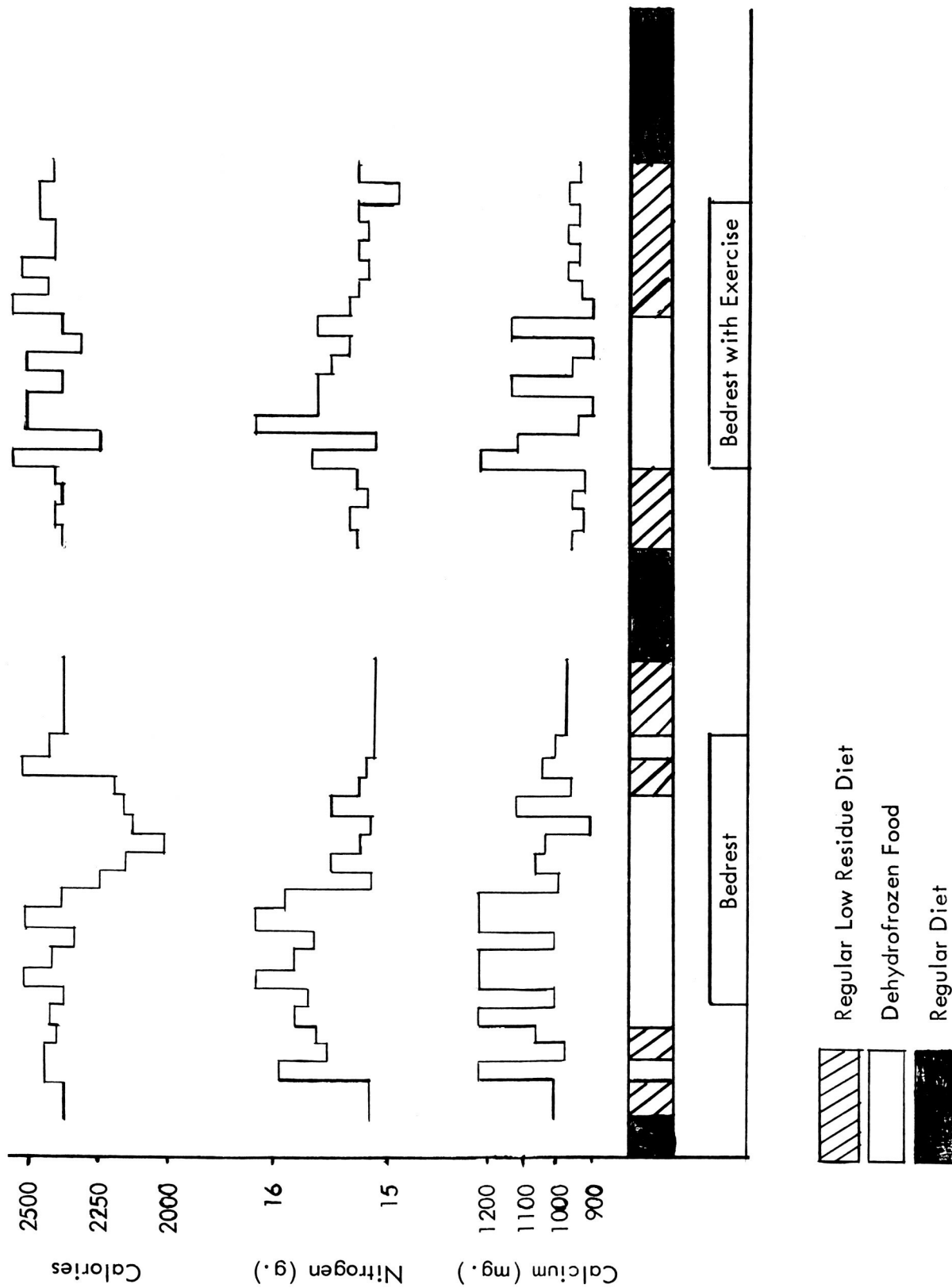


Figure 2. Dietary characteristics of the 14-day experiment.



Figure 3. Exercise apparatus for use in the reclining position.

surface of the bed. This thrust rack consisted of a piece of plywood 3/4" x 24" x 78", covered with padding and Naugahyde. Devices were fastened on the surface to provide an adjustable end thrust abutment between the shoulders and heels. Rigid shoulder braces presented non-yielding resistance. The foot pressure resistance surface was adjustable by a link chain in accordance with the subject's height. An axial tensiometer was activated by the thrust force developed between heels and shoulders by muscular effort of the subject. An ink-writing recorder, as output of the myodynagraph,¹⁴ registered the time course of the subject's forceful effort during each trial. Figure 4 shows a sample record taken during an exercise trial on one of the subjects.

The thrust rack was moved from bed to bed for each subject's exercise period. The subject was rolled over on his side toward one edge of the bed surface. The rack then was placed in position on the bed. The subject was rolled back onto the rack surface, and the rack was centered on the bed. The padded shoulder braces were adjusted laterally and at an angle to conform with the subject's contours. Care was taken to insure that the resisting pressures occurred over the region of the acromion processes and not over the soft tissue areas of the trapezius muscles. The feet were held in dorsiflexion at the ankle, and resistance forces were concentrated at the os calcis. The distance between shoulders and heels was adjusted individually for each subject so that under controlled forceful extensor action either of the following two conditions could be maintained.

1. Knees partially flexed position: The hips and knee joints are sustained by direct muscular action at approximately 15 degrees flexion.
2. Full extension position: In addition to elevation of the shoulders, all joints involved (knee, hip, and spine) are put into full extension.

A recording was made of every exercise routine for each subject. An auxiliary meter, synchronized with the myodynagraph, was placed for easy viewing of the subject to allow him to maintain the desired forces and thus perform the prescribed amount of exercise with each trial. The subjects were required to produce a thrust force in the range of 300-400 pounds, the desired value having been determined for each subject prior to immobilization. Maximal effort forces were variable for the different subjects, and the exercise levels prescribed were approximately 60 percent of maximum for each individual.

A standard exercise sequence was used as follows:

1. Knee partially flexed position: (a) Five sequential efforts for 5-seconds duration each, with 5-second intervals of rest between efforts, at prescribed level of force, (b) Two sequential maximum efforts, 5-seconds duration each, with 5-second intervals of rest

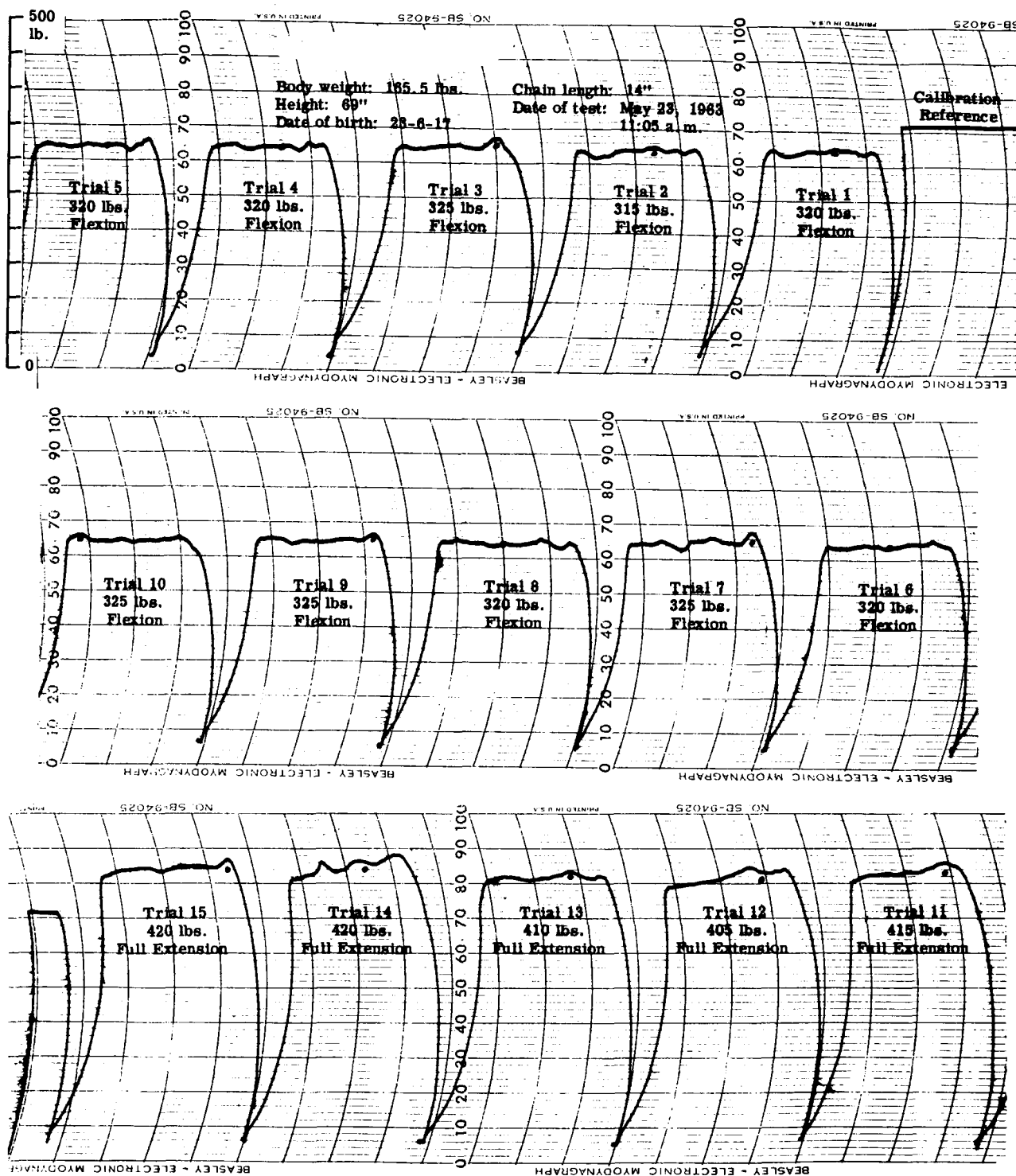


Figure 4. Example of graphic recording for one complete isometric tension exercise routine.

between efforts, and (c) Five sequential efforts, 5-seconds duration each, with 5-second intervals between efforts, at prescribed level of force.

2. Full extension position: Five sequential efforts, 5-seconds duration each, with 5-second intervals of rest between efforts.

E. Bone Densitometry

Bone mass has been measured in this investigation by means of the method of radiographic bone densitometry as developed and used by staff members of the Nelda Childers Stark Laboratory for Human Nutrition Research, Texas Woman's University.

The roentgenographic bone densitometric technique used in the reported studies was that developed by Mack and colleagues,¹⁵ and was reported first in 1939. The theoretical aspects of the method have been described by Mack, Brown, and Trapp,¹⁶ while its historical development up to 1950 was reported by Mack.¹⁷ Mack, Vose, and Nelson¹⁸ have covered the history of the tracing technique for determining bone mass from X-rays through the following decade, and have given a detailed description of the instrumentation employed in the current investigation.

The following presentation describes the instrumentation used in measuring bone mass in this study, as well as the method of its use. The technique of measuring bone mass from X-rays is based on the use of an aluminum alloy reference wedge with X-ray absorption characteristics similar to that of bone, which is X-rayed on the film with the bone to be assessed. Because the wedge presents approximately the same X-ray characteristics as bone, the preferential wave length absorption change produced by different amounts of bone is compensated by a similar wave length change in the wedge. The wedge pattern thus obtained on a roentgenogram reflects the results of possible differences in the film itself, in film exposure, and in processing conditions. Hence, although the wedge itself has a linear slope, the initial wedge density curve usually is non-linear. However, the wedge density trace can be linearized by the instrument assembly, thus correcting the trace of the bone section on the same film.

Proper positioning of a subject on the X-ray table is absolutely essential as the first step in determining bone mass from roentgenograms. Figure 5 shows the positioning of the os calcis on the X-ray film holder and the placing of the calibration wedge in the proper location.

A pre-determined anatomical site has been chosen for making a trace of a bone section from the same film as that on which the wedge curve has been linearized. For example, the mass of a central section of the os calcis is measured by making a scan from a posterior to an anterior landmark as shown in Figure 6. The os calcis possesses definite posterior and anterior landmarks which



Figure 5. Position of subject for making an os calcis roentgenogram, with the reference wedge being placed in correct position. The subject will be covered with lead sheeting except for the foot being x-rayed.



Figure 6. Roentgenograph of Os Calcis and wedge, showing line scanned by densitometer.

can be located accurately in repeated evaluations of the same subject. This section between these two landmarks consists largely of cancellous tissue, which is amenable to rapid change in skeletal mass. In addition to the central section which is scanned to determine bone mass changes in this one segment of the os calcis, traces may be made one millimeter apart parallel to this central trace over the entire bone image to obtain the mass of this bone as a whole.

Another skeletal location which reflects mineral loss quite readily is the lumbar spine. The correlation of radiographic density of the body of the third lumbar vertebra (X-rayed in situ, postmortem) with its actual ash density following autopsy of 60 cadavers has been presented by Vose, Hoerster, and Mack.¹⁹ The coefficient of correlation between this X-ray determined aluminum/bone equivalency and actual ash content was $r = 0.93$ and with its ash content in grams/cc. was $r = 0.85$. Both correlations are significant above the 0.01 level.

The technique for producing roentgenograms of the lower spine in which the effects of X-ray scattering are reduced has been developed at the Texas Woman's University. Figure 7 shows the positioning of a subject for the making of an X-ray of this anatomical site. Whereas the soft tissue above and below the os calcis is minimal with X-ray scattering not a problem, the tissue surrounding the lumbar spine is considerable. For exposures of the latter, a special wedge designed for use at this skeletal site is embedded in a plate of plastic which has an X-ray transmission similar to that of soft tissue. Plates of plastic are placed below the wedge to a height which places the wedge opposite the spine. Additional plates of plastic are placed above the wedge to approximate the total height of the soft tissue above the spine. In this manner, a roentgenogram is obtained of the bone and the wedge simultaneously.

The densitometric equipment shown in Figure 8 consists of four basic units as follows:

1. A modified Knorr-Albers scanning unit.
2. A Speedomax model G transmitting recorder which reproduces the film density curve and contains a potentiometer panel for adjusting the non-linear density curve to linearity.
3. A Speedomax model G recorder for displaying the linearized curve.
4. An Instron integrator geared to the output of the second recorder.

The sequence of operations required to achieve linearization of a density curve and to integrate the area under the curve of the corresponding scanned bone is as follows: The wedge pattern first is scanned in order to provide the density

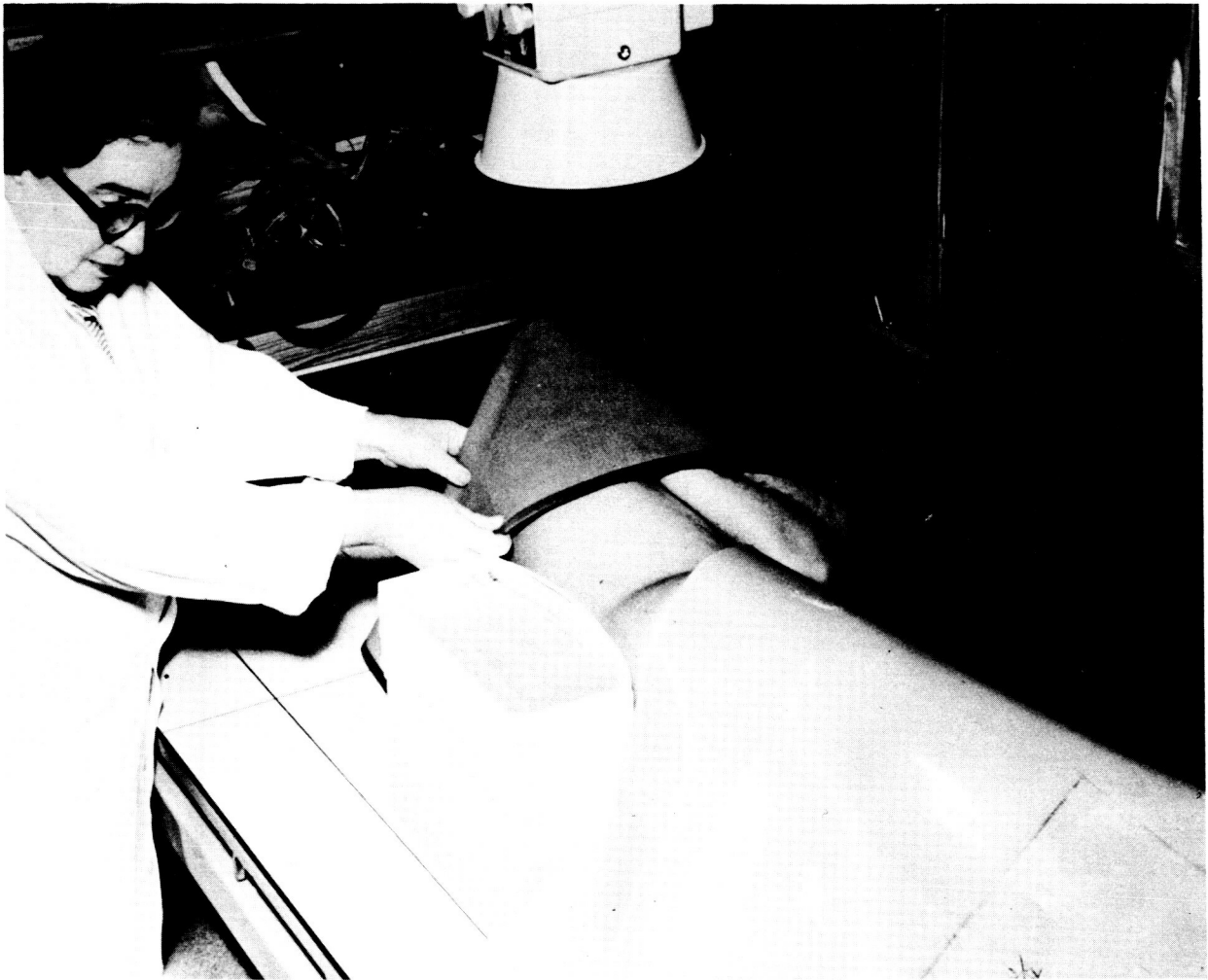


Figure 7. Position of subject for making lateral roentgenogram of the lumbar spine. The wedge is embedded in plates of plastic which have an X-ray transmission similar to that of soft tissue surrounding the spine. The wedge is located at the level of the spine. The subject is being covered with lead sheeting except for the area of the third and fourth lumbar vertebrae.

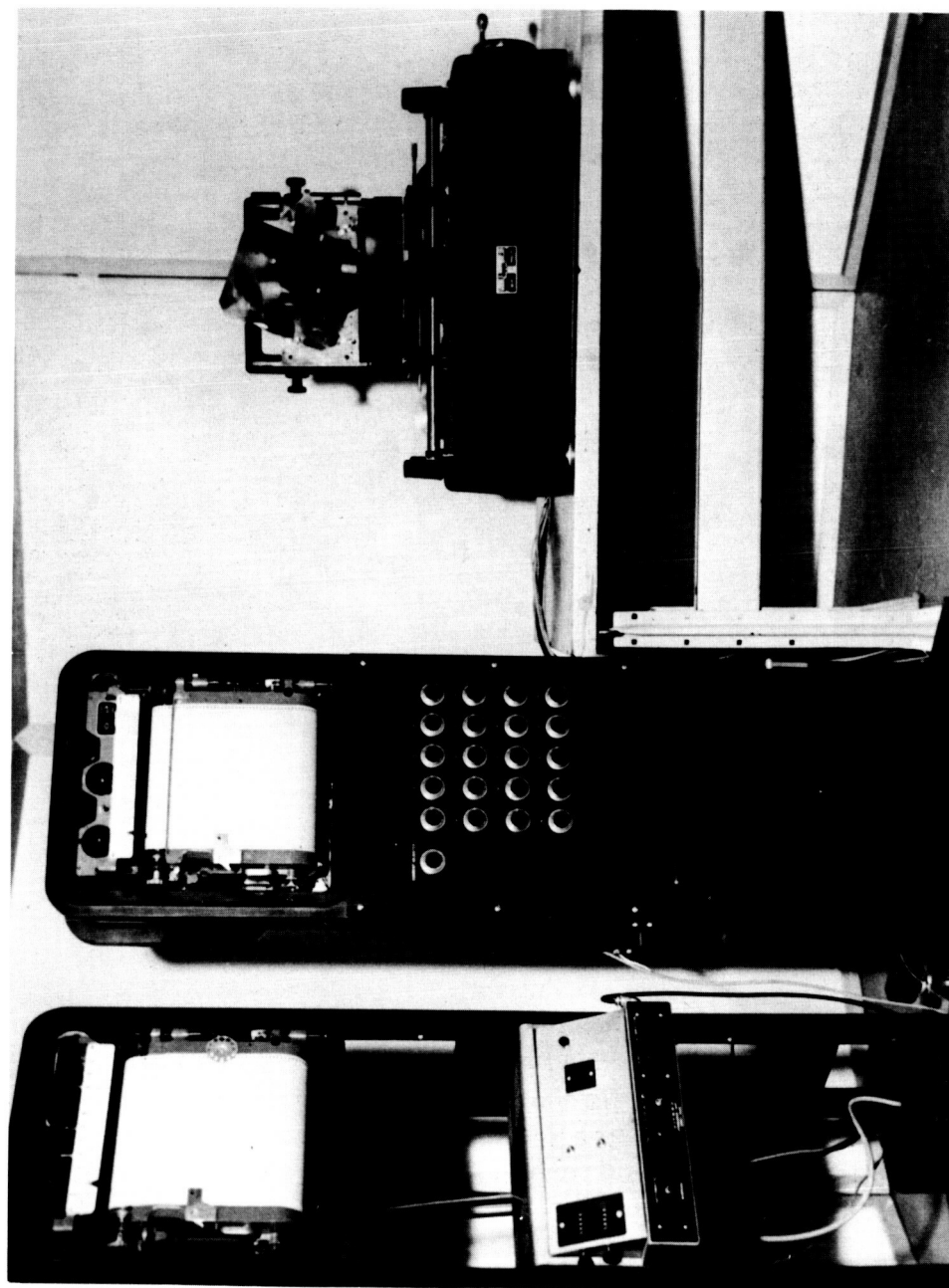


Figure 8. Densitometric assembly which is used to measure the equivalent calcium apatite mass in a section of bone penetrated by a scanning beam from a roentgenogram: right, densitometer; center, first recorder and potentiometer panel; left, second recorder and integrator.

curve of that film on the first recorder. Next the operator measures the curve displacement from linearity along the chart baseline at 20 points on the curve and sets the respective potentiometers in the panel associated with each of the points. This is facilitated by the use of a small scale which indicates the deviation of the scan from linearity at each point, thus indicating the correct potentiometer setting to obtain the needed resistance for each slidewire section. The wedge image on the X-ray is traced again in order to insure that a linear scan has been accomplished. This linearized tracing is displayed on the second recorder. The bone section is then scanned and is evaluated by means of a read-out count obtained from the Instron integrator situated in the second recorder panel of the instrumentation.

After the integrator count reading has been obtained for the corrected bone scan, it is converted into equivalent X-ray mass of calcium apatite by a conversion factor which has been derived from the following formula for determining equivalent wedge volume.

$$\text{Equivalent Wedge Volume} = \frac{S \cdot L \cdot V \cdot H \cdot C}{K} \text{ (cm.}^3\text{)}$$

Where	S	=	Slope of wedge (cm./cm.)
	L	=	Baseline length of wedge portion traced (cm.)
	V	=	Velocity of scan (cm./min.)
	H	=	Vertical dimension of the scanning slit (cm.)
	K	=	Counts obtained for 1 minute of full scale integration (counts/min.)
	C	=	Integrated counts (counts)

For the studies reported herein

S	=	0.100
L	=	13.00
V	=	2.00
H	=	0.130
K	=	5000

The equivalent X-ray mass of $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaCO}_3$, the calcium complex to which the wedges are calibrated for each section of bone traced on the same film, can be obtained as follows:

$$\text{Equivalent X-ray Mass of Apatite} = \frac{S \cdot L \cdot V \cdot C \cdot H \cdot D}{K} \times 1.075$$

Where D = Density of aluminum alloy wedge

and 1.075 = Ratio of wedge density to apatite density ($2.706/2.517 = 1.075$)

Roentgenograms of os calcis were made daily or on alternate days throughout this study. Those of the lumbar spine were made weekly while the bedrest phases were being investigated.

In order to evaluate the reproducibility of the technique (including positioning error, differences in exposure technique and in developing, as well as densitometer error), the os calcis of the same subject was filmed eight times, with the subject taken off the table, put back on the table, and repositioned for each exposure. In addition each film was read four times on the densitometer, with the film being removed from the densitometer, repositioned, and relinearized between the times for each tracing.

F. Biochemical Analyses

Urine was collected in iced containers during 12-hour time periods from 7 a.m. to 7 p.m. and from 7 p.m. to 7 a.m. Samples were frozen and saved for analysis of calcium and phosphorus at a later time. Fecal specimens were collected and frozen, and analyses were made of the calcium and phosphorus content of each stool specimen. Stool specimens were separated by dye markers (1.5 g. carmine dye) given on day 2, day 8, day 22, day 26, day 32, day 36, day 51, and day 55 of the experiment.

Some of the subjects were unable to void at exactly 7 a.m. or at 7 p.m. The exact times of voiding were recorded, and the samples analyzed. An "equivalent" 12-hour value was determined by multiplying the actual measurement by an appropriate correction factor. For example, if a subject voided at 7 p.m. and then again the next morning at 8 a.m., the urine content of calcium represented a value for 13 hours. Therefore, this value was multiplied by 12/13 to get an equivalent 12-hour measurement that would allow comparison of data in different subjects, as well as on the same individual for different collection periods. The 24-hour values presented under results represents the sum of the two 12-hour samples for a given day.

Calcium was determined by the technique of Ferro and Ham.²⁰ Phosphorus was determined by the technique of Fiske and Subba-Row.²¹

RESULTS

Table II gives the values for the mass equivalency in grams of calcium apatite of the central os calcis section for the individual subjects determined from X-rays taken during the experiment. Day 1 represents the time at which the subjects were admitted to the study unit; day 8, the first day of bedrest; day 22, the last day of bedrest; day 36, the first day of the second bedrest period (with exercise); day 50, the last day of the second bedrest period; and day 54, the day final X-rays were taken on the subjects. Subject T.O. did not participate in the second bedrest period because he had to return to school. He was replaced by subject A.I. Subject C.B. did not participate in the second post-bedrest observation period because of illness in his family.

TABLE II

X-RAY DENSITOMETRY RESULTS

MASS (GRAMS) OF CALCIUM APATITE EQUIVALENCY OF CENTRAL
OS CALCIS SECTION

Fourteen-Day Bedrest Experiment

A. Bedrest Period								
Day	A.L.	T.O.	M.O.	D.C.	C.B.	C.P.	A.I.	Average
1	2.06	2.12	----	2.06	2.31	2.32	----	2.17
7	2.16	2.17	2.66	2.20	2.36	2.41	----	2.32
8*	2.20	2.36	2.80	2.22	2.34	2.46	----	2.37
9	2.21	2.21	2.83	2.12	2.34	2.31	----	2.34
10	1.98	2.28	2.59	2.05	2.21	2.40	----	2.25
11	1.92	2.24	2.56	2.01	2.23	2.27	----	2.21
12	2.08	2.22	2.54	2.12	2.25	2.28	----	2.25
15	2.02	2.16	2.53	2.15	2.17	2.23	----	2.21
18	1.86	2.09	2.68	2.10	2.24	2.30	----	2.21
22**	2.10	2.16	2.64	2.15	2.16	2.29	----	2.25
26	2.08	2.24	2.50	2.06	2.33	2.47	----	2.28
29	2.22	2.34	2.61	2.12	2.46	2.36	----	2.35
B. Bedrest With Exercise Period								
36	2.08	----	2.58	2.13	2.42	2.25	1.84	2.22
37	2.19	----	2.53	2.17	2.65	2.47	1.91	2.32
38	2.10	----	2.53	2.24	2.20	2.41	1.89	2.23
39	2.05	----	2.56	2.36	2.16	2.54	1.82	2.25
40	2.08	----	2.53	2.29	2.34	2.52	1.98	2.28
42	2.03	----	2.57	2.28	2.35	2.49	1.99	2.22
44	2.06	----	2.51	2.10	2.35	2.48	2.03	2.27
46	1.98	----	2.58	2.33	2.30	2.52	1.92	2.27
48	2.21	----	2.53	2.20	2.28	2.52	1.94	2.28
50**	1.97	----	2.66	2.25	2.31	2.30	1.76	2.21
52	2.19	----	2.50	2.18	----	2.47	1.79	2.23
54	2.08	----	2.61	2.07	2.19	2.39	1.77	2.17

* First day of bedrest.

** Last day of bedrest.

Figure 9 shows the average daily bone mass of the subjects throughout the experiment. There was an increase in bone mass in the pre-bedrest period, at which time the subjects were given diets containing 1.0 - 1.1 grams of calcium daily. This level of calcium intake was greater than they had been consuming at home, as determined by calculations of the average diets which they recalled eating. After the subjects were put to bed, the average bone mass progressively decreased for 3 days, showed a slight increase on the fourth day, and then returned to a lower level until the end of the bedrest period.

The average mass increased in the interim between the two bedrest periods and showed fluctuations during the second bedrest period to which controlled exercises were added. The average level of bone mass was higher in the second bedrest period, compared to the first. There was not an initial progressive decrease in mass as was seen in the first bedrest period. After the second bedrest period, the average mass decreased further below the level found during the second period of recumbency.

Figures 10 through 16 are separate plots of the mass equivalency of a central section of the os calcis for the individual subjects throughout the experiment. All six subjects showed an increase in mass during the initial pre-bedrest period, with subject C.B. showing the least change. After being put to bed, all six subjects showed a decrease in bone mass which was progressive over the first 3 or 4 days of bedrest. Then the pattern became more variable, with the subject exhibiting individual differences during the remainder of the first bedrest period. However, all subjects had one thing in common; the final measurement of the 14-day bedrest period was lower than the measurement made the first day of this period.

Considerable variability was found in bone mass results obtained during the interim between the two bedrest periods when the subjects were carrying on their own individual activities. The pattern of bone mass changes in an individual during the bedrest with exercise period was more variable than the response to bedrest alone. All of the subjects except C.B. showed values of bone mass which were at times higher during the second period than were found when starting the period.

Although the subjects did not show homogenous changes in their day-by-day bone mass, certain definite trends emerged during the study. A statistical analysis of the bone mass data revealed the following: In the first pre-bedrest period, an increase in mass of the central section of the os calcis was found which was highly significant for all subjects except C.B. ($p < 0.001$ in all except C.B.). Subject C.B. had a higher calcium intake before entering the study than did the other subjects, as judged from previous diets given by recall during the preliminary interview with the subjects.

When the bone mass data for the central section of the os calcis were pooled for the pre-bedrest period and were compared statistically with the

MASS (GRAMS) OF CALCIUM APATITE EQUIVALENCY OF
CENTRAL OS CALCIS SECTION

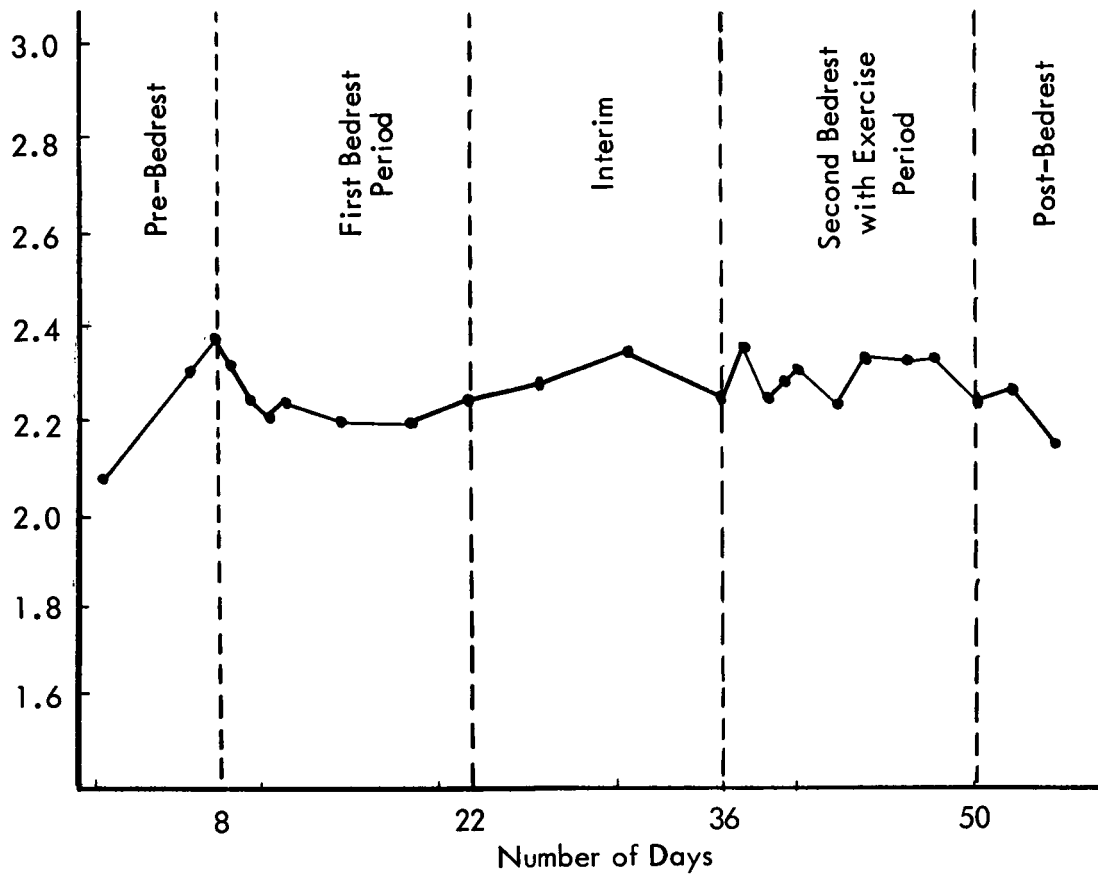


Figure 9. Bone mass changes in fourteen-day bedrest studies.
(Daily averages for six subjects)

MASS (GRAMS) OF CALCIUM APATITE EQUIVALENCY OF
CENTRAL OS CALCIS SECTION

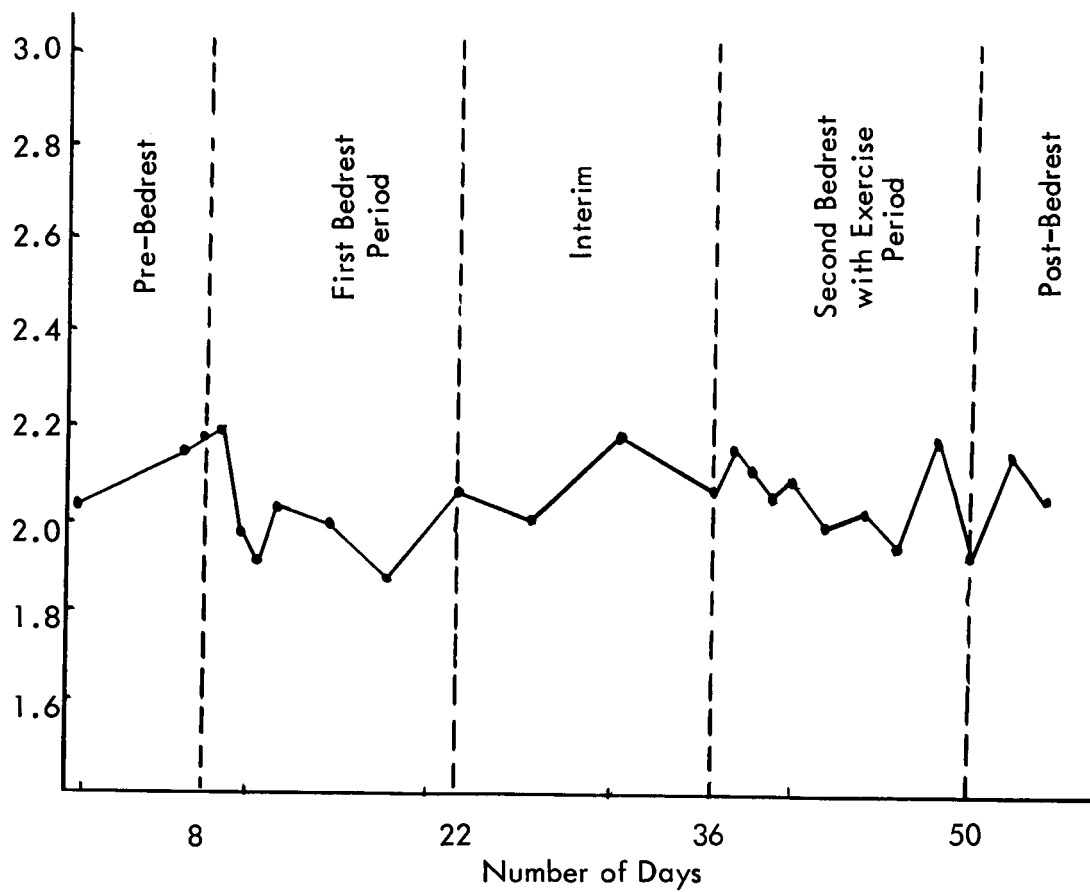


Figure 10. Bone mass changes in fourteen-day bedrest studies.
(Subject A.L.)

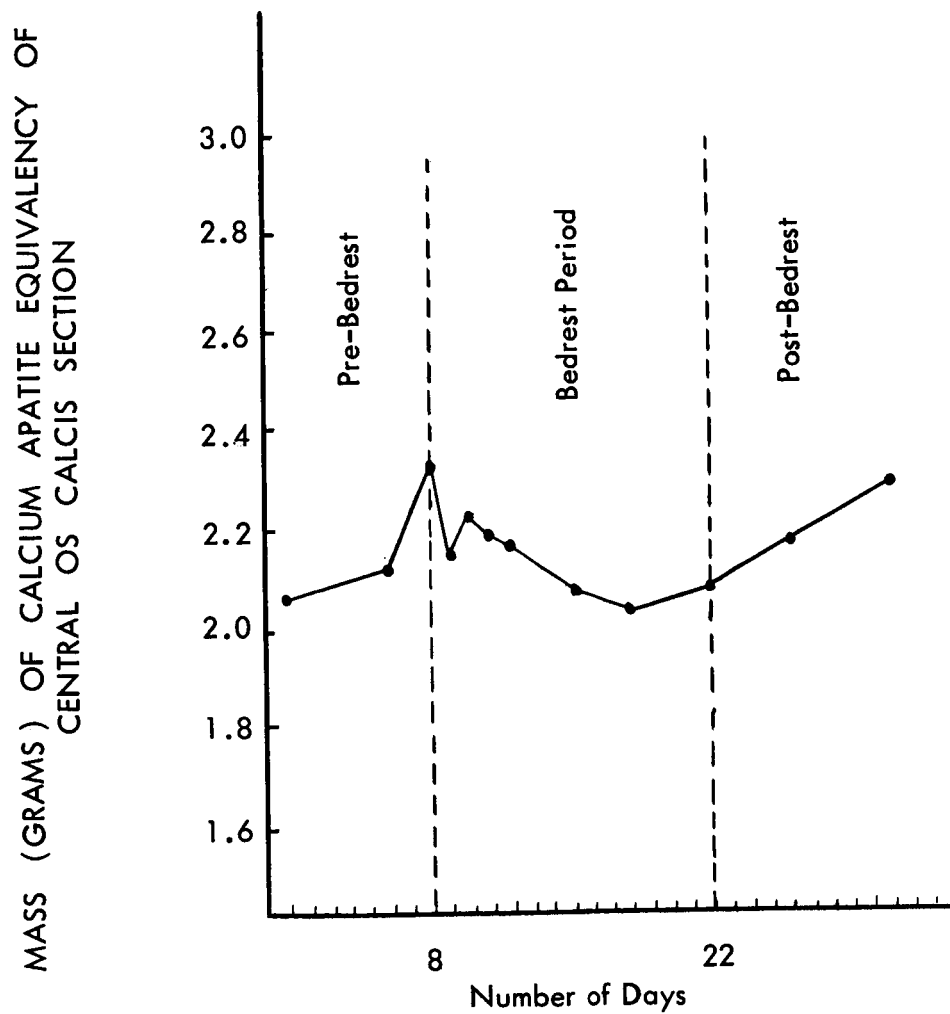


Figure 11. Bone mass changes in fourteen-day bedrest studies.
(Subject T.O.)

MASS (GRAMS) OF CALCIUM APATITE EQUIVALENCY OF
CENTRAL OS CALCIS SECTION

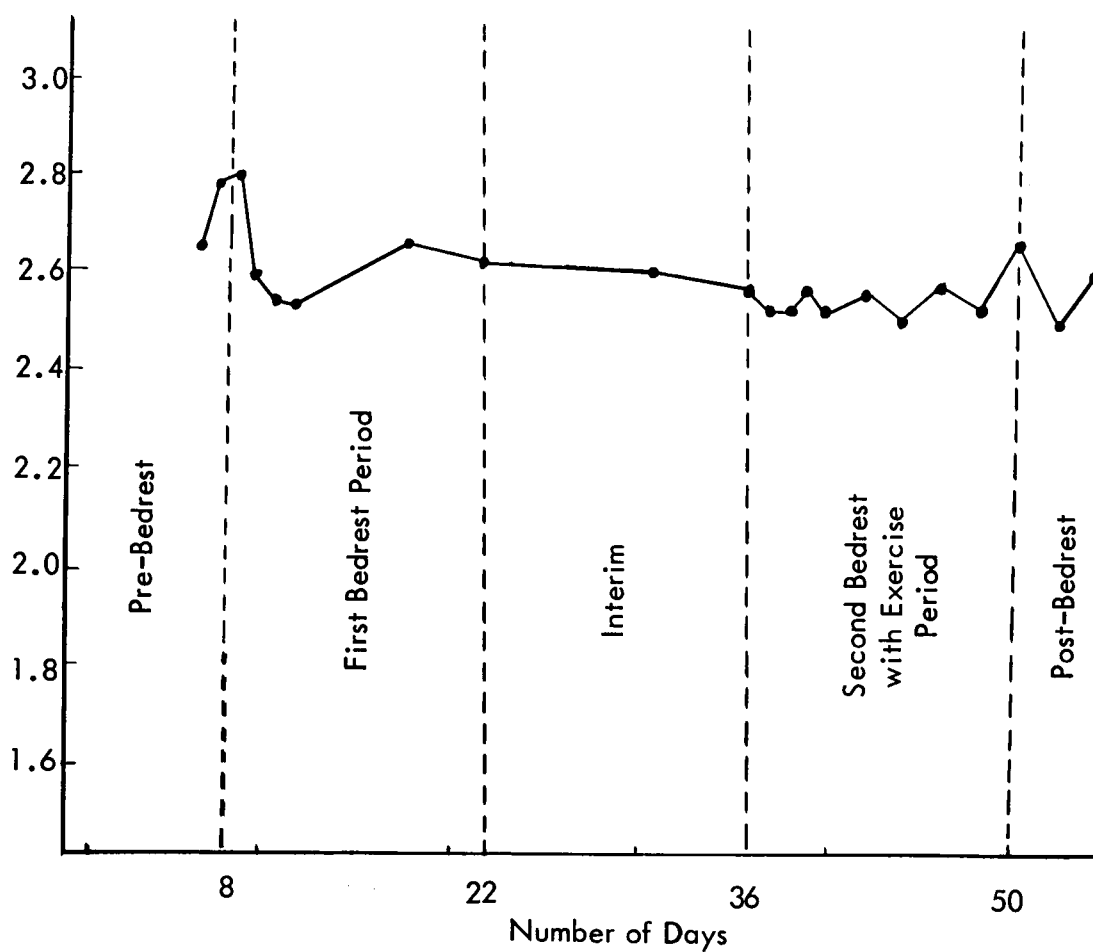


Figure 12. Bone mass changes in fourteen-day bedrest studies.
(Subject M.O.)

MASS (GRAMS) OF CALCIUM APATITE EQUIVALENCY OF
CENTRAL OS CALCIS SECTION

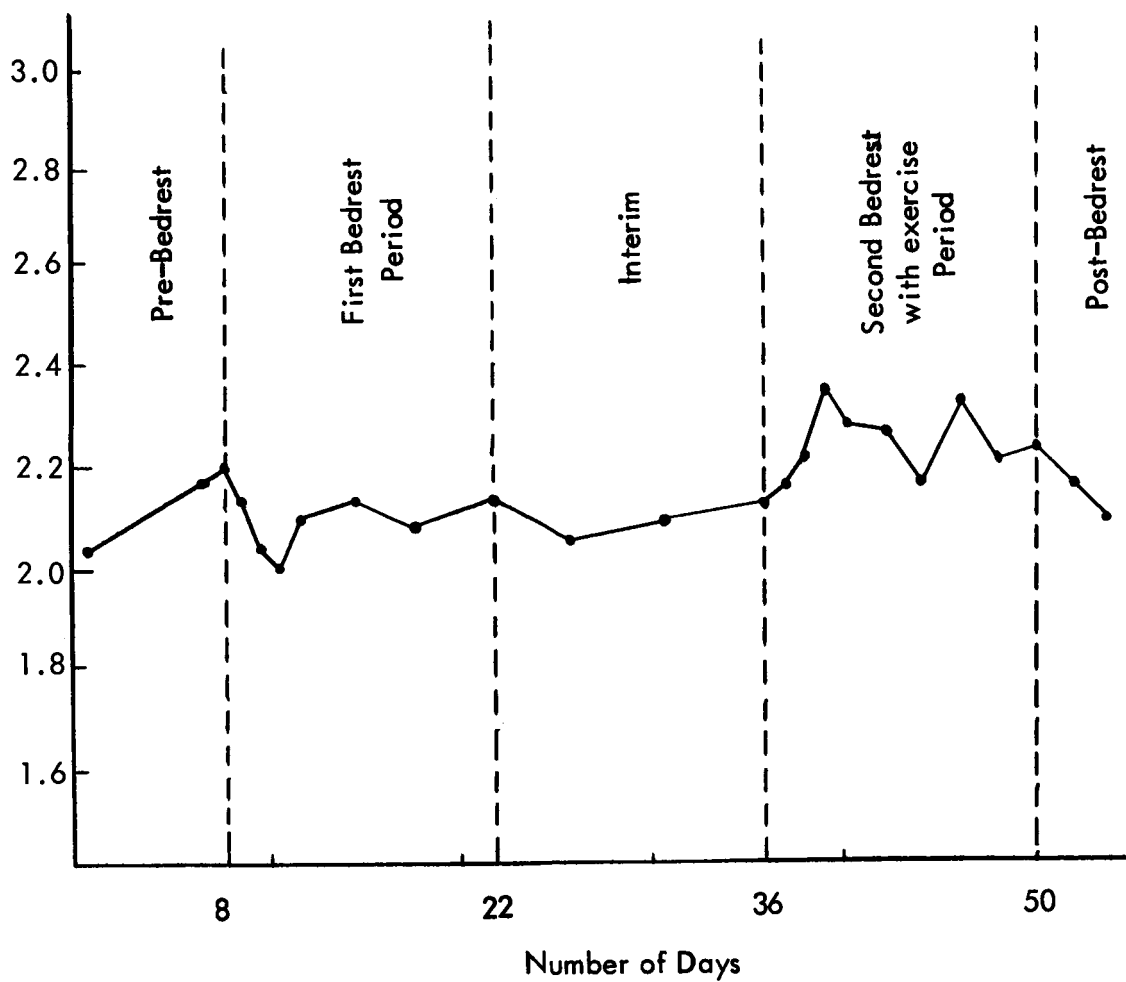


Figure 13. Bone mass changes in fourteen-day bedrest studies.
(Subject D.C.)

MASS (GRAMS) OF CALCIUM APATITE EQUIVALENCY OF
CENTRAL OS CALCIS SECTION

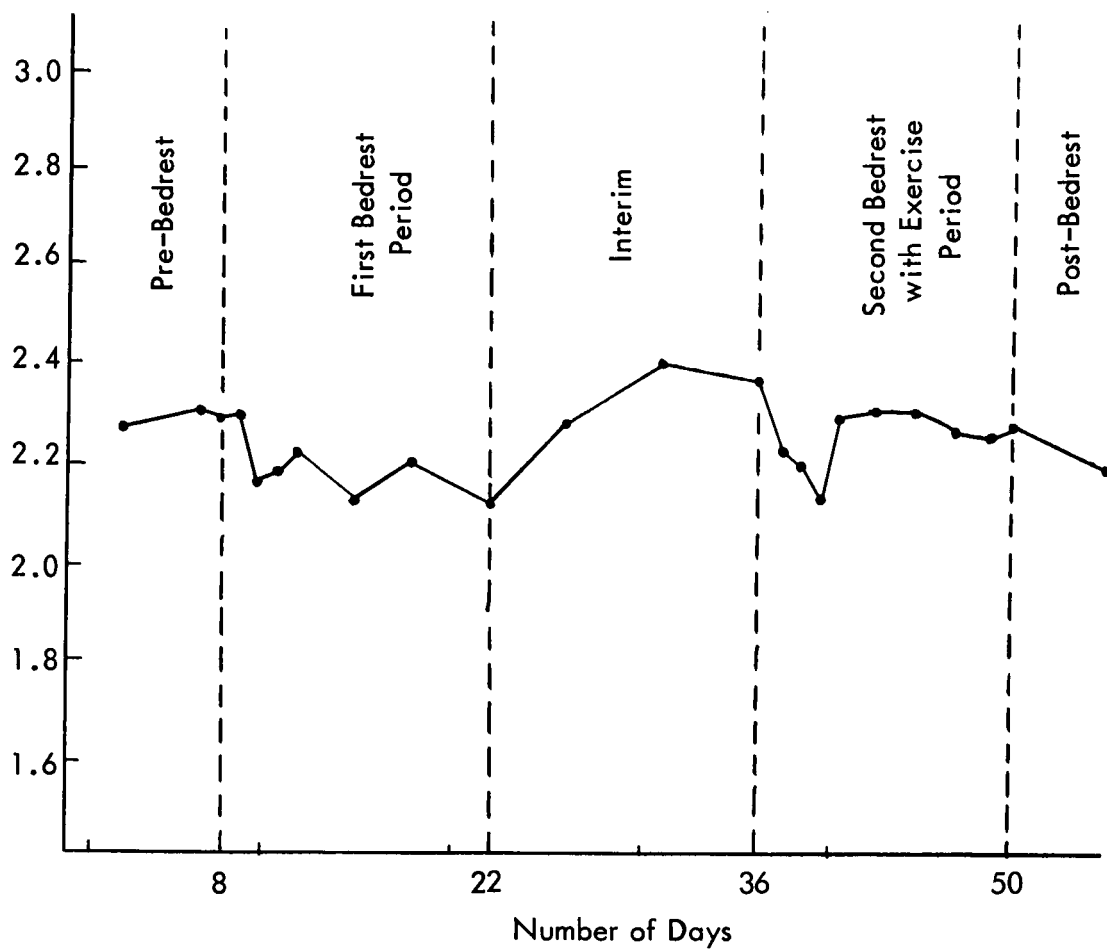


Figure 14. Bone mass changes in fourteen-day bedrest studies.
(Subject C.B.)

MASS (GRAMS) OF CALCIUM APATITE EQUIVALENCY OF
CENTRAL OS CALCIS SECTION

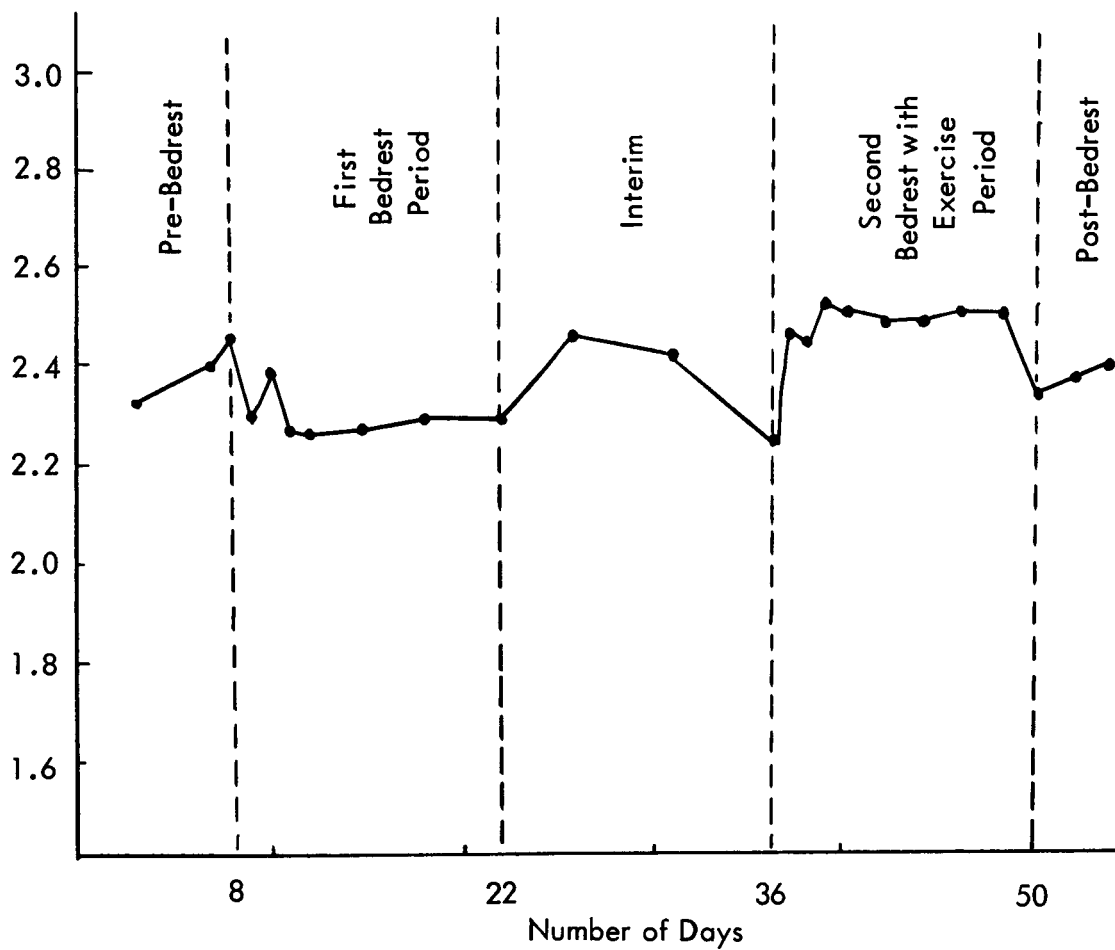


Figure 15. Bone mass changes in fourteen-day bedrest studies
(Subject C.P.)

MASS (GRAMS) OF CALCIUM APATITE EQUIVALENCY OF
CENTRAL OS CALCIS SECTION

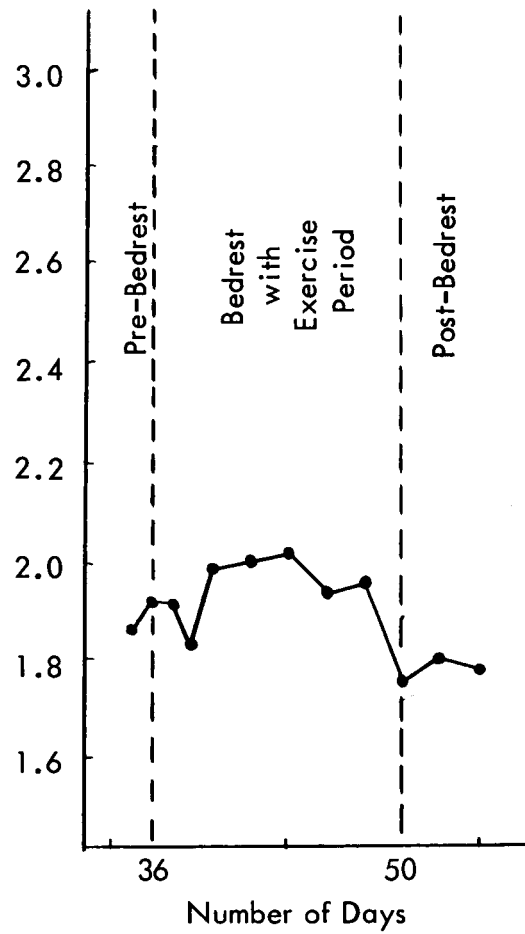


Figure 16. Bone mass changes in fourteen-day bedrest studies .
(Subject A.I.)

pooled data for all roentgenograms made during the first bedrest period, the former surpassed the latter by a significant difference ($p < 0.01$) for all subjects grouped together. The differences also were significant for each individual subject, with $p < 0.10$ to $p < 0.001$ representing the range for the individuals.

The results obtained during the bedrest with exercise period showed bone mass values which exceeded those found in the same subjects during the first bedrest period when exercise was not administered. When the data were pooled for all subjects for each of the two periods, the bedrest with exercise bone mass surpassed that of the period without exercise significantly ($p < 0.05$). When the data for individual subjects were considered for the two respective periods, the same findings occurred with $p < 0.10$ to $p < 0.001$ denoting the range of statistical significance found for the individual subjects.

Table III presents the 24-hour urinary calcium values for the individual subjects. Figure 17 shows a plot of the average daily 24-hour urine calcium for all subjects. Averages of these values have been determined for the pre-bedrest, first week of bedrest, second week of bedrest, and post-bedrest intervals of time for both periods of recumbency.

A pre-bedrest average 24-hour urinary calcium of 287 mg. for the six subjects rose to an average 24-hour urinary calcium of 340 mg. during the first week of bedrest, of 328 mg. during the second week of bedrest, and 335 mg. in the post-bedrest period. During the second bedrest study period, the pre-bedrest average 24-hour urinary calcium of 272 mg. rose to 304 mg. the first week of bedrest with exercise, rose further to 322 mg. the second week of bedrest with exercise, and fell to 295 mg. during the post-bedrest period with exercise.

Thus, there was a greater average urinary calcium output during both bedrest periods compared to the pre-bedrest value. The average 24-hour urinary calcium was also greatest the first week of the bedrest period (without exercise) which corresponds to the time period in which the X-ray mass of the os calcis showed the most marked losses. The pattern of daily variation in urinary calcium excretion for the individuals does not appear to have a close relationship to the daily variations in bone mass, although the over-all loss of bone mass was associated with increased urinary excretion of calcium.

Table IV presents the 24-hour urinary phosphorus values for the individual subjects. Figure 18 is a plot of the daily average of phosphorus for all six subjects. There was an increase in the average daily excretion of phosphorus during the periods of recumbency. Urinary volume also was found to increase during the periods of recumbency; these data are presented elsewhere in detail (Vogt et al.²²).

Table V gives the calcium content of each stool specimen obtained on the individual subjects. The number of values presented is thus an indication

Table III

TWENTY-FOUR HOUR URINARY CALCIUM (mg.)

Fourteen Day Bedrest Experiment

A. Bedrest Period

Day	AL	TO	MO	DC	CB	CP	AI	Average
3	251	484	284	205	---	---	---	306
4	290	427	265	166	336	---	---	297
5	294	361	218	249	317	176	---	269
6	287	362	227	158	276	197	---	251
7	311	493	270	---	362	121	---	311
8 *	377	479	179	132	602	219	---	331
9	367	475	361	256	452	258	---	362
10	403	538	254	233	400	321	---	358
11	373	453	311	196	443	220	---	333
12	400	393	324	236	402	228	---	331
13	---	535	355	196	391	289	---	353
14	318	464	321	172	344	250	---	312
15	403	403	369	233	357	279	---	341
16	394	242	362	226	349	213	---	297
17	338	523	312	172	314	213	---	313
18	337	464	387	245	364	266	---	344
19	323	362	346	174	343	226	---	296
20	362	410	333	213	390	240	---	325
21	376	498	425	283	438	246	---	378
22 **	462	519	409	251	496	264	---	400
23	352	470	276	213	362	212	---	314
24	346	376	307	200	502	226	---	326
25	383	334	258	227	385	214	---	300
Average	335	438	311	211	372	232	---	324

* First day of bedrest.

** Last day of bedrest.

Table III
 TWENTY-FOUR HOUR URINARY CALCIUM (mg.)

Fourteen Day Bedrest Experiment

B. Bedrest with Exercise Period

Day	AL	TO	MO	DC	CB	CP	AI	Average
32	391	---	191	204	353	210	222	262
33	338	---	287	---	344	235	385	318
34	374	---	245	192	279	127	282	250
35	323	---	220	187	310	197	323	260
36 *	348	---	317	221	322	247	267	287
37	361	---	313	194	353	253	445	320
38	321	---	275	180	304	188	340	268
39	396	---	342	---	342	---	397	369
40	392	---	316	194	290	235	419	308
41	339	---	288	205	244	189	378	273
42	355	---	356	183	353	193	403	307
43	407	---	338	213	392	234	442	338
44	337	---	256	175	279	213	384	274
45	336	---	391	236	294	266	422	324
46	412	---	324	226	360	218	417	326
47	387	---	322	243	355	253	389	325
48	404	---	299	235	408	225	396	328
49	384	---	379	270	---	203	440	335
50 **	334	---	374	158	---	228	364	292
51	314	---	230	173	---	116	364	239
52	281	---	217	150	---	176	360	237
53	280	---	119	162	---	148	344	211
Average	355	---	291	200	328	207	372	295

* First day of bedrest.

** Last day of bedrest.

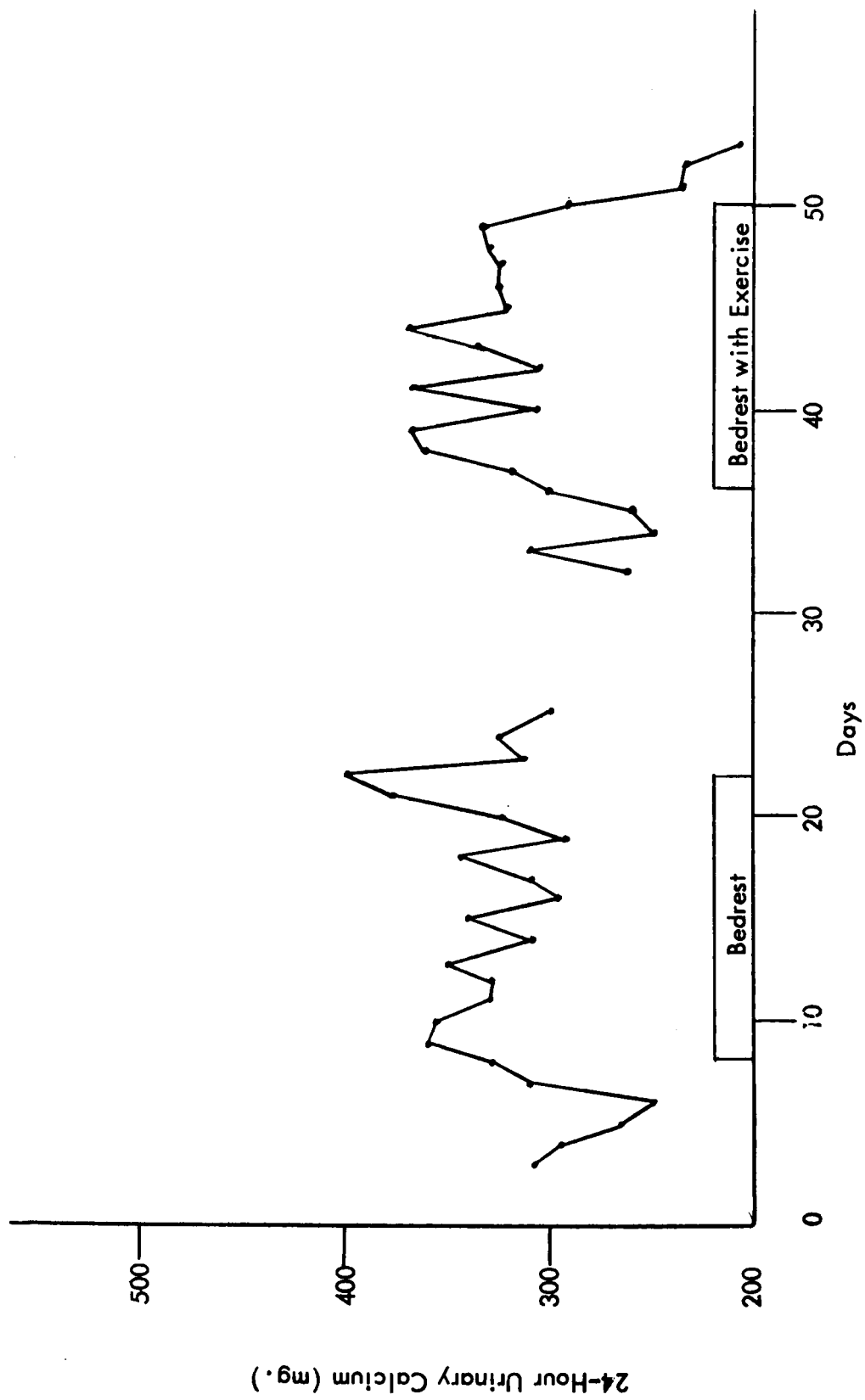


Figure 17. Average daily urinary calcium.

Table IV
 TWENTY-FOUR HOUR URINARY PHOSPHORUS (mg.)

Fourteen Day Bedrest Experiment

A. Bedrest Period

Day	AL	TO	MO	DC	CB	CP	AI	Average
3	1172	1019	1294	1173	---	---	---	1165
4	1322	1298	1682	1453	1247	---	---	1400
5	980	1101	1196	971	1303	1078	---	1106
6	917	1039	1092	1134	1062	940	---	1031
7	781	1102	1181	1196	1179	1021	---	1077
8 *	868	1201	1644	1271	1230	1096	---	1218
9	722	1158	1234	1143	1061	1072	---	1065
10	1057	1141	1169	1248	1216	1022	---	1142
11	1132	1185	1566	1238	1150	1146	---	1236
12	1182	1114	1122	1301	1082	1040	---	1150
13	968	1385	1206	1175	1170	1133	---	1163
14	1711	1291	1138	1284	1301	1221	---	1325
15	1131	1290	1567	1259	1249	1001	---	1250
16	1144	916	1381	1279	1288	1107	---	1186
17	1099	1062	1349	1164	1290	1148	---	1185
18	1044	1421	1064	1226	1113	1008	---	1146
19	965	1099	1235	1159	1131	1041	---	1105
20	1061	1222	1304	1143	1197	966	---	1149
21	1166	1155	1121	1131	1141	853	---	1095
22 **	1003	1179	1267	1177	1102	997	---	1121
23	995	1096	1024	1092	998	1073	---	1046
24	1043	1026	1371	1138	1388	986	---	1160
25	1078	987	934	1187	1004	939	---	1022
Average	1241	1152	1267	1197	1173	1042	---	1179

* First day of bedrest.

** Last day of bedrest.

Table IV

TWENTY-FOUR HOUR URINARY PHOSPHORUS (mg.)

Fourteen Day Bedrest Experiment

B. Bedrest with Exercise Period

Day	AL	TO	MO	DC	CB	CP	AI	Average
32	1270	---	920	1040	784	959	---	995
33	1250	---	1103	1204	872	1034	1272	1123
34	1241	---	1007	1389	888	900	1131	1093
35	1125	---	917	1240	1453	1002	1127	1144
36 *	1301	---	1048	1234	1209	1146	1221	1193
37	1409	---	1142	1379	1182	1093	1182	1231
38	1239	---	1116	1272	1192	1016	839	1112
39	1289	---	1035	1212	1298	1002	1433	1211
40	1055	---	1381	1198	1198	996	1091	1153
41	1443	---	1137	1288	1136	996	1250	1208
42	922	---	1226	1108	1078	1688	1220	1207
43	1285	---	1362	1234	1240	1498	994	1269
44	808	---	1252	1369	1033	1062	1020	1091
45	1127	---	1437	1104	787	1057	888	1067
46	709	---	965	1027	1000	1518	1067	1047
47	1116	---	1266	1106	1122	1064	985	1110
48	1016	---	1064	1078	1291	1047	1276	1130
49	1320	---	1145	1326	---	1052	1104	1189
50 **	1340	---	1363	1244	---	576	1126	1130
51	803	---	940	1068	---	402	1201	883
52	1019	---	873	1275	---	1213	1151	1106
53	306	---	523	1229	---	1120	1443	924
Average	1112	---	1101	1210	1104	1066	1144	1123

* First day of bedrest.

** Last day of bedrest.

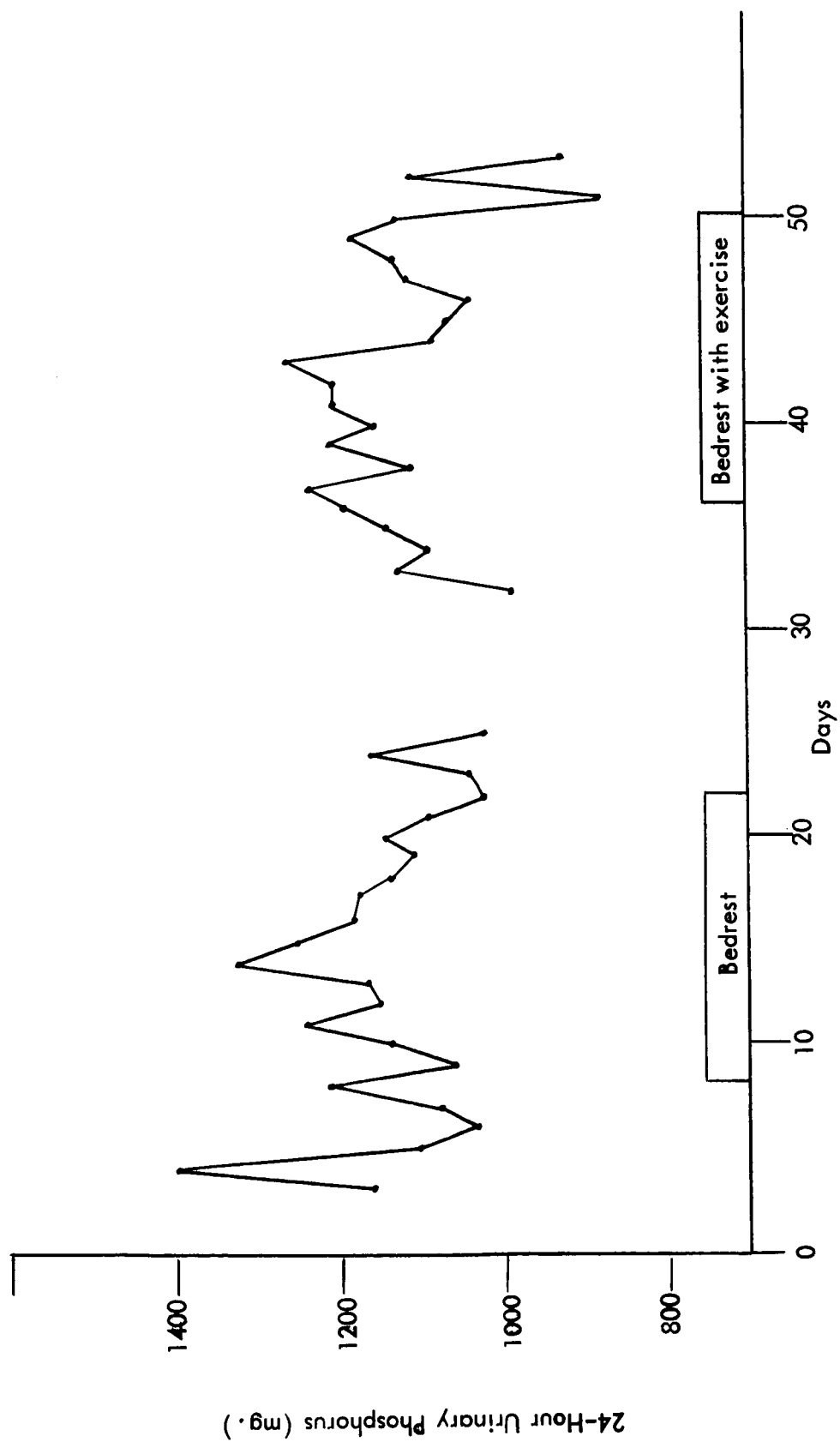


Figure 18 Average daily 24-hour urinary phosphorus.

Table V
FECAL CALCIUM PER STOOL (grams)

Fourteen-Day Bedrest Experiment

A. Bedrest Period

Day	AL	TO	MO	DC	CB	CP	AI
2 *							
4		0.36 0.03					
5	0.10	0.35	2.00	1.38	0.95		
6	0.58	0.69 0.61	2.58	2.11	0.56 0.42	0.27	
7				0.33	0.56	0.62	
8 **	1.80	0.85		1.19	0.94 0.06	0.37	
9	0.84				0.41		
10	2.06	0.63			0.58	0.38	
11		0.50		1.29	0.68	0.58	
12		0.93	1.24 1.06		0.92 0.33	0.63	
13	0.12				0.56		
14	1.73	0.20			0.78	1.14	
15		0.15			0.68		
16	0.51	0.71			0.64	1.66	
17	1.32		3.82		0.52	2.74	
18	0.62	1.10			0.98		
19		1.50		1.63			
20	1.47	3.20	1.64		1.02		
21					1.26	2.74	
22 ***	2.30		3.39	2.34	0.70	2.46	
23			1.00	1.01 1.84 1.17	0.60	0.37	
24	2.15	1.17	2.25 0.08	1.76	1.23	1.24	
25		0.74		2.96	0.68	0.93	
26 *	3.22 0.64	0.71	3.30 1.06	2.35	0.38 0.51	1.12 0.67	
27	0.81	0.39		1.08	0.29 0.60	1.36	
28	1.18	0.56		3.02			
29	0.55	0.87	2.31	0.66 1.88			
30	0.15	0.46 0.84		0.69			
31				1.42			
Average	0.923	0.714	1.072	1.255	0.780	0.876	

* Dye marker given. ** First day of bedrest. *** Last day of bedrest.

Table V
FECAL CALCIUM PER STOOL (grams)

Fourteen-Day Bedrest Experiment

B. Bedrest with Exercise Period

Day	AL	TO	MO	DC	CB	CP	AI
32 *	0.24			0.96		0.36	
	1.23			3.12			
33					0.42	1.45	
					0.30		
34	1.34			0.39	0.35	0.35	1.73
35	1.02		0.78	0.89	0.31		
					0.40		
36 **				1.21	0.64	0.38	0.66
37	1.10		0.45			1.00	
38	0.79				1.02	0.96	2.02
					0.66		
39				1.07	0.51	0.73	
40	1.12			1.59	0.68	0.58	1.59
41	1.01		2.32	0.93	0.98	0.93	
42	0.68			1.06	0.65	0.73	1.04
43					0.52	0.65	
44			3.89	1.17	0.67	0.36	1.09
45	0.86				0.58		
	1.22						
46					0.85	1.59	
47	1.42				0.88	0.34	1.33
48				2.29	0.59	1.16	1.90
49	0.86		4.24	1.31	0.96	0.49	
50 ***	1.40		2.93	1.82		0.35	1.70
						0.30	
51			1.26	1.49		0.71	2.50
			0.63	0.62			
52	1.94			0.61		1.09	
53	1.31			0.73			
54			1.31	1.84		0.71	2.46
55	0.77		1.06	1.06		0.77	1.19
56				1.06			
57				0.60			
Average	0.793		0.820	1.166	0.748	0.888	0.835

* Dye marker given. ** First day of bedrest. *** Last day of bedrest.

of the frequency of defecation. The first stool value represents the stool which contained the dye marker given on day 2 of the study. The last stool calcium value in the first bedrest period represents the stool that contained a dye marker given on day 26. The first stool in the second bedrest period is that which contained a dye marker given on day 32, and the last stool contained the marker given on day 55. Subject C.B. had to leave the experiment prematurely, and his stools were obtained only through day 49. The average value for stool calcium represents the sum of the calcium values for each stool sample divided by the number of days in the collection period, which was 24 in the first bedrest period and 23 in the second bedrest (with exercise) period. These values thus represent the average stool excretion of calcium for the total collection period. The periods of observation before and after bedrest were too short to provide representative data for these intervals since the stool collection was not available daily, and the dye markers given in these periods did not provide as distinct an indication as would be needed to separate the stool for such short periods.

Subjects A.L., M.O., D.C., and C.B. showed a greater stool calcium excretion in the first bedrest study (including pre-bedrest and post-bedrest periods) compared to the second. Subject C.P. showed a slightly smaller excretion in the first period.

Table VI gives the phosphorus content of each stool specimen obtained on the individuals. Subjects A.L. and M.O. showed a greater stool phosphorus excretion in the first study period compared to the second study period; subjects D.C. and C.B. showed a slightly smaller excretion in the first period; and subject C.P. showed approximately the same excretion for the two periods.

Table VII presents the data representative of the calcium balance for the periods of recumbency. Sweat calcium losses were not measured. The data presented compare the recumbency periods of bedrest and bedrest with exercise. Calculations are not made of the calcium balance prior to and after bedrest since the short duration of these intervals would introduce a large error in the stool calcium values because of difficulty in recognizing and separating stool specimens using dye markers. The average daily urine calcium given is the average value of the 24-hour urinary calcium excretion for each individual during the 14-day period of recumbency. The fecal value given is the daily average determined for a collection period that included the pre-bedrest and post-bedrest intervals because of difficulty in separating stool specimens with dye markers. The total loss of calcium represents the sum of the average urinary and fecal losses for the individuals. The average dietary intake of calcium is subtracted from this to give the average balance calculated on a 24-hour basis.

All subjects showed a negative calcium balance for both periods of recumbency determined by this technique. Subjects A.L., M.O., D.C., and

Table VI
FECAL PHOSPHORUS FOR STOOL (gm. PO₄)

Fourteen-Day Bedrest Experiment

A. Bedrest Period

Day	AL	TO	MO	DC	CB	CP	AI
2 *							
4		0.36 0.03 0.02				0.17	
5	0.06	0.43 0.19	1.29	0.67	0.46	0.25	
6	0.39	0.36	1.31	0.97	0.22 0.18		
7				0.15	0.19	0.26	
8 **	1.80	0.49		1.12	0.47 0.04	0.28	
9	0.49				0.17		
10	1.24	0.53			0.24	0.19	
11		0.37		0.65	0.30	0.33	
12	0.73		0.72 0.63		0.19	0.39	
13	0.09				0.35		
14	1.06	0.15			0.40	0.58	
15		0.07			0.46		
16	0.32 0.02	0.47				1.03 0.37	
17	0.91		1.97		0.46	1.46	
18	0.36	0.82			0.68		
19		0.94		0.86			
20	0.78	1.90	0.94		0.58		
21					0.79	1.38	
22 ***	1.27		1.87	1.27	0.28	1.51	
23			1.16	0.59 1.09 1.17	0.28		0.37
24	0.73		1.12 0.06		0.53		
25	1.66	0.72			0.19	0.67	
26 *	1.61 0.35	0.73	1.58 0.77	1.17	0.19 0.26	0.66 0.40	
27	0.38	0.28			0.12 0.52 0.27	0.62	
28	0.60	0.34					
29	0.28	0.67	1.37	1.59 0.69			
30	0.10	0.39 0.64		0.42			
31				0.93			
Average	0.63	0.45	0.61	0.56	0.38	0.50	

* Dye marker given. ** First day of bedrest. *** Last day of bedrest.

Table VI
FECAL PHOSPHORUS FOR STOOL (gm. PO₄)

Fourteen-Day Bedrest Experiment

B. Bedrest with Exercise Period

Day	AL	TO	MO	DC	CB	CP	AI
32 *	0.67 0.16			0.63 1.46	0.32		
33					0.66 0.16	0.69	
34	0.79			0.18	0.30	0.17	1.36
35	0.42		0.57	0.55	0.31 0.17		
36 **				0.72	0.40	0.15	0.28
37	0.63		0.40			0.52	
38	0.37				0.92 0.41	0.51	1.15
39				0.84	0.31	0.50	
40	0.85			0.78		0.41 0.31	1.00
41	0.56		1.30	0.47	0.50	0.57	
42	0.93			0.53	0.48	0.52	0.72
43					0.36	0.36	
44			1.90	0.60	0.37	0.22	0.96
45	0.52 0.68				0.42		
46					0.33	0.37 0.53	
47	0.73				0.55	0.17	0.78
48				1.09	0.37	0.81	0.99
49	0.45		1.57 2.48	1.05 1.17	0.84	0.17	
50 ***	0.77					0.18 0.18	0.97
51			0.68 0.35	0.67 0.64		0.41	1.32
52	0.99		0.40			0.48	
53	0.73			0.40			
54	0.42		0.99	0.94		0.90	2.75
55 *			0.58	0.42		0.45	0.89
56				1.06			
57				0.37			
Average	0.46		0.46	0.66	0.51	0.53	0.57

*Dye marker given. ** First day of bedrest. *** Last day of bedrest.

TABLE VII
CALCIUM BALANCE

Fourteen-Day Bedrest Experiment
(Calcium in Grams)

SUBJECT:	A.L.	T.O.	M.O.	D.C.	C.B.	C.P.	A.I.
A. BEDREST (Average Daily Values)							
1. Urine calcium	-0.367	0.446	0.331	0.212	0.399	0.247	-----
2. Fecal calcium*	-0.923	0.714	1.072	1.255	0.780	0.876	-----
Total output	1.280	1.160	1.403	1.467	1.179	1.123	-----
Average Intake	1.083	1.060	1.098	1.096	1.098	1.097	-----
Balance	-0.197	-0.100	-0.305	-0.371	-0.081	-0.026	
B. BEDREST WITH EXERCISE (Average Daily Values)							
1. Urine calcium	-0.369	-----	0.322	0.213	0.330	0.224	0.396
2. Fecal calcium*	-0.793	-----	0.820	1.166	0.748	0.888	0.835
Total Output	1.162	-----	1.142	1.379	0.078	1.112	1.231
Average Intake	1.042	-----	1.042	1.022	1.032	1.046	1.025
Balance	-0.120	-----	-0.100	-0.357	-0.046	-0.066	-0.206

* Average daily fecal calcium excretion determined from stools obtained during period of recumbency and the pre-bedrest and post-bedrest observation periods.

C.B. were in slightly greater negative balance during the first bedrest period compared to the period in which exercise was added. Subject C.P. showed the opposite effect.

No information is available as to the balance status at the start of the recumbency periods. A comparison is made only of the two periods of bedrest which used the same experimental design except for the addition of isometric exercises to the second period of study.

The reproducibility study, in which the same subject (Mack) was repositioned and X-rayed eight times and each film read four times in the densitometer, yielded a mean bone mass of 1.89 grams of calcium apatite equivalency with a standard deviation of 0.03. The details of this study are presented elsewhere by Mack and Vogt.²³ The bone mass of 1.89 grams of calcium apatite equivalency is lower than that found in the young males of the experimental groups and is a reflection of the smaller bone of the female subject (Mack) as well as a possible lower mineral content per unit volume of bone.

The X-ray mass of the third lumbar vertebra of the experimental subject has not been completed at the time of this report and will be given later.

DISCUSSION

A precise technique of bone X-ray densitometry has been used to evaluate mass changes in the os calcis of normal adult males kept at prolonged bedrest. Significant losses of bone mass of the os calcis have been found to occur with 14 days of bedrest. There also has been found a suggestive protective effect of isometric exercises added to the bedrest routine. The results of this study also confirm that calcium balance is affected by the physical activity of a person, there being a negative balance with prolonged periods of recumbency.

The os calcis was selected for study because of the previous experience with X-ray densitometry on this bone, because of the minimal soft tissue surrounding the bone, because it has precise anatomical sites to locate an X-ray scanning line, and because it seemed that this bone should be the most likely to show changes with bedrest. During normal activity the os calcis is subjected to the stresses of walking and bears the weight of the body. With recumbency, all weight-bearing requirements are removed from this bone, even if the subject is allowed to turn from side to side in bed.

All subjects showed an increase in bone mass of a central section of the os calcis in the pre-bedrest period. It is thought that this resulted from the diet which was greater in calcium than they had been consuming at home. After starting bedrest, the subjects showed a marked and progressive decrease in bone mass. This initial type response had been observed in 3-day bedrest studies (Vogt et al.²⁴) done previously at the TIRR.

It is not possible to define the exact mechanism of this marked change in bone mass of the os calcis during the initial days of bedrest. Such changes in X-ray mass are greater than would be expected from reports of previous studies.^{7,8,9,10}

Independent bedrest studies were conducted at Texas Woman's University in which attention was directed specifically to the measurement of calcium balance and bone X-ray mass. These studies at TWU were of a more simplified nature than those conducted at TIRR and did not involve tilt table studies before and after bedrest, or the involved measurements during the period of recumbency. Table VIII presents the average bone mass of the subjects at TWU and TIRR, respectively for different levels of calcium diets and for the experimental condition of 14 days of bedrest. Figure 19 is a plot of these data. X-rays were taken and densitometry measurements were made by the same personnel (from TWU) in both the TWU and TIRR studies.

The subjects in both the TWU and TIRR studies were young healthy males. The bone mass of the os calcis at the start of bedrest was comparable in both cases. The bone mass of the TWU subjects at the end of 2 weeks of bedrest was significantly lower for both dietary levels of calcium with the lower bone mass occurring in the subjects while on the lower calcium diet. The average bone mass of the TIRR subjects at the end of bedrest was not significantly different from the final average bone mass observed in the TWU subjects.

There was a very apparent decrease in bone mass the first several days of the bedrest studies at TIRR. Similar findings were noted²⁴ in earlier studies at TIRR where a comparable experimental protocol was used in 3-day bedrest studies. At present, the mechanism for these changes cannot be defined specifically. It is thought that it may be a reflection of the additional stresses imposed by the complex experimental protocol and tilt table studies performed on the subjects. It is possible that this would be the type of response that may be seen in astronauts participating in space flights since they will be exposed to a complicated and stressful experimental circumstance.

The variability in bone mass during the interim between periods of recumbency is beyond interpretation since controlled experimental conditions did not exist.

An isometric exercise schedule was added to the second bedrest period because it was thought that mechanical stresses are important in attaining and maintaining skeletal mineralization. The findings of a significantly higher bone mass during this period would seem to confirm such an hypothesis. Still, consideration must be given to the effect that may result from adaptation of the subjects to the experimental conditions and to the effect of exposure of the subjects to the higher calcium diet during the bedrest study which has immediately preceded the bedrest with exercise period. Initial plans included a third

TABLE VIII
AVERAGE MASS (GRAMS) OF CALCIUM APATITE EQUIVA-
LENCE OF CENTRAL OS CALCIS SECTION

Day	TWU*		TIRR**
	1.5 g. Ca	0.7 g. Ca	1.0 g. Ca
1	2.42	2.42	2.37
2	2.40	2.38	2.34
3	2.35	2.36	2.25
4	2.38	2.35	2.21
5	2.36	2.34	2.25
6	2.34	2.33	***
7	2.34	2.30	***
8	2.40	2.32	2.21
9	2.35	2.32	***
10	2.30	2.29	***
11	2.36	2.27	2.21
12	2.33	2.28	***
13	2.29	2.25	***
14	2.31	2.25	***
15	2.28	2.24	2.25

* Independent bedrest experiments conducted at TWU under NASA Grant NsG-440. Four subjects were in each group. Cardiovascular studies were not performed in conjunction with this bedrest study. (Unpublished data)

** Conducted under Contract NAS 9-1461. Six subjects were in the group. A combined cardiovascular and metabolic experiment was conducted in this study.

*** Bone x-rays were not made on these days.

MASS (GRAMS) OF CALCIUM APATITE EQUIVALENCY OF
CENTRAL OS CALCIS SECTION

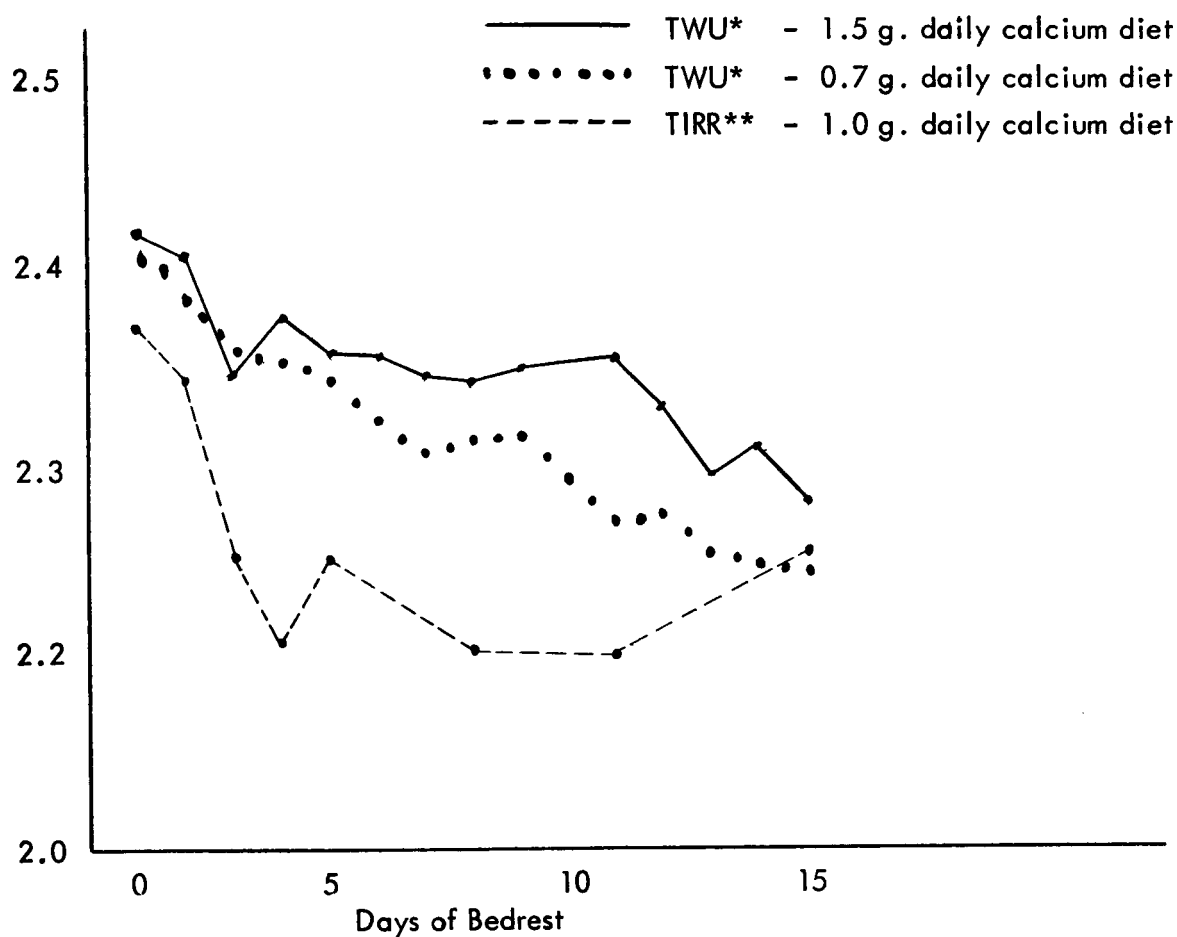


Figure 19. Comparative os calcis mass data from bedrest studies with different levels of calcium diet and experimental conditions.

* Average mass of os calcis section on four healthy subjects participating in bedrest study to determine metabolic and skeletal change with recumbency.

** Average mass of os calcis section on six healthy subjects participating in complex bedrest study to determine cardiovascular and metabolic changes with recumbency. Tilt table studies performed immediately before start of bedrest.

bedrest (without exercise) period to follow the bedrest with exercise period, but this was not possible to complete. Future studies should give consideration to this.

The bone mass fell following the bedrest with exercise period. This may have resulted from the cessation of the isometric exercises which were directed to the os calcis, or may have resulted from the stress of tilt table studies which were performed on the last day of recumbency.

The reproducibility of measurements for the technique²³ of bone densitometry used in this study confirms its usefulness in such studies where progressive changes in bone mass are evaluated in a given subject. Since the bone size in an adult is not likely to change in studies of 2-weeks duration, the change in bone mass is an accurate representation of the dynamic changes in bone mineral. Dynamic changes in bone mineral are usually accompanied by changes in protein matrix, but the contribution of protein density relative to mineral density is negligible as shown on an X-ray film. X-ray absorption is related to the atomic number of the elements comprising the substances being X-rayed. Omnell²⁵ gives the mass absorption coefficients of the elements involved in bone and in protein as presented in Table IX, at the X-radiation energy levels designated.

The changes in bone mass of the os calcis did not necessarily reflect changes in mass of other bones. The os calcis was selected for study because it was thought to be the bone most likely to change over short periods of recumbency. Data on the changes in the third lumbar vertebra will be reported when analyses are complete. It is likely that other bones will not show as pronounced changes as seen in the os calcis. This is suggested further by the amount of calcium loss from the body, which was not nearly as great as would be expected if similar losses of minerals were found in all bones of the body.

The observations made of urinary calcium loss, fecal calcium loss, and dietary calcium intake in this study do not represent a true metabolic balance study. The pre-bedrest periods of 1 week were too short to assure complete stabilization on the prescribed dietary intake. Further, the separation of fecal collections by dye markers does not provide distinct enough separation to allow consideration of the pre-bedrest period as a separate fecal collection interval. For this reason, fecal specimens were pooled in the pre-bedrest, bedrest, and post-bedrest periods. Since there was no reason for the subjects to be in negative calcium balance prior to bedrest, and since bone mass was increasing in this interval, one can conclude that the average fecal loss for the period of recumbency would have been even greater than is represented by the average, where given. There is justification in comparing one bedrest period with the other, since the experimental design was the same except for the addition of isometric exercise. An interpretation cannot be made that there is less negative calcium balance in the period of recumbency to which an exercise procedure is added, because the inherent error in a balance study is as great as the changes observed.

TABLE IX.
X-RAY ABSORPTION COEFFICIENTS*

Element	Atomic Number	Energy in KeV		
		10	20	30
Calcium	20	29.6	12.9	6.8
Phosphorus	15	12.2	5.3	2.8
Oxygen	8	1.83	0.86	0.52
Nitrogen	7	1.25	0.62	0.40
Carbon	6	0.84	0.45	0.32
Hydrogen	1	0.42	0.40	0.39

* From Omnell²⁵

REFERENCES

1. Allison, N. and Brooks, B.: Bone Atrophy--An Experimental and Clinical Study of Changes in Bone which Result from Non-use. *Surg. Gynec. Obstet.* 33: 250, 1921
2. Chor, H. and Dolkart, R.D.: A Study of Simple Disuse Atrophy in the Monkey. *Amer. J. Physiol.* 117: 626, 1936
3. Stevenson, F.H.: The Osteoporosis of Immobilization in Recumbency. *J. Bone and Joint Surg. (Amer.)* 34: 256, 1952
4. Geiser, M. and Trueta, J.: Muscle Action, Bone Rarefaction and Bone Formation: An Experimental Study. *J. Bone and Joint Surg. (Amer.)* 40: 282, 1958
5. Armstrong, W.D., Knowlton, M., and Gauze, M.: Influence of Estradiol and Testosterone Propionates on Skeletal Atrophy from Disuse and on Normal Bones of Mature Rats. *Endocrinology* 36: 313, 1945
6. Cuthbertson, D.P.: The Influence of Prolonged Muscular Rest on Metabolism. *Biochem.J.* 23: 1328, 1929
7. Deitrick, J.E., Whedon, G.D., and Shorr, E.: Effects of Immobilization Upon Various Metabolic and Physiologic Functions of Normal Men. *Amer. J. Med.* 4: 3, 1948
8. Whedon, G.D., Deitrick, J.E., and Shorr, E.: Modification of the Effects of Immobilization upon Metabolic and Physiologic Functions of Normal Men by the Use of an Oscillating Bed. *Amer.J. Med.* 6: 684, 1949
9. Brannon, E.W., Rockwood, C.A., and Potts, P.: Prevention of Debilitating Musculoskeletal Disorders: Physiological Conditioning for Prolonged Weightlessness. *Aerospace Med.* 34: 900, 1963
10. Birkhead, N.C., Blizzard, J.J., Daly, J.W., Haupt, B., Isschutz, B., Myers, R.N., and Rodahl, K.: Cardiodynamic and Metabolic Effects of Prolonged Bed Rest. Technical Documentary Report No. AMRL-TDR-63-37, May, 1963
11. David, H.: Russians Discuss Space Radiation Findings at Conference. *Missiles and Rockets*, October 21, 1963. p. 34

12. Berry, C.A., Minners, H.A., McCutcheon, E.P., and Pollard, R.A.: Aeromedical Analysis. Results of the Third United States Orbital Space Flight. October 3, 1962. NASA SP-12
13. Catterson, A.D., McCutcheon, E.P., Minners, H.A., and Pollard, R.A.: Aeromedical Observations. Mercury Project Summary, Including the Results of the Fourth Manned Orbital Flight. May 15 and 16, 1963. NASA SP-45
14. Beasley, W.C.: Instrumentation and Equipment for Quantitative Clinical Muscle Testing. Arch. Phys. Med. 37: 604, 1956
15. Mack, P.B., O'Brien, A.T., Smith, J.M., and Baumann, A.W.: A Method for Estimating the Degree of Mineralization of Bones from Tracings of Roentgenograms. Science 89: 467, 1939
16. Mack, P.B., Brown, W.N., Jr., and Trapp, H.D.: The Quantitative Evaluation of Bone Density. Amer.J. Roentgenology and Radium Therapy 61: 808, 1949
17. Mack, P.B.: Results from the Study of Bone Density in the Appraisal of Calcium Status. Papers presented at the 1949 Conference of the Milbank Memorial Fund, November 16-17, 1949. Published by the Milbank Memorial Fund, 1950
18. Mack, P.B., Vose, G.P., and Nelson, J.D.: New Development in Equipment for the Roentgenographic Measurement of Bone Density. Amer.J. Roentgenology, Radium Therapy, and Nuclear Med. 82: 303, 1959
19. Vose, G.P., Hoerster, S.A., and Mack, P.B.: New Technique for Radiographic Assessment of Vertebral Density. Amer. J. Med. Electronics (To be published.)
20. Ferro, P.V. and Ham, A.B.: A Simple Spectrophotometric Method for the Determination of Calcium. Am.J. Clin. Path. 28: 1957
21. Fiske, C.H. and Subba-Row, Y.: J. Biol. Chem. 66: 375, 1925
22. Vogt, F.B., Spencer, W.A., Cardus, D., and Vallbona, C.: The Effect of Bedrest on Blood Volume, Urinary Volume, and Urinary Electrolyte Excretion. Report XI, NASA Contract NAS 9-1461, January, 1964

23. Mack, P.B. and Vogt, F.B.: The Roentgenographic Assessment of Bone Density: Reproducibility of Measurements. (In Preparation to be submitted for publication)
24. Vogt, F.B., Mack, P.B., Beasley, W., Spencer, W.A., Cardus, D., Vallbona, C.: The Effect of Three Days of Bedrest on Bone Mass and Calcium Balance. (Unpublished data)
25. Omnell, Karl-Ake: Quantitative Roentgenographic Studies on Changes in Mineral Content of Bone in Vivo. Acta Radiologica Supplementum 148: 1957 (Stockholm)