NASA SP-269

HYPOGRAVIC AND HYPODYNAMIC ENVIRONMENTS

A symposium held at
French Lick, Indiana
June 16–18, 1969

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

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HYPOGRAVIC AND HYPODYNAMIC ENVIRONMENTS

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PREFACE

Man has now ventured into space and returned. These efforts not only have ushered in a new era of science and technology in the areas of engineering and the physical sciences but have changed our focus in the areas of biology and human research. Man has demonstrated that he can survive spaceflight and be an integral and necessary part of the spaceflight complex by adding elements of judgment and reliability not available from engineering hardware alone. With the passage of the historic Gemini and early Apollo flights, we now enter a new phase of endeavor directed at knowledge that will allow man to live, work, and explore in extended spaceflight. This new endeavor is associated with unique unanswered questions concerning man's capability as worker, crew member, and explorer, and with the need to create a strong biomedical research capability.

In the coming decade, studies must be conducted to determine man's relationship to gravity, his ability to accomplish complex tasks in space, and his ability to utilize this new and unique environment with innovation and creativeness. These tasks can be accomplished only if they are associated with a carefully planned inflight experiment program supplemented with meaningful related ground-based studies. The workshop conducted at French Lick, Indiana, was directed toward investigating this latter point. It brought together for the first time in one place most of the principal investigators, in this country and abroad, interested in the use of bed rest to simulate weightlessness. Members of the National Aeronautics and Space Administration, universities, and industry were asked to evaluate the ability of bed rest to duplicate the findings of manned spaceflight. The results of this workshop are extremely encouraging to date. Physiological findings during bed rest parallel most measurements obtained during or after manned spaceflight. More importantly, studies during recumbency point to problems in metabolism and circadian dysrhythmia that were not anticipated as areas for concern in spaceflight.

A well-structured ground-based program for human study is anticipated to be of great service to all individuals associated with manned flight. Such studies will obviously save considerable funds compared to the costs for inflight measurements, provide a means of testing remedial measures for problems encountered during flight or on reentry into the Earth's atmosphere, and serve as a means for comparison against inflight measurements to isolate changes induced by weightlessness from those caused by nongravity-related phenomena. The conclusion from this workshop is that bed rest is an excellent means for simulating the medical and physiological problems to be encountered during spaceflight. This technique promises wider use as NASA enters a new phase requiring information concerning human physiology, performance, and habitability during extended space missions.

I would like to personally extend my gratitude to all participants and particularly to Indiana University whose efforts made this symposium possible.

WALTON L. JONES, Director
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INTRODUCTION

This report contains papers and discussions presented at the Symposium on Hypogravic and Hypodynamic Environments, sponsored by the National Aeronautics and Space Administration and conducted by Indiana University at the Sheraton Hotel, French Lick, Indiana, June 16-18, 1969.

Since the time of Claude Bernard, physiologists have emphasized the study of the mechansims by which organisms maintain the relative constancy of their internal environment in response to stress or change in the external environment. The sum of these mechanisms is homeostasis. A biological stress is almost always considered an increase in the magnitude, variety, or duration of inputs to the system. Little attention has been given to the biological consequences of the absence of stress or the reduction of input stresses. The recent problems of the manned spaceflight environment characterized by physical inactivity, confinement, isolation, and weightlessness have emphasized our lack of knowledge in these areas. The problem has been considered in sensory deprivation research and classic clinical studies of disuse atrophy of muscle and bones. More recently, in an attempt to simulate the inactivity and weightlessness components of the spaceflight environment in the laboratory, prolonged bed rest, immobilization, and water immersion have been used as tools by aerospace medical scientists, and systematic physiological studies in these environments are now under way. The term "hypodynamic" is used to describe any state of reduced physical input to, or reduced activity of, the subject; "hypogravic" refers to a specific hypodynamic condition in which the reduced input is the acceleration due to gravity.

In modern physiology a great deal is understood about muscular exercise but very little about inactivity. In recent years there has been growing medical concern that the sedentary aspects of our modern society may predispose us to cardiovascular disease. Many public health studies have demonstrated an inverse relationship between the degree of physical activity and the mortality from coronary heart disease. The consequences of prolonged bed rest – muscle atrophy, osteoporosis, renal calculi, phlebothrombosis, and hypostatic pneumonia – are recognized clinically, and early ambulation of the hospitalized patient has been emphasized since World War II. Physiological results from recent manned spaceflights emphasize the debilitating effects of the spaceflight exposure, particularly the cardiovascular deconditioning syndrome. Weightlessness, confinement, and inactivity are specific examples of low input environments in that their physiological effects are closely related and, at present, nearly indistinguishable and perhaps inseparable.

Recent concern with hypodynamic and hypogravic aspects of the spaceflight environment, particularly inactivity, confinement, and weightlessness, has resulted in the support by NASA and the U.S. Air Force of a number of studies utilizing bed rest, immobilization, water immersion, and similar analogs of weightlessness. Many of these reports are large, recently completed, and essentially unreported to the scientific community. Many of these data represent applied research and do not readily lend themselves to publication in the usual professional journals. For these reasons, a number of concerned investigators involved in these programs informally discussed the need for a symposium or workshop at which the investigators themselves, and a limited number of other participants, could: (1) review their data and discuss their ideas and mutual problems at length and in detail; (2) discuss the physiological mechanisms of response and adaptation to these hypodynamic and hypogravic environments; (3) provide correlations, where possible, between laboratory and spaceflight results; and (4) establish the current state of knowledge in these areas. Other goals included: (5) identification of the need and direction of future work in this area;

(6) suggestions for more uniform methods for the conduct of prolonged bed rest and immersion studies; and (7) discussion of the need for inflight countermeasures and conditioning programs. This symposium was a response to these objectives.

The format was patterned after the Gordon Conference series. Scientific sessions were held morning and evening; the afternoon was free for informal discussion. Each session was introduced by a keynote paper that attempted an overview and brief literature survey of the subject area. These papers were prepared and circulated to the participants in advance of the meeting to establish a common body of information among all attendees. In addition to the five formal sessions, a sixth session entitled "Research Directions," gave the session chairmen an opportunity to summarize their groups' papers and address the goals of the meeting.

We wish to express our sincere gratitude to the many people who contributed to the success of this undertaking: the speakers who took the time and effort to produce thoughtful manuscripts containing the most recent data from their laboratories, and, in particular, the authors of the keynote papers who undertook major literature reviews and research summaries; the chairmen who organized their sessions, kept speakers on time, and directed the discussion in a stimulating and thoughtful fashion; and all of the participants in the stimulating discussions who also edited their comments. Special thanks are due to Mrs. Ruth Rotunno for her excellent help in the final editing, to Mrs. Helen Jelenchick who acted as conference secretary, and to Daniel Benford who handled administrative arrangements. Armour Pharmaceutical Company; Lederle Laboratories; Merck, Sharp and Dohme; and Sandoz Pharmaceuticals all contributed invaluable financial support to the conference. Finally, speaking for ourselves as directors and for all of the participants in this most successful meeting,we would like to express our thanks to the NASA Ames Research Center, Moffett Field, California, and to Harold Sandler of that organization for providing the funds that made this conference possible.

RAYMOND H. MURRAY

MICHAEL McCALLY

Indianapolis, Indiana
January 1970

Keynote Paper

1 SPACEFLIGHT DECONDITIONING: AN OVERVIEW OF MANNED SPACEFLIGHT RESULTS

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INTRODUCTION

The scientific questions appropriate to manned spaceflight research are as diverse and inexhaustible as those common to earthbound laboratories. There is a series of milestones, however, that must be achieved sequentially if manned spaceflight research is to proceed with justifiable expectation of success. At least three major advances have developed from the program of ground-based laboratory studies, combined with observations made from Project Mercury and the Gemini and Apollo programs.

Investigations of null gravity, which led to the first major breakthrough in manned spaceflight research, really began with the animal flights. The null gravity state cannot be achieved for any appreciable length of time short of actual orbital spaceflight. The first orbital flights of animals by the United States and Russia were necessary to refute untested, but plausible, theories of catastrophic failures of various vital functions in an organism suddenly thrust into an environment without gravitational force. The results from Project Mercury showed that man could expect to remain alive and operationally effective for brief periods in space. The durations of the Mercury flights were cautiously extended and terminated with the 1½-day flight of the Mercury-Atlas 9 flight of Schirra. However, by the end of Project Mercury, there was positive evidence of significant physiological changes similar to those observed in man during bed rest or water immersion.

The studies conducted during the Gemini program were directed toward evaluating the magnitude of flight-related changes first noted in the Mercury project, and other physiological changes that might occur in manned spaceflights of up to 2 weeks' duration. Heavy emphasis was given the evaluation of the cardiovascular system, since the principal changes observed during Mercury involved alterations in cardiovascular reflexes that regulate the flow of blood against the hydrostatic gradient in the gravity field. Evaluation of physiological systems in an operational program is simply a field study to obtain the most accurate data possible, and in no way resembles the

capability of a physiological laboratory to make multiple sophisticated measurements on animals or humans. The pre- and postflight measurements and the inflight evaluations during the Gemini program were qualitative and intended to detect only gross alterations in the functional status of the principal human systems with increased flight duration.

The Gemini studies also included evaluation of conflicting reports of possible central nervous system and cardiac disturbances. These reports resulted in part from isolated interpretations of scattered and incomplete data from Russian manned spaceflights.

STUDY RESULTS AND DISCUSSION

The results of the Gemini studies (refs. 1–6) indicated that some of the major human physiological systems undergo consistent and predictable changes as a result of spaceflight. Moreover, the changes observed in flights lasting as long as 14 days are unlikely to degrade human performance significantly during missions required to achieve the goals of the Apollo program. Changes were detected in the cardiovascular and musculoskeletal systems; in the composition and quantity of body fluids, including the blood; and in certain hormone and blood cell levels. If changes occurred in other systems, they were not of sufficient magnitude to be detected by the methods used. It was interesting to compare the magnitude of the changes observed after the 14-day Gemini flight with those observed after the 8-day Gemini flight. An initial conclusion might be that most of these changes developed during the first week of flight, with little if any progression during the second week. However, there were significant differences in diet and degree of exercise as well as in the astronauts' suits, which may account for the observed findings. Although the Gemini data are significant, they are insufficient to permit extrapolation to major extensions in mission duration. Confirmation of the rate of onset and the possible stabilization of observed changes is one of the most important objectives of the proposed Apollo applications program, particularly the proposed biomedical studies.

Project Mercury and Gemini Results

Figure 1.1 shows the relative sizes of the launch vehicles and space capsules used in Project Mercury, Gemini, and Apollo programs. Contrary to popular belief, the launch accelerations of Saturn 5 (Apollo) are much less than those of either the Titan or the Atlas.

Table 1.1 outlines the principal environmental stresses to which the astronauts are exposed. Weightlessness is of particular interest; other stresses under study include radiation and atmospheric composition.

Table 1.2 shows the cardiovascular data for the longest Mercury flight, MA-9. Note the low supine heart rate, a higher heart rate in the erect position, and some diminishing of pulse pressure in the postflight tests. Table 1.3 shows the peak heart rates on launch and reentry in Gemini flights; these rates were somewhat reduced in the Apollo flights. Table 1.4 outlines the biomedical measurements performed in the Gemini program. Primarily because of their duration, the 8- and 14-day flights were of the greatest medical and physiological significance. Figure 1.2 shows an astronaut using the exerciser in the left-hand cockpit with the hatch open; with the hatch closed, there is very, very little room. Figure 1.3 shows the resting pulse rate plotted against time during flight. Note the almost linear increase in heart rate up to the end of the 8-day flight, and marked improvement

during the 14-day flight. The improvement is probably due to better food intake, water intake, and exercise, and to being out of the spacesuit for a large part of the flight time. Figure 1.4 shows the tilt-table pulse rate changes at bed rest and postflight levels. These values are for the final 5 min of the tilt procedure and again show a linear percentage increase in heart rate, as well as a marked improvement during the 14-day flight. Figure 1.5 shows the pulse pressure decrease with days of exposure to weightlessness. Of special interest is the lowering of the pulse pressure, even during the 14-day flight in which the heart rate response appeared to improve. Figure 1.6 outlines the detailed plotting of one tilt test, the astronaut's first after the Gemini 7 flight. Note the marked increase in heart rate during the first postflight tilt compared with that recorded during the preflight test. There is also a narrower pulse pressure, although there was no syncope or presyncope in the astronaut. The Whitney strain gauge tracing at the bottom of figure 1.6 estimates the leg-girth change during tilt and may indicate an increase in volume. Compared to preflight measurements, postflight findings show an increase in leg girth, suggesting that a greater amount of blood has been pooled. Figure 1.7 shows a similar tilt test 8 hr after hydration, rest, and some exercise; the tilt heart rate change has improved markedly, as have the pulse pressure and the leg circumference.

Table 1.5 shows some of the results of the hematologic studies. There was a decrease of up to 20 percent in red cell mass and a slight decrease in plasma volume. The total blood volume decreased, except in the 14-day flight when there was essentially no change. All the astronauts lost weight in about the same pattern as in the Apollo series (ref. 3). One of the astronauts has constitutional hyperbilirubinemia, which could account for some of the aberrant values found. The table also shows other hematological measurements: a decrease in the red cell survival time, and a decrease in the total body hematocrit that parallels the decrease in red cell mass. There was an increase in red blood cell fragility and in the mean corpuscular volume, with a decrease in mean corpuscular hemoglobin concentration. The spleen/liver ratio counts were elevated significantly here, but no reticulocytosis was demonstrated. Figures 1.8 and 1.9 summarize the red cell mass and plasma volume changes in the Gemini flights. Figure 1.10 shows some of the other pre- and postflight determinations.

After the 14-day flight, the sodium excretion in the urine was quite low. Initially, there was a marked postflight increase in aldosterone, with return to normal on hydration and ingestion of adequate electrolytes.

Table 1.6 reviews the radiation dosages in the Gemini program. These doses are miniscule (in millirads) and apparently innocuous. Figure 1.11 shows the time course of some of the physiological changes observed in Gemini studies.

Apollo Results

Apollo flights 7, 8, 9, and 10 have been accomplished successfully. Although the manned Apollo flights have been shorter than the longest Gemini flight, they account for most of the man-hours of spaceflight thus far. Also, the host of biochemical studies performed with Apollo programs far exceeded those in the Gemini and Mercury programs.

Several important observations have been made. For example, the crew performed with gratifying efficiency when allowed extensive movement in the larger work area of the Apollo

spacecraft. Further, no serious problems have arisen in the physical or psychological integrity of the crew. The crew members of all Apollo flights have reported the feeling of fullness in the head that is noticeable on attaining weightless flight. They are aware of this sensation for varying lengths of time, but always for at least several hours. This finding was reported previously in the Gemini program and was misinterpreted at that time as disorientation and/or motion sickness. The astronauts have adapted quite well to weightlessness within a very short period of time. Minimal movement effort was required, and the total lack of weight of objects and clothing became an advantage in the weightless environment. The astronauts utilized this environment for passing materials to each other and for placing items in a holding or stationary situation for short periods of time.

In the Gemini program, some of the extravehicular activity (EVA) tasks outlined for the astronauts were found to be overly ambitious; the EVA program was later changed considerably, and the tasks less ambitious and paced more evenly. In view of the Gemini experience, intravehicular activity (IVA) was of particular interest in the Apollo program. Training was begun in an underwater facility, which closely simulated the weightless environment, despite the viscosity of the water. Apparently, IVA demands no extra work; no abnormal sensations or increase in work loads or heart rates were noted, with the exception of the experience of the Apollo 8 and 9 crewmen early in the mission. The flight plan of Apollo 8 and 9 required that the crewmen leave their couches to prepare for translunar injection. All three Apollo 8 crewmen noted some motion sickness (stomach uneasiness, nausea, and vomiting), confined generally to the first day of the flight. In the Apollo 9 series, this condition lasted considerably longer and, in the case of one astronaut, necessitated a postponement of the flight plan. No response of this type had been noted before in manned spaceflight. The Apollo 7 crewmen noted some back muscle soreness that developed during the early portion of the flight; the problem was relieved by hyperextension and exercise. (It is fortunate that the Gemini astronauts did not experience this difficulty, because there was not sufficient room in their spacecraft for hyperextension.) The Apollo 7 crewmen felt muscle soreness was caused by, or related to, the fetal position they assumed in the weightless state, particularly when sleeping. On returning to the 1-G environment on earth, they reported that the clothing they had worn inflight (with no sensation of having it on) now felt extremely heavy, as if they had weights in their pockets.

Sleep in the weightless state continued to be a problem on the Apollo flights, including flights 9 and 10. The crewmen required approximately three nights to adapt to sleep in the weightless state. The astronauts continued to want physical contact with some object; they tried to wrap an arm or leg around something even though they were floating relatively free in the sleep station. The longest sleep period was about 5 hr.

In the Gemini program, major interference with work/rest cycles was successfully avoided by having the sleep and meal periods coincide with sleep and meal periods at Cape Kennedy time, regardless of the mission plan. Apollo flights require that one crewman be on watch at all times. The 20-hr lunar orbit period, which necessitated activity by all three men, was a particularly difficult factor in the flight planning for these missions. The circadian biorhythms of all of the crewmen were disrupted. The effect of weightlessness, combined with the noise made by the other crewmen in the cabin and by communications with ground-base stations, produced a most unsatisfactory inflight

sleep situation. All the crewmen became fatigued; the fatigue problem was most acute on the Apollo 8 mission because the crewmen had not had adequate rest prior to the 20-hr lunar orbit period, and it interfered with their ability to perform assignments optimally, and this will probably be true on the lunar flight. According to the flight plan, when the two astronauts descend in the Lunar Excursion Module (LEM) to the lunar surface, they are supposed to sleep for several hours. This is probably unlikely. Following this sleep period, one (and possibly both) of the astronauts are to exit to the moon's surface.

Inflight illnesses occurred during Apollo 7, 8, and 9 missions and, although successfully dealt with, interfered with flight operations and planning. Increased attention to preflight preventive medicine procedures—which at best are limited in the prelaunch period at the Cape—and some sort of modified isolation (which is currently imposed) are indicated. However, Apollo 7 and 8 took place in the fall and winter when respiratory illnesses are at their peak; the preflight program is such that absolute prevention of infection will not be possible, and the possibility of illness must be considered on flights of long duration.

Apollo Cardiovascular Studies

In the Apollo program, lower body negative pressure (LBNP) evaluations have been conducted pre- and postflight; in the Gemini program, the tilt table was used. The use of LBNP was predicated on long duration flights, such as the Apollo applications program, where the LBNP technique is suitable for following the time course of cardiovascular deconditioning. The LBNP test can be used inflight, and it provides better controlled cardiac stress than the tilt table. Three baseline LBNP procedures are conducted preflight, and the postflight determinations are continued until they compare favorably with the preflight baselines. After 5 min of baseline data, the astronauts were exposed to 5 min each at −30, −40, and −50 mm Hg pressure. The crewmen had highly significant increases in heart rate and decreases in pulse pressure during the immediate postflight LBNP test. Contrary to the findings in the Gemini tilt-table studies, no postflight increase in leg circumference was noted in most of the six individuals tested. Two of the Apollo 8 crewmen developed presyncopal symptoms during the −50 mm Hg portion of the test. All the Apollo 7 postflight LBNP test results returned to within the preflight or normal range within 24 hr after recovery. After the Apollo 8 flight, the LBNP test values returned to levels well within the preflight range at the 51- to 53-hr postlanding period. In the case of one astronaut, LBNP test results were remarkably similar to the tilt-table results of the Gemini series; results for most of the other astronauts, however, show little resemblance. It is difficult to draw any final conclusions from these data. However, the spaceflight situation does cause some modification in the cardiovascular system that results in an increased pulse rate and a decreased pulse pressure response to LBNP stress.

Table 1.7 outlines the purposes of the Apollo manned flights. Table 1.8 shows the physiological parameters monitored during the Apollo 7, 8, and 9 flights; note there are fewer parameters than in the Mercury and Gemini programs. There is no blood pressure monitored during the Apollo missions. The peak heart rates during launch and reentry on these flights, shown in Table 1.9, are considerably lower than those from the Gemini program. Apollo launch accelerations are less than those experienced by the Mercury and Gemini astronauts.

Radiation doses on the Apollo missions (table 1.10) are considered very small, particularly since the flights were made at a time when solar flares were very likely. The lunar surface operations may be compromised somewhat in the event of major flare activity.

Table 1.11 details crew weight losses in Apollo 7, 8, and 9. One astronaut lost 10 lb, yet he did not have presyncope during the LBNP test in the postflight period; he also had diarrhea for several days during this flight period. The others' weight losses were from 4 to 10 lb, essentially the same as those observed in the Gemini flights. Figure 1.12 shows the LBNP set up aboard a recovery carrier. The work area is almost as confined as the Gemini spacecraft.

Figure 1.13 is a schematic diagram of the cardiovascular system and its hydrostatic columns. During the tilt test, the force affecting the blood vessels is predominately from within the capacitance vessels; during the LBNP exposure, on the other hand, a negative pressure is applied externally to the vessels. This difference in the application of pressure may cause somewhat different physiological responses and is under study in several laboratories; it will take some time, however, to discern the exact relationship between the two modes of stressing.

Figure 1.14 shows the control supine heart rates following Apollo flights 7, 8, and 9. There is a slight increase in resting heart rate, but it is less than that observed in the Gemini series. Figure 1.15 shows the maximal heart rate during the LBNP exposure; in postflight studies, heart rate generally increases. Figure 1.16 demonstrates the resting supine calf circumference both pre- and postflight; there is essentially no change. Figure 1.17 shows the LBNP maximum change in leg volume. In most cases, there is actually a decrease in the leg circumference as compared with the preflight condition. Other methods, including capacitance, of measuring the circumference or volume of the leg are under consideration.

Figure 1.18 shows control supine pulse pressures pre- and postflight (immediately on recovery). In most cases, there is a decrease in pulse pressure.

Figure 1.19 is a plot of astronaut Borman's Gemini 7 and Apollo 8 LBNP measurements. These two responses match very nicely, but this is the only pair that does match so well. Unfortunately, only a limited number of astronauts have flown in both the Gemini and Apollo programs.

In another cardiovascular study at the Massachusetts General Hospital, Haber and Sanders have measured renin activity during routine cardiac catheterization tests by taking samples from the renal vein. They have shown that during a head-up tilt, renin activity increases up to 50 times the control values; this increase occurs during the first 5 to 10 min of the tilt and reaches a maximum at the 10- to 20-min period (Haber and Sanders, personal communication). Accompanying this increase in renin activity is an increase in diastolic pressure of some 20 mm Hg. Maximum renin response is usually coincident with maximal diastolic pressure. Two individuals who had syncopal episodes during testing demonstrated a very minimal increase in renin activity. These observations may be helpful in the interpretation of postflight tilt-table results.

Apollo Ergometry Studies

Figure 1.20 shows the postflight ergometry testing carried out in the Apollo program on the

recovery carrier. Figure 1.21 demonstrates the heart rate during a controlled exercise using this special ergometer; work loads necessary to produce heart rates of 120, 140, and 160 beats/min (bpm) were used, now modified to include a maximal heart rate of approximately 180 bpm. Table 1.12 shows the Apollo test protocol, and table 1.13 shows the measurements made during this test. Table 1.14 shows the test schedule, and table 1.15 summarizes the results in Apollo 7, 8, and 9 for a work load of 120 bpm. Oxygen consumption, shown in figure 1.22, corresponds to an approximate 70 percent decrease in work performed for the 120-bpm heart rate. Figure 1.23 shows the oxygen consumption at the 140-bpm work load, equivalent to an approximate 40 percent decrease in work performed at this heart rate. Figure 1.24 shows a decrease of 20 percent in the work capacity or work performance at the higher heart rate of 160 bpm. Figure 1.25 summarizes these findings. In eight of the nine astronauts, there was a decrease in work capacity and oxygen consumption at submaximal heart rates. The percentage decrement is less apparent at higher heart rates.

SUMMARY AND CONCLUSIONS

It is not possible to extrapolate the findings from a 10-day space mission to one many times that duration. It is evident, however, that if crewmen are given a reasonable workspace, interesting work to perform, an opportunity to rest when not working, and an adequate and acceptable diet, all should do well and spaceflight may not be as formidable and devastating to mind and body as anticipated. Man does accommodate to weightlessness and IVA incurs little physiological cost. However, sufficient accommodation time must be allowed to prevent motion sickness. Lack of confinement in the Apollo series did not preclude the development of some cardiovascular deconditioning, as determined by LBNP and by the reduction of exercise capacity. Exercise and intake of adequate calories, vitamins, and minerals may be expected to ameliorate, to a large measure, the threat of calcium immobilization from the bones. However, this is yet to be demonstrated in flight.

The red cell losses that were observed in earlier flights appear to be minimized or attenuated by a mixed gas atmosphere, that is, nitrogen and oxygen. The illnesses observed have been the result of ground-base exposures and require preflight preventive medicine measures and provision for inflight treatment. The microbiological changes that occur among the crewmen in flight should be watched with continuing interest; they appear to be a fruitful area for research. There is dissatisfaction with waste management and body hygiene methods available for the present flight program, but the spacecraft configuration and other constraints permit very little latitude in improving the present facilities.

Noise levels in the Apollo spacecraft are occasionally high but are not intolerable. The crew members have been able to enjoy some restful sleep, but sleep in the weightless spacecraft has been of concern and requires further investigation. Radiation levels in the spacecraft have been extremely low thus far, and adequate monitoring should be available in future missions to assure knowledge of potential problems.

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Table 1.1 Principal environmental factors (stresses) in manned spaceflight

Weightlessness
Ionizing radiation
Temperature and humidity
Accelerations
Circadian rhythm disruption
Noise and vibration
Atmospheric composition

Table 1.2 Summary of cardiopulmonary data, flight MA-9

Event	*Pulse*	*Blood Pressure*	*Respiration Rate*
Prelaunch	72	113/79	19
Orbital	89	119/81	15
Postflight	83 (supine)	89/64	–
(1 to 7 hr)*	123 (erect)	90/73	–
(18 hr)	58 (supine)	98/61	–
	80 (erect)	94/68	–

Flight time: 34-1/3 hr
Weight loss: 7-3/4 lb
Postflight temperature: 99.4° F (oral)
Hematocrit: 43 to 49
Subjective symptoms: dizziness

*Subject under residual effects of 5 mg D-amphetamine sulfate.

Table 1.3 Peak heart rates of command pilot and pilot during Gemini launch and reentry

Mission	*Peak Rates during Launch, bpm*	*Peak Rates during Reentry, bpm*
3	152/120	165/130
4	148/128	140/125
5	148/155	170/178
6A	125/150	125/140
7	152/125	180/134
8	138/120	130/90
9A	142/120	160/126
10	120/125	110/90
11	166/154	120/117
12	136/110	142/137

Table 1.4 Medical experiments on Gemini long-duration missions

Code	*Experiment*	*Gemini 4, 4 days*	*Gemini 5, 8 days*	*Gemini 7, 14 days*
M-1	Cuffs		X	X
M-2	Tilt table		Include as medical operations procedure	
M-3	Exercise tolerance	X	X	X
M-4	Phonocardiogram		X	X
M-5	Body fluids			X
M-6	Bone densitometry	X	X	X
M-7	Calcium and nitrogen balance study			X
M-8	Sleep analysis			X
M-9	Otolith function		X	X

Table 1.5 Gemini hematologic studies

Factor	*Gemini 4, 4 days* CP	P	*Gemini 5, 8 days* CP	P	*Gemini 7, 14 days* CP	P
Δ Red blood cell mass, %	−12	−13	−20	−20	−19	−7
Δ Plasma volume, %	−4	−13	−8	−4	+15	+4
Δ Total blood volume, %	−7	−13	−14	−13	+1	−1
Δ Nude body weight, (lb)	−4.5	−8.5	−7.5	−8.5	−10.0	−7.0
Δ Total body hematocrit, %	—	—	−3	−5	−8	−2
Δ RBC survival half-life (days)	—	—	−6	−9	−6½	0
RBC fragility	—	—	—	—	++	+
Δ Mean corpuscular volume, 3%	—	—	+4	+1	+9	+13
Δ Mean corpuscular hemoglobin concentration, %	—	—	—	—	−3	−5
Δ Spleen/liver ratio counts, %	—	—	—	—	+30	+13
Δ Percent reticulocytes per 1000 RBC	—	—	−1.2	−1.2	+0.05	−0.08

CP = Command Pilot
P = Pilot

Table 1.6 Radiation doses on Gemini missions*

Mission	*Duration hr:min*	*Mean Cumulative Dose, mrad* Command Pilot	Pilot
3	4:52	<20	42 ± 15
4	96:56	42 ± 4.5	50 ± 4.5
5	190:56	182 ± 18.5	170 ± 17
6A	330:35	25 ± 2	23 ± 2
8	25:53	155 ± 9	170 ± 10
8	10:41	<10	10
9A	73:04	17 ± 1	22 ± 1
10	70:46	670 ± 6	765 ± 10
11	71:17	29 ± 1	26 ± 1
12	94:37	<20	<20

*Dosimeters located in helmet, right and left chest, and thigh.

Table 1.7 Apollo manned flights

Mission	*Crew*	*Description*	*Duration, hr:min*
7	Schirra Eisele Cunningham	Earth orbital checkout of CSM systems	260:9
8	Anders Borman Lovell	Lunar orbital mission of CSM systems (TLI, LOI, TEI checkout)	147:0
9	McDivitt Scott Schweickart	Earth orbital checkout of CSM-LM systems (LM-CSM separation and docking)	241:0
		Total	1944:27
		Manned Space Flight Total	3937:37

Table 1.8 Physiologic parameters monitored during Apollo

Data Retrieval System	*Parameters*	*Instrumentation*
Apollo 7/8 one crewman selectable	ECG sternal lead respiratory rate	Bipolar electrode impedance pneumograph
Apollo 9 three crewmen simultaneous	ECG sternal lead respiratory rate (oral temperature on demand)	Bipolar electrode impedance pneumograph

Table 1.9 Peak heart rates during launch and reentry

Apollo Mission	*Crewman*	*Peak Rates during Launch,* bpm	*Peak Rates during Reentry,* bpm
7	Cmdr	94	*
	CMP	*	*
	LMP	*	*
8	Cmdr	118	92
	CMP	*	*
	LMP	*	*
9	Cmdr	145	110
	CMP	135	82
	LMP	95	82

*Data not available.

Table 1.10 Radiation doses on Apollo missions*

Mission	*Duration hr:min*	*Commander*	*CM Pilot*	*LM Pilot*
7	260:09	154	157	158
8	147:00	151	163	138
9	241:00	199	211	195

*Dosimeters located on chest, thigh, and ankle.

Table 1.11 Flight crew weight loss to the nearest half pound

Gemini Mission	*Command Pilot Weight Loss, lb*	*Pilot Weight Loss, lb*
3	3	3.5
4	4.5	8.5
5	7.5	8.5
6A	2.5	8
7	10	6
8	*	*
9A	5.5	13.5
10	3.0	3.0
11	2.5	0
12	6.5	7

Apollo Mission	*Commander Weight Loss, lb*	*Weight Loss, lb* CMP	LMP
7	6.5	10	8
8	9	9	4
9	5.5	6	6

*Not available.

Table 1.12 Apollo exercise response test

Test Protocol	
Resting	1 min
Light work (120 heart rate)	6 min
Moderate work (140 heart rate)	3 min
Heavy work (160 heart rate)	3 min
Very heavy work (180 heart rate)	3 min
Recovery	3 min

Table 1.13 Apollo exercise response test

Measurements
ECG
Work load
Oxygen consumption
Carbon dioxide production
Minute volume
Blood pressure
Respiration rate
Pretest pulmonary function

Table 1.14 Apollo exercise response test

Test Schedule	
Preflight	F – 30
	F – 14
	F – 4
Postflight	R + 0
	R + 1

Table 1.15 Apollo exercise response test

Summary of Changes
Eight out of nine flight crewmen have demonstrated a significant decrease in work performed and oxygen consumed at submaximal heart rate levels.
Percent decrement appears to be less at higher heart rates.
Response has returned to preflight values within 24 to 36 hr.
No significant changes in vital capacity, forced expired volumes, or peak expiratory flow rate have been observed.

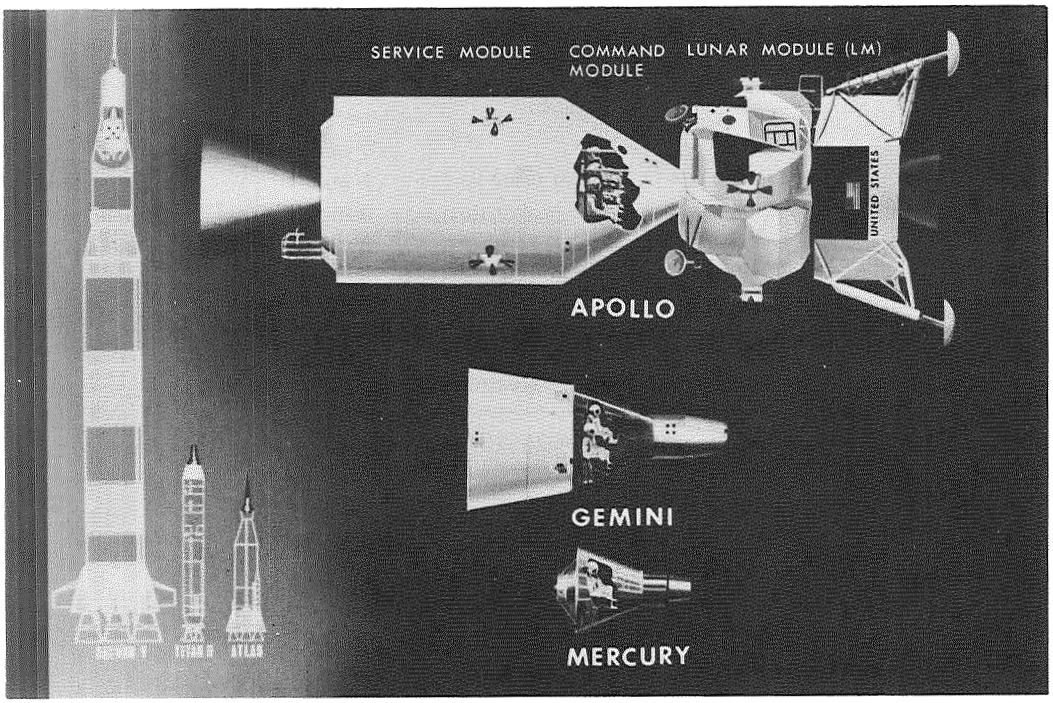

Figure 1.1 Comparison of spacecraft and launch vehicle configurations.

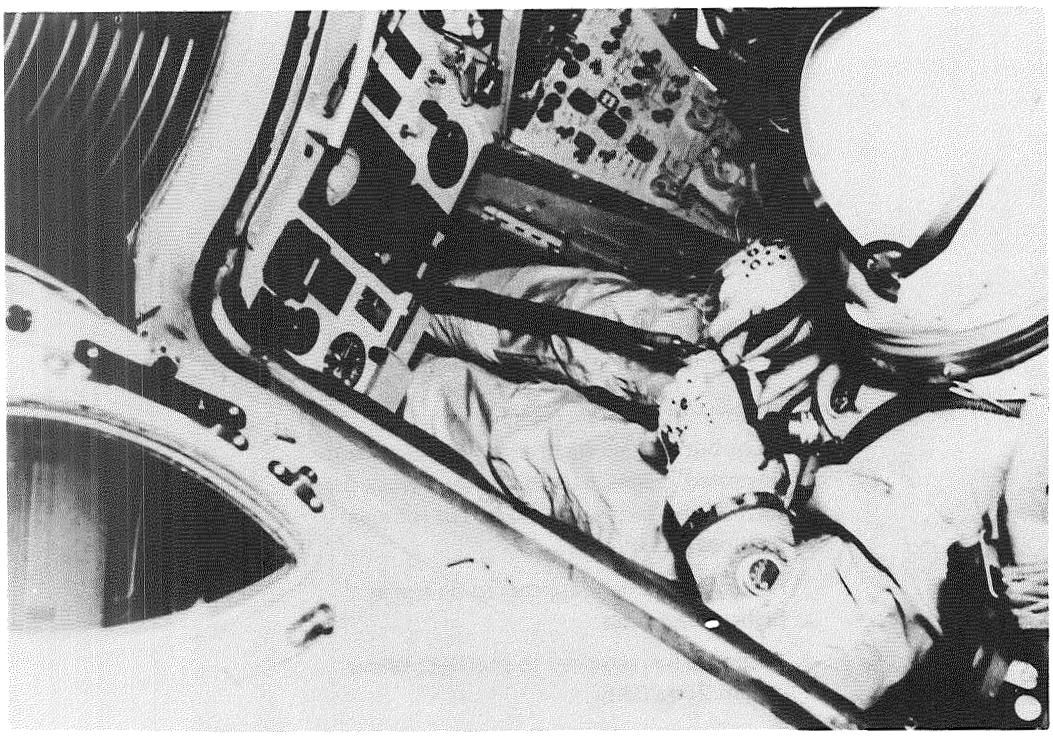

Figure 1.2 Inflight exerciser in use by astronaut.

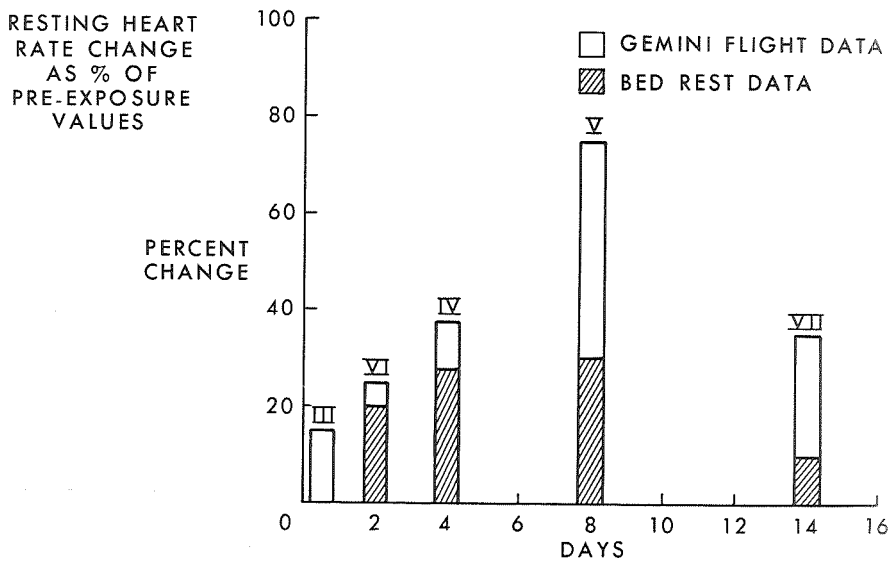

Figure 1.3 Gemini missions and bed rest; resting pulse rate vs time

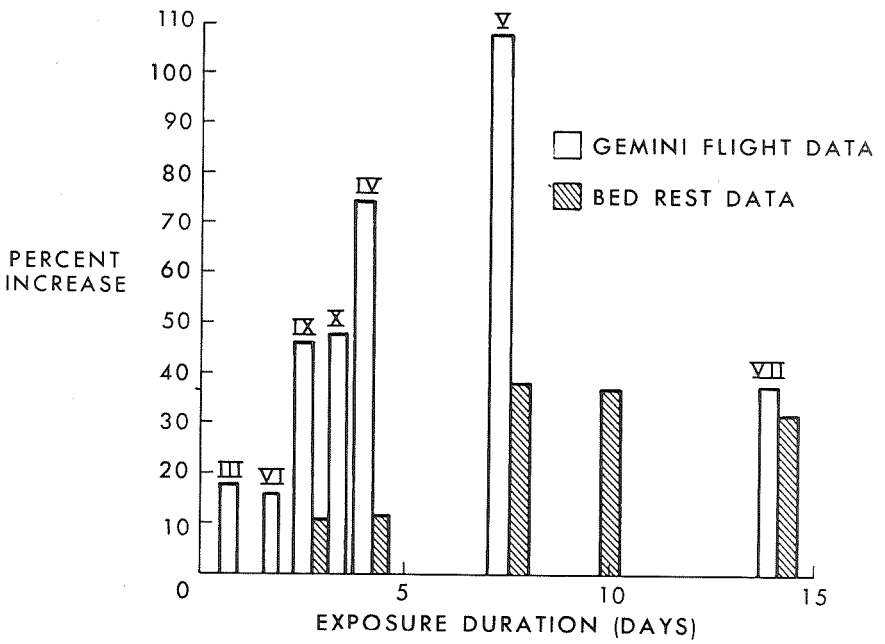

Figure 1.4 Effects of spaceflight compared with bed rest. Tilt heart rate change as percent of pre-exposure tilt mean values

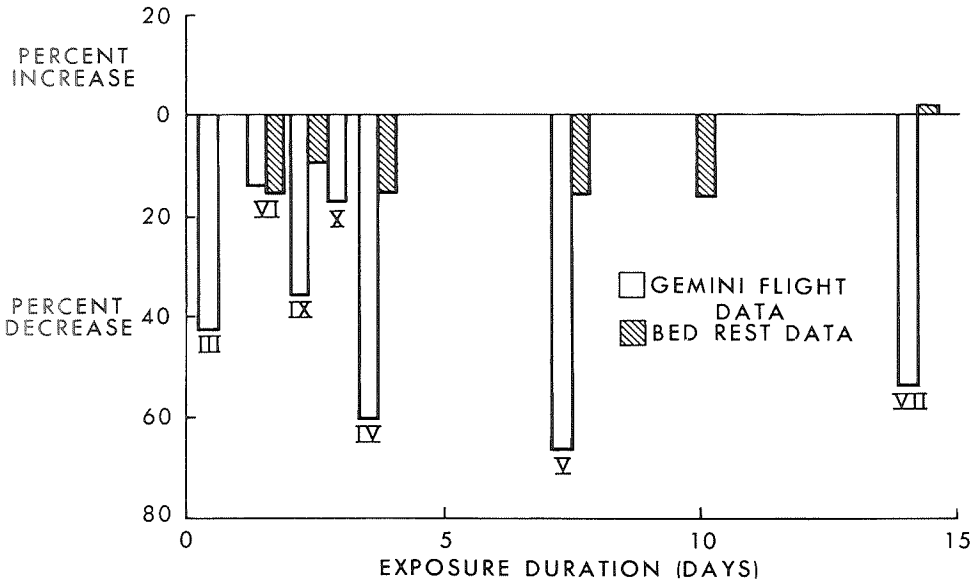

Figure 1.5 Effects of spaceflight compared with bed rest. Tilt pulse pressure change as percent of pre-exposure tilt mean values

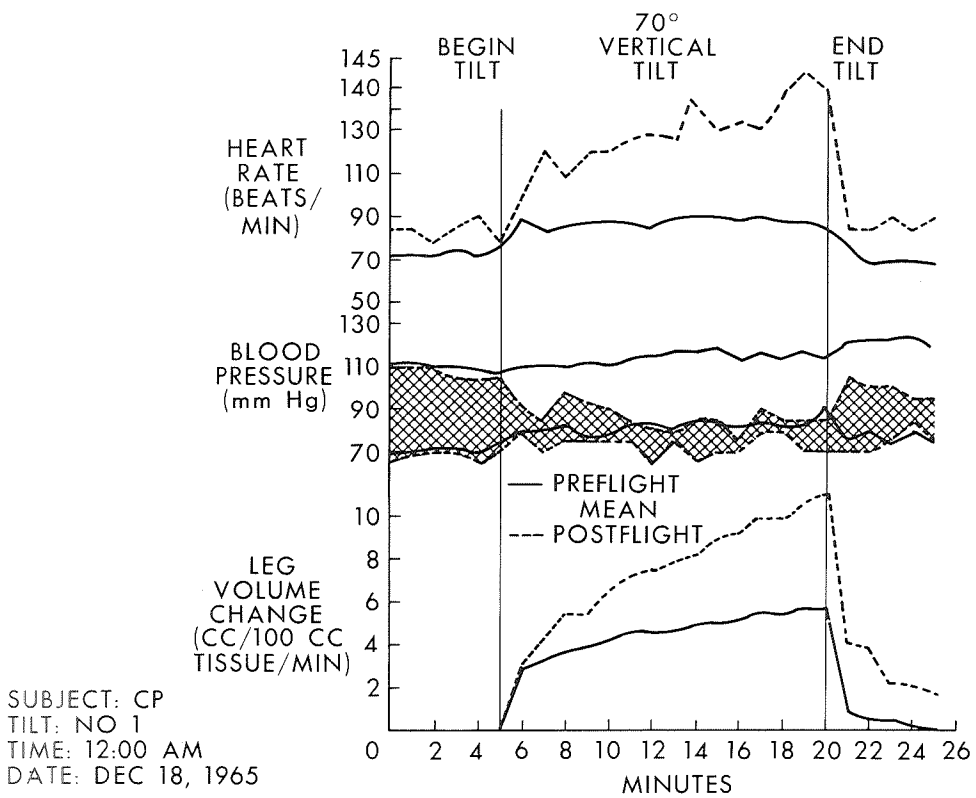

Figure 1.6 Gemini 7 tilt-table data-1

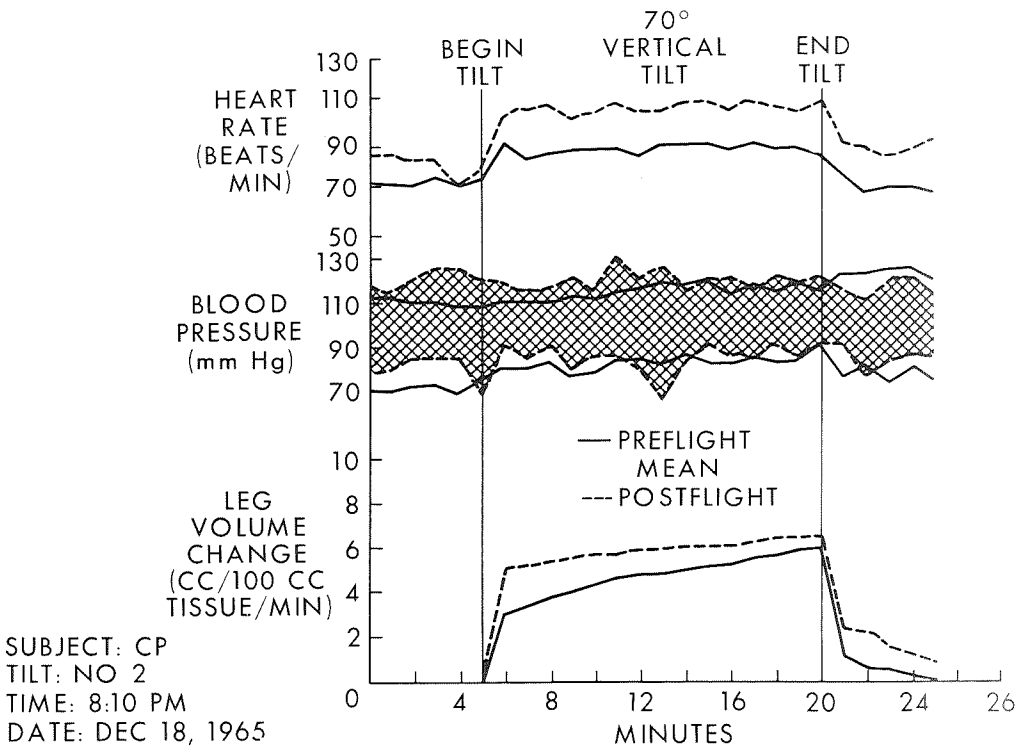

Figure 1.7 Gemini 7 tilt-table data-2

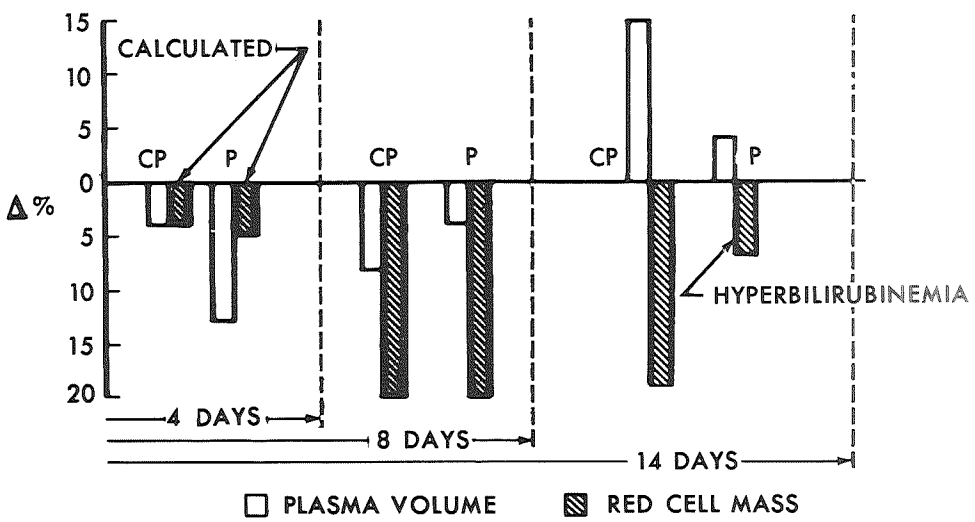

Figure 1.8 Red cell mass and plasma volume, Gemini

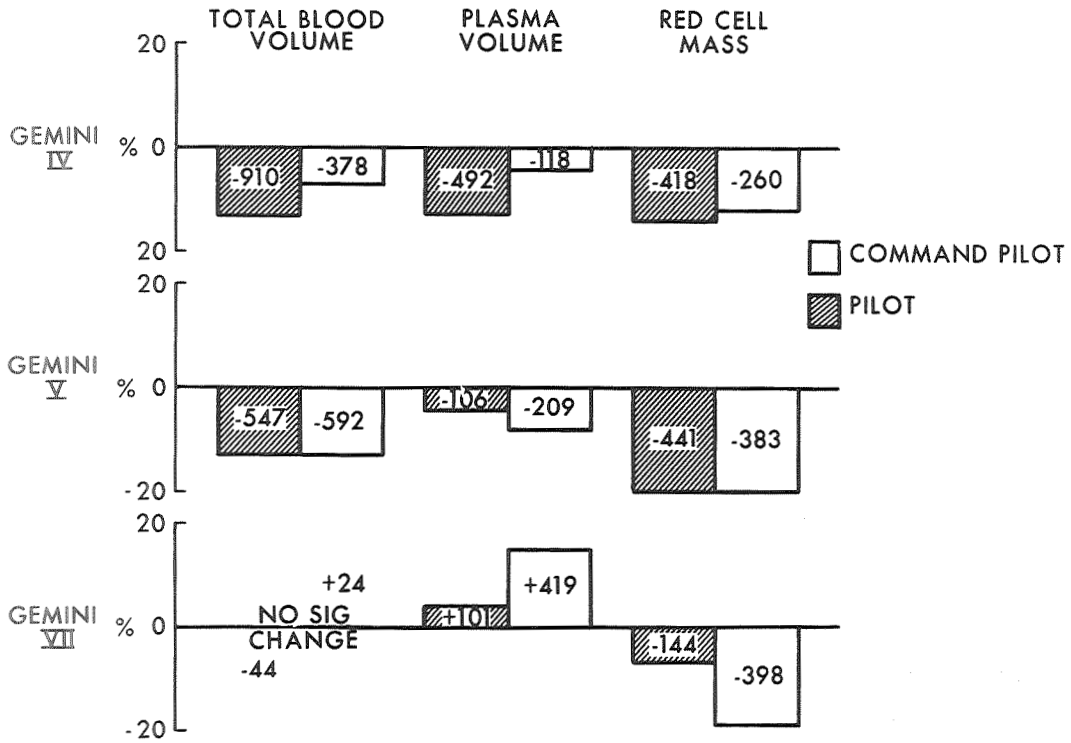

Figure 1.9 Gemini blood volume studies

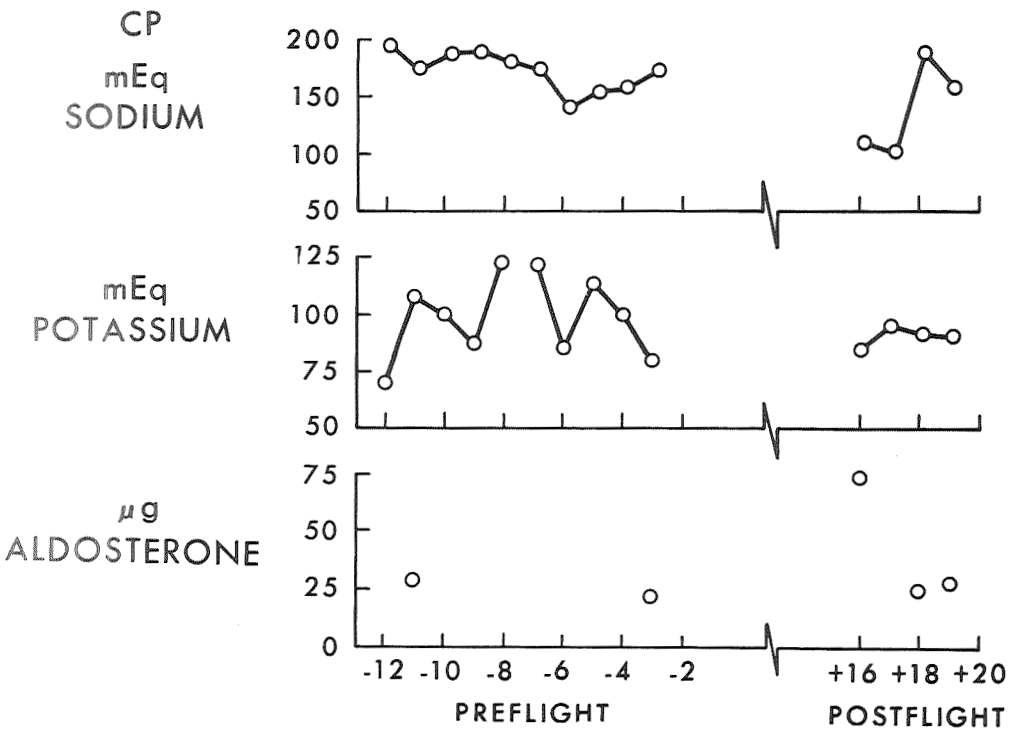

Figure 1.10 Gemini 7 calcium balance experiment

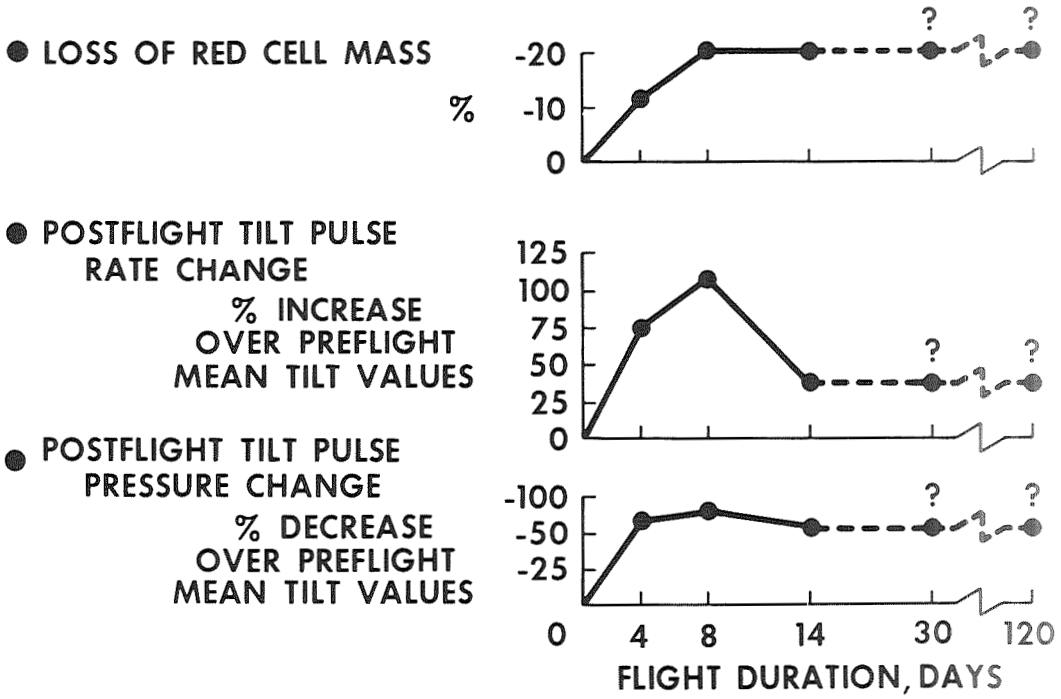

Figure 1.11 Time course of observed physiological changes in Gemini missions

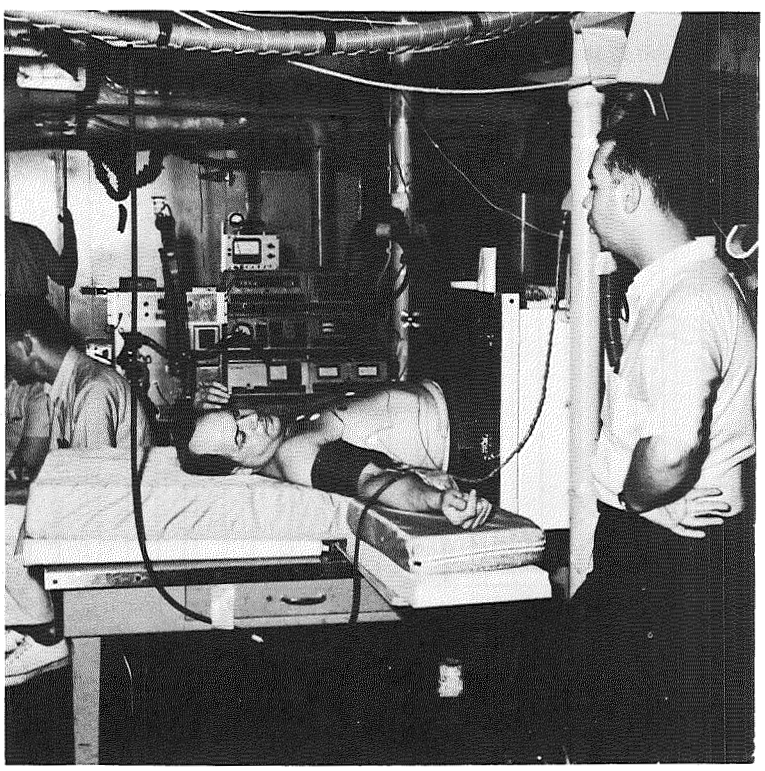

Figure 1.12 LBNP on recovery carrier

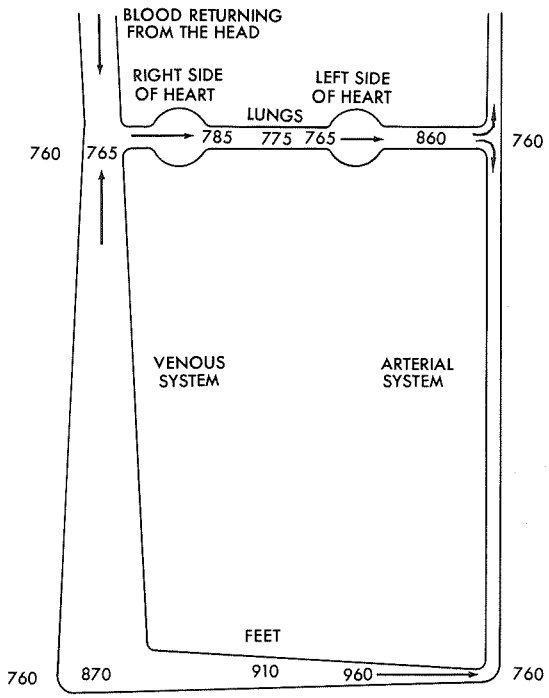

Figure 1.13 Standing in air before immersion

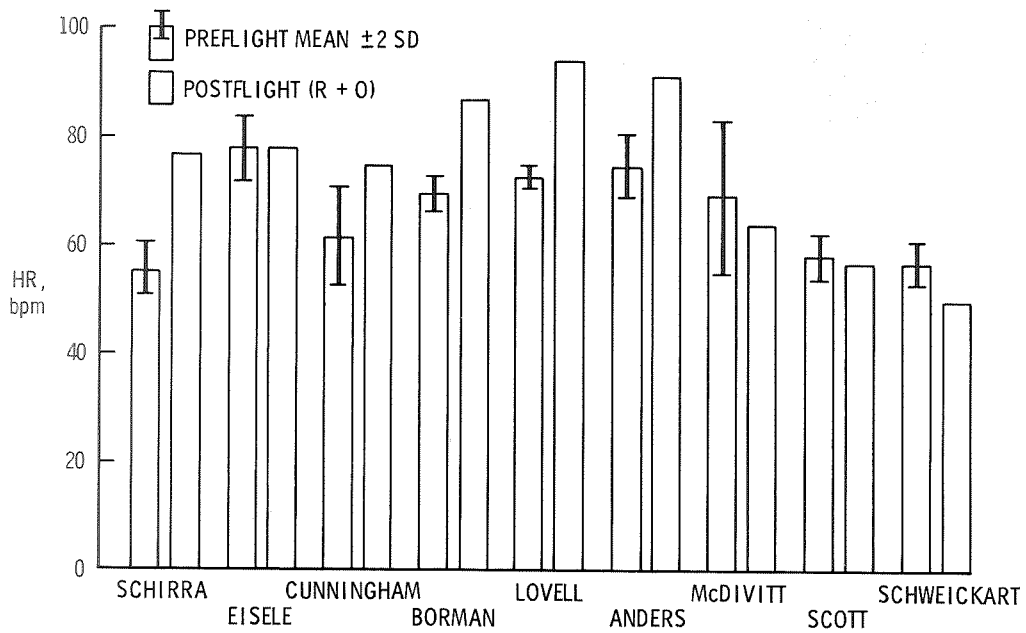

Figure 1.14 LBNP control supine heart rate

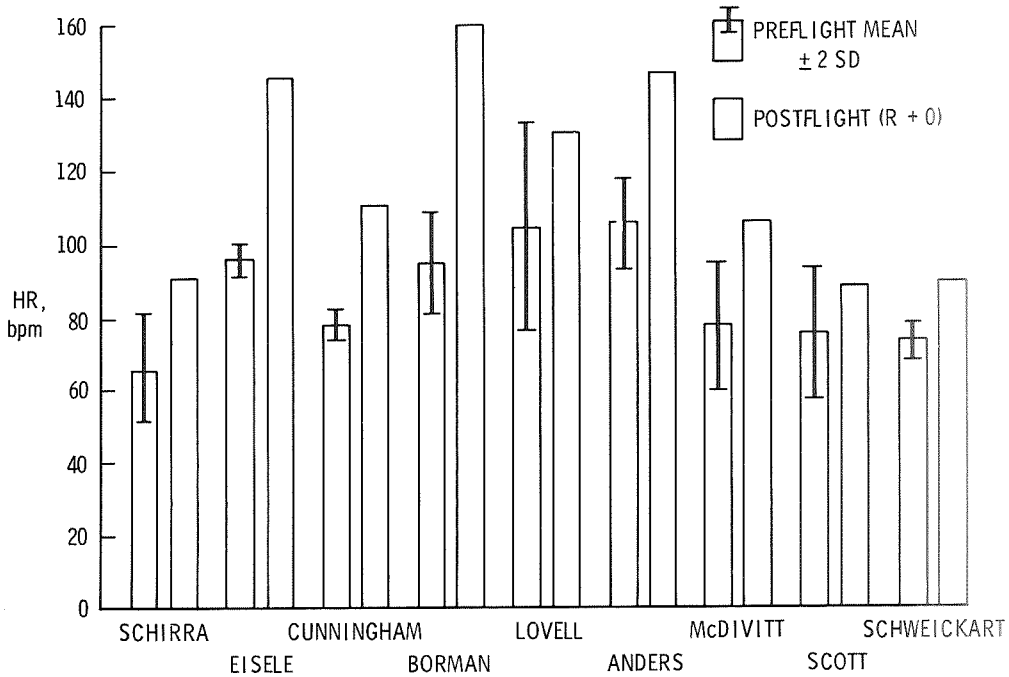

Figure 1.15 LBNP maximal heart rate

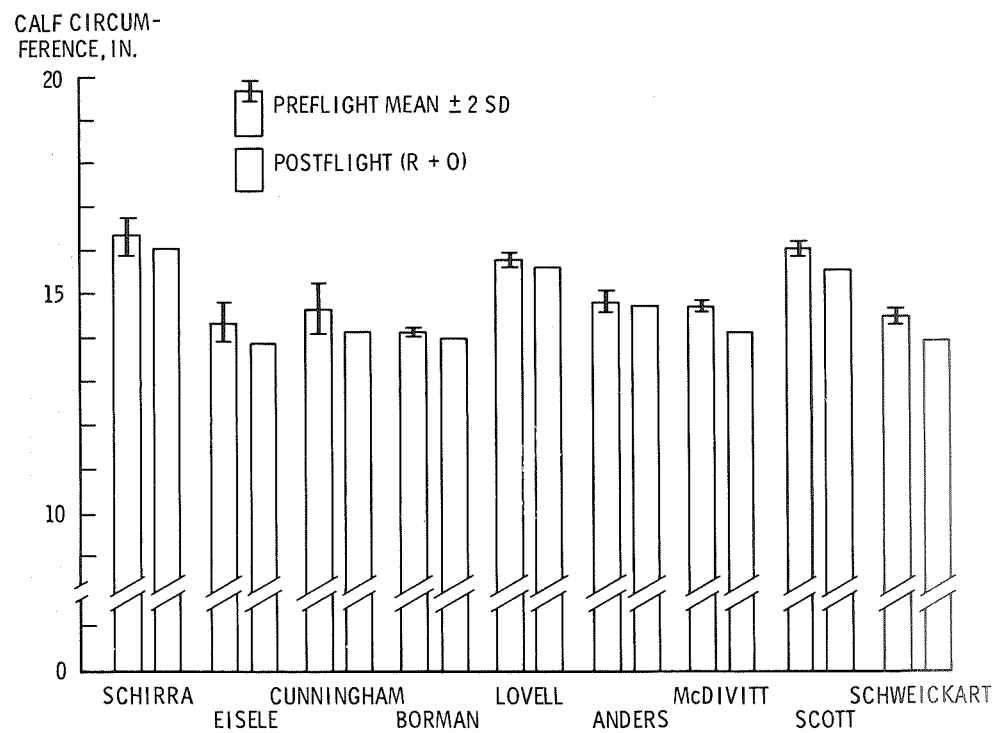

Figure 1.16 Resting supine calf circumference

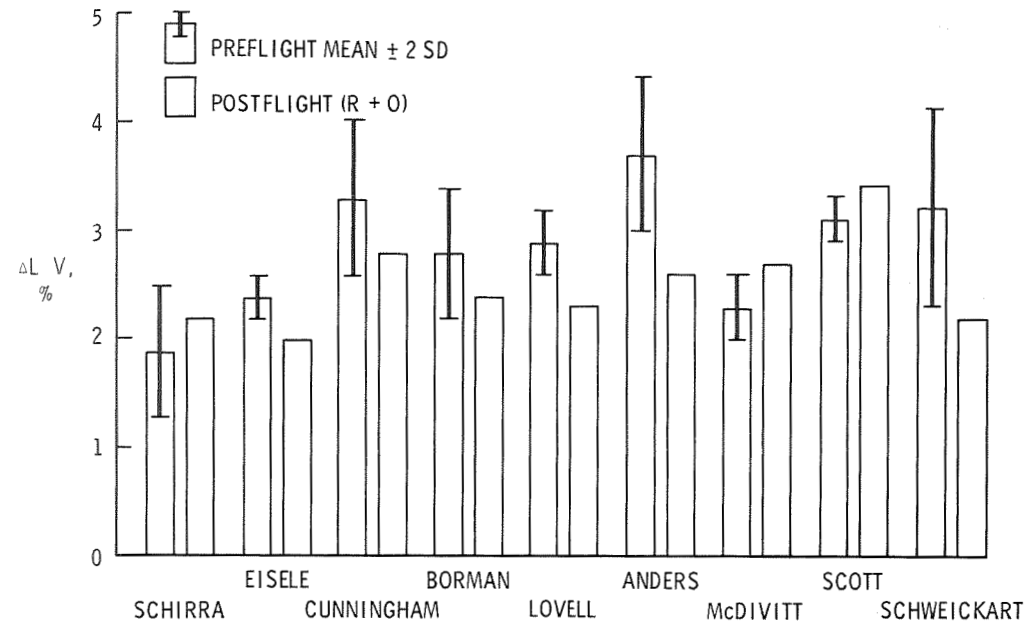

Figure 1.17 LBNP maximal change in leg volume

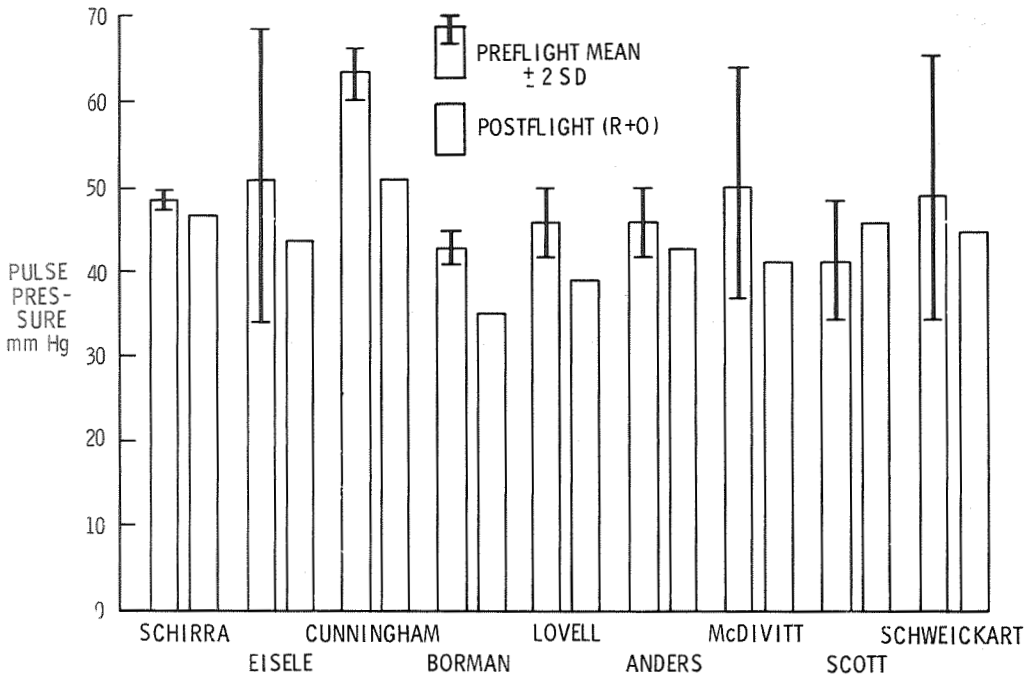

Figure 1.18 LBNP control supine pulse pressure

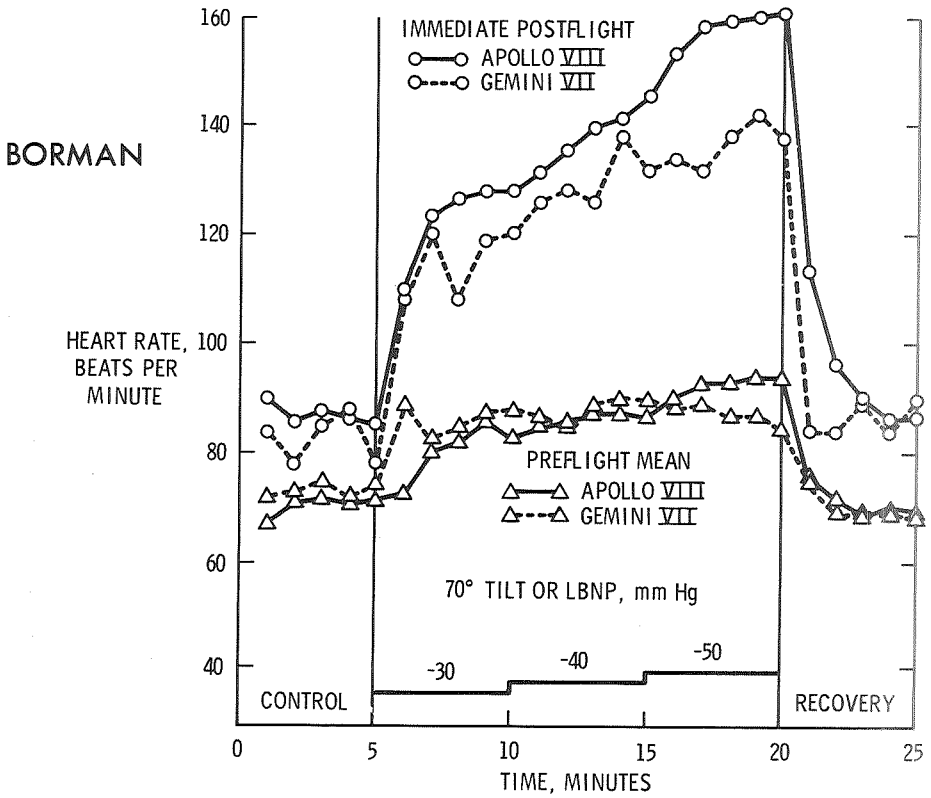

Figure 1.19 Heart rate, bpm

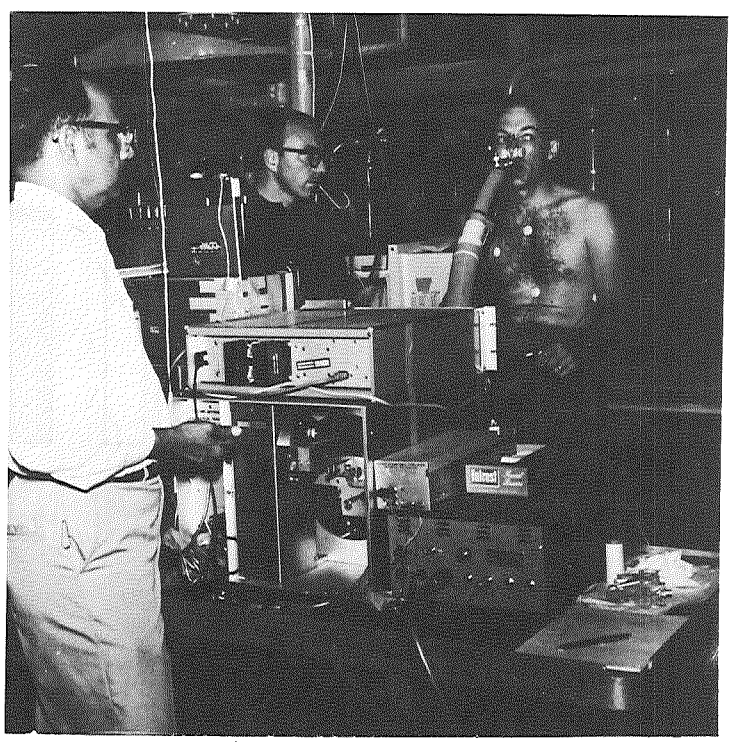

Figure 1.20 Ergometry testing on recovery carrier

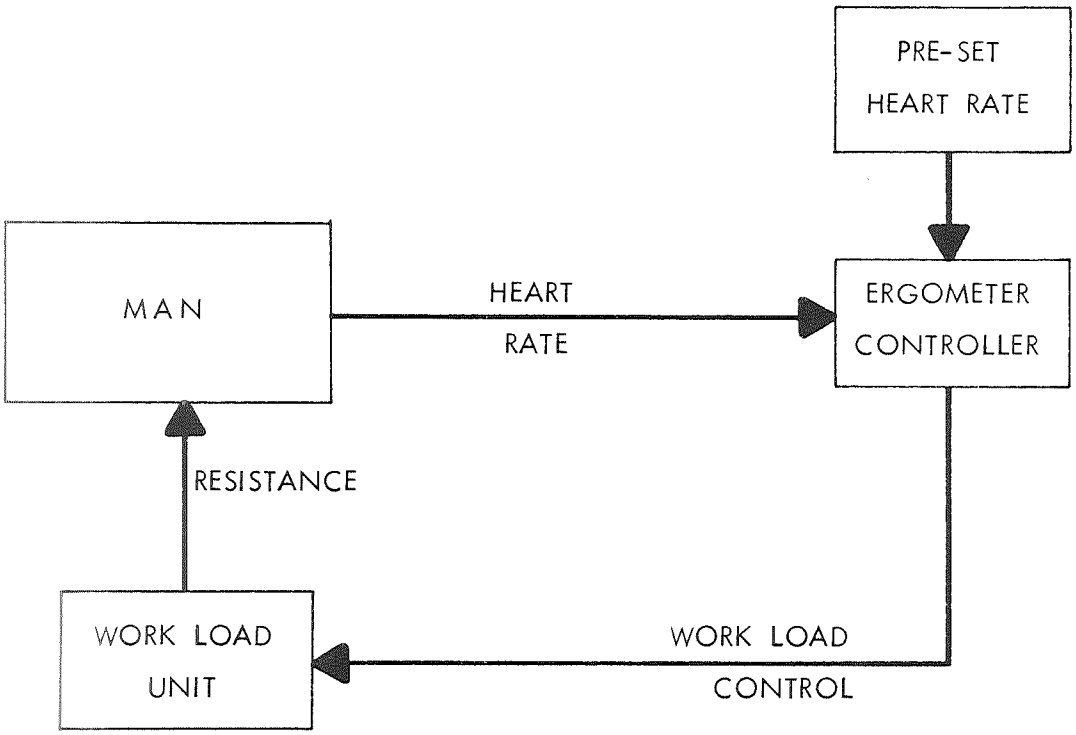

Figure 1.21 Heart rate controlled bicycle ergometer

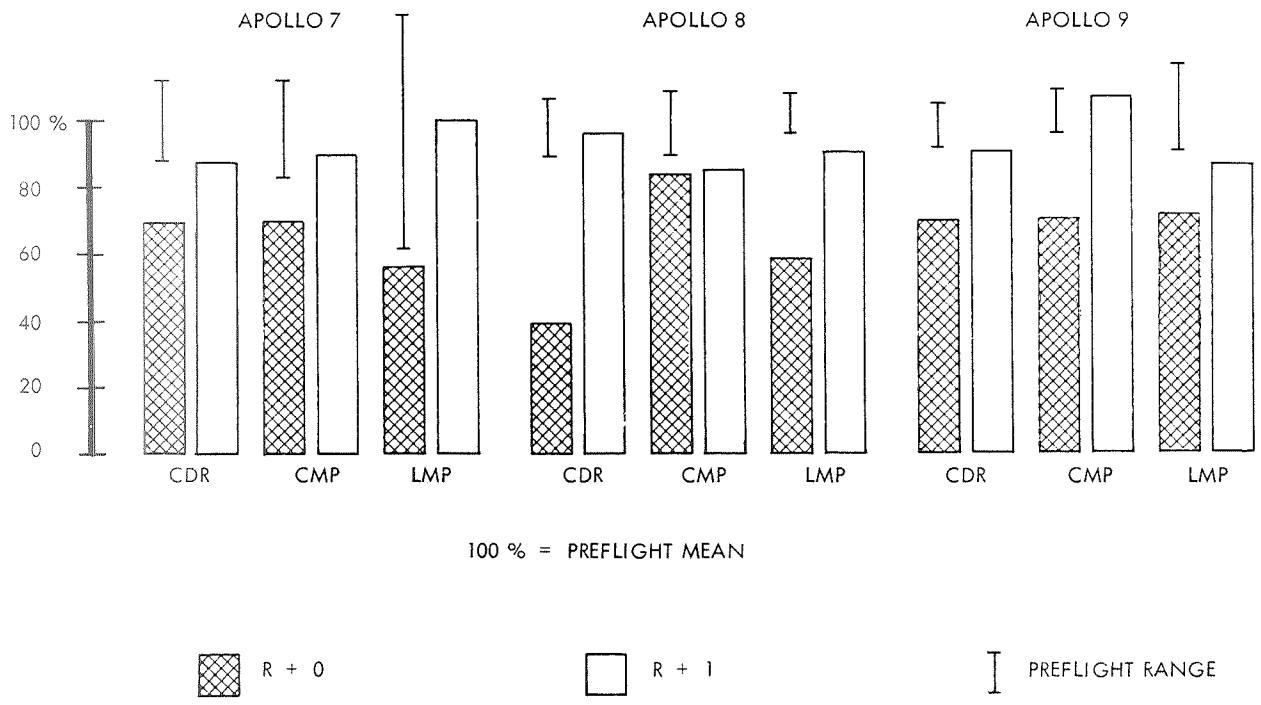

Figure 1.22 Changes in oxygen consumption. Heart rate: 120 bpm

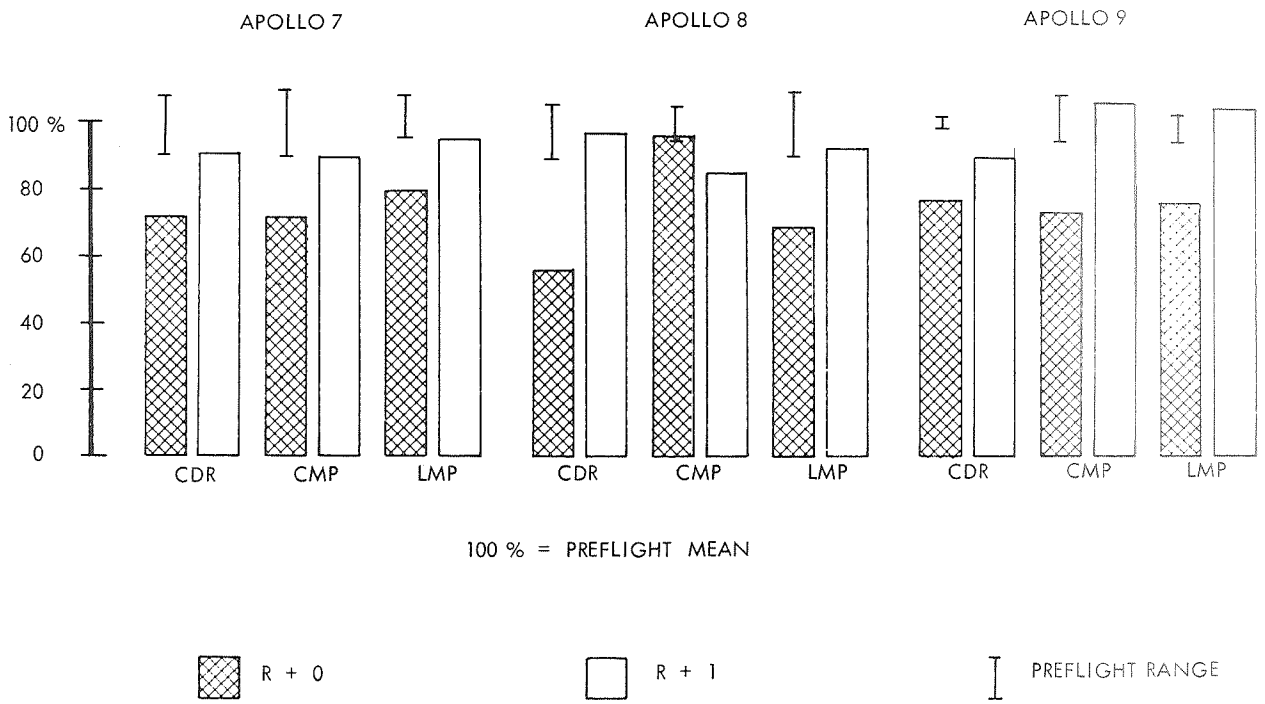

Figure 1.23 Changes in oxygen consumption. Heart rate: 140 bpm

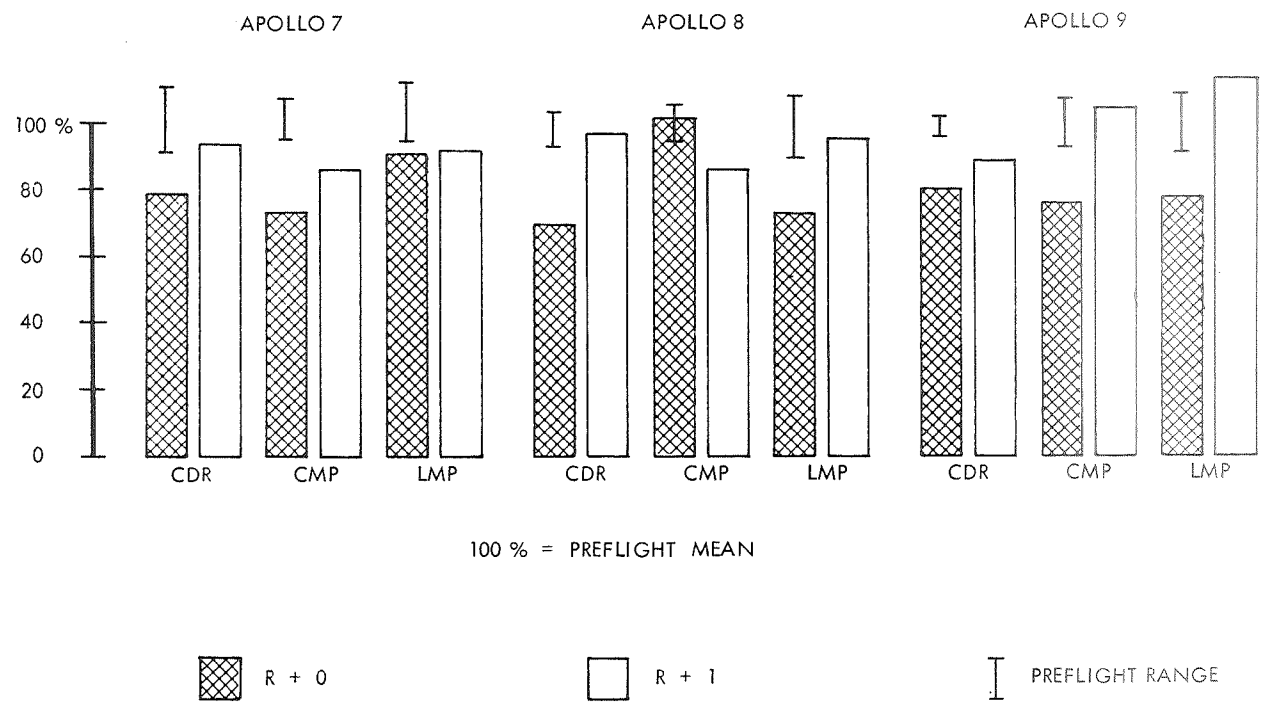

Figure 1.24 Changes in oxygen consumption. Heart rate: 160 bpm

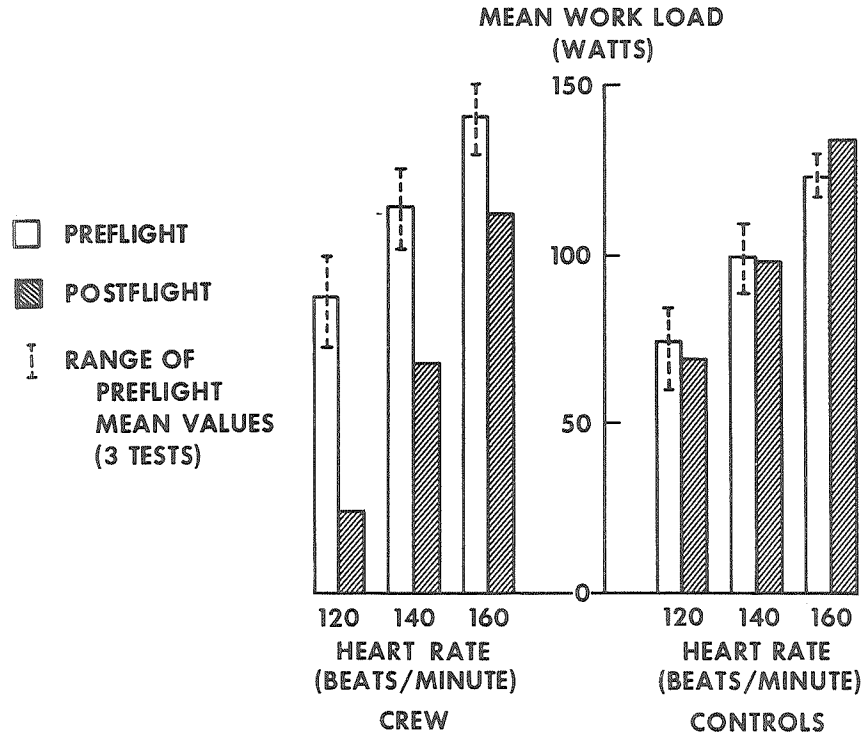

Figure 1.25 Summary of work load change, Apollo 7

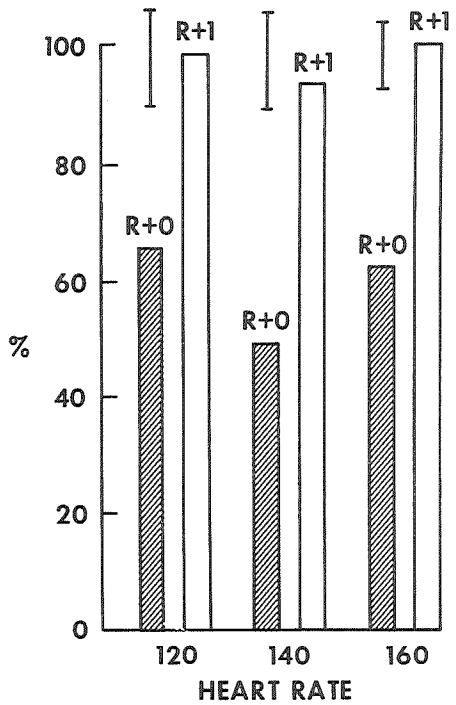

Figure 1.26 Apollo 8 changes in oxygen consumption 100 percent-mean of 3 preflights

2 HEMATOLOGIC IMPLICATIONS OF HYPODYNAMIC STATES

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INTRODUCTION

A review of the medical findings from the Gemini series indicated that only one-third of the 33 potential medical problems predicted or suspected prior to orbital flights were actually reported by the astronauts or their medical monitors. For example, cardiovascular deconditioning was expected, probably related to either zero G or the physical inactivity of confinement in a small spacecraft. From the results of bed rest and water immersion studies, it was predicted that plasma volume would be decreased after the flights and that this decrease would be one cause of cardiovascular intolerance. This effect indeed was noted in Gemini missions 4 and 5. However, several other physiological changes occurred during the missions that had not been predicted on the basis of medical knowledge available prior to the flights, e.g., the decrease in red cell mass found after Gemini missions 5 and 7.

This paper reviews the red cell mass and plasma volume changes noted in the hypodynamic states of bed rest and water immersion, and compares these changes with the hypodynamic- and hypogravic-state characteristics of earth orbital missions.

PLASMA VOLUME

In health, the plasma volume is fairly constant from day to day. Plasma volumes were recorded under standard conditions for normal subjects on two occasions one month apart. These studies show that a plasma volume is technically reproducible to about 1 percent and that day-to-day variations probably do not exceed 2 percent. However, there are environmental, functional, and pathological factors that do influence plasma volume. These are: (1) altitude, (2) season, (3) hydration, (4) posture, (5) physical activity, (6) blood loss, and (7) disease.

Two environmental factors affecting plasma volume are altitude and season. Plasma volume decreases at higher altitudes, while plasma volumes are higher in hot weather than cold.

Four endogenous factors also affect plasma volume. Dehydration results in a lowered plasma volume. Changes in posture with respect to gravity also cause changes in plasma volume. Eisenberg noted that plasma volume decreased 7 percent below recumbency values during 20 min of quiet standing (ref. 1). This decrease has been postulated to result from electrolyte and plasma protein effusion from the dependent vascular spaces, due to the increased hydrostatic pressure.

*Paper read by Dr. Carolyn S. Leach

It is accompanied by increased serum protein concentration. Asmussen et al. have measured the volume change produced by a 45° tilt and have found an increase in total volume of 475 cc in 1 hr (ref. 2). Postural effects are the major cause of diurnal changes in plasma volume. After vigorous exercise, plasma volume may decrease 10 percent if the subject is upright, but we found no changes when subjects performed recumbent exercise. Physical conditioning increases plasma volume, presumably because fatty tissue, which is avascular and therefore contains less plasma, is replaced with muscular tissue, which is highly vascular and has a high plasma volume.

Acute blood loss is usually followed by shifts in the distribution of the extracellular fluid that increases the plasma volume, tending to compensate for losses in both plasma and red cells.

The factors outlined above are primarily homeostatic, tending to maintain a constant internal environment. However, there also are certain pathological situations that affect plasma volume, for increases or decreases in this parameter are characteristic of various diseases.

Most of the environmental functional factors mentioned, and hence plasma volume changes, could be associated with spaceflight. In the past few years, NASA has initiated various studies with spaceflight simulations, such as bed rest and water immersion, to investigate the cause and extent of plasma volume changes. Our studies were typical of these. Young, healthy males were used as substitute astronauts. The subjects were admitted to a metabolic unit, maintained on a constant diet and activity during the pretest procedures, and subjected to bed rest. Bed rest was maintained as strictly as possible with no subject being allowed to sit or stand during the experimental periods.

Figure 2.1 presents the combined results from published studies to show how plasma volume changes over time with bed rest (refs. 3-8). There was a mean 10 percent decrease in plasma volume by the second day of bed rest. By the 27th day, plasma volume had decreased to 20 percent of the control value. The ranges surrounding each mean represent the minimum and maximum change found in the experimental subjects, which approaches a standard deviation of 2. The standard deviation was not calculated, because some of the subject groups were relatively small and each study was somewhat different from the others.

Figure 2.2 shows the recovery of the plasma volume after periods of bed rest. The mean values are somewhat more variable following bed rest, possibly because various control periods of recumbency preceded these experiments. However, these results show that it takes nearly 20 days for the plasma volume to return to the control value. Thus, the recovery rate is slower than the rate of decrease.

In water immersion, the changes are of similar magnitude and differ only in the rate at which the decreases occur. For example, during 6 hr of water immersion, there was a 12 percent decrease in plasma volume. In a recent study, nine normal subjects were immersed to the neck for 12 hr in water maintained at a comfortable temperature. On the average, they lost 563 ml of plasma during the period. This compares with 318 ml during a similar period of bed rest and 151 ml during chair rest (ref. 9). These decreases in plasma volume were associated with a loss of albumin from

the vascular space. The mean albumin losses equaled 53 gm during water immersion, 32 gm during bed rest, and 2 gm during chair rest. Thus, water immersion, like quiet standing, is associated with a loss of both fluid and protein from the vascular space.

Table 2.1 shows the plasma volume changes from three Gemini and Apollo missions. The percentage of change found is compared to that predicted from the bed rest studies. Plasma volume decreases were found after all but the longest (14-day) mission. Thus, plasma volume changes in spaceflights of up to 14 days will not necessarily exceed those predicted from bed rest study results, and comparisons of flight- and ground-based data do not indicate that a combination of null gravity and hypoactivity are additive or synergistic. At present, there is no good explanation for the increase in plasma volume after GT 7; however, it probably represents a compensatory response to changes in the red cell mass.

As in bed rest and water immersion studies, the plasma volume changes noted in earth orbital missions were not great enough to be the major cause of orthostatic intolerance found in the first 24-hr post-mission. Because plasma volume increases during the Gemini 7 mission resulted in essentially normal blood volumes, hypovolemia prior to tilt could not have contributed to orthostatic intolerance noted in these crewmen.

RED BLOOD CELL VOLUME

The red blood cell mass is a more stable parameter than is the plasma volume. Red cells cannot be stored and mobilized as plasma proteins can and therefore losses are replaced slowly. Red cells have a fairly finite life span and live approximately 120 days. Factors influencing red cell production and destruction rates can obviously affect the total circulating red cell mass. The higher production rate of red cells, and hence increased red cell mass, associated with decreases in oxygen partial pressure is well known and adequately documented by studies conducted at high altitudes or in altitude chambers.

Some diseases affect production and some the destruction of red blood cells, and thus cause changes in the red cell mass. In polycythemia, an overactive bone marrow produces too many red cells. In certain types of malignancy and anemias, red cell production is inhibited. Hemolytic anemias result in early cell death and, if not compensated, lower the red cell mass.

Certain drugs also cause changes in red cell mass. For example, polycythemia can be produced by the administration of cobalt chloride, while anemia may result from administration of certain other drugs.

Hypodynamic states probably cause decreases in red cell mass. Changes in red cell mass during bed rest are not as well documented as plasma volume changes; there has been only one published bed rest study in which red cell mass has been determined (ref. 8). Other investigators have derived their red cell masses from blood volumes determined from peripheral vessel hematocrits and plasma volumes. It is known that such a calculation contains an inherent and unpredictable error, since the peripheral vessel hematocrit never equals the total body hematocrit. Wide and unpredictable swings in the ratio of total body to peripheral hematocrit occur in normal, and

particularly in pathological, conditions. Thus, the statement of Miller et al. that no change in red cell mass occurs during bed rest is not proven, since only derived red cell masses were obtained in their studies (ref. 3). Vogt and Johnson's bed rest studies showed a 15 percent drop in red cell mass during the first 4 days of the testing interval (ref. 8). However, blood was also drawn for other studies, raising the possibility that this value is too high and does not reflect the effects of the bed rest alone. Oberfield et al. noted an 8.6 percent mean increase in red cell mass in orthopedic patients who were ambulated after prolonged immobilization (ref. 10). The mechanism for these changes is unknown.

There is no proof that hypodynamic and nullgravic situations have resulted in a decreased red cell mass. Red cell mass measurements made after three Gemini and three Apollo missions (ref. 11) are shown in table 2.2. Of the 16 crew members in the Gemini and Apollo series, 15 have undergone decreases in red cell mass during the flight interval. Current explanations are that the decreases resulted from the spacecraft atmosphere rather than from inactivity and null gravity. In two of the Gemini missions and the Apollo 9 mission, the red cell mass changes were greater than those found after Apollo 7 and 8. The Apollo 7 and 8 missions differed from the previous Gemini missions in that the spacecraft's atmosphere never reached 100 percent oxygen at 5 psi.

The peripheral venous hematocrit values in four of the six crew members from the Gemini missions decreased postflight, indicating that red cell mass decreases were proportionately greater than those of plasma volume. These findings are contrary to results found with bed rest, where plasma volume decreases have been noted with associated rises in peripheral venous hematocrit values.

There are four mechanisms that could explain the decrease in red cell mass observed in the Gemini and Apollo 9 studies: (1) increased intravascular destruction, (2) decreased production rate, (3) external or interstitial bleeding, or (4) incomplete mixing of the radioactive ^{51}Cr cells used in postflight measurements of red cell mass.

Internal bleeding is neither plausible nor supported by the clinical findings. There is no evidence to suggest incomplete mixing or sequestration of the red cell mass. Guaiac tests of fecal specimens saved throughout the Gemini 5 mission were negative, and negligible amounts of ^{51}Cr were found in them.

The red blood cell survival data in the Gemini 5 and 7 missions indicate increased red cell destruction. An increased erythrocyte production rate ordinarily would follow a decrease in erythrocyte survival. No direct inflight measurements were made that provide information about changes in the production rate of erythrocytes. However, there is evidence that no changes in production rate occured: the red cell mass decreased, and the dilution of the ^{51}Cr with newly released red cells would have resulted in an abnormally low level of remaining ^{51}Cr. The reticulocyte data indicated that the red cell production rate at the end of the flight was probably close to the preflight rate. Iron turnover data from the recent Apollo missions rule out increased production as an operating factor during flight.

Several explanations for this spaceflight-related hemolysis have been offered, most of them related to the hyperoxic environment. Increased oxygen tension is known to cause red cell abnormalities—specifically, inclusion bodies, hemolysis, changes in red cell volume, and altered enzyme activity. These changes may also be a result of vitamin E deficiency. However, these abnormalities have been reported only at oxygen tensions higher than 100 percent O_2, 5 psi. It has been postulated that the observed hemolysis results either directly from damage to erythrocyte lipoprotein membrane or indirectly from interference with other metabolic systems by lipid peroxides (refs. 12-14). The decreased red cell mass, especially in Apollo 9 where measurements with ^{51}Cr indicated a normal rate of red cell survival, suggests a possible decreased production rate during that mission. Teleologic reasoning suggests that this might be a homeostatic mechanism. Just as the red cell production rate increases after exposure to high altitude or low oxygen tensions, thus increasing the amount of hemoglobin and the oxgen-carrying capacity of the blood, so red cell production rate could be expected to decrease with high oxygen tensions as less hemoglobin is needed to carry the oxygen.

Although previous investigations have assumed a decrease in circulating red cell mass due to the hypodynamic and nullgravic states inherent in orbital flight, our experience with these mission situations does not support this assumption.

CONCLUSIONS

Hypogravic and hypodynamic situations cause decreases in plasma volume of at least 10 percent. This loss of volume is recovered after return to normal activity. Available data suggests that hypogravic and hypodynamic states may cause a decrease in red cell mass. The available data are not sufficient, or are too complicated by other factors (e.g., increased O_2 pressure), to allow a final conclusion concerning this possibility.

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Table 2.1 Plasma volume changes

		Percent Change			
Mission	*Days*	*1*	*2*	*3*	*Predicted*
GT4	4	−4	−13		−7
GT5	8	−9	−5		−10
GT7	14	+18	+4		−12
AP7	11	−5	−4	+1	−11
AP8	7	−16	−15	−8	−9
AP9	11	−4	−7	−13	−11

Table 2.2 Red cell mass changes

		Percent Change		
Mission	*Days*	*1*	*2*	*3*
GT4	4	−12	−13	
GT5	8	−20	−22	
GT7	14	−19	−8	
AP7	11	−1	−9	0
AP8	7	+2	−2	−4
AP9	11	−4	−7	−10

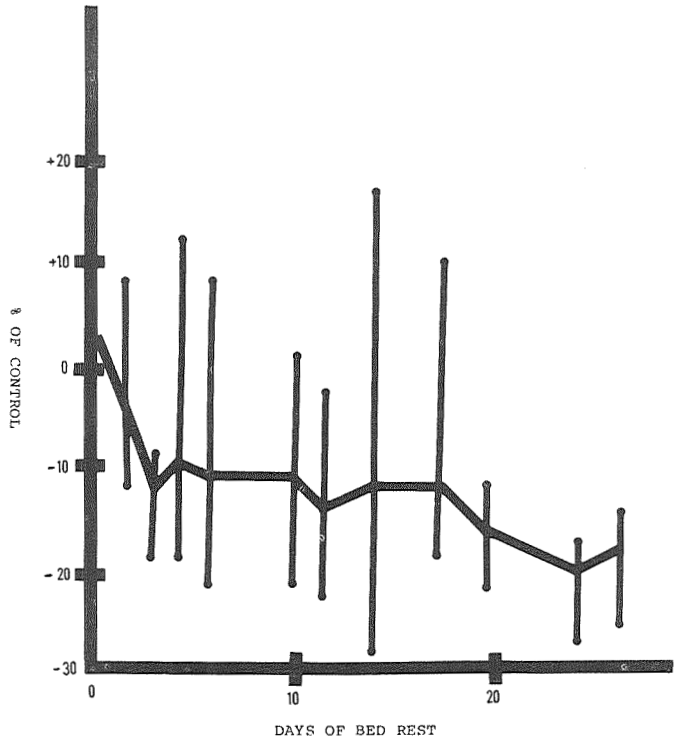

Figure 2.1 Changes in plasma volume with bed rest over time; the combined results of published studies (refs. 3-8)

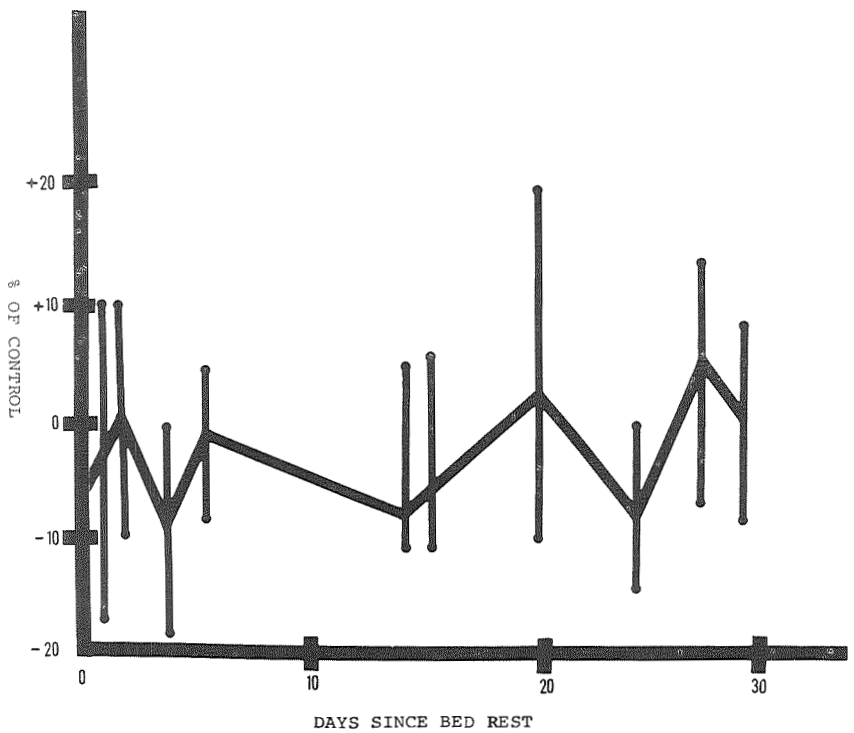

Figure 2.2 Plasma volume recovery after periods of bed rest

3 BONE DENSITY CHANGES IN THE ASTRONAUTS DURING SPACEFLIGHT

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INTRODUCTION

Russian scientists attending a conference in 1963 (ref. 1) reported loss of urinary calcium by the cosmonauts of Vostoks 2 and 3. The Russian scientist Gurvowsky (at a symposium organized by the International Astronautics Federation in behalf of the World Health Organization, Geneva, Switzerland, November 19-22, 1968) reported the effects of prolonged weightlessness on the two dogs flown for 3 days in the Cosmos 110 flight. He stated that roentgenographs made of the heel bones of the animals showed a loss in mineral density of 10 to 11 percent. The method used in evaluating the bone density was not reported (private communication from W. Ross Adey, MD, to Gurvowsky).

The first human studies to evaluate the loss of bone density in weightlessness were performed on the astronauts of the Gemini-Titan 4, 5, and 7 missions in 1965 (refs 2-5). Similar investigations were made during the Apollo 7 and 8 missions in 1968 (ref. 6). In these studies, the method of roentgenographic bone density as developed by Mack and associates was followed.

It has long been known that immobilization induces a decrease in mineralization of the skeletal system (refs. 7-12). Recumbency investigations involving subjects in the horizontal position in bed rest, sufficiently supervised to approach null gravity, have been conducted in various laboratories (refs. 13, 14).

The astronaut bone density studies described in this report were designed to determine: (1) the extent of bone density loss experienced during spaceflight, (2) possible means of reducing such losses, and (3) the rate of postflight recovery of any bone mineral loss.

MISSION SCHEDULES

Gemini Missions

Gemini 4, Launch 6/3/65; Recovery 6/7/65 *Preflight,* 9 days and 3 days before liftoff at Kennedy Space Center, and on the morning of liftoff, also at the Space Center; *postflight,* immediately after recovery on the aircraft carrier USS Wasp, and at Manned Spacecraft Center, Houston, 16 days and again 50 days following recovery. The length of this orbital flight was 4 days.

Gemini 5, Launch 8/21/65; Recovery 8/29/65 *Preflight,* 10 days, 4 days, and 2 days before liftoff, and on the morning of liftoff at Kennedy Space Center; *postflight,* immediately after recovery; again 24 hr later on the aircraft carrier USS Lake Champlain, and at Manned Spacecraft Center, Houston, 10 days and 66 days following termination of the flight. The length of this orbital flight was 8 days.

Gemini 7, Launch 12/4/65; Recovery 12/18/65 *Preflight,* 10 days, 3 days, and on the day of launch at Kennedy Space Center; *postflight,* immediately after recovery and again 24 hr later on the aircraft carrier USS Wasp, and at Manned Spacecraft Center, Houston, 11 days and 47 days following recovery. The length of this spaceflight was 14 days.

Apollo Missions

Apollo 7, Launch 10/11/68; Recovery 10/22/68 *Preflight,* 14 days and 4 days before liftoff at Kennedy Space Center; *postflight,* immediately after recovery on the aircraft carrier USS Essex, 32 hr later at Kennedy Space Center, and 9 days postflight at Manned Spacecraft Center, Houston. The length of the spaceflight was 11 days.

Apollo 8, Launch 12/21/68; Recovery 12/27/68 *Preflight,* 14 days before liftoff at Manned Spacecraft Center, Houston; 5 days preflight and the day of launch at Kennedy Space Center; *postflight,* immediately after recovery on the aircraft carrier USS Yorktown; 3, 6, and 27 days postflight for two members of the crew, 3, 6, 27, and 45 days postflight for the third crew member, all at Manned Spacecraft Center, Houston.

TEST CONDITIONS FOR BONE DENSITY DETERMINATIONS

Roentgenographic Method

The bone densitometric method used in these studies was first reported by Mack and associates in 1938 and 1939 (refs. 15, 16). Later reports describe further developments in the techniques (refs. 17–22).

Densitometric Assembly

The densitometric assembly used in evaluating the films for the Gemini missions was based on an analog system as described by Mack (refs. 20, 21). The addition of a digital computer to the analog assembly eliminated certain manual operations in the former system (ref. 22). Figure 3.1 shows the digital-analog computer assembly used in scanning sections of bone radiographs in order to evaluate their density in the Apollo 7 tests. At the extreme right is the densitometer through which the calibration wedge is scanned by means of a standard light beam, after which the bone section to be measured is scanned. The two central sections are a part of the analog computer assembly. At the extreme left is the digital computer, by means of which the test is programmed so that the density of the bone section is obtained without manual work on the two central recorders. The entire analog assembly is used only periodically for calibration purposes.

Exposed films are calibrated by placing an aluminum alloy reference wedge on each film adjacent to the bone to be evaluated. The alloy in the wedge was selected because it exhibited an X-ray absorption coefficient close to that of bone. Thus, the wedge serves as a calibration device by showing, through its scan, any deviations from the correct slope of the wedge itself caused by slight differences in X-ray film and exposure and development techniques, which thereafter are corrected.

Procedure

A specific sequence of operations is required for calibrating a density curve and integrating the area under the curve of a bone on the same radiograph.

In the new assembly, the output of the scanning unit is connected through an amplifier (Astrodata Model 885) to a digital computer (Digital Equipment Computation PDP-8 supplied by Kaman Instruments).* An analog-to-digital (A/D) converter formats the data for direct processing by the computer, and the computer samples the data at specific intervals of time. Programming and operating control is provided by a teletypewriter unit (TTY) that includes a paper-tape input/output (I/O) feature.

The computer has performed computations similar to those performed by the previous analog system. First, the wedge image is scanned and the resulting light transmission data are stored according to distance along (or thickness of) the wedge. Second, the bone image is scanned and the resulting light transmission data are stored. After both the wedge and bone scan data have been completed, the computer calibrates the stored bone scan data in terms of equivalent wedge thickness by means of the sotred wedge scan data. The calibrated bone data then are integrated along the scan by means of a trapezoidal approximation integration formula.

The advantage of the present system over the earlier system is that it provides an automatic means for calibrating the film in terms of the light transmission of the calibration wedge, the roentgenogram of which is taken simultaneously with the roentgenogram of the bone of interest.

Comparison of the analog and digital-analog systems as to the reproducibility of successive scans shows that the two systems provide essentially the same results. The digital technique, however, offers a more rapid procedure for analyzing the films; it also reduces the chance of technical error by the operator in measuring the heights of the uncorrected initial wedge tract at 20 points and adjusting the corresponding potentiometers. The analog system is used at intervals for comparative calibration purposes.

GEMINI MISSION FINDINGS

Spaceflight Duration

One of the most outstanding findings from the Gemini missions was that spaceflight duration was not a major factor in bone density losses. Losses in all the anatomic sites evaluated were pooled for all astronauts in each of the three orbital flights. Results were as follows:

1. Lowest negative bone mass changes, crew of Gemini 7
2. Intermediate negative bone mass changes, crew of Gemini 4
3. Highest negative bone mass changes, crew of Gemini 5

The 4-day mission of the Gemini 4 astronauts resulted in major reductions in radiographic bone density, particularly in the fingers and wrist. The reductions were markedly higher in the astronauts of Gemini 5, who made an 8-day orbital flight. The men of 14-day Gemini 7 mission, however, experienced the least negative change in bone density of any of the three crews. Table 3.1 compares the bone density changes in the major anatomical sites of the astronauts of Gemini 5 and 7.

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* Kaman Instruments, Austin, Texas; now the Columbia Scientific Industries.

Dietary Intake

A comparison of crew diets during these missions showed, for the command pilot and the pilot of Gemini 5, a daily mean calcium consumption of only 375 mg and 313 mg, respectively, representing between one-third and one-fourth of the calcium provided for them. The two astronauts of Gemini 7, on the other hand, consumed 945 mg and 921 mg of calcium per day, respectively, of the approximately 1,200 mg in their spacecraft. Calories and other nutrients were ingested proportionately.

Supporting Bed Rest Trials

Following the analysis of bone density and dietary results from the Gemini 5 mission, a 14-day bed rest study was conducted with four men; the only change in the basic TWU bed rest diet was the daily calcium provision of 300 mg for each subject. During the 14 days of the experiment, there was a mean change of -12.35 ± 0.25 SD in the central os calcis bone density of the four men in terms of calcium hydroxyapatite equivalency, which verified the adverse effect of low calcium on the maintenance of skeletal mineral.

During the 14-day Gemini 7 mission, a planned exercise program was used for the first time in a space mission. The program centered around the use of an inflight exerciser developed by Dietlein and Rapp of NASA Manned Spacecraft Center (ref. 23). The crew used the exerciser with a program of isometric and isotonic exercises to provide a means of determining the response of the cardiovascular system to a calibrated work load on a day-to-day basis.

Results of a previous study of the effect of calibrated exercise on bone density during recumbency (ref. 13) support the possibility that the exercise program, in combination with the improved dietary intake, contributed to the low bone density losses by the Gemini 7 astronauts. To test this hypothesis, two similar 14-day bed rest studies were conducted at TWU with the same subjects. The first bed rest period included no exercise, while an exercise program was followed during the second. There was a period of reconditioning, with the subjects ambulatory, between the two bed rest periods. The exercise program was carried out four times daily, as in the Gemini 7 orbital flight, and followed the same routine with the same exercisers. The bone density losses in the os calcis during the bed rest period with exercise were significantly lower than during the bed rest with no exercise ($p < 0.05$). In addition, the sum of the urinary and fecal calcium excreted by the subjects during during the first bed rest period was significantly higher than that during the second bed rest period, which included exercise ($p < 0.01$).

It should be emphasized that accurate determinations of fecal calcium have shown that urinary calcium has not been the major avenue of calcium loss in balance studies. The amount of calcium in the feces has averaged 70 percent of intake in recumbency studies when the subjects received 1,000 mg of calcium daily. The percent of fecal calcium has been found to increase when higher levels of calcium were ingested per day.

The dietary intake of calcium during the two bed rest periods was constant and was similar to that of the Gemini 7 astronauts. Diet, therefore, was ruled out as a variable contributing to the lower losses in bone density following exercise.

BED REST STUDIES PRIOR TO APOLLO

Because programmed exercise appeared to reduce bone density loss in the astronauts of Gemini 7, the Manned Spacecraft Center planned a carefully controlled bed rest study, involving exercise, at TWU before the Apollo 7 and 8 missions. If from study results it seemed advisable, special exercisers could be provided on these flights.

The six subjects, lying in a horizontal recumbent position, first underwent a 28-day bed rest period with no exercise. As in previous TWU bed rest studies, bathing, tooth brushing, and all other hygienic needs were cared for by trained male orderlies around the clock, and all meals were spoonfed by dietitians. Radiographs of the foot and hand were taken daily. The subjects then underwent an interim equilibration period followed by a second bed rest period, which included exercise, and finally by an ambulatory reconditioning period.

Throughout the study, including the exercise period, the subjects were in a completely recumbent, supine position.

The Exercise Program

The Exer-Genie and hand gripper were used, with the Exer-Genie set at 8 lb during bed rest. The program included the following steps:

1. Isometric exercise with strap of Exer-Genie pulled up to the chest, with subject stretching, 10 sec
2. Leg exercise (Exer-Genie), 6 min
3. Rest, 2 min
4. Hand, fingers squeezing gripper, 1 min
5. Rest, 2 min
6. Isometric exercise (Exer-Genie), 10 sec
7. Arm exercise (Exer-Genie), 6 min
8. Rest, 2 min
9. Hand, fingers squeezing gripper, 1 min
10. Rest, 2 min
11. Isometric exercise (Exer-Genie), 10 sec
12. Leg exercise (Exer-Genie), 6 min

    Total isometric exercise, 30 sec

    Total isotonic exercise, 20 min

During the bed rest period in which the exercise program was administered, three men chose to exercise four times daily under direction, with time kept by a metronome while the Exer-Genie was used. The other three men chose to exercise at will, calling the director whenever they decided to use the exerciser, and continuing for as long or as short a time as they desired.

Results

A comparison of the differences in bone density changes during Bed Rest I (no exercise) and Bed Rest II (exercise programmed or "at will") based on results from the os calcis, is shown in the following:

Subject	*Change during Bed Rest I, No Exercise, %*	*Change during Bed Rest II, Exercise, %*
Programmed exercise		
BB	– 10.7	– 0.295
FF	– 15.7	– 0.21
HH	– 12.3	– 3.20
"At Will" exercise		
GG	– 8.7	– 11.98
AA	– 5.7	– 0.780
EE	– 10.2	– 10.03

The results of metabolism tests made on these men were the same as those in the Gemini 7 tests. The diet was kept constant throughout the bed rest study so that exercise constituted the only variable. Highly significant differences were found among the men who exercised regularly, both for decreases in bone density loss and increases in excretion of calcium and phosphorus. The maintenance of bone mineral in the foot bones surpassed that in the hands, wrist, and lower arm, even of those who exercised. Undoubtedly, this was because the exercise did not involve the hands as much as the feet. Even with respect to the hands, however, the regular exercise group surpassed those who exercised only to a limited extent.

APOLLO MISSION FINDINGS

As a consequence of the results obtained with the bed rest study exercise program, the Exer-Genie was placed aboard the Apollo 7 and 8 spacecrafts and the astronauts of both missions were briefed concerning its use.

Apollo 7

Bone Density Table 3.2 and Figure 3.2 summarize the bone density changes calculated through the prelaunch, the flight, and the postrecovery periods, as well as the differences between the initial and the final tests for all major anatomical sites for each astronaut of Apollo 7.

Astronaut 1. Table 3.2 shows that Astronaut 1 exhibited some minor changes in the various skeletal sites prelaunch, with the changes ranging from less than + 1.0 percent to – 4.92 percent; the latter in a section across the distal end of the ulna.

During the flight period, astronaut 1 sustained somewhat greater losses in every site than were found during prelaunch; the greatest negative change being found in hand phalanx 4-42 (–9.30 percent). During the postrecovery period, all changes in bone density were positive. Moreover, the final bone density status of every skeletal site considered surpassed that of the initial status of the same site. In short, all bone mineral lost during the mission was regained by the end of the post-recovery period.

Astronaut 2. This astronaut experienced a very mild illness during the prelaunch period and lost some bone density in five of the seven anatomic sites tested. During the flight period, he

regained all of the bone density lost. This peculiar phenomenon is discussed later. During the post-recovery period this man gained bone mineral in all skeletal sites except one, and had regained initial bone density status, except in one site, by the close of the study.

Astronaut 3. This astronaut suffered losses in two sites during the prelaunch period. During the flight, he sustained some losses in the wrist, hand, and lower arm, but none in the foot. During the postrecovery phase of the study, astronaut 3 regained lost bone density in all anatomic sites, with marked increases in some skeletal locations tested.

Exercise. None of the Apollo 7 astronauts kept a log of his exact exercise activities. From information given by them during debriefing and personal conversations, however, the three men appeared to rank as follows in the extent to which they exercised during flight.

Rank	*Astronaut*
1	Astronaut 2
2	Astronaut 3
3	Astronaut 1

This ranking is consistent with the findings in Table 3.2 and in Figure 3.2, and discussed earlier. The findings also point up an important conclusion concerning the different results found in the feet and hands of astronauts 2 and 3. The hand gripper used in the preliminary bed rest study was found to be made of inflammable material and therefore was not put aboard the space vehicle. As an alternative, the astronauts were instructed to flex their fingers periodically, preferably while grasping a small rod or a pen or lead pencil. Conversations with the men indicated that astronaut 2 followed this advice to a greater extent than did the others.

At the close of the study, when the final bone density evaluations were compared with those made in the prelaunch test, the values in all anatomic sites, except two minor values close to or below the reproducibility of the method (±0.75 percent, ref. 20), together with the hand phalanx of astronaut 2, met or exceeded the prelaunch bone density levels (Table 3.2). In view of the general loss of bone density in most skeletal sites of astronaut 2 during the prelaunch period, it is remarkable that this subject made slight positive gains during flight in all sites. The bone density level of his hand phalanx 4–2 was higher at the close of the study than at the launch date, and was higher than the mean of the prelaunch values for all three astronauts.

Apollo 8

Bone Density Table 3.3 summarizes the bone density values of the major skeletal sites evaluated for the three astronauts of Apollo 8. This table gives the computer readings for the designated skeletal sites at the designated test periods. The columns on multiple sections of the os calcis contain the values for the sums of the various sections, based on 41 scans for astronaut 4, 47 for astronaut 5, and 39 for astronaut 6. The number of sections depended on the respective bone sizes.

The data for multiple sections of hand phalanx 4–2 also consist of the sums of sections across the indicated bone. All other values related to the computer counts are scanned across one section of bone with the width of the densitometer scanning beam.

Astronaut 4. Table 3.3 shows that during the prelaunch period, astronaut 4 suffered negative changes in bone density only in the hand, wrist, and distal ends of radius and ulna. This astronaut also exhibited negative changes in every anatomic site tested during the mission. During the post-recovery period, the direction of bone density change was reversed and positive changes occurred at all sites. At the close of the study, his bone density status was as good as, or better than, it was when the first prelaunch roentgenogram was taken.

Astronaut 5. This astronaut showed negative changes in bone density in all skeletal sites during the prelaunch period, with even larger losses in some sites during the flight period. During the postrecovery period, however, he gained markedly in bone density in all sites, reaching final values equal to those of the first test in all skeletal locations except one, in which the negative value was very small.

Astronaut 6. This astronaut showed negative changes in bone density throughout the anatomic sites investigated during the prelaunch period, and continued to lose during the mission itself. During postrecovery, he gained significantly in all sites. At the close of the study, his skeletal density was virtually the same as at the beginning of the study in four sites, with some gains in three locations.

The highest negative change in any skeletal site during flight was: –9.60 percent in the central wrist bone for astronaut 4; –12.41 percent in the distal end of the ulna for astronaut 5; and –16.17 percent, also in the distal end of the ulna, for astronaut 6.

It should be noted that, in all anatomic sites tested for bone density by the roentgenographic method in this study, none of the three astronauts was lower at the close of the study than at the beginning. Further, the time that elapsed before the final tests was not necessarily the number of days required for a return to the initial bone density status. The three men were traveling for different periods of time, and the dates of the final X-rays were those on which the men were available. Note, however, that on January 2, 1969, six days after recovery, when all the men were tested, none had fully recovered lost bone mass.

Exercise The Apollo 7 astronauts exercised with reasonable regularity, while those of Apollo 8 used the exercisers very little, if at all. A comparison of the changes in bone density during the periods of the two missions show distinct differences between the two groups of men (Table 3.4).

Numerous investigators have shown that "pull" on the muscles related to bone produces a stress that stimulates circulation in the bone, and thereby assists in the transport of nutrients needed to maintain the integrity of the skeleton (refs. 24–30).

COMPARISONS OF GEMINI 7 AND APOLLO 8 MISSION FINDINGS

It is interesting to compare the bone density level of the central os calcis of astronauts Borman and Lovell, who participated in both Gemini 7 and Apollo 8 missions. Comparisons of the relevant data, given below, are astonishingly close. The same similarities in the final values after the post-recovery periods for Gemini 7 and Apollo 8 have been discovered for other sites.

		Integrator Counts
Borman		
Initial bone density	Gemini 7, 11/24/65	11,973
Initial bone density	Apollo 8, 12/7/68	11,900
Final bone density	Gemini 7, 1/3/66	12,823
Final bone density	Apollo 8, 1/23/69	12,429
Lovell		
Initial bone density	Gemini 7, 11/24/65	13,367
Initial bone density	Apollo 8, 12/7/68	13,093
Final bone density	Gemini 7, 1/3/66	13,984
Final bone density	Apollo 8, 1/23/69	13,425

SUMMARY

For each of the three astronauts of the Apollo 7 mission, seven skeletal sites were evaluated in a total of approximately 100 sections at each test. In these tests, astronaut 1 sustained losses during orbital flight, at each site, in amounts ranging from –3.02 to –9.30 percent. Astronaut 2 made slight gains in bone density during flight in all seven skeletal locations. Astronaut 3 experienced minor gains in the bone density of sections of the os calcis and talus, with losses ranging from –3.41 to –6.50 percent in the skeletal sites evaluated in the hand, wrist, and distal bones of the lower arm.

In the Apollo 8 mission, approximately the same number of sections of bone were scanned as for the Apollo 7 astronauts; the exact number of sections evaluated for each man depended on the bone sizes.

Astronaut 4 experienced losses during the flight period in all skeletal sites evaluated, in levels ranging from –2.13 to –9.60 percent. Astronaut 5 similarly showed bone density losses in all sites varying from –2.81 to –12.41 percent. Astronaut 7 exhibited losses ranging from –2.93 to –16.17 percent in the seven anatomic sites evaluated. In most cases, losses were greater in the hand, wrist, and distal bones of the lower arm than in the feet. Negative changes in bone density of the astronauts of Apollo 8 were markedly greater than in those of Apollo 7, apparently because of the greater regularity of exercise by the Apollo 7 crew.

Throughout the study of both missions, losses in bone density in the hand, wrist, and lower arm tended to surpass that in the feet, even when exercise was taken. An exception was found in the case of one man who regularly exerted pressure on the palms of the hand and the finger in an exercise routine.

The value of exercise in a collateral study involving extreme recumbency in strictly controlled, horizontal bed rest tended to support the conclusion that exercise was at least one of the major causes of the difference in bone density loss in the two Apollo missions studied. The beneficial effect of exercise to the skeletal system during weightlessness is believed related to "pull" on the muscles attached to bone which, in turn, stimulates circulation through the bone.

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Table 3.1 Changes in bone density at major anatomic sites of Gemini 5 and 7 astronauts (percent)

	Gemini 5 (8/21/65-8/29/65)		*Gemini 7 (12/4/65-12/18/65)*	
Section Evaluated	*Command Pilot*	*Pilot*	*Command Pilot*	*Pilot*
Conventional os calcis section	−15.10	−8.90	−2.91	−2.84
Multiple os calcis sections	−10.27	−8.88	−2.46	−2.54
Section of talus	−13.24	−9.87	−7.06	−4.00
Multiple sections of hand phalanx 5-2	−23.20	−16.98	−6.78	−7.83
Multiple sections of hand phalanx 4-2	−9.86	−11.80	−6.55	−3.82
Section of capitate	−17.10	−16.80	−4.31	−9.30

Table 3.2 Changes in specified skeletal sites in bone density of Apollo 7 astronauts at designated study periods (percent)

Period	*Central Os Calcis*	*Multiple Os Calcis*	*Central Talus*	*Capitate*	*Distal Radius*	*Distal Ulna*	*Hand Phalanx 4-2*
Astronaut 1							
9/27 to 10/11/68 (prelaunch)	+0.81	+0.43	+0.25	−2.29	−2.53	−4.92	−3.32
10/11 to 10/22/68 (flight)	−5.53	−4.10	−3.60	−4.07	−3.25	−3.02	−9.30
10/22 to 11/1/68 (postrecovery)	+8.75	+5.59	+4.96	+11.96	+14.32	+17.45	+15.32
9/27 to 11/1/68 (overall study period)	+3.69	+1.69	+0.93	+4.94	+7.81	+8.30	+8.31
Astronaut 2							
9/27 to 10/11/68 (prelaunch)	−10.26	−9.70	−7.98	−1.10	+0.92	+4.76	−8.85
10/11 to 10/22/68 (flight)	+0.74	+1.19	+1.75	+3.31	+3.34	+2.12	+2.04
10/22 to 11/1/68 (postrecovery)	+9.63	+9.10	+6.34	−0.74	+2.55	+1.01	+3.13
9/27 to 11/1/68 (overall study period)	−0.88	−0.32	+0.43	+1.42	+6.95	+8.29	−4.08
Astronaut 3							
9/27 to 10/11/68 (prelaunch)	+1.42	−0.05	−1.35	−3.05	−8.83	−0.77	+0.33
10/11 to 10/22/68 (flight)	+2.27	+0.85	+2.89	−3.44	−3.64	−3.41	−6.50
10/22 to 11/1/68 (postrecovery)	+2.66	+5.35	+3.92	+7.06	+4.55	+1.28	+4.27

Table 3.3 Changes in specified skeletal sites in bone density of Apollo 7 astronauts at designated study periods (percent

Period	*Central Os Calcis*	*Multiple Os Calcis*	*Central Talus*	*Capitate*	*Distal Radius*	*Distal Ulna*	*Hand Phalanx 4-2*
Astronaut 4							
9/27 to 10/11/68 (prelaunch)	−0.37	+3.00	+1.69	−5.76	−6.81	−9.23	−1.79
10/11 to 10/22/68 (flight)	−2.13	−7.08	−2.62	−9.60	−8.76	−6.42	−2.19
10/22 to 11/1/68 (postrecovery)	+7.12	+12.12	+7.27	+18.71	+17.79	+18.93	+6.63
9/27 to 11/1/68 (overall study period)	+4.45	+7.31	+6.22	+1.13	+0.15	+1.02	+2.43
Astronaut 5							
9/27 to 10/11/68 (prelaunch)	−3.02	−4.59	−5.15	−3.11	−1.46	−4.16	−7.32
10/11 to 10/22/68 (flight)	−6.95	−6.04	−2.81	−12.11	−11.06	−12.41	−2.41
10/22 to 11/1/68 (postrecovery)	+13.63	+9.97	+11.52	+17.60	+14.15	+22.87	+10.72
9/27 to 11/1/68 (overall study period)	+2.54	−1.41	+2.79	+0.14	+0.04	+3.14	+0.14
Astronaut 6							
9/27 to 10/11/68 (prelaunch)	−8.88	−2.51	−5.31	−4.72	−7.22	−8.06	−9.13
10/11 to 10/22/68 (flight)	−2.93	−6.50	−3.18	−6.65	−11.39	−16.17	+4.81
10/22 to 11/1/68 (postrecovery)	+16.95	+12.72	+19.69	+13.25	+21.98	+29.34	+5.00
9/27 to 11/1/68 (overall study period)	+4.57	+2.74	+9.73	+0.73	+0.23	−0.33	+0.004

Table 3.4 Changes in bone density at major anatomic sites of Apollo 7 and 8 astronauts during flight (percent)

	Astronaut 1	Astronaut 2	Astronaut 3
Apollo 7			
Central os calcis	-5.32	+0.74	+2.27
Multiple os calcis	-4.10	+1.19	+0.85
Central talus	-3.60	+1.75	+2.89
Hand phalanx 4-2	-9.30	+2.04	-6.50
Capitate	-4.07	+3.31	-3.44
Distal radius	-3.25	+3.34	-3.64
Distal ulna	-3.02	+2.12	-3.41
Apollo 8			
Central os calcis	-2.13	-6.95	-2.93
Multiple os calcis	-7.08	-6.04	-6.50
Central talus	-2.62	-2.81	-3.18
Hand phalanx 4-2	-2.19	-2.41	+4.81
Capitate	-9.60	-12.11	-6.65
Distal radius	-8.76	-11.06	-11.39
Distal ulna	-6.42	-12.41	-16.17

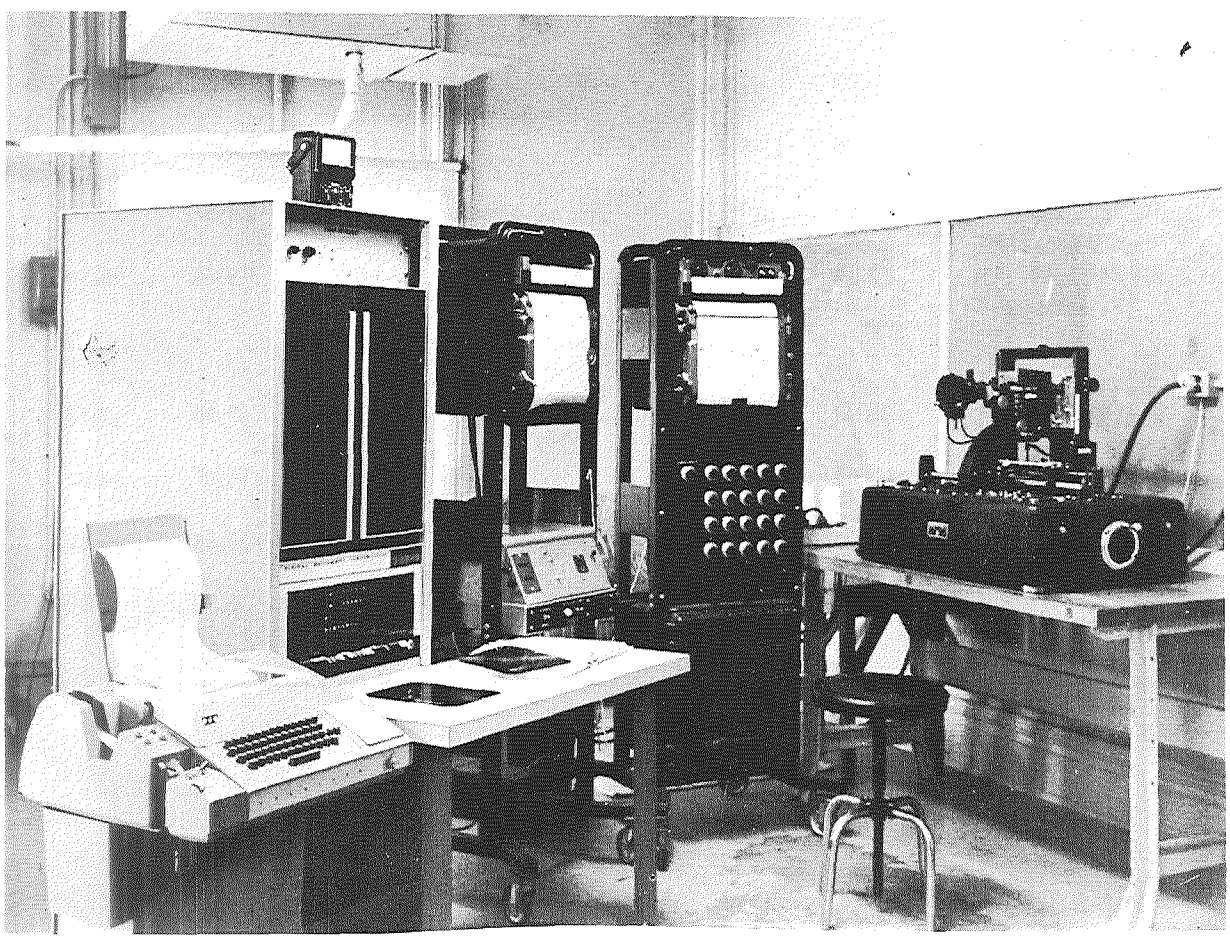

Figure 3.1 Digital-analog computer assembly.

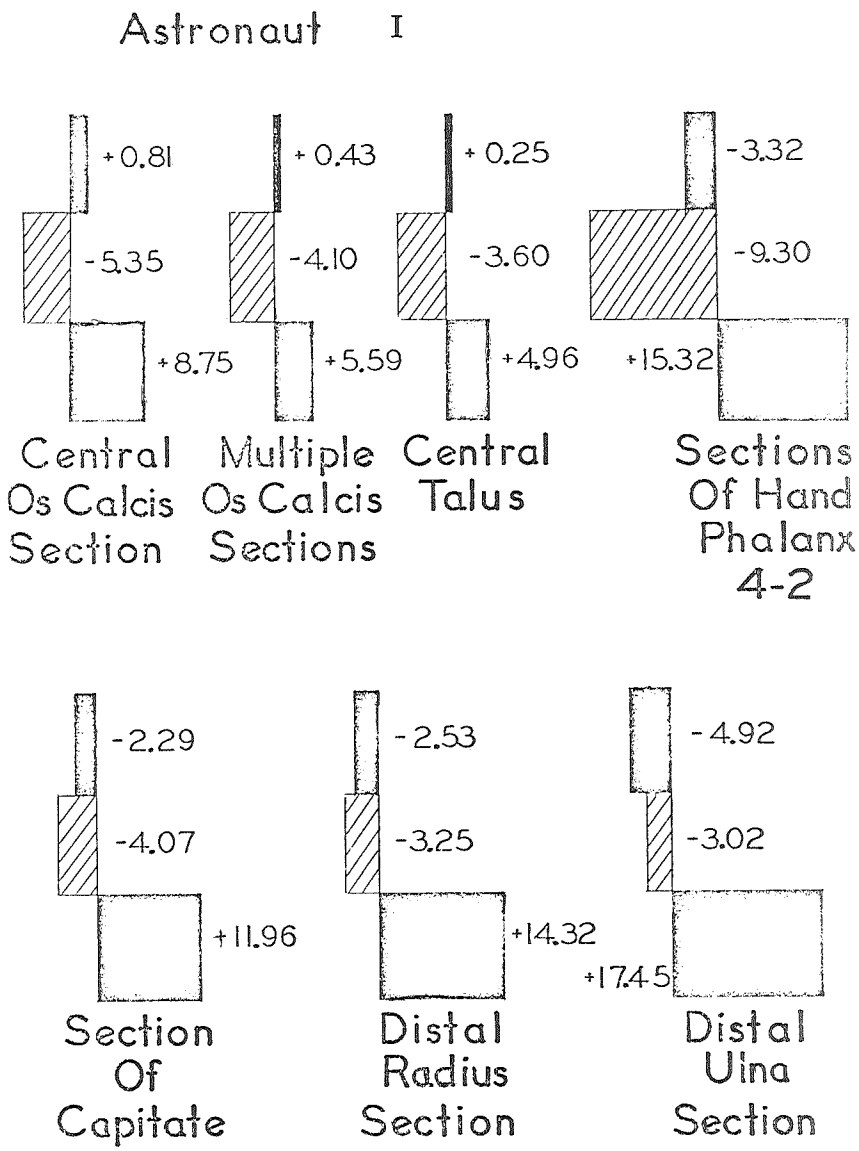

Figure 3.2 Percent change in overall bone density values in seven skeletal sites during (a) prelaunch, (b) orbital flight, and (c) postrecovery of astronaut Schirra.

4 METABOLIC STUDIES OF THE GEMINI 7 14-DAY ORBITAL SPACEFLIGHT

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INTRODUCTION

This report describes an effort to perform complete metabolic balance studies of two astronauts during a 10-day preflight control phase, 14 days of orbital spaceflight (NASA Gemini 7), and a 4-day postflight recovery phase. The studies included measurement of dietary intakes and excretions of calcium, magnesium, phosphate, sulfate, nitrogen, sodium, potassium, and chloride; urinary excretions of 17-hydroxycorticosteroids, aldosterone, and catecholamines were also measured.* Study conditions were far from auspicious for precise and definitive results, but the information gained has great potential importance.

In the environment of spaceflight development to date, with its necessary initial emphasis on engineering technology, only fragmentary data bearing on metabolic function have been obtained on the several flights of both the United States and Russia. Previous inflight measurements consisted of very few urine collections. Collections from the astronaut of Mercury MA-9, a flight of 34 hr, revealed no changes in any of the constituents measured, including calcium. To the best of our knowledge, the Russians have made no collections of any kind. Thus, the 14-day Gemini 7 flight constitutes the first, and to date only, effort to carry out a detailed, extensive metabolic study.

This unique study had to be planned within the rigorous constraints of the technical characteristics of the flight itself. These characteristics included astronaut training, geographic and temporal aspects of the flight plan and schedule, and the limited volume of the space vehicle. In addition, the medical observations, including certain hematologic and cardiovascular measurements, as well as the metabolic study, were but a part of the total effort, including extensive operational and physics-oriented experimental activities required of the astronauts before, during, and after the flight.

Among the possible effects of long-term spaceflight on human physiology, the metabolic response of the musculoskeletal system was considered a probable major stress reaction, with weightlessness the principal physical stress. For this reason, a number of ground-level studies of normal subjects (principally on the effects of bed rest) were done in an attempt to predict the

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* These data were presented in preliminary form by Dr. Lutwak at the annual meeting of the Endocrine Society in June 1966, six months after the flight, and the definitive paper (ref. 1) has just appeared.

effects of weightlessness on nitrogen and mineral metabolism. It is well to bear in mind, however, that spaceflight involves many additional actual or potential physical stresses such as hyperoxia, diminished atmospheric pressure, changes in levels of physical activity and dietary intake, and, possibly to a lesser degree, changes in atmospheric temperature, sound levels, vibration, and circadian rhythms. Psychologic stresses on physiology must also be considered.

Several studies of immobilized or inactive bed rest, as the nearest simulation to weightlessness for long-term measurements, are discussed in later sections. For this presentation, useful background information is provided in figure 4.1; the balance chart for calcium of the Cornell Study (ref. 2) of the 1940s; and table 4.1, which shows the percentile change in urinary calcium in the normal subjects during the second week in plaster casts in bed. The only other particularly relevant study is that of Lynch et al. (ref. 3), whose investigation of the role of simulated altitude in modifying the effects of bed rest showed that urinary losses of calcium were significantly less with the decreasing atmospheric pressure of higher altitudes.

PROCEDURE AND METHODS

The astronauts in this study were two 37-year-old healthy males in an excellent state of physical fitness. As part of astronaut training, the subjects engaged daily in vigorous *physical exercise,* including calisthenics, for approximately 1 hr, and running up to approximately 2 mi. A similar level of activity was resumed the second day after flight. During flight, the astronauts were mainly occupied in manual tasks required for controlling the spacecraft, observing the physical science experiments, keeping records, etc. A program of exercise was planned around a Bungee spring-cord exerciser, which provides approximately 70 lb tension (through a distance of 1 ft) on arm, back, and leg muscles; the program included one pull per second for 30 times, three times a day during flight, but it was not rigorously followed because of the press of other activities.

In addition to weightlessness, the traveling spacecraft provided several other physical *environmental conditions* different from earth environment: atmospheric pressure of 5 psi (one-third of earth sea level), 100 percent oxygen (approximately 258-mm partial pressure versus earth sea level of approximately 159 mm), very high sound levels and marked vibration on liftoff, silence through remainder of flight, periods of night and day alternating continuously in cycles of 90 min, and confinement within a volume of 40 ft^3 per man. Temperature and humidity were maintained well within the comfort range.

The preflight control phase began 12 days before the flight and continued for 10 days. Dietary control was maintained, but collections of excreta had to be interrupted for two days prior to liftoff because of the intensity of flight preparations at that time. This phase was carried out entirely at Cape Kennedy Space Center, primarily in the astronauts' quarters where special facilities were installed; flight training and preparation proceeded without significant interruption. The orbital, or inflight, phase lasted 14 days while the astronauts were orbiting in the Gemini 7 spacecraft. The postflight phase lasted four days; the first portion of this phase was carried out on board the recovery vessel for approximately 12 hr, and thereafter at the facility at Cape Kennedy.

Dietary Intake

To permit optimum interpretation of metabolic data on the effects of factors under study, diets through all three phases were as similar and constant in content and composition as possible. During the preflight phase, a diet, constant in composition, was planned by a metabolic dietitian and prepared and served under her supervision in the crew quarters. No additional snacks were permitted, and complete ingestion of prepared menus was reasonably well achieved. A three-day rotation of menus was set up for each individual. The actual intakes of the major components of the diet, as determined by analyses of duplicate menu items, are given in reference 1. Further details on dietary control during this phase are provided in reference 4.

Orbital dietary intake was controlled by the feeding of precooked, inflight foods of the Gemini program (ref. 5). Initially it was planned that each man would eat his meals in a predetermined sequence so as to provide identical 24-hr consumption of each of the dietary factors; however, because of the problem of management of food packs in the limited space of the cabin, the meals were eaten at random. Inflight calcium intake was maintained at a level close to that of control phases; but the intake of other elements, notably nitrogen and phosphorus, was considerably reduced in flight. To minimize the effect of adjustment on calcium balance, the subjects agreed to drink two glasses of milk daily, as regularly as possible, for the 5 months preceding the flight. The calcium intake selected for these studies was the level least likely to affect the amounts excreted in the urine.

Postflight menus were identical to those during the preflight phase and prepared under similar conditions. The mean values for intake of the major dietary constituents are given in table 4.2

Urine Collections

During the pre- and postflight phases, collection facilities, including individual refrigerators, labeled containers, and commodes, were established in the crew quarters and at various sites at Cape Kennedy where the crew was undergoing training and preparation.

Orbital flight collection was accomplished by a specially designed urine transport system that permitted calculation of total urine volume of each voided sample by means of tritium dilution. In brief, at each voiding, a condom-like receiver was fitted over the end of the penis from which urine passed through a valve into a 500-ml plastic bag containing a known quantity of tritium injected from the valve assembly at each voiding. After the astronaut mixed urine and tritium by kneading the collection bag, he turned the valve and expressed a urine aliquot into a 75-ml plastic sample bag. Thereafter, the valve was turned again and the remaining urine vented out of the spacecraft. A fourth and final turn of the valve reloaded the large collection bag with a sample of tritium and returned the valve to the open position. Because storage space was sharply limited, only 75 ml of each voided sample could be stored (in plastic bags, without refrigeration, but with benzoic acid as a preservative). Despite best efforts to design a satisfactory system for collection and aliquoting urine in the weightless situation, significant and frequent leakage and loss of samples occurred. This was not only detrimental to metabolic study but a source of chronic inconvenience to the astronauts. At the recovery of the spacecraft, an aliquot of each voiding was preserved by refrigeration for analysis.

Stool Collections

Carmine red and brilliant blue were given orally to the subjects for separation of stool periods. Pre- and postflight stool collections were carried out by using the previously described toilet facilities at Cape Kennedy. Orbital stool collections were made with specially designed, inflight plastic defecation bags; the entire sample was stored in the cabin without refrigeration but with a mixture of phenylphenolate derivatives in propylene glycol (provided by NASA) as a preservative.

Sweat Collections

During the pre- and postflight phases, two 24-hr sweat collections were carried out in each phase. During these periods, the subjects, after complete washing down of the entire skin surface, donned previously extracted flight underwear suits and wore them for a continuous 24-hr period. At the end of this period, another washing down was carried out. The wash water and suits were combined and the total fluid concentrated for analysis. During the orbital phase, a total 14-day collection was carried out with extraction of the inflight underwear removed immediately after recovery and combined with wash water of the whole skin surface.

Fluid Intake

Fluid intake was *ad libitum,* but the quantities ingested during the pre- and postflight phases were recorded. The majority of the fluid intake was obtained with the diet. Inflight fluid intake was estimated from a water-dispensing device on board the spacecraft; this information was not sufficiently precise to permit calculation of fluid balance data.

Details of the methods of specimen preservation and analyses are provided in reference 1.

RESULTS

The validity of any metabolic study rests heavily on the completeness of specimen collections for which investigators rely mainly in the expertness of their collection techniques and implementation. The 24-hr excretion of creatinine, though not exactly infallible, has long been a useful guide in evaluating 24-hr urine collection. The preflight values for 24-hr excretion of urinary creatinine were quite constant for each individual (except for the first two days of J. L.), indicating accuracy of collection procedures. During the orbital or inflight phase, however, the creatinine values were quite erratic (for both men, inflight variance was significantly greater than preflight variance), primarily due to difficulties in utilizing the inflight urine collection and transport system. Leakage occurred during collection of *many* specimens; some aliquots were lost; some aliquots possibly were mixed inadequately with tritium. It was decided that inflight urine volumes were not sufficiently reliable as a basis for calculation. Therefore, to provide data hopefully more indicative of the true state of metabolic indices, with due acknowledgment of possible error and with the necessary assumption that *renal clearance was not significantly altered by the space environment,* it was decided to correct all inflight urinary excretion values on the basis of presumed unchanged urinary creatinine excretion. This latter value was calculated as the mean of urinary creatinine excretion of the 10 preflight control days plus the 4 postflight control days for each of the two astronauts studies in flight. Thus, the urinary metabolic data of the inflight phase are all reported as corrected on the basis of measured versus expected 24-hr creatinine excretion.

Mineral Metabolism Data

Metabolic balance data for the two subjects are given in tables 4.3 and 4.4. Urinary excretion of calcium did not change significantly during the first 7 days of spaceflight in either man, but a definite increase occurred starting about the eighth day for astronaut F. B., and persisted during the 4 days of observation after flight. As shown in figure 4.2, the mean increase in urinary calcium during the second week in flight was 23 percent for F. B. and 9 percent for J. L.; the latter increase is not significant.

The phosphate date obtained for the astronauts showed an increase in urinary phosphate over the first 9 days of spaceflight, occurring during the time when dietary phosphate was half the control values. Thereafter, despite relatively constant dietary intake, urinary excretion dropped nearly to control values.

The net balance of calcium during flight (fig. 4.3) was distinctly less positive for both men: in the case of J. L., this was due to an increase in fecal calcium. Dermal losses of calcium, listed as "sweat," were low for both men in all phases and slightly higher during the relatively inactive postflight recovery days. Despite decreased fecal excretion, the phosphate balances became more negative during the flight, returning to control levels during the recovery phase.

In both astronauts, urinary nitrogen fell during flight and returned to preflight values during the postflight phase. Dietary nitrogen was significantly less during the flight, with the result that nitrogen balance became negative during this phase. Sulfate excretion data tended to resemble those of nitrogen.

Changes in magnesium balance and urinary excretion were similar to those for calcium (fig. 4.4)

Electrolyte Data

In potassium metabolism, response varied between the two astronauts (fig 4.4). F. B. showed an initial decrease in urinary potassium inflight in the presence of a marked decrease in dietary potassium. During the second week of flight, urinary potassium rose (a change that correlated with a simultaneous modest decrease in urinary sodium). Immediately postflight, in F. B., potassium excretion fell to preflight values as the dietary intake was increased. J. L., on the other hand, demonstrated only a slight fall in urinary potassium in the first week of flight despite a marked restriction in intake. During the second week, his excretion decreased further and then rose to preflight values during the recovery phase.

The two astronauts studied showed different patterns of urinary sodium excretion (fig. 4.5). In F. B., despite a slight decrease in dietary sodium, there was natriuresis during the first week of flight, a return to control values during the second week, and significant sodium retention in the early postflight period. Conversely, J. L. demonstrated no change in sodium excretion during the first part of the spaceflight, an increase thereafter, but then, similary to F. B., marked sodium retention postflight. The salient feature of urinary chloride excretion was a marked reduction during the first 10 days of flight in F. B., and during the recovery phase in J. L.

Hormone Excretion

The urinary excretion of metabolites of adrenal gland hormones showed very definite changes in relation to the flight (figs. 4.6–4.8). In J. L., epinephrine excretion and, more variably, norepinephrine excretion were highest on the two days of greatest predicted "stress"—liftoff and splashdown. In F. B., catecholamine excretion approximated this pattern, but the values were not significantly different from the control, preflight phase. The excretion of 17-hydroxycorticosteroids, regarded as representing chronic adaption to stress, was surprisingly low during the entire orbital flight phase. In both subjects, this measurement was elevated on the day of splashdown. The few values obtained for urinary aldosterone were elevated during and immediately following flight.

Blood Analyses

Chemical analyses of blood have been reported by Dietlein and Harris (ref. 6). Analyses of pre- and postflight serum and plasma for calcium, phosphorus, sodium, potassium, chlorine, urea nitrogen, total protein, and albumin showed no changes as a result of the flight. Immediately postflight, plasma 17-hydroxycorticosteroids were elevated and plasma uric acid slightly reduced.

DISCUSSION

The principal goal of these studies was to measure changes, if any, in the total body metabolism related to the musculoskeletal system as a result of a period of weightlessness in space. The presence of additional stresses, besides weightlessness, has been clearly indicated. There were many problems, some foreseen and others not, associated with the conduct of this experiment. These problems may account, in part, for some of the differences in metabolic responses between the two subjects. Despite these inadequacies, however, the "experiment" was of value in that it represented the first attempt to obtain information on possible metabolic changes in man during spaceflight.

A crucial point in connection with the relative validity of the data is the correction of the inflight, or orbital, urinary excretion values on the assumption of unaltered 24-hr renal clearance and urinary excretion of creatinine, ascribing the low and variable urinary creatinine values mainly to *known* incomplete and variable urine collections in flight. This correction procedure cannot be validated short of a repeat study with accurate collections that might rule out the possibility of decreased renal clearance. The lowered inflight creatinine value cannot be explained partly on the basis of muscle wastage, for during the several weeks of extreme immobilization of bed rest in casts (ref. 2), urinary creatinine did not decline. This latter finding may also be cited as some reassurance that glomerular filtration rate (GFR) and creatinine clearance do not change greatly, if at all, in weightlessness; GFR increases briefly on shift of body position from vertical to horizontal on earth and it may decrease slightly during sleep, but a characteristic of GFR is its ability to adjust promptly to the initiation of stresses and return to its original level.* That the correction was reasonable was supported by the pattern of urinary calcium excretion. The intake of this element was least changed between control and experimental phases, and its urinary

* Since this paper was submitted, creatinine clearances have been measured weekly in three normal subjects before, during, and after 30 weeks of bed rest; they did not change (C. S. Donaldson, unpublished observation).

excretion is only sluggishly alterable by most influences, including dietary. The corrected inflight values for urinary calcium during the first few days of flight were closely similar to those preflight; those near the end of flight were at the same level as, and certainly no higher than, the recovery phase values. For J. L., the recovery phase values were virtually the same as preflight, and for F. B. they were above the preflight values. Finally, correction on the basis of urinary creatinine was the only method available in this situation for obtaining meaningful data. The conclusions must be tempered by this clearly stated reservation.

Calcium Metabolism

The trend in urinary calcium excretion during flight was similar to that seen in immobilization but much less in degree. In the Cornell Study (ref. 2), mean urinary calcium increase over control levels during the second week of bed rest was 107 percent (ranging from 53 percent to 130 percent among the four subjects), whereas the increase for the same period was 23 percent for F. B. and 9 percent for J. L., the latter not significant. The changes in calcium balance were appreciable, however, assuming, as indicated by fecal nitrogen values, that fecal period separations between preflight control and inflight values were accurate.

Interpretation of this moderate negative shift involves consideration of the influences of various factors interplaying in this study. The bases for predicting that weightlessness would increase losses of calcium are evident. With regard to the gaseous atmosphere of the spacecraft, 100 percent oxygen at 5 psi, it has been shown in tissue culture studies that high oxygen atmosphere leads to increased bone resorption and, therefore, hyperoxia might contribute further to losses of calcium. On the other hand, high altitude has been demonstrated to decrease or suppress the losses of calcium at bed rest, suggesting that decreased atmospheric pressure could be protective—a possible partial explanation for the modest degree of calcium loss in this study. In addition, the isometric exercise program and the continuous activity in the subjects' flight work may have acted further to decrease calcium losses. Finally, the recently observed direct relationship between calcinuria and dietary protein intake raises the possibility of another protective influence against urinary calcium loss, i.e., the inadvertent, sharp reduction in protein intake in flight in this study. In view of the expected individual variability of response to each of these influences—weightlessness, high oxygen atmosphere, high altitude, exercise, and dietary protein reduction—the differences between F. B. and J. L. may not be surprising. Clearly, many additional ground-based clinical and animal studies are needed to sort out the validity and relative importance of these various possible influences on calcium metabolism..

The Relationship of Phosphate and Nitrogen to Muscle

The marked increase in urinary phosphate excretion was one of the more striking changes observed, particularly since it occurred in spite of an inflight decrease in phosphate intake of approximately 1 g/day. This change was reminiscent of the early peak of urinary phosphate excretion with nitrogen in immobilization (ref. 1) and must have reflected some significant metabolic derangement. Since the loss of calcium was modest, focus turned from bone to muscle. Although urinary sulfate and nitrogen excretion were less in flight than in control, they did not fall nearly as much as would have been expected from the uncontrollable decrease in dietary intake of these two elements. This observation suggests the loss of substantial muscle tissue, corroborated subjectively by one of the astronauts who commented on the "flabbiness" of his leg muscles on his return to Cape Kennedy.

Electrolyte Metabolism

Urinary sodium is a function of dietary intake, aldosterone activity, and glucocorticoid secretion; fecal losses of sodium usually are very small and relatively constant. Correlation of the observations of urinary sodium excretion with measurements of urinary adrenocortical metabolites suggested a relationship to 17-hydroxycorticosteroid but not to aldosterone excretion.

Urinary excretion of potassium may reflect protein metabolism, aldosterone secretion, and glucocorticoid action. There is no ready explanation for the variability between the two astronauts in their response to potassium excretion.

J. L. showed a pattern of chloride excretion parallel to that of sodium excretion, while F. B. excreted chloride in parallel with potassium. The reason for this discrepancy was not apparent. Balances of chloride were not calculated because of technical difficulties in measurement of dietary chloride.

Hormone Excretion

One of the most striking findings in the study was the consistently low urinary 17-hydroxycorticosteroid values during flight. The expected high values were seen on the first postflight day. Since deterioration of corticosteroids in unrefrigerated urine, with or without benzoic acid crystals, is generally less than 1 mg/24 hr over a 2-week period, * the observation in flight appears to be valid. Urinary sodium excretion was well correlated with 17-hydroxycorticosteroid excretion for both men; sodium retention occurred with the increased excretion of these hormonal metabolites, as would be expected. Various physiological bases for the low inflight corticosteroids may be listed:

1. Suppression of the hypothalamo-pituitary-adrenal axis (unlikely in view of the brisk rise in urinary values demonstrated by both crewmen immediately on reentry)
2. *In vivo* steroid interconversions, either enzymatic or mechanistic and related to altered transcortin levels (not measured)
3. Alterations in renal tubular function (not measured)
4. A defect in erythrocyte membranes due to pO_2 excess, allowing steroid entrapment within red blood cells (a speculative possibility since erythrocyte fragility was indeed increased)
5. Losses of steroids in sweat (but even in induced sweating, losses are minimal, rarely exceeding 4–8 μ/100 ml of eccrine sweat)

In brief, of these possible causes, two are unlikely and the others have not been studied in sufficient depth to explain the experimental findings.

In J. L., the pattern of catecholamine excretion was reasonably well correlated with times of greatest stress—the beginning and end of the flight. J. L.'s data also showed a correlation between the corticosteroids and both potassium excretion and the ratio of urinary sodium to potassium. The urinary excretion of aldosterone did not correlate with electrolyte excretions.

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* This point was kindly checked for us, through a closely similar method, by E. M. Cotlove, D. Young, and D. Dorow, Clinical Center, Department of Clinical Pathology, National Institutes of Health.

CONCLUSION

In view of the numerous influences of many kinds in spaceflight, notably 100 percent oxygen atmosphere, one-third atmospheric pressure, and relatively uncontrolled physical activity, in addition to weightlessness, the metabolic effects of weightlessness per se could not be determined in this study. The various metabolic changes observed undoubtedly represented the net effect of several different concurrent and counteracting factors, predominantly physical in nature. Determination of the true effects of weightlessness will require the careful sorting out of the effects of these other factors in appropriate, well-controlled, ground-based studies.

Within the rather broad limits of precision of this first metabolic study in space, the changes in calcium metabolism and in other indices were sufficiently modest to support, from the metabolic point of view, the decision that a voyage to the moon and return would be medically safe, since the time involved would be no more (and, in fact, less) than was assigned to Gemini 7. For assessment of the physiological safety and performance of astronauts on future, much longer flights, however, additional space metabolic observations must be planned with better control, despite operational constraints. Such studies will provide more reliable information for accurate prediction of the extent of mineral and other metabolic changes to be expected in long-duration spaceflight, and a basis for judging the necessity for development and assessment of corrective or protective measures.

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Table 4.1 Effects of immobilization on urinary calcium (7-day periods)

	Experimental Periods (mg/24 hr)				
Subjects	*Mean of Last Two Control Periods*	*1st Immobilization*	*2nd Immobilization*	*Increase of 2nd Immobilization over Mean of Control*	*Increase, Percent*
E.M.	42	85	87	45	107
C.O.	116	200	254	138	119
A.S.	120	162	184	64	53
S.W.	211	322	486	275	130
Mean	122	192	253	131	107

Source: Reference 2.

Table 4.2 Summary of daily dietary intake

Phase	*Calcium, g*	*Phosphate, g*	*Nitrogen, g*	*Calories*
F.B.				
Preflight	1.103	2.548	24.78	2771
Orbital	1.042	1.362	15.81	1789
Postflight	1.102	2.424	22.82	2665
J.L.				
Preflight	1.108	2.373	22.33	2838
Orbital	1.042	1.362	15.81	1789
Postflight	1.090	2.376	22.02	2838

Table 4.3 Metabolic balance data, F.B.

Phase	Duration, days	Tests	Calcium, g*	Magnesium, g	Sodium, meq	Potassium, meq	Phosphate, g	Sulfate, g	Nitrogen, g	Chloride, meq
Preflight	10	Diet*	1.103	0.388	151.7	128.9	2.548	2.737	24.78	–
			±.044	±.011	±15.7	±22.8	±.239	±.368	±2.36	
		Urine*	.215	.117	172.4	98.9	1.323	1.344	22.83	145.3
			±.024	±.014	±16.8	±17.0	±.091	±.292	±2.65	±10.9
		Feces	.765	.221	3.0	7.9	.557	.182	1.78	18.4
		Sweat	.026	.007	24.7	10.4	.000	.004	0.19	0.1
		Balance	+.097	+.043	−48.4	+11.7	+.668	+1.207	−0.02	–
Orbital	14	Diet*	1.042	.198	145.1	36.8	1.362	.874	15.81	–
			±.251	±.040	±28.4	±8.9	±.161	±.163	±2.85	
		Urine*	.238	.129	196.3	93.4	1.741	1.254	17.90	65.7
			±.032	±.033	±41.1	±41.6	±.442	±.210	±2.27	±42.5
		Feces	.796	.115	2.3	1.1	.311	.127	1.31	1.4
		Sweat	.014	.006	18.6	6.9	.000	.003	0.03	0.2
		Balance	−.006	−.052	−72.1	−64.6	−.690	−.510	−3.43	–
Postflight	4	Diet*	1.102	.371	167.2	122.9	2.424	2.655	22.82	–
			±.111	±.052	±29.3	±20.9	±.292	±.276	±3.36	
		Urine*	.286	.093	140.1	90.3	1.563	1.689	25.34	126.7
			±.002	±.011	±34.8	±3.9	±.286	±.453	±3.97	±48.6
		Feces	.769	.148	6.5	9.6	.503	.124	1.21	0.3
		Sweat	.043	.015	12.0	11.0	.000	.004	0.26	15.1
		Balance	+.004	+.115	+8.6	+12.0	+.358	+.838	−3.99	–

* Means of daily values, ±sd

Source: Reference 1.

Table 4.4 Metabolic balance data, J.L.

Phase	Duration, days	Tests	Calcium, g*	Magnesium, g	Sodium, meq	Potassium, meq	Phosphate, g	Sulfate, g	Nitrogen, g	Chloride, meq
Preflight	10	Diet*	1.108	0.366	123.6	116.0	2.373	2.562	22.32	–
			±.048	±.018	± 9.8	±15.5	±.150	±.304	±1.44	
		Urine*	.159	.101	143.7	74.6	1.259	1.077	20.36	129.3
			±.017	±.015	±26.3	±7.6	±.133	±.433	±2.20	±23.0
		Feces	.431	.173	4.9	6.9	.407	.090	1.22	16.1
		Sweat	.023	.006	25.2	14.4	.000	.005	0.36	0.5
		Balance	+.495	+.086	−50.2	+20.1	+.707	+1.390	+0.38	–
Orbital	14	Diet*	1.042	.198	145.1	36.8	1.362	.874	15.81	–
			±.251	±.040	±28.4	±8.9	±.161	±.163	±2.85	
		Urine*	.162	.097	181.8	50.9	1.577	1.019	16.24	146.4
			±.019	±.016	±22.6	±6.3	±.155	±.102	±1.86	±22.1
		Feces	.766	.109	10.3	7.2	.289	.096	0.87	0.2
		Sweat	.016	.007	2.9	1.6	.000	.002	0.04	2.2
		Balance	+.098	−.015	−49.9	−22.9	−.504	−.243	−1.34	–
Postflight	4	Diet*	1.090	.359	125.3	117.6	2.376	2.588	22.02	–
			±.119	±.035	±30.4	±12.5	±.168	±.191	±2.48	
		Urine*	.172	.093	74.5	63.4	1.296	1.529	19.92	65.5
			±.014	±.006	±21.8	±14.1	±.542	±.104	±2.91	±26.8
		Feces	.766	.109	10.3	7.2	.289	.096	0.97	0.2
		Sweat	.045	.017	9.8	11.4	.000	.010	0.29	15.3
		Balance	+.107	+.140	+30.7	+35.6	+.791	+.953	+0.84	–

* Mean of daily values, ±sd

Source: Reference 1.

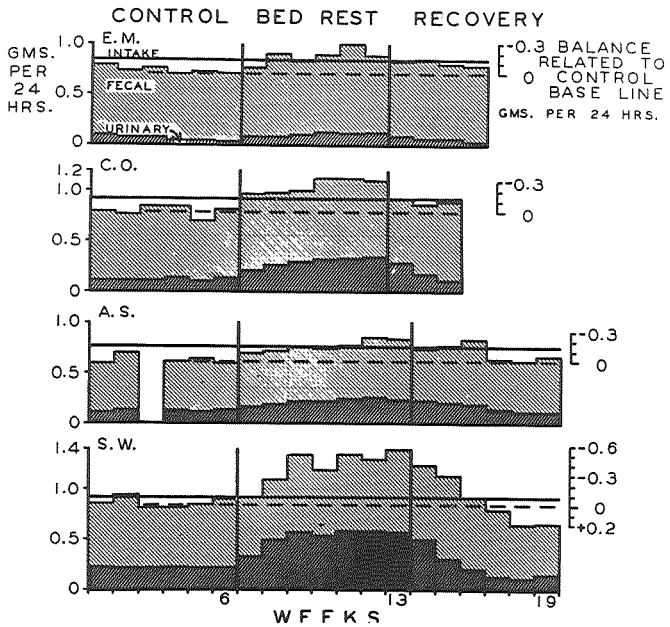

Figure 4.1 Effect of immobilization in plaster casts for 6 to 7 weeks on the calcium metabolism of four normal male subjects (ref. 2). In each subject the daily calcium intake was kept constant throughout all periods of the experiment. For each subject the control baseline (interrupted horizontal line) is an average of the total outputs of the last four control weeks. (Reproduced by permission of Medical Clinics of North America.)

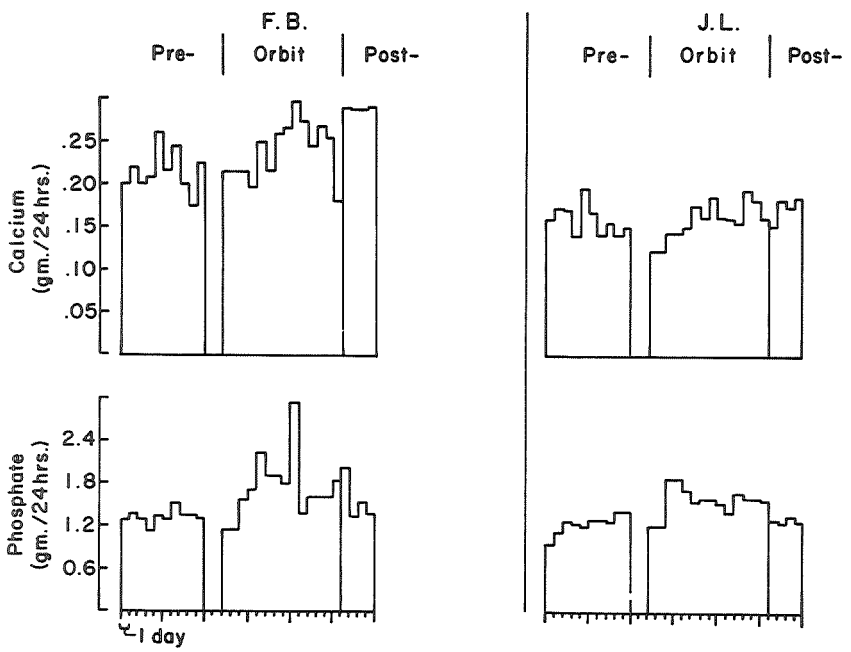

Figure 4.2 Urinary excretion of calcium and phosphate, astronauts F. B. and J. L., before, during, and after 14-day orbital spaceflight. (From reference 1, reproduced by permission of J. Clin. Endocrinol. and Metab. and J. B. Lippincott Co.).

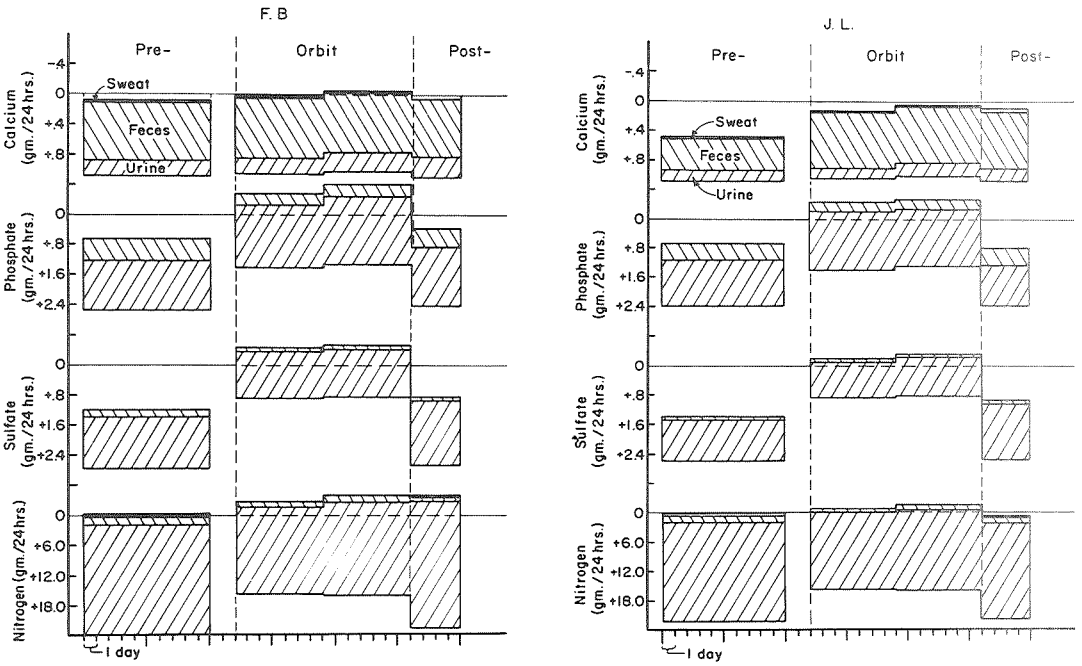

Figure 4.3 Metabolic balance data, plotted according to Reifenstein et al. for calcium, phosphorus, sulfate, and nitrogen; astronauts F. B. and J. L. before, during, and after 14-day orbital spaceflight. (From reference 1, reproduced by permission of J. Clin. Endocrinol. and Metab. and J. B. Lippincott Co.)

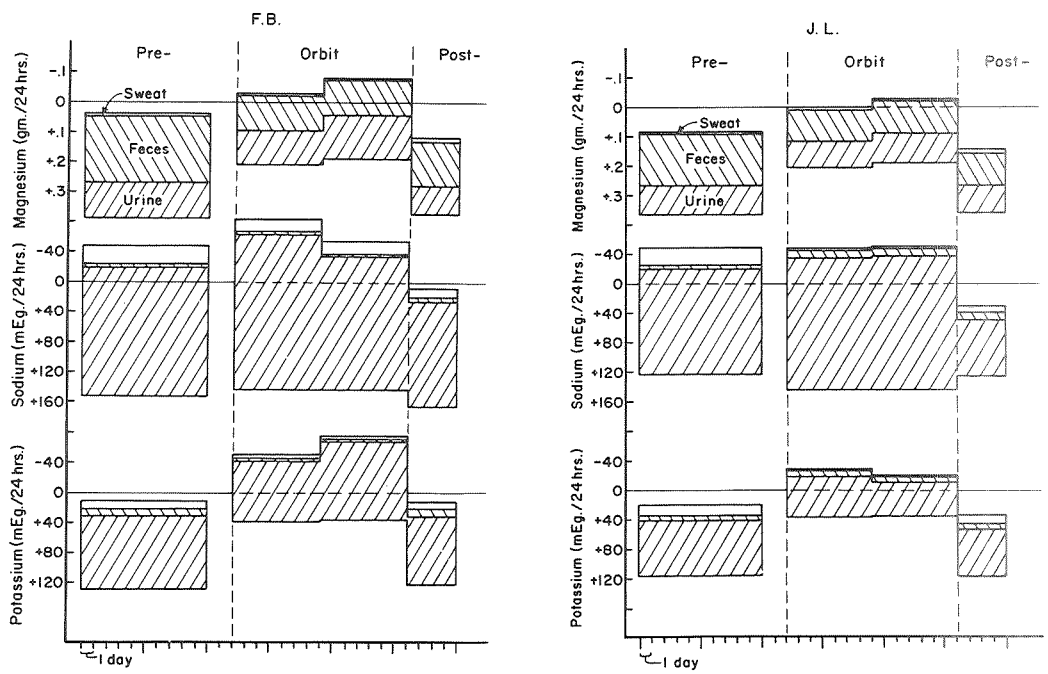

Figure 4.4 Metabolic balance data, plotted according to Reifenstein et al. for magnesium, sodium, and potassium; astronauts F. B. and J. L. before, during, and after 14-day orbital spaceflight. (From reference 1, reproduced by permission of J. Clin. Endocrinol. and Metab. and J. B. Lippincott Co.)

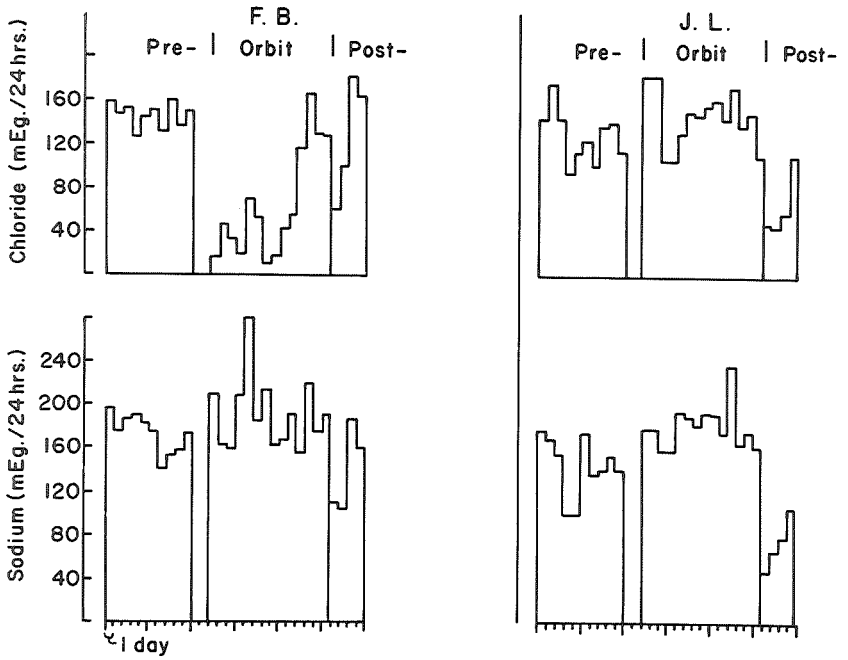

Figure 4.5 Urinary excretion of sodium and chloride; astronauts F. B. and J. L., before, during, and after 14-day orbital spaceflight. (From reference 1, reproduced by permission of J. Clin. Endocrinol. and Metab. and J. B. Lippincott Co.)

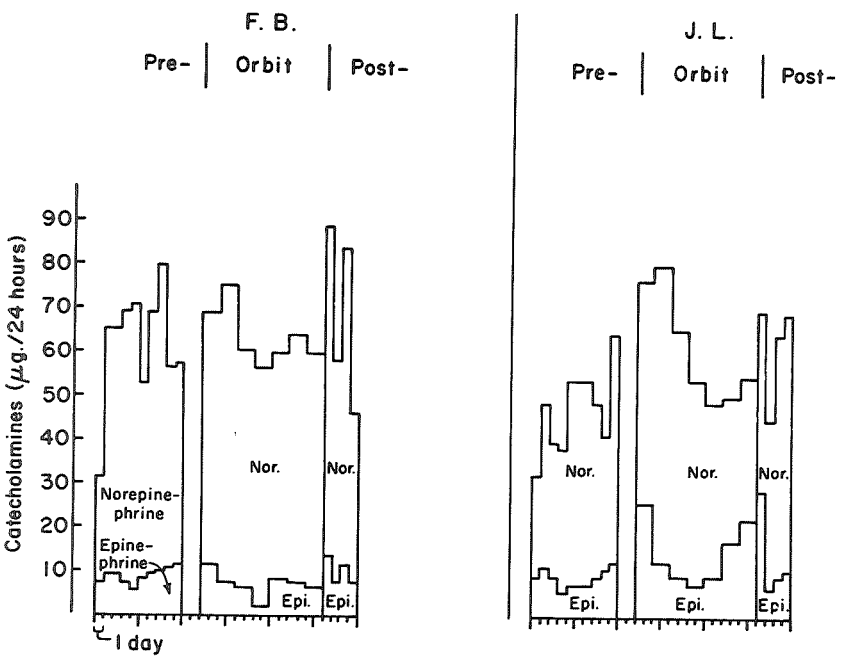

Figure 4.6 Urinary excretion of epinephrine and norepinephrine; astronauts F. B. and J. L., before, during, and after 14-day orbital spaceflight. (From reference 1, reproduced by permission of J. Clin. Endocrinol. and Metab. and J. B. Lippincott Co.)

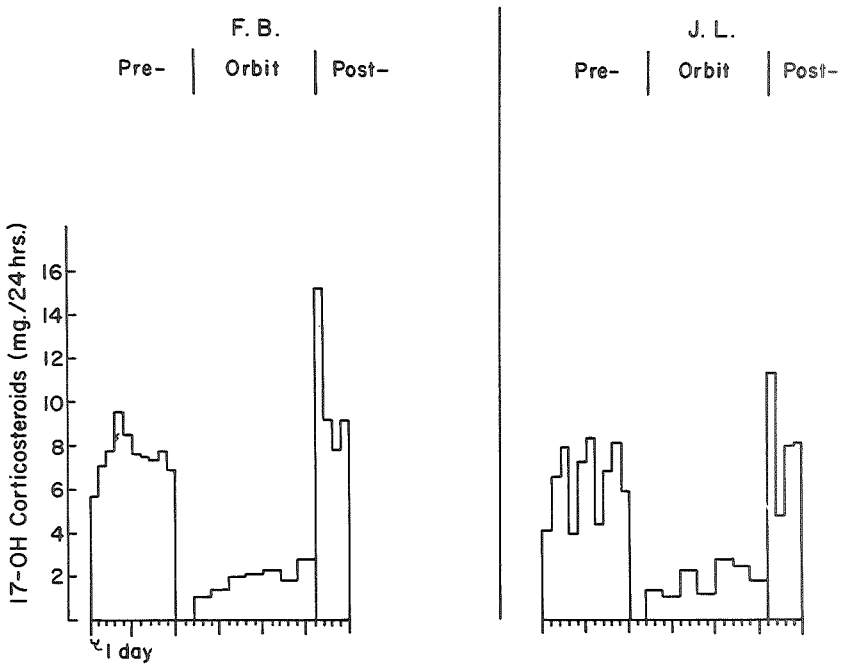

Figure 4.7 Urinary excretion of 17-hydroxycorticosteroids; astronauts F. B. and J. L., before, during, and after 14-day orbital spaceflight. (From reference 1, reproduced by permission of J. Clin. Endocrinol. and Metab. and J. B. Lippincott Co.)

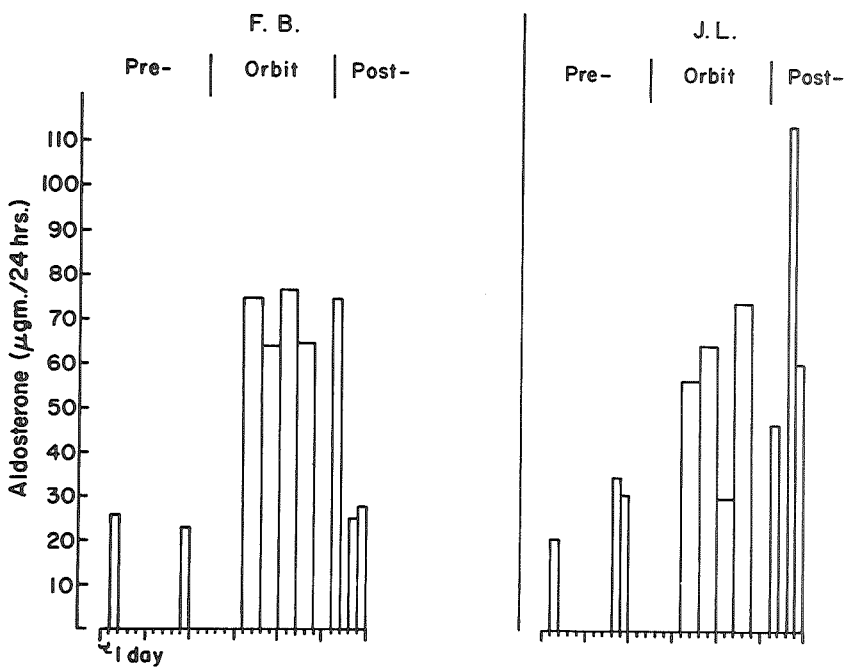

Figure 4.8 Urinary excretion of aldosterone, astronauts F. B. and J. L., before, during, and after 14-day orbital space flight. (Lutwak, L., et al. J. Clin. Endocrinol. and Metab. 29, 1139, Sept. 1969. Reproduced by permission of the J. Clin. Endocrinol. and Metab. and J. B. Lippincott Co.)

5 DISCUSSION

Dr. Vinograd: I am a rather strong believer in defining the anticipated end products of a discussion. I think a discussion should be generally pointed toward something without being yoked to it in too formal a manner. Looking at the goals established by Drs. Murray and McCally for this session (see Introduction), and after examining our own objectives in the manned spaceflight medical experiments program, it seems to be that they correspond very well.

The following four goals have been our objectives for the past 5 or 6 years: (1) to determine the effects of spaceflight on man and the time course of these effects; (2) to find the mechanisms by which these effects become manifest and their specific etiologies; (3) to learn the means of predicting the onset and severity of these effects; and (4) to find the best remedial measures in terms of either prevention or therapy.

We should attempt to determine a consensus of the present understanding of these areas and their research requirements. It would be desirable to view the product of these discussions in terms of such factors.

Dr. Dietlein: A point I neglected to mention is that intestinal gas and abdominal distention often have been a problem. Much of the drinking water is mixed with hydrogen that comes from the fuel cells under great pressure. Water ingestion is associated with the intake of large amounts of this gas. Additional factors are gum chewing and consequent air swallowing, and the use of drugs to decrease bowel activity; at least two of the astronauts had no bowel movements in flight. This distention may explain the heart palpitations noted on at least one flight, and may have contributed to the motion sickness. The Apollo 10 crew reported no motion sickness and no vomiting; however; one of the fecal bags contained material that looked and smelled like vomitus, and had a pH of 2. We have not determined if this occurred in flight, in the sea, or after landing.

There is at least one other bed rest study in which red cell mass has been measured (Section 14). In contrasting the increased plasma volume in Gemini 7 with that of Apollo 7, 8, and 9, I think that the dietary factors may be very important. In the Gemini 7 flight, the astronauts ate and drank on command practically everything that they were asked to because of Dr. Whedon's calcium balance experiment. This may have had a salutary effect on plasma volume; every other flight crew fell behind in their food and fluid intake.

Another point of importance is that the atmospheric oxygen concentration in the Apollo 8 flight reached only 93 percent; the remainder of the gas mixture was nitrogen with a small amount

of hydrogen. A 100 percent oxygen atmosphere was present throughout all of the Gemini missions and in the later part of Apollo 9.

Dr. Behn: You mentioned that there was a loss of albumin as well as water from the intravascular space during water immersion, and we can infer that this may have occurred in spaceflight. Wouldn't such a loss of albumin tend to make plasma volume measurements inaccurate when albumin-bound indicators are used?

Dr. Leach: We injected radioactive albumin. Thus, unlike nonradioactive Evans blue dye methods for measuring plasma volume, our determinations were not dependent on albumin binding.

Dr. Behn: Therefore, the values obtained might not be real losses but, instead, effects produced by simultaneous protein losses from the intramuscular space.

Dr. Leach: We know of no data showing that protein loss from the muscle mass affects the plasma volume. The radioactive albumin used to measure plasma volume hardly enters the extravascular spaces during the time of the measurement. We found that the radioactivity per cc of plasma decreased less than 1 percent between the 15- and 30-min specimens. This is good evidence that nearly all of the albumin remained in the vascular space during the measurement. If, as in bed rest or inactivity, there is a greater loss of muscle than there is of other tissues, we would find a proportionately greater decrease in plasma volume, especially if it is equated on the basis of total body weight or surface area, since muscle is a relatively vascular tissue. No measurements were made of muscle volume or lean body mass. Therefore, we are unable to rule out this possibility.

Dr. Dietlein: I think it is possible that it might be a factor, but we certainly have not measured plasma volume redistribution. This possibly could be done by some external radioactive counting or scanning, pre- and post-tilt, or something of that sort. This has not been done.

Dr. Murray: How many samples did you obtain for your mixed blood sample after the isotope injection?

Dr. Leach: Two samples were taken, one at 15 min and the other 30 min after isotope injection. These two blood samples were used for both the plasma volume and red cell mass determinations.

Dr. Murray: If a line is extrapolated back to so-called "zero" time through these points, the rate of loss from the vascular space should not interfere with the accuracy.

Dr. Leach: Right.

Dr. Hyatt: I am a little disturbed by your statement that plasma volume levels are reproducible within 1 percent when determined at 1-month intervals, yet the day-to-day variation may be as

much as 6 percent. Even with very careful technique, our laboratory cannot achieve a 1 percent reproducibility. We have a large amount of data accumulated over the past few years which indicates that repeated sampling at 10-min intervals during the first half hour of mixing is an inaccurate method because there is considerable variation in the plasma ^{125}I level during this period. In order to get a truly accurate plasma volume, we have had to sample at 1-hr intervals for long periods of time and then derive a least-squares regression line to zero time.

What was your method for the calculation of red cell mass? Were the isotope concentration values averages of the two samples, or did you derive a regression line?

Dr. Leach: To answer your question about reproducibility, this study was conducted by Dr. Johnson and Dr. Fischer, and I'm sure they would be interested in your data. Our data show that the plasma volume is technically reproducible to 1 percent. Thus, two volumes done simultaneously should be within 1 percent. However, consecutive plasma volumes done daily will vary up to 6 percent. The 5 percent difference is the true biological variation in plasma volume that occurs due to standing, exercise, etc. As Dr. Hyatt knows, albumin first mixes with the plasma volume. In health, there is nearly complete mixing of the injected radioactive albumin in 3 to 5 min. Simultaneously with intravascular mixing, albumin is being lost to the extravascular spaces, such as the spinal fluid, joint fluid, gastrointestinal tract, etc. Protein from these spaces is entering the vascular space at the same rate. As it enters, it decreases the specific activity of the circulating albumin. Because these are more slowly equilibrated spaces, they are seen in the die-away plot to a greater extent at later times after injection. Curves extrapolated after 1 hr would be affected by albumin exchange with these extravascular spaces. To measure plasma volume, it is necessary to separate the two curves. Obtaining samples several hours after injection would not improve the estimate of the slope of the early phases of the curve. Therefore, the determination of circulating plasma volume should be made after adequate mixing and before the time that decreasing specific activity due to vascular leakage occurs. The only possible exception to this is the remote possibility that there was postflight intravascular trapping of the plasma. Our relatively crude external counting techniques tend to rule out this possibility.

Dr. Zollinger: Have you performed the red cell mass studies close enough to preflight time that you could follow urinary ^{51}Cr output? Do you carry out external scanning over the liver and spleen postflight?

Dr. Leach: The closest time to liftoff that we were allowed to determine red cell mass was 4 days preflight in the Apollo missions. In the Gemini program, red cell mass was determined 7 days and 10 days prior to the mission. We did not receive inflight urines so that we were unable to measure ^{51}Cr excretion. However, as far as I know, radioactive chromium excretion in the urine is not an adequate measure of radioactive chromium red cell half life. When inflight fecal samples were analyzed, very little ^{51}Cr was found, indicating that no GI bleeding had occurred during the mission. In the Gemini series, liver, spleen, and heart counts were obtained as soon as possible. These measurements were relatively crude, but they did not suggest elevated splenic uptake of chromium or an abnormally slow distribution of the injected tracer.

Dr. Zollinger: What do you use as a baseline for your weight and plasma volume values? We find a great difference between evening and morning samples, and believe the fasting morning state is the best baseline value for weight, plasma volume, and extracellular fluid determinations.

Dr. Leach: All of our blood work is done in a fasting state in early morning.

Dr. Jensen: There have been a great many constraints in the testing of the astronauts. It is equally important to point out that we do not yet have sufficient data for broad conclusions. The Gemini life-span studies using ^{51}Cr were based on six cases and two or three samples per test; of course, a straight-line was obtained. Certainly, looking at the other data available, one can properly infer that these subjects had hemolysis in the earlier Gemini flights. The chromium red cell survival data are not complete enough to prove the presence of hemolysis. As Dr. Whedon will tell you, the urine collections were highly unsatisfactory.

Dr. Leach: We agree with Dr. Jensen and feel it is important to point out again that the Gemini red cell data are too imcomplete to rigorously prove or disprove the presence of hemolysis. We are willing to consider other possible mechanisms or a combination of mechanisms as the cause of the postflight decreases in red cell mass. Additional and more complete studies will answer this question.

Dr. Taylor: I didn't understand exactly how long and how often the subjects exercised in the bed rest simulation.

Dr. Mack: They exercised four times a day for approximately 30 min each time; it was designed to be as close as possible to the Gemini exercise program.

Dr. Jurist: What was the volume of fluid ingested by each astronaut?

Dr. Whedon: We do not have any definitive information on the amount of fluid consumed by each astronaut. We have the gross intake for both, but these data are too inexact to permit calculation of fluid balance data.

Dr. Behn: We have just examined the sodium excretory mechanism during water immersion and found an increased sodium excretion, a constant or sometimes decreased glomerular filtration rate, as measured by inulin and creatinine clearances, and decreased tubular reabsorption of sodium.

Dr. Whedon: The assumption that creatinine clearance or glomerular filtration rate does not change in spaceflight is based mainly on long-term bed rest studies in which the 24-hr excretion of creatinine did not change. We found increased urinary sodium excretion in the spaceflight study. As I recall the data, there was a rather gradual increase over a few days, followed by a more or less stable excretory rate. What was the duration of your immersion studies?

Dr. Behn: Between 4 and 8 hr.

Dr. Whedon: This fits in with my point about the timing of the measurements of glomerular filtration. It seems reasonable that, in the initial few hours of immersion or bed rest, one could see changes in glomerular filtration, but it would tend to be normal over many days or longer. I should also like to mention information obtained from renal physiologists that the tendancy in most studies of this sort is for the glomerular filtration rate to come back to, or toward, control levels in periods of study extending over several days or more.

Dr Nordin: Just for the record, I think it ought to be stated that creatinine output does not bear any relationship to renal function. If renal function falls, the plasma creatinine level rises, but the total output of creatinine per day remains unchanged, as it does for urea and many other things. You cannot extrapolate from output per day of creatinine to level of renal function.

Dr. Whedon: Well, then, you force me to call upon data that have been gathered by Drs. Donaldson and Hulley and which will be presented subsequently. In essence, they will show that creatinine clearances done weekly do not change throughout 30 weeks of bed rest.

Dr. Barzel: Is it true that chloride concentration in the urine was lower than that for sodium?

Dr. Whedon: In one astronaut, F. B., there was an increase in urinary sodium during the first week in flight and a subsequent return to control, whereas his urinary chloride decreased throughout the first 10 days. The other astronaut had no change in urinary sodium excretion throughout the first phase of the flight, and then an increase later. His urinary chloride did not change significantly during flight but was reduced during the recovery phase.

Dr. Piemme: Some investigators have assumed that the sodium losses seen in the hypodynamic environments were due to an inhibition of aldosterone excretion, and yet your aldosterone excretion rate was very high. Will you comment on what might induce the sodium losses in these environments in the face of these high aldosterone levels?

Dr. Whedon: We really don't have any solid information on which to base an interpretation.

Dr. Lind: How much do the astronauts sweat?

Dr. Whedon: They apparently sweat very little, as judged by the analyses we ran of their undersuits for sodium, chloride, nitrogen, calcium, etc., in the 14-day flight. This is in contrast, as I understand it, to the situation in earlier flights where the control of temperature and humidity was not nearly as good as it has been in later flights.

Dr. Lind: Everyone seems to be placing a good deal of importance on the fact that the astronauts are in negative calcium balance, but the data seem to show that this has been very variable from subject to subject and that, in the earlier studies you mentioned, at least one subject seemed to remain in calcium balance throughout the bed rest study. In Dr. Donaldson's paper, which will be presented later, the average balances for calcium were always negative throughout the bed rest period. What then is the real significance of a negative calcium balance, and what happens if men are starved?

Dr. Whedon: Clearly, the level of calcium balance is responsive to several factors, not excluding the level of dietary intake of calcium. I may have neglected to mention that there could have been a protective influence on calcium loss by the reduction of dietary nitrogen during flight. It does seem reasonable that increasing the dietary calcium might protect to some extent against the calcium-losing effects of confinement, immobility, and possibly weightlessness. The real significance of a negative calcium balance is that, if it continues long enough, over several weeks to months, enough mineral will be lost from the skeleton to weaken it and make it susceptible to fracture under stress.

Dr. Lind: Precisely what is the relationship between the densitometry changes found by Dr. Mack and the calcium balances in your studies and that of others?

Dr. Whedon: I think it relates to the reproducibility and precision of Dr. Mack's method, and whether the os calcis is representative of changes in the skeleton as a whole. At the present state of our knowledge and with these meager data, we can make no correlations.

Dr. Mack: We don't pretend that the os calcis represents the entire skeleton. It does represent types of skeletal tissue that are found throughout the body. We've tried numerous times to measure the reproducibility of our method, and we think that the error is less than 1 percent in the same person tested repeatedly within a short period of time. I cannot say what it might be between different individuals. We have had our method reviewed by the Data Corporation. They made 800 scans of the films in each direction and have come out with results very similar to ours. That's as far as we can go at present.

Dr. Hulley: Dr. Donaldson and I will be presenting more data tomorrow on the correlation between calcium balance and bone densitometry. With regard to Dr. Whedon's paper, it should be noted that there is an inverse relationship between dietary phosphate and urinary calcium excretion. Since the phosphate intake was quite low during spaceflight, the urinary calcium increases might possibly be on this basis.

Dr. Whedon: We too are in the process of studying the potential usefulness of increasing the phosphate intake to prevent calcium loss.

Dr. Hulley: I was surprised at the reduced urinary 17-hydroxycorticosteroids during flight, since, as I recall it, studies in previous flights did not show this.

Dr. Whedon: The only inflight urines collected prior to this study were those on MA 9, and in this flight, only a few inorganic elements were measured.

Dr. Dietlein: Dr. Chris Eichness did carry out 17-hydroxysteroid determinations during the Mercury series, but to my knowledge these data have not been published yet. I don't recall the inflight determinations, but immediately postflight the values were generally increased, except for one subject in whom it was low.

Dr. Henry: The 17-hydroxysteroids were elevated during at least one flight. You mention that you have four explanations for the observed changes. Did any of these include the possibility that the *control* 17-hydroxysteroid levels are high (perhaps associated with the emotional tension) and that during flight, emotional tensions and 17-hydroxysteroids fall?

Dr. Whedon: This was not included in my four explanations, but it's certainly a valid point to raise. However, the control 17- hydroxycorticosteroid excretions were normal.

Dr. Henry: There have been some interesting observations that suggest that in unpleasant circumstances there is actually depression of the 17-hydroxy levels. I think it's interesting that in this flight 17-hydroxy levels were low until the recovery period.

Dr. Whedon: This is an interesting point, but I would point out that the catecholamine excretions, particularly the urinary epinephrine values, were very high immediately after launch, came down later, and were elevated again just before reentry and after landing.

Dr. Henry: The catecholamines fit perfectly with the 17-hydroxycorticosteroid data values, which were high during and after recovery.

Dr. Whedon: Precisely, but they don't fit with the 17-hydroxy values *in flight,* which were low up to the very end of the flight.

Dr. Lecocq: Tomorrow I'll present some of our data on the 2-deoxyglucose studies during bed rest. We have found that, after 2 weeks in bed, the adrenals do not respond appropriately to stress. These data seem to fit in very well with what Dr. Whedon has presented, if bed rest can, in fact, be taken as a model for spaceflight.

Dr. Taylor: In the presence of normal plasma constituents, I'd be curious to know how much calcium the astronauts could lose before you would suggest that their physical condition might be jeopardized; where are we on this good versus bad scale?

Dr. Whedon: That question is very pertinent to this whole business. If the values obtained by Dr. Mack for the os calcis were to be considered representative of the whole skeleton, we should be in great fear of some weakening of bone structure. However, if one were to extrapolate from the balance studies we've done thus far, the astronauts could go a great deal longer (at least several weeks) before reaching a point of real concern.

Dr. Jurist: There is more information on roentgenograms than one can obtain by densitometry. Have densitometric studies been supplemented with computer readout images that magnify and amplify structural changes? The trabecular bone structure of the os calcis would be remodeled in areas corresponding to areas of bone resorption. Correlated with morphologic and Ca^{47} kinetics methods, could questions be answered about the validity of present densitometric and metabolic balance data on inactivity and weightlessness?

Dr. Mack: My only comment is that the films have been viewed and kept in reserve, and we have retraced films after 3 and 4 years and we get the same results. I would be very glad to turn the films over to you or to anyone else for further study of the trabeculae.

Dr. Taylor: Dr. Jurist, in clinical situations, what kind of total losses of calcium do you see in situations where bone is known to be deteriorating?

Dr. Jurist: Clinicians deal with symptoms of rarefying diseases of bone that are associated with losses of bone mineral of about 30 percent or 600 g of the total body calcium. These orders of magnitude are associated with grossly visible roentgenographic evidence of remodeling of the trabecular and cortical bone structure. There is no clinical bone disorder that can be diagnosed by densitometric methods, or metabolic balance, or radioisotope kinetic studies alone; clinical roentgenograms and bone biopsy are the bedrock foundation of treatment in every known bone disease or disorder in all age groups.

Dr. Whedon: We can get some help from the quantitative measurement of calcium loss in conditions like paralytic polio where we had measurements of calcium balance for 6 months and compared them with the X-ray films. Osteoporosis, demonstrable to the ordinary eye, occurred in the tibiae in 3 months with a measured total body calcium loss of only 2 percent (ref. 1).

Dr. Piemme: Dr. Whedon seems to be drawing a fair amount of the fire here this morning. I, for one, would like to state that the mere fact that Dr. Whedon can draw *any* conclusions under the adverse conditions in which he worked is a testimonial to his abilities and herculean efforts. Arguing with his conclusions is like complaining that the Aegean stables weren't clean enough. I would like to direct a question now, though, to Dr. Dietlein. Every bed rest study, every water immersion study, and all but one of the spaceflights demonstrated plasma volume loss. I do not understand the plasma volume gain on Gemini 7. Was there any difference in the way these studies were carried out on that flight? Were these astronauts engaged in any activities that could account for the aberrant result?

Dr. Dietlein: I cannot really account for the differences, nor can I give you many details of the postflight activities on each individual crew. In general, after they left the spacecraft and came aboard the carrier, they were hustled down to sick bay, and the studies were carried out there as soon as possible. You do have to allow for some outside pressures, which are sometimes beyond control. Generally, the same techniques were used for all the tests, and great effort was made to carry out these studies according to protocol. They are supposed to be recumbent 30 min before the start of the study.

I have a comment regarding the accuracy of the 17-hydroxycorticosteroid determinations in the first Gemini flights. These samples were not refrigerated, and all of them had benzoic acid added, which may be detrimental to the steroid assay.

Another point I'd like to make is that, in addition to the potential weakening of the bony structures, the demineralization associated with deconditioning environments may promote the

formation of renal stones. The dehydration often accompanying spaceflight also may be a predisposing factor. Indeed, in one of the bed rest studies in San Francisco, one of the subjects with no previous history of urological disease developed a calcium oxalate stone following 14 days of bed rest. There was, however, an intercurrent viral infection that may have played some role.

Dr. Whedon: Initially, we were inclined to attribute the low 17-hydroxycorticosteroid values to the lack of refrigeration and the addition of the benzoic acid preservative, so we analyzed fresh urine samples for 17-hydroxysteroids.

Dr. Hyatt: If I may jump the gun on my presentation tomorrow, I'd like to show some data relative to sodium and aldosterone excretion during bed rest. Figure 14.14 of my paper presents data from 14 subjects. The lower left-hand corner shows weekly mean values (with standard errors) for aldosterone excretion during a control period of ambulation, 4 weeks of bed rest, and 2 weeks of recovery with ambulation. The only other noticeable changes in aldosterone excretion are between the last day of the control period (marked "Tilt Day") and the first day of the recovery period (an identical day). This difference was quite significant statistically. In addition, there appears to be a higher excretion during the first week after resuming ambulation. This did not quite reach levels of significance, but the change correlates well with other measurements such as those on sodium excretion, weight change, fluid balance, and plasma and extracellular volumes.

Dr. Nordin: The great majority of renal stones in immobilization are due to urinary tract infection, and most of these are triple-phosphate stones; simple hypercalcuria does not produce stone formation. You can add an almost infinite amount of calcium to urine without producing precipitation, so long as the oxalate content is low. In the presence of a high urinary calcium, it's very dangerous to have a high oxalate. You can control the urinary oxalate by modifying the oxalate in the diet, so that I would advise Dr. Dietlein that the most important measure to prevent stones in the astronauts is to keep their dietary and urinary oxalates low. Dr. Mack, what is the correction for the presence of soft tissues in the X-rays of the os calcis?

Dr. Mack: There is no correction unless there is a change in size. We measured the width of the bone under study before and after spaceflight. If the size had changed significantly, we make a correction according to the actual dimensional thickness of the soft tissue.

Dr. Nordin: I think this is very important. We are all concerned about the discrepancy between the apparent densitometry changes in the os calcis and the phalanx particularly, and the negligible rise in urinary calcium. As we all know, these two things are internally incompatible. I want to suggest the possibility that the X-ray changes are due to soft-tissue changes. I noticed particularly that the Gemini flights, which had the biggest changes in plasma volume, body weights, and fluid shifts, were the ones where there were the biggest X-ray changes; and, in the Apollo flights, with their much smaller changes in these factors, there were small X-ray changes. It's very common to think that densitometry measures the absorption of X-rays by bone. Although bone ash has a higher coefficient of absorption than soft tissue, it is not so much higher that soft tissue can be neglected. In trabecular bone, only about 20 percent of the bone is, in fact, bone. (The volume:volume ratio of trabecular bone is about 20 percent.) When you allow, therefore, that

80 percent of the os calcis is soft tissue, and add to that the fact that there is muscle on both sides of the bone, I estimate roughly that only 10 percent of the volume there is actually bony tissue and that soft tissue is contributing equally with bone in the X-ray absorption. If that is the case, then a significant shift of fluid from the heel would give a significant change in the overall density of the heel. Generally, in clinical medicine, when we get rapid changes in phenomena, such as rapid changes in body weight, we look for changes in water balance and water distribution. I find it inconceivable that there could have been such a rapid loss of bone as you observed and such a rapid restitution. I wonder if the explanation could lie in shifts of fluid.

Dr. Mack: We've stated in all our reports that we were measuring the whole bone and not just the calcium phosphate in the bone. However, we've done many hundreds of measurements of soft tissue versus bone, and we find that 0.70 versus 0.17 is the ratio of what we get from soft-tissue measurements versus bone measurements, taking bone as it is with its protein and fluid content.

Dr. Nordin: You mean an equal volume of bone has four times the absorption of the same volume of soft tissue?

Dr. Mack: The ratio is 0.17 to 0.70.

Dr. Nordin: Well, I accept that. But allowing for these figures, I'm still trying to make the point that there's so much more water in the soft tissue in the heel than there is actual bone, because it's a trabecular bone, and the volume:volume ratio of trabecular bone is about 20 percent. If you consider the amount of soft tissue and water in and around that trabecular bone, you will find that approximately half your X-ray absorption is due to water and soft tissue, and half is due to bone mineral. Now, if this half that is due to water and soft tissue loses 10 percent of its water from fluid shifts, that would give you a 5 percent change in the density of the os calcis. There is surely a 10 percent shift of fluid when you elevate your feet.

Dr. Mack: Well, there is a band of cortical tissue at the bottom of the os calcis, and we have traces through that which don't substantiate your suggestion that these density changes could be due to fluid shifts. Moreover, we have sacrificed numerous primates and have studied the bone (usually the spine) carefully with all available X-ray techniques. Initially we X-ray the bone with whatever over- and underlapping of soft tissue there is, and with interstitial soft tissue. Then, we've dried the bone and X-rayed that, and ashed the bone and X-rayed that, and finally analyzed the ash for calcium.

Dr. Nordin: But, do you get a 5 percent loss of density in that cortical bone at the base of the os calcis?

Dr. Mack: I have no data with me, but we do not have as much loss as we have in the trabecular of cancellous bone.

Dr. Nordin: This is because it's not filled up with water and marrow as trabecular bone is.

Dr. Mack: I believe that there are changes that you're not taking into account when you assume that all of the os calcis is trabecular bone; it's not.

Dr. Vogt: The X-ray densitometry changes took a very long time to return to normal values—as long as 30 to 50 days. We, too, have been concerned with the effect of fluid shifts and conducted a study to answer this question. We inflated extremity cuffs to 60- to 70-mm Hg pressure and kept them inflated constantly for days at a time, making measurements daily to see what effect the impounded fluid would have on the densitometry changes. We did not find a significant change in the density of the os calcis.

Dr. Nordin: You say the fluid shift changes are not significant, but most of the changes you are reporting are not in themselves significant but are merely trends. Did you get a change of 1 or 2 or 3 percent?

Dr. Vogt: Some were increases and some were decreases, as you would expect in any data like these.

Dr. Lancaster: Briefly, let me show some additional preliminary hematologic data from our current bed rest studies involving exercise. This study will be described in more detail later in the conference. Eight subjects were studied for 16 weeks using 600 kcal of recumbent exercise per day on a special exercise unit. During a period of 35 days of bed rest, after a 35-day control period, four subjects continued on exercise and four subjects were not exercised. Figure 5.1 shows total blood volume data in the two groups over the study period. There are no significant differences ($p > 0.05$) in total blood volume expressed as total milliliters between the fourth week of control, first week of bed rest, and fifth week of recovery for the exercise group; while the no-exercise group showed a significant decrease ($p < 0.05$) of –312 ml in the bed rest period compared to control, and a decrease ($p < 0.05$) of –251 ml in the bed rest versus recovery periods. The first week of recovery showed an increase ($p < 0.05$) of +315 ml compared to bed rest. There was no difference between the control and recovery periods for the no-exercise subjects. When viewed as milliliters per kilogram body weight, the total blood volume in the exercise group showed an increase ($p < 0.05$) of +3.7 ml/kg in the bed rest versus control period, and a +5.0 ml/kg increase ($p < 0.01$) for recovery versus control. There was no significant difference between bed rest and the fifth week of recovery. The no-exercise group showed a decrease ($p < 0.05$) of –4.3 ml/kg for the bed rest versus control period, but no significant difference between recovery and control. There was an increase ($p < 0.05$) of +3.8 ml/kg in the no-exercise group's first week of recovery compared to the second week of bed rest.

Figure 5.2 shows plasma volume determined by ^{131}I-RISA. The exercise group showed no significant difference for the mean of all determinations during the control and bed rest periods, an increase ($p < 0.01$) of +277 ml of the recovery compared to the control means, and an increase ($p < 0.01$) of +338 ml of the recovery versus the bed rest period means. The no-exercise group showed a decrease ($p < 0.001$) of –387 ml for the bed rest versus the control period means, no significant difference in the control versus recovery period means, and an increase ($p < 0.001$) of +434 ml in the recovery versus bed rest period means. Plasma volume measured during the first

week of bed rest compared to the fifth week of control showed a decrease ($p < 0.001$) for both groups. The decrease was –383 ml for the exercise group and –584 ml for the no-exercise group. Plasma volume measured during the first week of recovery compared to the fourth week of bed rest showed significant increase ($p < 0.01$) in both groups. The increase in the exercise group was +613 ml and in the no-exercise group, +633 ml. On the basis of milliliters per kilogram, the exercise group demonstrated no significant difference for means of control versus bed rest periods, but a significant increase ($p < 0.001$) of +5.9 ml/kg for the mean of the recovery versus the control period. The no-exercise group showed a decrease ($p < 0.01$) of –5.0 ml/kg for the bed rest versus control period means, and an increase ($p < 0.001$) of +5.9 ml/kg for the recovery versus bed rest period means. The plasma volume measured during the first week of bed rest compared to the fifth week of control showed a decrease ($p < 0.01$) of –5.1 ml/kg for the exercise group and –8.2 ml/kg ($p < 0.001$) for the no-exercise group. Plasma volume measured during the first week of recovery compared to the fourth week of bed rest showed an increase ($p < 0.001$) for both groups. The exercise group showed an increase of +10.1 ml/kg and the no-exercise group, +9.2 ml/kg.

Figure 5.3 shows red blood cell mass measured by the ^{51}Cr method. The comparison of the control, bed rest, and recovery period means shows a decrease ($p < 0.01$) of –178 ml during bed rest versus control, a decrease ($p < 0.01$) of –297 ml during recovery versus control, and a decrease ($p < 0.05$) of –119 ml during recovery versus bed rest for the exercise group. The no-exercise group showed a mean decrease ($p < 0.01$) of –186 ml for the bed rest versus control, a decrease ($p < 0.001$) of –204 ml in the recovery versus control, and no significant difference between the recovery and bed rest periods. When the second week of bed rest was compared to the fourth week of control, red cell mass measurements showed no significant difference in the exercise group but a decrease ($p < 0.01$) of –161 ml for the no-exercise group. The values obtained during the first week of recovery compared to the fourth week of bed rest showed a decrease ($p < 0.05$) of –117 ml for the exercise group but no significant difference for the no-exercise group. On the basis of milliliters of red cells per kilogram body weight, there was a decrease ($p < 0.01$) of –1.9 ml/kg in the mean for the bed rest period versus mean for the control period, a decrease ($p < 0.001$) of –3.3 ml/kg for recovery versus control, and a decrease ($p < 0.05$) of –1.4 ml/kg for the recovery versus the bed rest period in the exercise group. The no-exercise group showed a decrease ($p < 0.001$) of –2.7 ml/kg in the bed rest versus control period, a decrease ($p < 0.001$) of –3.2 ml/kg in the recovery versus control period, and no significant difference between the recovery and bed rest periods. When the first week of bed rest was compared to the fourth week of control, the exercise group showed no significant difference, while the no-exercise group showed a decrease ($p < 0.01$) of –2.3 ml/kg. The first week of recovery compared to the fourth week of bed rest showed a decrease ($p < 0.05$) of –1.7 ml/kg in the exercise group, while the no-exercise group showed no significant difference.

Neither the total blood volume, plasma volume, nor the red cell mass showed any significant difference between the two group means for each individual week or between the means for the two groups for each particular experimental period.

In summary, these data support our previous observations that show relatively consistent decrease in red cell mass associated with bed rest, which we believe may be related to a decrease

in erythropoietin production. During the bed rest or recovery phase, we have not found evidence for hemolysis. Recovery of the diminished red cell mass is incomplete 35 days post-bed rest.

Dr. Vogt: I'm concerned about the plasma volume changes you reported on a per kilogram basis. Could your exercised subjects have had the same caloric intake but lost weight, which would make for an apparent increase in plasma volume?

Dr. Lancaster: We did have a gradual significant weight loss in all of the exercise subjects shown in figure 5.4, with the mean for the group of –2.2 kg ($p < 0.001$) while there was no significant change in the no-exercise group means. Both groups consumed diets of equal caloric content. The largest weight loss was 6 kg in one subject, while two of the no-exercise group gained weight slightly in the bed rest period. There was, however, a significant increase of 277 ml in the mean value for the recovery versus control period plasma volumes, as well as an increase of 5.9 ml/kg. I feel that this latter figure may be exaggerated by the weight loss, but that there is also a real increase.

Dr. Schmid: How were the erythropoietin determinations compared; was there a decline in every subject during bed rest, or were there some variations?

Dr. Lancaster: During bed rest, there was a decline in every determination. In the postbed rest determinations, unfortunately, there was one odd value (of the eight) that was high, which increased the mean value.

Dr. Schmid: I think that is a meaningful trend that would prove to be significant statistically.

Dr. Lancaster: We feel that the trend is probably significant, but these values were still all within the range of error of the mouse assay method. We're reluctant to attribute significance to this as yet, particularly because of the technical problems associated with the mouse assay technique.

Dr. Piemme: The bed rest studies we've done in Pittsburgh confirm that the red cell survival is unchanged in bed rest, and the fragilographic studies demonstrate no evidence of hemolysis.

Dr. Hyatt: Our red cell mass measurements declined during bed rest, and this seemed to be attributable to a diminished production of red cells and nothing more. It is important to correct for the red cells lost through repeated phlebotomies.

Dr. Lancaster: I noticed in your figures that you had corrected for this. We have not corrected for this source of red cell loss, but our control subjects, who were bled in exactly the same amount and had the same studies carried out, did not show this change during the same period, so we feel that the changes we observed are not due to blood letting. Iron stores are a problem. We do not know the answer to whether these subjects should be given supplementary iron before and/or during the study. In another related study, we have had two of eight subjects develop a definite iron deficiency following the same amount of blood letting that was carried out in this program.

Dr. Mc Cally: You show that the red cell mass increases in the recovery phase, which might be considered a compensation for decreased production and would go along with your proposed mechanism. Did the reticulocytes increase?

Dr. Lancaster: Yes.

Dr. Mc Cally: There was a description by Dr. Broun some years ago of a hemolytic response in exercising animals after prolonged confinement. Does this reticulocytosis indicate hemolysis or increased production?

Dr. Lancaster: Dr. Broun's work, of course, gave us a lot of solace for some time. We thought that we were demonstrating mild hemolysis until we corrected for the red cell mass changes, and then, of course, there was no evidence of hemolysis. We do see petechiae in the feet uniformly in these subjects, as I believe most everyone else has. The rise in reticulocytes, we think, is in response to the previous decrease in the red cell mass.

Dr. Mc Cally: Do you have the other studies evaluating the possibility of hemolysis, such as plasma haptoglobins or bilirubins?

Dr. Lancaster: Bilirubins have not shown any change, and the haptoglobin values are not available to us as yet.

Dr. Jensen: Dr. Hyatt's study has another interesting aspect to it: the state of iron stores during his period of repeated phlebotomies during bed rest. In essence, he's challenged the animal by placing him at bed rest and then taking large amounts of blood from him. Instead of the normal response, which would be a regeneration of the lost red cells, the animal failed to respond. This gives us another bit of confirmation for your thesis that the fundamental problem here is really a lack of ability to generate red cells and has nothing to do with hemolysis. I predict that, when all the evidence is in, there will be no significant evidence of hemolysis. I think you already have the best data in support of this in the red cell life span data, which is more critical than haptoglobins, etc. What do you think is happening to the astronauts? Obviously, they do have some hemolysis in addition to what may be a decreased rate of formation of red cells. What happened on the Gemini flights?

Dr. Lancaster: I can't answer that. If you reconstruct the data from the Gemini flights and compare them to this study, obviously there is a marked difference in the amount of red cell loss per period of time. The most logical explanation seems to be that there has been red cell destruction in addition to a decreased production rate.

Dr. Hyatt: We have carried out plasma haptoglobin and hemoglobin determinations on four of our bed rest subjects and, thus far, have not seen any change.

Dr. Webb: Dr. Dietlein, I noticed that in the Apollo program, the average weight loss was approximately 7 lb for the nine men who have flown so far. The average weight loss in the Mercury and Gemini flights was about 5 lb (ref. 2). What is causing this weight loss?

Dr. Dietlein: Probably two-thirds of it is due to dehydration and water and electrolyte loss, and the other one-third is due to not eating enough.

Dr. Webb: It can't be caloric loss since the weight is restored so quickly. Do we know why they are dehydrating?

Dr. Dietlein: There is a considerable period of time when they are in spacesuits and sweating quite a bit. In addition to that, some of the astronauts do not feel like eating or drinking, and this may be related to the distention and a "belchy" feeling that I described before. Apparently, they can not eructate normally in weightlessness.

Dr. Webb: That's not very healthy, is it? Dehydration will aggravate the orthostatic intolerance of weightlessness.

Dr. Lind: The calf volume changes that Dr. Dietlein found are quite explicable. The apparent anomaly between the volumes on tilt and in the lower body negative pressure chamber may depend entirely on where he placed the one Whitney gauge that was used. In order to get a good measurement, one should place a number of gauges along the calf. When a subject goes from the horizontal to the upright position (particularly if a footboard is used, but also with a saddle), the gastrocnemius and soleus muscles tend to shift up under the contraction and shorten. If so, they increase the calf volume. This is a false increase as far as the cardiovascular system is concerned. You don't get this with lower body negative pressure stress as far as I know, unless, of course, the subject is "sucked in" hard enough so that his feet are pushed against the bottom of the chamber. I was puzzled by the fact that you found a decreased calf volume with lower body negative pressure stress postflight.

I was also interested very much in your general comments about fatigue, which obviously can't be measured properly. I assume that you are using fatigue here in a general sense. How much of it was muscular? It would appear from the postflight studies on exercise, where you get apparent differences in the relationship between heart rate and oxygen uptake, that there is a sliding scale of inefficiency, decreasing as you increase the exercise load. This would suggest more of a cardiovascular contribution than a metabolic contribution to the general fatigue problem you mentioned, and I wonder if you have any supporting evidence on that particular point.

Dr. Dietlein: The only study that I can cite is one carried out by the Federal Aviation Administration, with which you are probably familiar. That involved a study of exercise tolerance before and after sleep deprivation. Subjects felt quite confused and very weak, and yet their actual performance with ergometry was essentially unchanged.

Dr. Lind: Have you made any measurements of maximum capacity of a suitable group of muscles such as with a hand grip?

Dr. Dietlein: No, this has not been done, but is being planned in forthcoming long-term bed rest studies. Before we can really apply this to an operational flight situation, we must demonstrate

to the satisfaction of ourselves and our consultants that this is a worthwhile study and that it is feasible in flight.

Dr. Gauer: I am going to show evidence in my paper on Wednesday that 6- to 8-hr immersions reduce the working capacity in the same way that the working capacity of the astronauts seems to be reduced. Dr. Stegemann's group (Cologne) demonstrated a drastic reduction in the maximum oxygen uptake and a steeper increase in heart rate at a given work load; so I feel that this is a cardiovascular effect.

Dr. Lind: In the spaceflight situation, it is not difficult to imagine that a portion of it may be muscular, particularly as the calcium losses we hear about may affect the muscular function.

Dr. Lancaster: In our current bed rest study, we are using a speed control device for muscular strength testing that has been very reproducible for muscle group testing. The unit is a tension device tradenamed "Rybex." We have shown no change in the biceps, triceps, quadriceps, or gastrocnemius strength, or in plantar flexion during 5 weeks of bed rest when compared to the control period; this obtains in both the exercise and nonexercise group.

Dr. Taylor: Dr. Dietlein, have you calculated or measured the maximal oxygen consumption? The pulse rate and oxygen consumption data you have from several work levels permit you to extrapolate these points to a predetermined maximal pulse rate and obtain a fairly good estimate of maximal oxygen consumption.

Dr. Dietlein: I believe the oxygen consumption is measured up to a heart rate of 160 bpm only; in recent missions they have gone as high as 180 bpm.

Dr. Lind: It would be interesting to know the cardiovascular responses to submaximal static effort (isometric contraction), because then you might very well expect an interaction between muscle activity and cardiovascular activity. You might be able to sort out what proportion of the problem is invested in the cardiovascular system and how much in the muscle.

Dr. Gauer: May I remind you of the findings of Graybiel and Clark, who demonstrated that actual muscle power is unchanged after 8 hr of immersion but that the working capacity is reduced. This speaks in favor of a predominantly cardiovascular effect.

Dr. Lind: I would certainly like to argue this.

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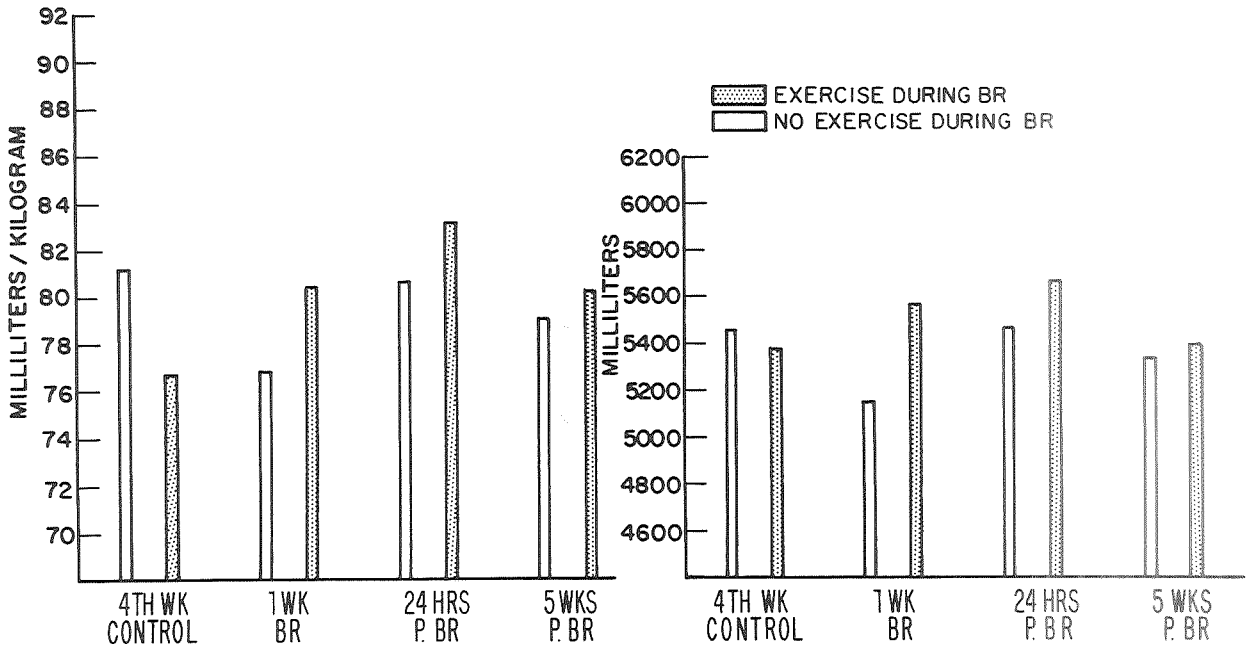

Figure 5.1 Bedrest Project: Blood volume

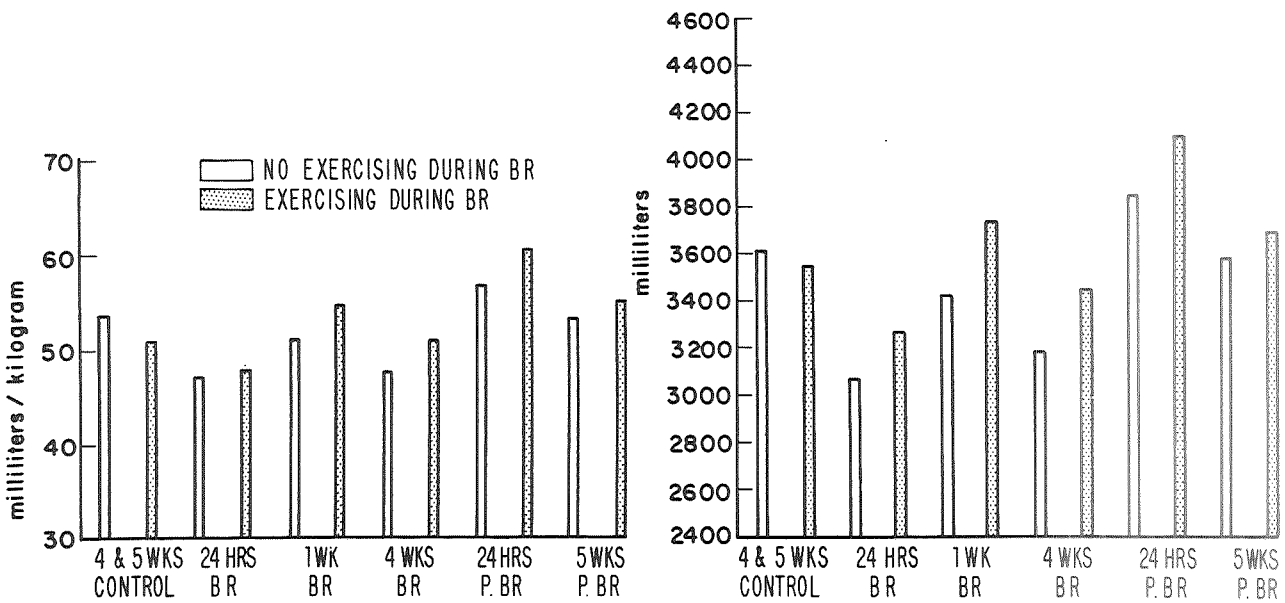

Figure 5.2 Bedrest Project: Plasma volume

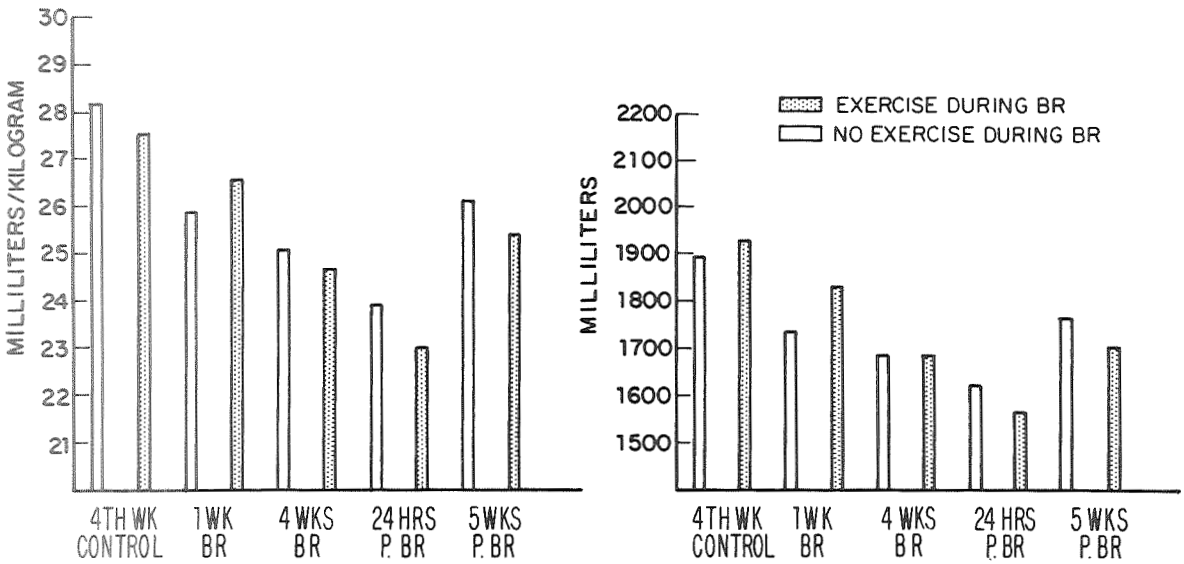

Figure 5.3 Bedrest Project: Red blood cell mass

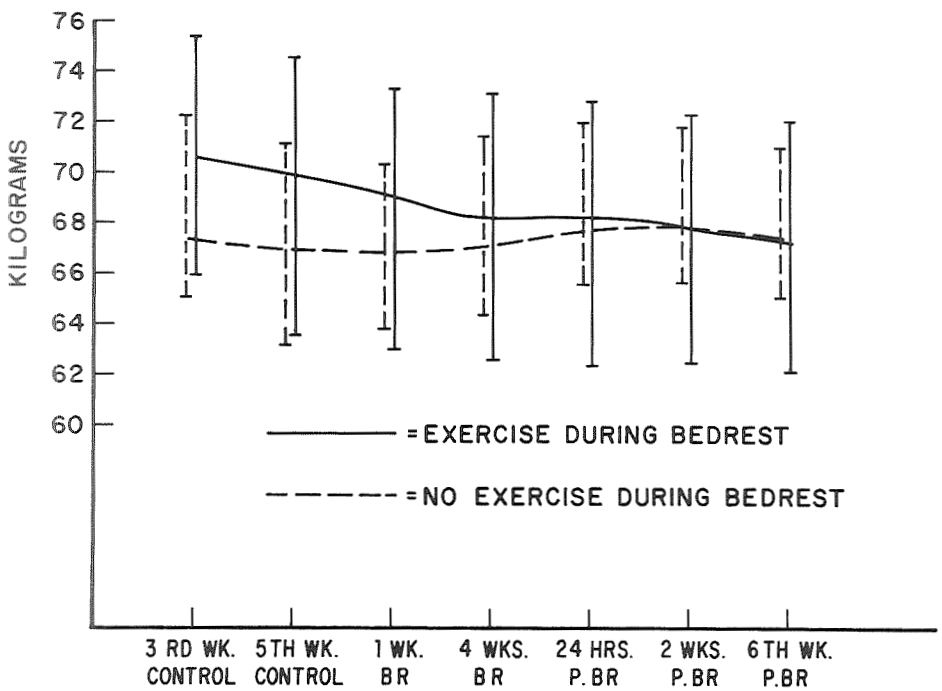

Figure 5.4 Bedrest Project: Body composition, total body weight

Keynote Paper

6 ASSESSMENT OF BONE MASS IN RELATION TO INACTIVITY

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INTRODUCTION

It is well established that inactivity and/or immobilization tends to produce "osteoporosis" in man and other vertebrates. This is seen as an actual loss of trabecular and/or cortical bone from the immobilized or inactive limb or limbs, sometimes associated with an appearance known as "spotty decalcification." The cause of the immobilization apparently is unimportant, and the "osteoporosis" may follow immobilization in plaster, traumatic paraplegia, poliomyelitis, and similar conditions (refs. 1–5). In experimental animals, denervation, disarticulation, and immobilization of the limb in plaster are the common procedures for producing bone atrophy (refs. 6–8).

Existing data on the histology of immobilization bone loss in man indicate that it is associated with osteoclastic bone resorption (refs. 3, 9) with little obvious change in osteoblastic activity. In experimental animals, it is also clear that the loss of bone is associated with a phase of osteoclastic resorption ending in a situation of rather quiescent bone in which both formation and resorption are probably low (refs. 6, 9).

Kinetic data of various kinds support this general concept. Heaney (ref. 1) found that bone resorption *and* formation were increased in the "acute" stage of immobilization but reduced (and in balance) in the "chronic" stage. Landry and Fleisch (ref. 8) made similar observations in rabbits using a tetracycline technique. All authors agree that during the acute stage of immobilization there is a substantial rise in fecal and urinary calcium (refs 1, 10, 11), which represents the net loss of mineral from the bones during the phase when bone resorption exceeds bone formation.

The cause of the increased bone resorption associated with immobilization is not established, but it appears to require the presence of the parathyroid and thyroid glands (ref. 12) and it tends to be inhibited by sex hormone administration (ref. 7). The resorptive process appears to be at least partially inhibited by various types of passive movement, especially if the "antigravity" muscles are brought into play (ref. 10). In fact, clinical and experimental data suggest that muscle tone is an important inhibitor of bone resorption and perhaps more important than weight-bearing per se. Restoration of bone destroyed during immobilization is seen, at least in part, in remobilized

experimental animals (ref. 6), but in human subjects significant replacement of bone lost during immobilization normally does not occur even when the original cause has been removed (ref. 4).

MEASUREMENT OF BONE MASS

Existing Techniques

Since immobilization leads to loss of bone, one way of detecting the process would be to determine changes in bone mass in all or part of the skeleton. Unfortunately, such methods have a disadvantage in comparison with measurement of excreta because of the relative amounts of calcium in the skeleton and in the excreta. On a normal diet, skeletal calcium amounts to about 1,200 g and excreted calcium to only about 1 g/day. Thus, the relative change in the skeleton produced by a given rate of bone loss over any reasonable period of time must be far smaller than the relative change in excreted calcium. Nonetheless, it is desirable to review available techniques for the estimation of skeletal mass, and consider their errors as well as their potential contributions in this field. The techniques concerned are morphometry, X-ray densitometry, γ-ray densitometry, and neutron activation (table 6.1).

Morphometry There are a number of bones (only cortical, not trabecular) on which it is possible to make simple measurements from X-ray films. The bones most commonly studied in this way are the second metacarpal of the right hand and the femur (refs. 13–15). These measurements provide a basis for calculating a number of parameters: cortical thickness and area, shaft diameter and area, and the ratios of the cortical to the shaft measurements. Several workers have determined the error (reproducibility) of the cortical/shaft ratio to be about 10 to 12 percent.

X-Ray Densitometry This involves taking a film of the limb alongside a standard, usually made of aluminum, and scanning the images in a densitometer. It has been applied to the metacarpal (ref. 16), the ulna (ref. 17), the radius (ref. 18), the spine (refs. 19, 20), the distal radius and ulna (ref. 21), the calcaneum (ref. 22), and other bones. Most workers find that the 1 SD error is at least 3 to 4 percent, although Mack et al. (ref. 22) claim an error of less than 1.5 percent based on a study of eight films on one subject.

γ-Ray Densitometry In this procedure, the bone is scanned with a γ source (usually americium-241 or iodine-125) and a crystal detector. The technique was pioneered by Cameron (ref. 23), who reports an error of ±2 percent on the radial shaft. In applying this technique with the greatest care, we have determined an error of from 3 to 9 percent on the lower end of the radius and ulna, depending largely on the cooperation of the subject in positioning the arm.

Neutron Activation The measurement of whole-body calcium by neutron activation was first reported in 1964 (ref. 24). The error at that time was very high, but one group (ref. 25) has reduced it to ±3.7 percent, which makes this procedure potentially useful.

These data, summarized in table 6.2, suggest that the measurement of changes in bone mass is probably subject to a 1 SD error of about 3 percent at best. Such procedures, therefore, must be much slower than measurement of excreted calcium in detecting the onset of negative bone balance. This should be compared with the observation by Whedon (ref. 5) that in acute poliomyelitis, bone loss could be seen in the lower limbs when about 2 percent of total skeletal calcium had

been lost in about 3 months. Presumably the loss from the immobilized limbs was proportionally greater than in the rest of the skeleton.

Prediction of Skeletal Weight

Apart from the work of Trotter et al. (ref. 26), little is known about the relation between the mass of individual bones and that of the skeleton as a whole. We have performed an experiment designed to establish whether skeletal weight can be predicted from a combination of X-ray measurements on selected bones; most of these measurements can also be performed on the living subject.

Procedures Twenty cleaned, dried skeletons were examined and measured as follows:

1. Total skeletal weight was determined to the nearest 10 g.
2. The second metacarpal, femur, ulna, radius, and third lumbar vertebra were weighed to the nearest gram. No distinction was made between right and left.
3. The maximum lengths of the second metacarpal, ulna, radius, and femur were determined to the nearest millimeter in a plane parallel to the long axis.
4. The bones listed in (2) were then X-rayed at 52 kVp at 40-in. film-focus distance using nonscreen film. The bones were placed directly on the film cassette alongside an aluminum stepwedge.
5. The radius, ulna, and metacarpal were also scanned at the same sites as the X-ray images (see below) using an americium source according to the technique of Cameron and Sorenson (ref. 23).
6. The diameter and cortical thickness of the metacarpal were determined with calipers at the midpoint of the bone on the X-ray films. The same measurements were made on the femur at the thickest point of the cortex.
7. The metacarpal and femoral films were then scanned in a Joyce-Loebl recording densitometer at the sites of measurement. The radius and ulna films were scanned 1 cm proximal to the joint surface and at right angles to the long axis of the bones. The lateral vertebral image was scanned across its center in the vertical plane. All scans were related to the appropriate stepwedge.

X-Ray Calculations *Metacarpal.*—Cortical and total cross-sectional areas were calculated on the assumption that the bone was a cylinder. Metacarpal cortical density (MCD), i.e., ash per unit volume of cortex, was calculated from the standard aluminum equivalent at the center of the trace, divided by cortical thickness (ref. 16), and converted into ash per ml using a conversion factor of 147 mg of ash per cm^2 for each mm of aluminum. Metacarpal ash per cm (MAC) was derived from MCD by multiplying by the cortical cross-sectional area (MCA). Metacarpal total density (MTD), i.e., ash weight per unit volume of anatomical bone, was derived from the ash per cm by dividing by the total area (MTA).

Femur.—The corresponding calculations were applied to the femoral data in the same way as the metacarpal to yield femoral cortical density (FCD), femoral ash per cm (FAC), and femoral total density (FTD).

Radius and ulna.—The aluminum equivalent thickness of the radius and ulna scans was determined at the midpoint of the traces and converted into ash per cm^2 (UAC2 and RAC2) using

the same aluminum-to-ash conversion factor. The values were also expressed as ash per cm^3 (UD and RD) by dividing the lateral depths determined from the lateral radiographs.

Vertebra.—The aluminum equivalent thickness of the vertebral traces was determined at the midpoint of the scan and converted into ash per cm^2 (VAC2), which was converted into ash per cm^3 (VD) by dividing by the depth of the vertebra at the same site.

γ-Ray Calculations *Metacarpal.*—MAC was derived from the scan by direct integration. MTD and MCD were derived from this value by dividing by the total and cortical areas calculated from the actual scans.

Radius and ulna.—RAC and UAC were also derived by direct integration in the same way.

Bone Size versus Porosity A review of the data indicated that the bone measurements were a function of bone size (table 6.2), bone porosity (table 6.3), or both size and porosity (table 6.4).

Table 6.2 shows the coefficients of correlation of skeletal weight with the metacarpal and femoral total areas (calculated from the X-ray films) and with the lengths of the bones as determined by direct measurement. The bone area measurements are not significantly related to skeletal weight, but all the length measurements are related significantly (at the 1 percent or 5 percent level) to skeletal weight.

Table 6.3 shows the relatively weak relationship of porosity measurements to skeletal weight, although significant correlations are observed with metacarpal and femoral total density. This indicates that, as expected, skeletal weight is a function of the porosity as well as the size of the bones. It will be noted that ulnar and radial density do not correlate with skeletal weight, possibly because of the error introduced by measuring lateral depth on the X-ray film, since there is no way of establishing that our depth measurement corresponds to the bone depth at the point on the X-ray scan where the density is determined.

Table 6.4 shows, as expected, that mixed (both porosity and size-dependent) measurements generally correlate better with skeletal weight than either the size or porosity measurements alone. Thus, the combination of metacarpal total area ($r = 0.33$) with metacarpal total density ($r = 0.57$) yields metacarpal ash per cm with a correlation coefficient of 0.72 (X-ray) and 0.84 (γ-ray). Similarly, femoral total area ($r = 0.43$) combines with femoral total density ($r = 0.62$) to yield femoral ash per cm ($r = 0.88$). However, even such simple quantities as metacarpal and femoral cortical area are significantly related to skeletal weight. It is also noteworthy that the measurements of radial and ulnar ash per cm are more strongly correlated with skeletal weight than the corresponding ash per cm^2, almost certainly because the former account for the whole size of the bone and the latter only the area density at the midpoint.

The weights of individual bones correlate highly with skeletal weight, but since these measurements cannot be obtained in life, they are of only limited interest.

These observations simply emphasize that the weight of a given skeleton is a function of both its size (i.e., external volume) and density or porosity. So far as the relative contributions of these two components are concerned, the coefficients of correlation suggest that size and porosity are of comparable significance but vary somewhat from skeleton to skeleton. This is supported by the observed ranges of the variables. Thus, for instance, the size measurements (MTA and FTA) vary by approximately a factor of 2 between the smallest and largest; the corresponding porosity measurement (MTD and FTD) also vary by a factor of about 2. Since the range of skeletal weights (2.13 to 5.08 kg) cover a variation of about 2.5, the contribution of bone size and porosity to final skeletal weight in this series would appear to be of the same order.

Multiple Regression Analysis of Measurement Data The object of the work described above was to establish what combination of bone measurements, applicable to the living subject, might be used to predict skeletal weight. This was done by applying multiple regression analysis to the data.

Preliminary analysis of the internal correlations obtained showed that a number of the measurements could be omitted without loss of predictive value. By a process of trial and error, it was finally concluded that some combination of measurements on the femur, metacarpal, ulna, and vertebra would yield the best estimate of skeletal weight. The optimum combination of 4, 3, and 2 variables with the regression equations and error of estimate are shown in table 6.5. It will be seen that a combination of femoral, metacarpal, ulnar, and vertebral ash per cm provides an estimate of skeletal weight with an error of ±176 g or 5.2 percent (mean skeletal weight = 3.37 kg). Almost equally good results can be obtained by omitting the femur (error, 5.7 percent). If the metacarpal is omitted instead, the error rises to 6.0 percent. If the ulna is omitted, it is 5.4 percent; but if the vertebra is omitted, it is 6.7 percent. If only two bones are used, tolerable estimates can be obtained from one tubular and one trabecular bone. Thus, the error from combining femur and ulna is 6.9 percent; with femur and vertebra, 6.6 percent; with metacarpal and ulna, 7.5 percent; and with metacarpal and vertebra, 5.9 percent. If only one bone is used, the highest predictive value is provided by the femoral ash per cm (error, 9.2 percent). The error using metacarpal ash per cm alone is 10.5 percent; with ulna ash per cm, 9.6 percent; and with vertebral ash per cm, 10.9 percent. It thus appears that the minimal requirement for a reasonable estimate of skeletal weight is the combination of a tubular and a trabecular bone measurement.

From available X-ray data on living subjects, we have made some estimates of skeletal weight (y) *in vivo.* With the X-rays, these have been based on the following equation:

$$y = 3.67\ MAC + 2.47\ UAC2 + 0.402\ kg$$

In 30 normal premenopausal women, skeletal weight estimated from X-rays ranged from 2.85 to 4.40 kg and was found to be weakly related to body weight ($r = 0.43$, $p < 0.02$). Body weight is certainly not the ideal reference standard, and the immediate objective is to find a reference standard that more closely reflects skeletal size.

MEASUREMENTS OF CALCIUM LOSS IN THE EXCRETA

As noted, immobilization is associated with a rise in fecal and urinary calcium. Most studies have reported little if any change in serum calcium, though Whedon (ref. 5) found it to be raised.

It must certainly be inferred that the increased resorption of bone mineral raises the serum calcium concentration, albeit very slightly, and that the increased urinary calcium is the result of this rise in filtered load. It might be speculated that the predisposition to hypercalcemia would also reduce parathyroid activity, thus reducing tubular reabsorption of calcium and increasing the rate of calcium excretion relative to the serum concentration. It is possible that a reduction in parathyroid activity might also account for the associated malabsorption of calcium.

The measurement of urinary calcium and/or calcium balance would appear to be a sensitive way of detecting the onset of the process that leads to immobilization osteoporosis. The rise in urinary calcium occurs within a few days after the onset of paralysis or other form of immobilization (ref. 10), rises to a peak at about 4 to 8 weeks, and then slowly subsides. The level of detection will depend on the number and variance of the control observations available. While no such control values are obtainable in clinical cases of paralysis, experimental studies on normal people and observations in spaceflight can yield baseline data and should permit detection of a rise in urinary calcium of perhaps 20 percent. Since the urinary calcium in experimental immobilization rises by a factor of 2 to 3 (ref. 27), the changes should be very easily detected if the diet is held constant.

Fecal calcium, though an important factor in overall calcium balance, is a less satisfactory indicator of the osteoporotic process because of (1) the greater difficulty of collection and analysis, and (2) the smaller relative change in fecal calcium in immobilization than the relative change in urinary calcium. Thus, the data of Deitrick et al. (ref. 27) show a 115 percent rise in urinary calcium during immobilization of four normal subjects but only a 13 percent rise in fecal calcium (table 6.6). However, fecal calcium also is related more closely than urinary calcium to dietary calcium (ref. 28); at high calcium intakes, therefore, fecal calcium is high (net absorption is low) and changes in calcium absorption are relatively difficult to detect. The onset of malabsorption would be most easily detected on a low calcium diet, which is accompanied by high calcium absorption, but few studies have been done on such diets. Thus, although in absolute terms a full calcium balance should be determined for detecting the negative calcium balance of immobilization, in practice very useful information can be obtained from examination of the urinary calcium alone, if there is an adequate period of control observations and if the whole study is performed on a constant or near-constant calcium intake. As shown in table 6.7, the constancy of the intake is particularly important in the study of healthy young men in whom calcium absorption is good and the slope of urine on dietary calcium is steep. In pathological immobilization, comparison of the fecal and urine calcium data of Deitrick (ref. 27) and Heaney (ref. 1) with our normal regression lines suggests that the hypercalciuria and malabsorption are more severe than in experimental immobilization (fig. 6.1). Again, the rise in urinary calcium is relatively greater than the rise in fecal calcium.

It thus appears that measurement of urinary calcium, on a constant diet and preceded by an adequate control period, should be a sensitive indicator of the onset of the negative bone balance that leads to osteoporosis. It is possible, however, that the provision of a constant calcium intake is not a practical proposition in the conditions of spaceflight; therefore, we have examined the

possibility that the fasting morning urinary calcium, related for convenience to the urinary creatinine to reduce errors due to timing and bladder emptying, might be a valid measure of the basal urinary calcium output, independent of the calcium intake.

The 24-hr calcium/creatinine ratio (Ca/Cr) in normal subjects on free diets has been reported to range from 0.03 to 0.28 with a mean value of 0.16 (ref. 29). The fasting morning calcium/creatinine ratio in 39 normal subjects on free diets ranges from 0.01 to 0.15 with a mean value of 0.08. It is clear that the fasting Ca/Cr is appreciably lower than the 24-hr Ca/Cr, presumably because the effect of diet has been largely eliminated. When fasting and 24-hr Ca/Cr values on the same subjects on free diets are compared (fig. 6.2), the greater range of the 24-hr Ca/Cr is immediately apparent. Moreover, elevation of dietary calcium raises the 24-hr Ca/Cr but hardly affects the fasting Ca/Cr. Thus, the fasting Ca/Cr appears relatively independent of diet and should be a simple and sensitive way of detecting the onset of calcium loss in the urine.

From this starting point, we have examined the fasting and 24-hr Ca/Cr in 10 patients, on free diets, with recent traumatic tetraplegia or paraplegia. As shown in figure 6.3, seven of these patients showed a substantial rise in 24-hr Ca/Cr after their accidents to maximum values at about 8 to 12 weeks (ref. 30). The three whose urinary calcium remained low were tetraplegics, but they also were the oldest patients in the group (45, 69, and 78 years). Comparison of the 24-hr Ca/Cr and fasting Ca/Cr values in these subjects (fig. 6.4) shows that the fasting values rose as high as the 24-hr values. Thus, in these cases, diet was an unimportant factor in determining urinary calcium, which clearly was running at a high basal level. In fact, this hypercalciuria could be detected with greater certainty in the fasting than in the 24-hr urine because the normal fasting Ca/Cr is so much lower than the normal 24-hr Ca/Cr. Expressed in absolute terms, the mean maximum urinary calcium in these cases was about 600 mg/day, or a loss from the skeleton of about 0.05 %/day. It would be a long time before this loss would be detected by densitometry.

If the fasting urinary calcium is expressed as mg per 100 ml of GF [i.e., (urine Ca X plasma Cr)/urine Cr (ref. 31)], it can be related in a more meaningful way to the serum calcium. Figure 6.5 shows that the high urinary calcium of the paraplegia cases is associated with a low serum calcium, suggesting the reduced tubular reabsorption of calcium seen in parathyroid insufficiency. The relation between serum and urinary phosphorus in the same cases (fig. 6.6) indicates a tendency toward a high tubular reabsorption of phosphorus, also suggestive of reduced parathyroid activity (ref. 32).

It is difficult to reconcile a state of parathyroid insufficiency, presumably due to calcium resorption from bone, with relatively low serum calcium levels. Is it conceivable that this situation represents a parathyroid "overshoot" analogous to the high-normal serum calcium levels sometimes seen in continuing secondary hyperparathyroidism? Or could calcitonin be involved? If it were, one might expect tubular reabsorption of phosphate to be low rather than high.

CONCLUSIONS

The study indicates that no densitometric procedure is likely to be as sensitive in detecting the onset of "osteoporosis" as the chemical measurement of the loss of bone mineral in the excreta.

Although full metabolic balances with turnover studies represent the ideal way of observing the metabolic changes, the measurement of the rate of calcium excretion in the fasting state could be a valuable substitute under spaceflight conditions.

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Table 6.1 Errors in measurement of bone mass *in vivo*

Techniques	*Author*	*Bone*	*Units*	*Error (1 SD), percent*
Morphometry	Adams (33)	Metacarpal cortex/shaft		8-10
	Anderson (16)	Cortex/shaft		8.2
X-ray densitometry	Doyle (personal communication)	Ulna	$g\ cm^{-2}$	3
	Meema (18)	Radius	$g\ cm^{-3}$	3
	Mack (22)	Calcaneum	$g\ cm^{-1}$	1.5
	Anderson (16)	Metacarpal	$g\ cm^{-3}$	4.8
γ-ray densitometry	Cameron (23)	Radial shaft	$g\ cm^{-1}$	2
	Horsman	Distal radius	$g\ cm^{-1}$	4
Neutron activation	Anderson (24)	Whole body	g	16
	Chamberlain (25)	Whole body	g	3.7
	Palmer (34)	Whole body	g	8

Table 6.2 Skeletal size measurements, X-ray morphometry

MTA	0.33
FTA	0.43
ML	0.66**
UL	0.62**
RL	0.52*
FL	0.48*

Table 6.3 Skeletal porosity measurements

Morphometry	*Densitometry*	*γ-ray*
MCA/MTA 0.39	MTD 0.57**	0.48*
FAC/FTA 0.36	(MCD 0.43)	0.26
	FTD 0.62**	–
	(FCD 0.57**)	
	UD 0.17	
	RD 0.34	
	VD 0.44	

Table 6.4 Mixed measurements

X-ray Morphometry	*X-ray Densitometry*	*γ-ray*
MCA 0.59**	MAC 0.72***	0.84***
FCA 0.54*	FAC 0.88***	–
MWT 0.86***	UAC2 0.55*	–
FWT 0.92***	RAC2 0.65**	–
UWT 0.92***	VAC2 0.76***	–
RWT 0.91***	UAC –	0.87***
VWT 0.89***	RAC –	0.87***

Table 6.5 Best estimates of skeletal weight (y)

N	*a*	*b*	*c*	*d*	*e*	*E*	*F*
4	0.197	2.48	0.83	1.00	−0.105	0.176	5.2
3	–	3.83	0.95	1.16	−0.080	0.191	5.7
3	0.376	–	1.26	0.837	+0.218	0.203	6.0
3	0.214	2.96	–	1.29	−0.266	0.184	5.4
3	0.291	1.71	1.99	–	+0.270	0.226	6.7
2	0.410	–	2.16	–	+0.460	0.231	6.9
2	0.462	–	–	1.26	+0.052	0.221	6.6
2	–	3.67	2.47	–	+0.402	0.252	7.5
2	–	4.51	–	1.50	−0.263	0.199	5.9
1	0.688	–	–	–	+0.336	0.310	9.2
1	–	6.81	–	–	+0.196	0.354	10.5
1	–	–	3.91	–	+1.37	0.323	9.6
1	–	–	–	2.34	+0.982	0.366	10.9

$y = ax_1 + bx_2 + cx_3 + dx_4 + e \pm E$ (kg)

x_1 = FAC
x_2 = MAC (γ)
x_3 = UAC (γ)
x_4 = VAC
} g ash/cm

E = error of estimate
F = (E/mean skeletal weight) X 100
N = number of variables in regression

Table 6.6 Rise in fecal and urinary calcium during immobilization of four normal men

	Calcium Intake,	*Urine Calcium, mg/kg/day*			*Fecal Calcium, mg/kg/day*		
Subject	*mg/kg/day*	*Control*	*Immobilization*	*Rise, percent*	*Control*	*Immobilization*	*Rise, percent*
E.M.	13.0	0.76	1.54	104	10.2	11.7	16
C.O.	14.8	1.87	4.58	143	10.8	12.2	12
A.S.	15.3	2.05	3.55	73	10.7	11.7	12
S.W.	14.4	3.35	8.10	140	9.8	11.0	13
Mean rise				115			13

Source: Reference 27

Table 6.7 Regression of urinary calcium (y) on dietary calcium (x) in young and old men and women

Age group	*Source*	*MEN No.*	*Urine Calcium, mg/kg/day*	*r*	*WOMEN No.*	*Urine Calcium, mg/kg/day*	*r*
50	Literature	62	$y = 1.29 + 0.16x \pm 2.0$	0.66^{***}	92	$y = 0.34 + 0.16x \pm 1.4$	0.66^{***}
50	Own data	38	$y = 1.85 + 0.07x \pm 1.5$	0.46^{**}	68	ns	0.14

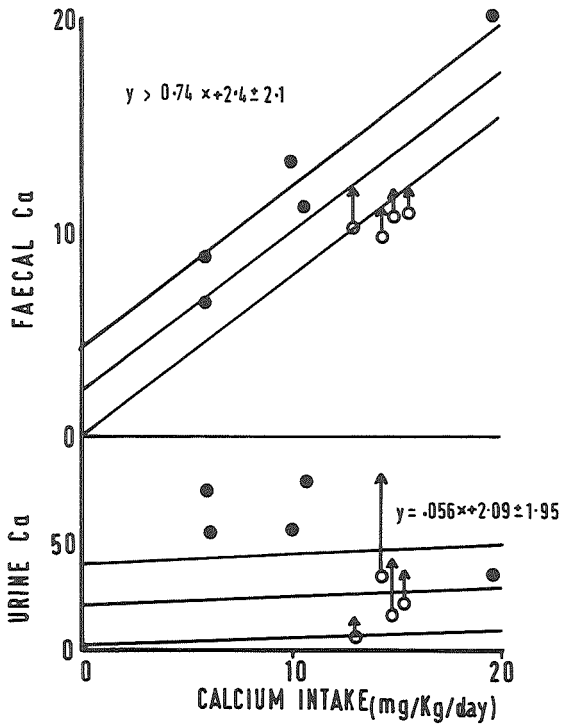

Figure 6.1 Fecal and urinary calcium in relation to normal limits in pathological immobilization (Heaney, ref. 1, solid circles) and in experimental immobilization (Whedon, ref. 5). Open circles indicate control values, and arrows denote experimental immobilization

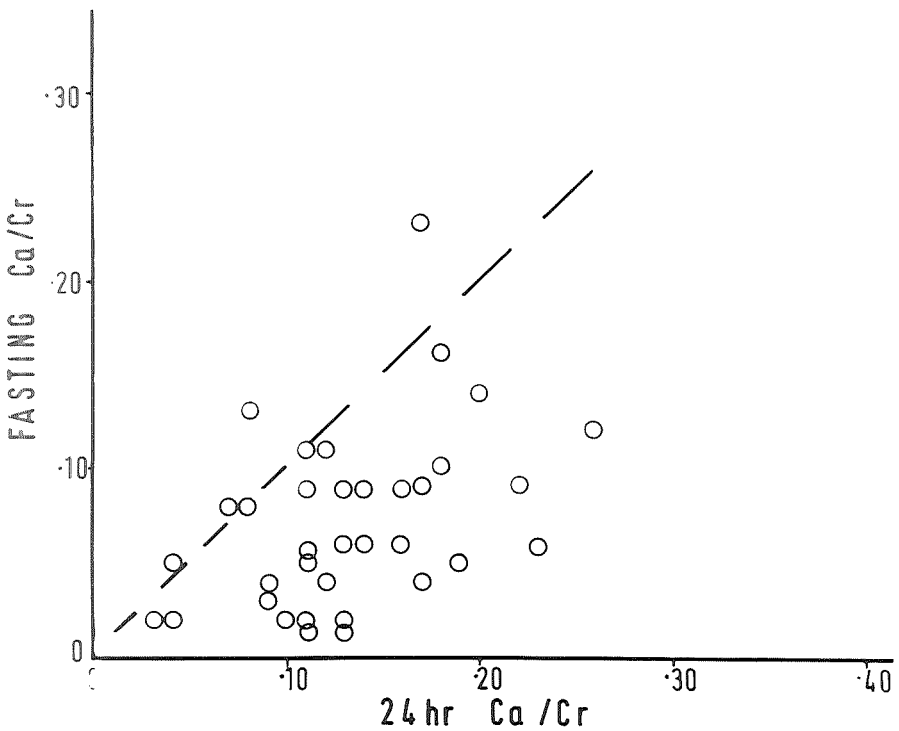

Figure 6.2 Comparison between fasting and 24-hr Ca/Cr values in the same subjects on the same day

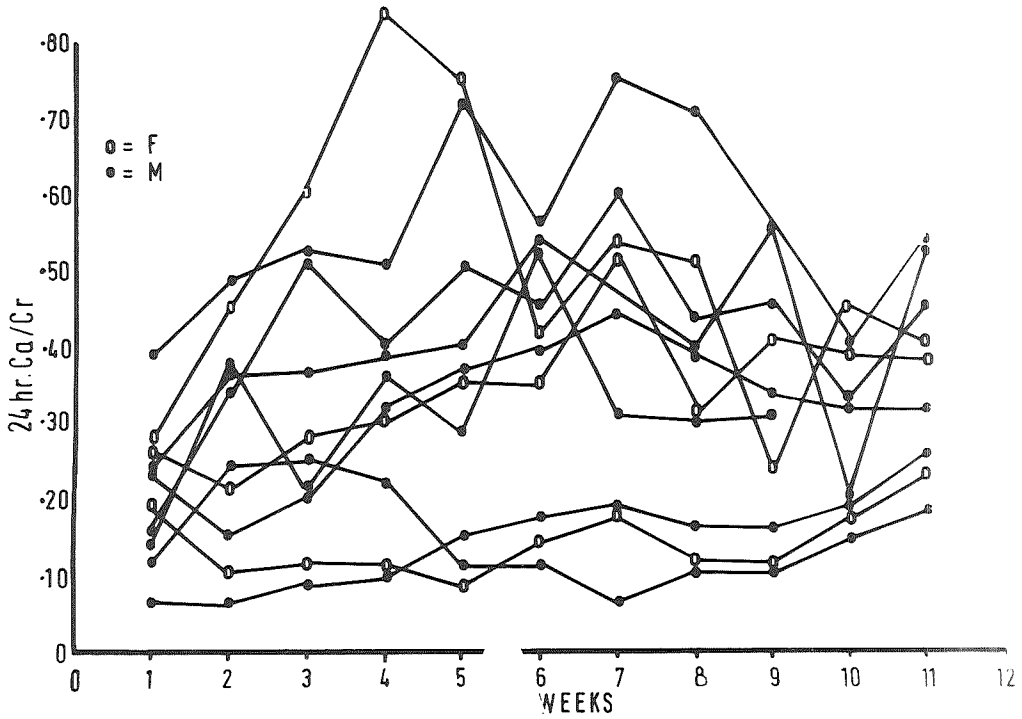

Figure 6.3 Twenty-four hour urinary Ca/Cr following traumatic paralysis in 10 patients

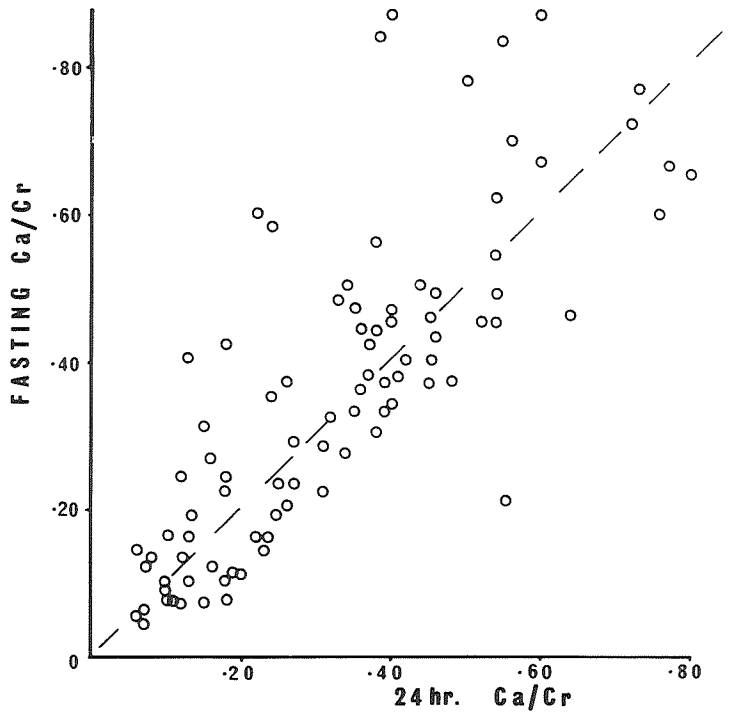

Figure 6.4 Comparison of the 24-hr and fasting Ca/Cr in cases of traumatic paraplegia

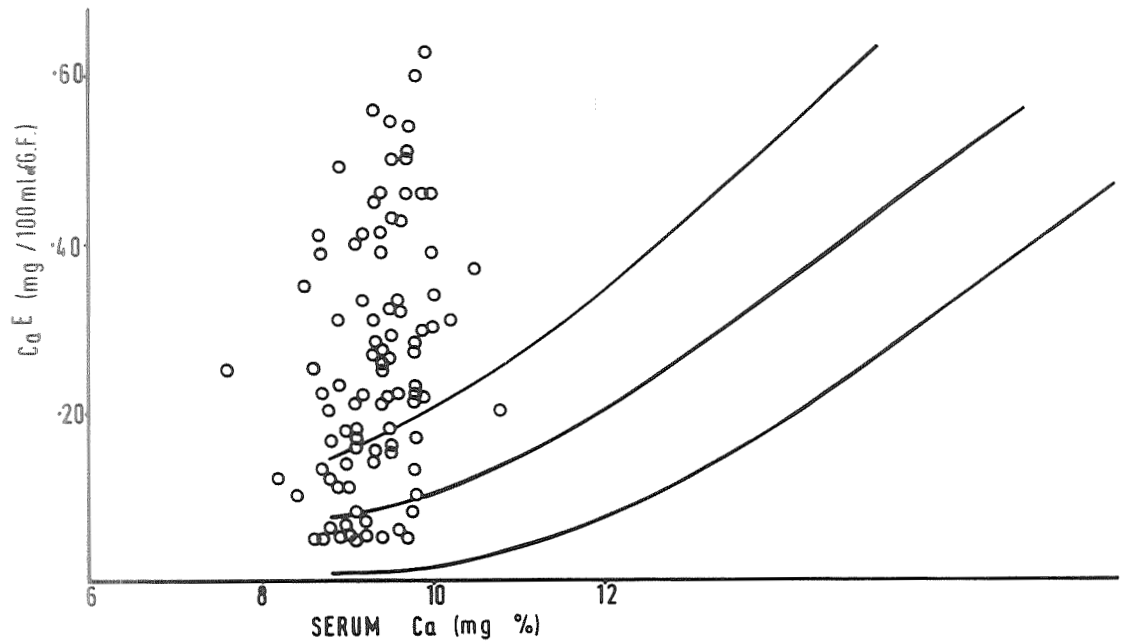

Figure 6.5 Relation between serum and urinary calcium in 10 cases of traumatic paraplegia; regression lines indicate normal range

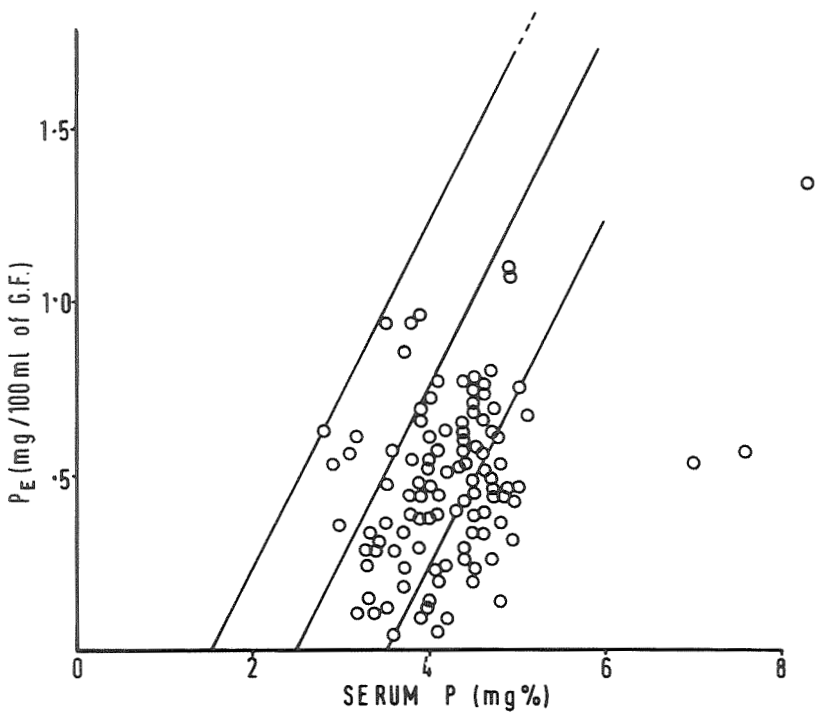

Figure 6.6 Relation between serum and urinary phosphorus in 10 cases of traumatic paraplegia; regression lines indicate normal range

7 SOME PHYSICAL METHODS OF SKELETAL EVALUATION

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INTRODUCTION

Changes in the musculoskeletal system occur in hypodynamic and hypogravic conditions; these changes, which occur in both bed-bound patients and astronauts, may lead to medical problems. This section summarizes some of the major changes, and reviews some newer physical methods useful in their measurement.

Disuse atrophy is a well-known clinical condition affecting both muscle and bone following denervation, prolonged bed rest, or immobilization. For many years, investigators have speculated that weightlessness would have an adverse effect, since the forces acting on the musculoskeletal system would be reduced, particularly in weight-bearing bones and postural muscles. Only recently have studies in weightless conditions been possible. The limited mobility of astronauts suggests that marked alterations may occur in prolonged spaceflight due to the "disuse" associated with both weightlessness and relative inactivity.

Bone loss poses two major hazards. First, ectopic mineral deposits may occur, particularly in the kidney, if bone loss is rapid or prolonged. Second, severe bone loss can compromise the structural integrity of the skeleton. These hazards necessitate sensitive measurements of the skeleton as a basis for evaluating the nature and extent of changes in exposed individuals, as well as preventive and curative actions.

CHANGES OF SKELETAL STATUS

Various medical conditions have been examined and several experimental techniques utilized for determining the characteristics of bone changes with inactivity. Information has been derived from the study of myopathies, tenotomies, denervation syndromes, prolonged bed rest, and fracture healing, as well as partial and total immobilization. In addition, weightlessness has been simulated by water or oil immersion for relatively short time periods. The major studies are reviewed in recent reports by other investigators (refs. 1–8). There is an almost uniform indication that hypodynamic conditions, of whatever sort, in both animals and man result in hypercalciuria, negative calcium balance, and bone loss. The extent to which these findings may be ascribed to generalized reduced muscle activity, reduced weight-bearing stress, or altered neural, humoral, or blood flow patterns is still unclear.

Similar results have been obtained in spaceflight experiments. Mack and coworkers (refs. 9–11) used radiographic photodensitometry pre- and postflight to evaluate bone mineral status. Average losses of 9, 12, and 14 percent were reported using os calcis radiographs of the Gemini 4, 5, and 7 crews. Losses in the metacarpals, oddly enough, were greater than those in the os calcis. The

reported losses were not proportional to flight duration (4, 8, and 14 days), and much of the variation between flights was attributed to differences of calcium intake. Estimated dietary calcium intakes for the Gemini flights were about 710, 350, and 930 mg/day. Bed rest studies made by these same investigators supported the role of dietary calcium in ameliorating bone loss. However, several other studies have indicated that dietary influences on calcium mobilization in recumbency are minimal (ref. 12). Calcium and nitrogen balance studies during the Gemini 7 mission provided limited information suggesting negative balance, particularly during the second week of the 14-day mission (ref. 13).

Average bone density losses have been reported for the three crew members of Apollo 7 and 8 (ref. 14); the average losses in the two flights were 0.8 and 4 percent for the os calcis, 1.2 and 10.4 percent for the distal radius, and 1.4 and 11.7 percent for the distal ulna. The individual bone changes ranged from +2 to −16 percent, and again the losses did not correspond to flight duration (260 hr for Apollo 7 and 147 hr for Apollo 8).

The extent of reported bone loss in the Gemini and Apollo crews was many times that expected for a comparable period of total immobilization. Further, the pattern of atrophy at different anatomical locations varied, and the losses in general did not correlate with duration of flight. Clearly, undetermined factors are affecting these bone measurements; it is possible that the radiographic method used was not sufficiently reliable.

If continued for long periods, the bone atrophy associated with hypodynamic conditions can reduce skeletal strength. This is a major problem for older individuals who are subject to the usual loss of bone with age. Bed rest appears to induce a negative calcium balance of approximately 200 to 300 mg/day, and total immobility, as in poliomyelitis, may double this loss (ref. 8). Under severe hypodynamic conditions, the loss might amount to 2 to 4 g/week, and at least one month would be required for depletion of 1 percent of the body calcium store. Although atrophy is slow, continued loss would result in a predisposition to fractures. For astronauts, the danger of this condition would be greatest during reentry deceleration. If the bone loss is irreversible, it also may lead to problems when superimposed on the usual aging loss. This mobilization of bone raises the concomitant problem of abnormal mineral deposition. Such deposits, particularly urinary calculi, commonly occur in accelerated bone resorption syndromes, and calculi often have been noted in recumbency and inactivity.

The implications of bone atrophy in spaceflight seem generally adverse; the extent of bone loss predicted from hypodynamic conditions should not exceed a few percent per month, even if loss rates during spaceflight are several times more severe than during total immobilization. Such bone loss, if uniform, need not affect the structural integrity of the skeleton, although it might stimulate extraskeletal mineralization. There are constrasting opinions of the risk and severity of possible kidney stone formation in spaceflight (refs. 15, 16); dehydration, metabolic alkalosis, and incomplete emptying of the urinary calyces may exacerbate this problem.

IN VIVO MEASUREMENT OF SKELETAL STATUS

There are several practical methods for measurement of skeletal status that do not require bone biopsy or blood samples. These methods include direct photon absorptiometry, radiographic photodensitometry, and measurement of bone vibratory properties.

Measurement of the small rates of bone loss in hypodynamic and hypogravic conditions necessitates precise, accurate, and sensitive methods, which also must demonstrate long-term reliability. Further, changes of soft tissue composition under these conditions may affect bone measurements. For example, shifts of body fluids occur with recumbency, and muscle atrophy accompanies disuse (refs. 17, 18). In spaceflight, these problems would be compounded by the marked water loss observed in crews of all flights to date (ref. 14). Alterations in the amount of soft tissue surrounding a bone, and changes in the fat, water, and muscle composition of that tissue, could affect measurements of bone mineral content determined by radiation absorption methods, and also might modify the measured vibratory properties of bone.

Direct Photon Absorptiometry

The photon absorptiometric method developed at the University of Wisconsin over the past 10 years uses the attenuation of a monoenergetic beam of low energy (20-100 keV) photons as a direct measure of bone mineral content (refs. 19–21). The basis principles of this method are illustrated in figure 7.1. The bone mineral mass (M_B) at any point in the photon beam is proportional to $\ell n(I_o^*/I)$ when the bone is embedded in a uniform thickness of soft tissue. The intensity of the beam is measured through the bone (I) and through the adjacent soft tissue (I_o^*). The bone mineral content can be determined at a single point, in a single path across a bone, or if serial scans are made, even over an entire anatomic area.

A low-energy radionuclide is used as a source of monoenergetic photons: ^{125}I(27.4 keV), ^{210}Pb (46.7 keV), and ^{241}Am (59.6 keV) have been used. Beam intensity is measured with a scintillation detector-pulse height analyzer system. Scattered radiation is essentially eliminated by using a narrow, well-collimated beam and detector. We usually scan across a long bone, typically the radius, to determine the mineral content of a given cross section. In such a case, the source and detector are mechanically linked and pass across the bone at a constant speed (fig. 7.2). The limb containing the bone of interest is surrounded by a soft-tissue-equivalent material to simulate uniform tissue thickness. We found that either water or Super Stuff works well. Super Stuff is approximately 95 percent water and is readily molded (available from Wham-O Corporation, San Gabriel, California, or at local toy stores).

The measured beam intensity depends on the absorption characteristics of the material in the scan path. It is necessary to integrate the change of beam intensity across the scan path to determine the mineral mass in the path. This is usually done by recording measurements at fixed intervals across the bone with a scaler timer, and analyzing them by computer. We also have developed a direct readout unit that uses analog techniques to measure the mineral content in the scan path as well as the path width. These values are presented immediately after completion of a scan; the unit is easily miniaturized and allows convenient monitoring of mineral content in patients, surveys, or in spaceflight.

The accuracy of the absorptiometric method has been demonstrated on ashed bone sections; section weight was predicted within about 3 percent over a wide range of values (refs. 22, 23). Varying the thickness and composition (relative amounts of muscle and fat) of surrounding tissue cover has little effect on the measurement (ref. 21). The method is highly reproducible over periods of many months. Typical measurement precision is 1 to 2 percent, which can be improved by scanning several times at a single location to obtain an average. We are developing new techniques, using casts of the area of interest, to ensure long-term precision and permit detection of small changes of mineral content.

The absorptiometric method is simple and involves a minimal radiation dose (10 mrad/scan). It is now used in at least a dozen medical centers for clinical and normative skeletal evaluation; the method has been used to evaluate changes resulting from bed rest (ref. 24) and immobilization (ref. 25).

The same equipment used for absorptiometric measurement of bone suffices to measure soft tissue composition (relative amounts of fat and muscle/water) as well. The composition of a two-component system can be determined by making absorptiometric determinations at two selected photon energies (refs. 22, 26) with ^{125}I and ^{241}Am. The measurement of the relative fat content of phantoms is accurate within about 2 percent, and measurement precision is about 1 percent. This method could be used to monitor dehydration or edema, fat changes, and muscle atrophy, and thus both soft tissue and bone changes occurring in hypodynamic or hypogravic conditions.

Radiographic Photodensitometry

The basic principles underlying radiographic photodensitometry are the same as for direct photon absorptiometry (refs. 27–29). The absorption of radiation by bone mineral is used as a measure of the mineral mass. However, instead of a monoenergetic radionuclide source, this method uses a conventional X-ray tube, and a radiographic film instead of a scintillation detector. A photodensitometer is used to measure the optical density of the radiographic bone image as an indication of the mineral mass. An extensive annotated bibliography on this method is available (ref. 30).

There are several problems in using this method that are not encountered with direct photon absorptiometry. The radiation from the conventional tube has a continuous energy spectrum, and absorption coefficients for such a beam cannot be well defined. In addition, the heterogeneous beam changes in quality (hardens) as lower energy radiation is preferentially absorbed during passage through soft tissue and bone. There is no hardening of a monoenergetic beam, and absorption coefficients are well defined. Furthermore, an undetermined and variable amount of scattered radiation from the surrounding soft tissues affects the radiographic bone image. This does not occur with narrow beam geometry. Also, film usually exhibits a nonlinear response to both radiation intensity and energy.

Precision of a photodensitometer scan is usually quite high and this may have given the deceptive impression that the method itself is highly precise. Variations in film, film development, and film exposure conditions may contribute to large systematic errors. Standard exposure is a

critical problem since the energy spectrum of a particular X-ray tube is poorly reproducible, and tubes differ greatly. These errors are partially reduced by using a calibrated wedge as a standard on each film. Investigators using this technique have sometimes erroneously failed to encase the bone of interest in a uniform thickness of tissue-equivalent material and to bury the calibration wedge in this material.

Accuracy of radiographic photodensitometry has not been completely evaluated. Workers using the Pennsylvania State University technique, largely developed by Mack and associates, have reported high accuracy on excised bones and on bones with slight tissue cover. However, on bones with substantial tissue cover, the error of the method is 20 to 50 percent (refs. 31, 32). Mayer et al. (ref. 33) concluded that scattered radiation was a major source of error. When appropriate methods are used to measure and compensate for scattered radiation (ref. 34), the predictive error of the measurement is reduced to about 6 percent on phantoms with moderate tissue cover (Colbert and Mazess, unpublished observations).

The overall utility of this method may be limited as much by long-term reliability as by accuracy. Numerous investigators report the usual precision to be approximately 4 to 8 percent (refs. 33, 35–38). Even larger systematic errors are possible unless great care is taken during film exposure and development (ref. 39).

Radiographic photodensitometry has been used in a variety of clinical and normative investigations. It has been used to assess bone changes during inactivity, bed rest, and spaceflight (refs. 3, 9–11, 40).

Measurement of the Speed of Sound in Bone

Measurement of bone vibratory properties provides a nonabsorptiometric method of skeletal evaluation that determines the functional characteristics of bone rather than its mineral mass. Several investigators have related the breaking strength of bone to its mineral content, density, elasticity, size and shape, as well as the age and sex of the propositus (refs. 41–45). Since the speed of sound in bone is related to its elasticity and density (ref. 46), several laboratories are studying acoustical or vibratory methods of skeletal evaluation. Rich and coworkers exploited the difference between the speed of sound propagation in bone and in soft tissue to build a scanner for measurement of bone quantity. This technique worked reasonably well for compact bone, but the attenuation of ultrasound in bone is so great, especially if any cancellous portions are present, that their technique was impractical. Smith and Keiper (ref. 48) measured the elasticity of excised bone segments by a vibratory technique. Hyatt and coworkers (ref. 49) obtain the speed of sound in excised bone by ultrasound propagation and relate this measurement to the density and elasticity of the bone sample (Abendschein and Hyatt, to be published). It is generally recognized that bone is somewhat nonhomogeneous with respect to elasticity (ref. 50), but an average or effective value may be measured.

Our laboratory is developing methods for measurement of the speed of sound in the ulna, tibia, clavicle, mandible, pelvis, and calvarium. We are studying the following approaches:

1. Timing of mechanical impulse or ultrasound propagation along the bone. If two accelerometers are strapped to a long bone, the speed of sound can be obtained by timing a short-duration mechanical impulse between the transducers. Alternatively, the speed of ultrasound propagation along the bone can be measured. However, the large attenuation of ultrasound in bone, mentioned previously, makes this approach difficult.
2. Measurement of the phase shift per unit length of bone for fixed vibrational frequencies. If a long bone, such as the ulna or tibia, is excited at one end by a sinusoidal vibration of known frequency, the relative phase difference between the outputs of two accelerometers placed on the bone, divided by the distance between the transducers, is proportional to the speed of sound in the bone at that frequency. Since shear waves are excited in the bone, the dispersion of phase velocity with frequency may be determined. This gives some information on the distribution of bone tissue.
3. Measurement of resonant frequency. The product of resonant frequency and length of a long bone is related to the speed of sound in the bone (refs. 51, 52). Apparatus used to measure ulnar resonant frequency in vivo is shown in figure 7.3. The resonant frequency is obtained from a recording of the amplitude response as a function of frequency (fig. 7.4). On repeated resonant frequency determinations, there is a standard deviation of 2 to 4 percent; the variation can be reduced by averaging several measurements. Determination of ulnar resonant frequency has been used for clinical investigations with promising results.
4. Incorporation of bone into oscillator circuit. This is an alternative method of measuring the resonant frequency of the bone. The output from an accelerometer that measures response of the bone is connected to the input of a power amplifier that powers the electromagnetic driver used to excite the bone. The bone is, therefore, essentially the frequency-controlling element of an oscillator circuit. The frequency of oscillation may be determined by using the oscillator output to drive a Schmitt trigger connected to a scaler.
5. Measurement of the transient response of the bone to application of a short-duration impulse. Analysis of the transient response by standard engineering methods allows determination of resonant frequency.

DISCUSSION AND CONCLUSIONS

Bone mineral losses in hypodynamic and hypogravic conditions probably are relatively slight compared to the total body mineral stores, and major alterations would require long-term exposure. Findings of major atrophy in pre- and postflight radiographs are exceptional, but probably are attributable to the large experimental error of photodensitometry. The methods used for measuring bone changes under such conditions must be accurate, sensitive, precise, and reliable. A medical hazard is presented by ectopic mineral deposition. This hazard could be magnified by conditions in spaceflight. In all situations, the decision for dietary change, medication, ambulation, or other physical activity should be based on reliable measurements of bone status. Direct photon absorptiometry and measurement of bone elasticity afford convenient measurement of these critical parameters, and in addition photon absorptiometry can be used to monitor fluid and tissue changes. In contrast, radiographic photodensitometry is inaccurate on bones with moderate tissue cover; the possibility of large systematic errors makes the method very unreliable, even though short-term precision may be high. We have not discussed the possibility of utilizing mineral balance as an indication of skeletal status because of the great difficulties attendant to reliable, complete

determinations. Balance studies are even difficult to use as research tools in metabolic wards. Although augmented calcium excretion may be a useful diagnostic tool, it need not reflect the extent of bone resorption. Neutron activation analysis may be another useful research tool (ref. 53), but the accuracy is unknown and the difficulties are great.

Newer physical methods are making nondestructive testing of the musculoskeletal system easier and more reliable. These methods will probably provide the most useful information when used in combination. The biomedical import of such information makes the exclusive use of any single method undesirable.

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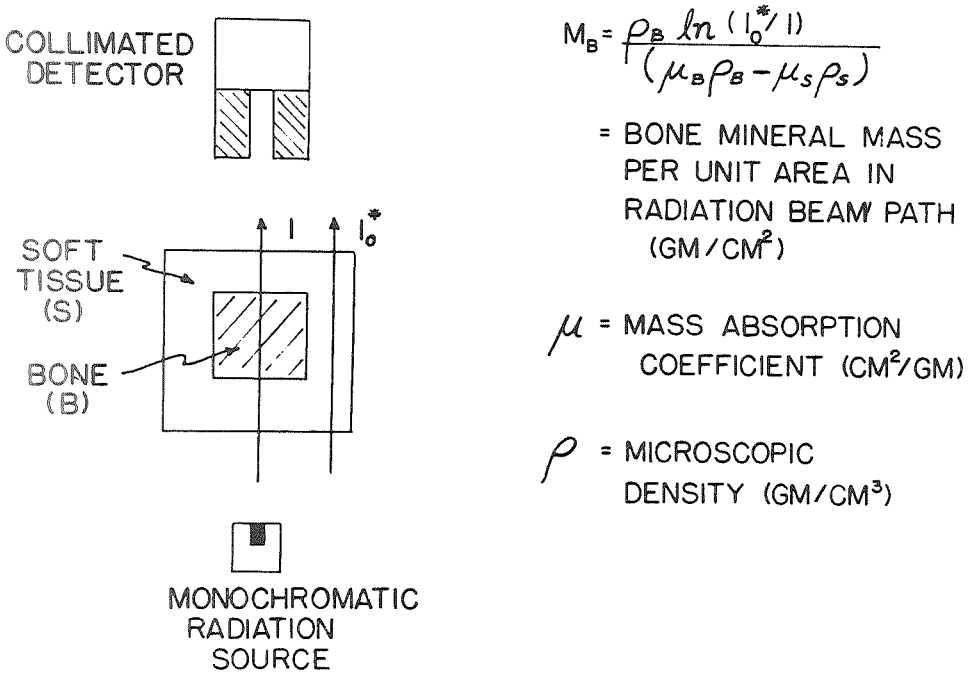

Figure 7.1 Basic principles used in measuring bone mineral with a monoenergetic photon source. The bone mineral mass per unit area in the beam path (M_B) is given by the equation. The measured quantities are I_o^*, the beam intensity through the soft tissue adjacent to the bone, and I, the beam intensity through the bone

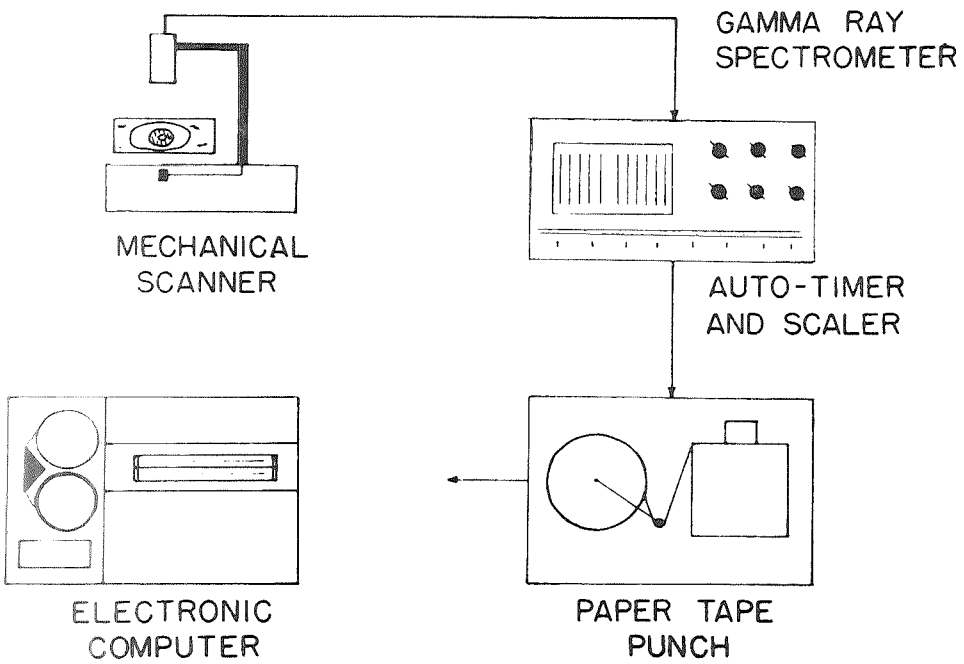

Figure 7.2 System for measuring bone mineral content. Our laboratory is testing a direct readout unit that would allow determination of bone mineral content immediately after completion of a scan

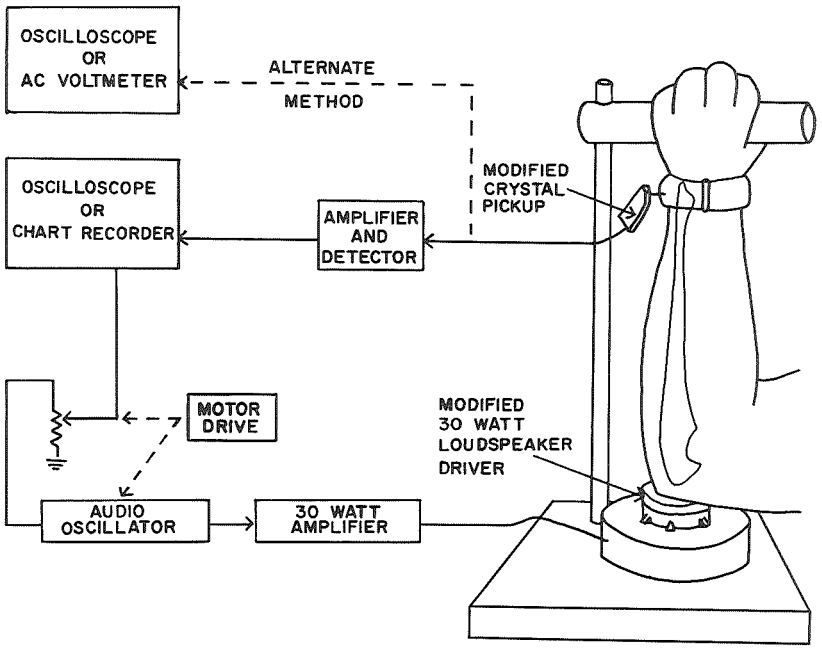

Figure 7.3 Apparatus used for ulnar resonant frequency measurement. The driver is modified by bonding a small lucite piston to the driver diaphragm. The piston contacts the elbow of the subject. The ulnar response is detected by a small crystal accelerometer strapped to the wrist. With changes in the driver support, the apparatus may be used for measurement of tibial resonant frequency (from Jurist, to be published).

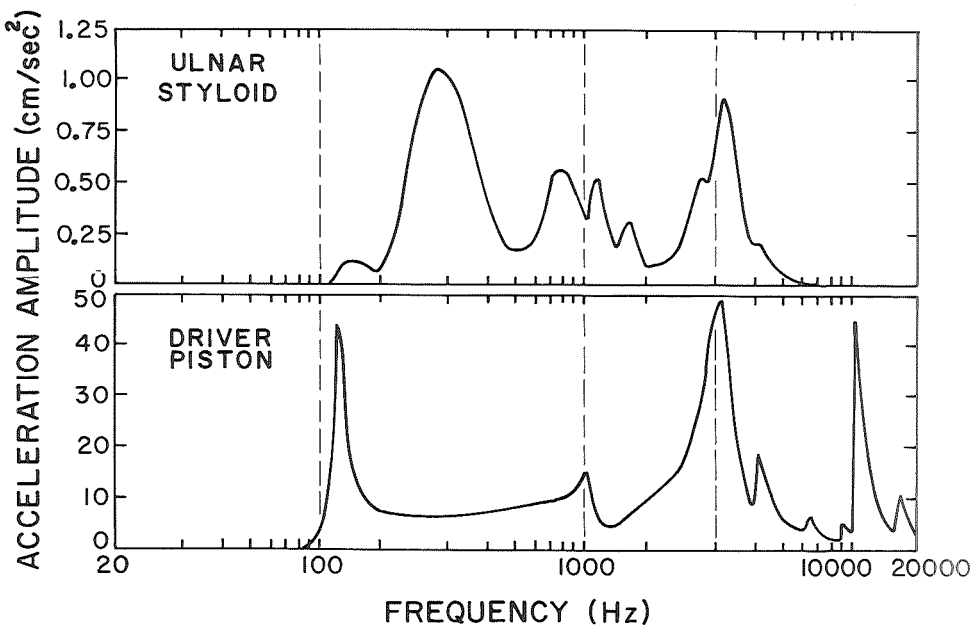

Figure 7.4 Acceleration spectrum of the driver piston (bottom) and of the ulnar styloid (top) for a typical adult. Note the ulnar resonance at about 290 Hz. The resonances at about 130 Hz, 1,000 Hz, and 3,000 Hz are driver resonances. The resonance at about 10,000 Hz is a resonance of the crystal accelerometer

8 BONE AT THE CELLULAR LEVEL: THE EFFECTS OF INACTIVITY*

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INTRODUCTION

It is known that bone is lost as a result of inactivity or immobilization. The mechanics of development of the osteopenia appear to be an increase in the amount of bone resorption. Some preliminary studies in animals have suggested that the increased resorption depends on the presence of both thyroid and parathyroid glands. However, since disuse atrophy can occur in a single bone, there must be a local phenomenon responsible for either increased sensitivity of the tissue to circulating hormones or an increase in the effectiveness of the hormones in their action on bone.

Various degrees of inactivity result in loss of bone known as osteopenia, ranging from severe, almost complete immobilization or paralysis, where severe osteopenia is the rule (refs. 1, 2), to more subtle bone loss, reported as a result of lack of gravitational force imposed on astronauts (refs. 3, 4). This section considers several questions that have arisen from the relatively few studies conducted to date.

WHAT IS THE MECHANISM OF BONE LOSS DEVELOPMENT?

Is there a failure of formation of new tissue or is there an increase in bone resorption? In so-called postmenopausal or senile osteoporosis, fractures secondary to bone loss are the main presenting features, and the bone turnover pattern is characterized by an increase in resorption of bone in the presence of normal levels of bone formation (fig. 8.1) (ref. 5). In disuse osteopenia, the pattern of bone turnover is distinct from that of senile osteoporosis and is a composite of the features of inactivity in normal people and osteoporosis. The specific question to be answered is: What is the effect of disuse alone on bone turnover? Data have been collected from four groups. Group 1 included ambulatory osteoporotic patients living a relatively active life but with the clinical symptoms of osteoporosis; that is, crush fractures of one or more vertebrae and back pain. Group 2 consisted of osteoporotic individuals who spent most of their time in bed. Group 3 included individuals without osteoporosis who had been in bed from 4 to 17 days for other conditions. Group 4 was the control group, that is, individuals who were not inactive and had no history of osteoporosis or bone disease. The majority of persons in group 4 had been run over in accidents or were suicide cases, and were used in age-matched groups for a comparison in each of the three experimental groups.

The results (figs. 8.2 and 8.3) show that extended rest in bed tends to result in decreased formation of bone in the presence of normal, or occasionally increased, levels of resorption. In

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osteoporotic individuals, who were also immobilized to the extent of rest in bed, bone resorption increased and formation decreased. Inactivity, therefore, appears to lead to a failure of new tissue deposition, possibly because osteoblastic activity is not stimulated. The effect is rapid, occurring after only a few days of inactivity. Much discussion of the effect of stress on cell activity strongly favors a mechanism whereby a force can influence osteoblasts to produce tissue. The mechanism suggested is the piezoelectric effect by which an electrostatic field may be responsible for controlling both osteoblasts and osteoclasts (refs. 6–8). A particularly relevant factor is the importance of some degree of stress in maintaining a level of bone formation considered normal.

IS INCREASED RESORPTION A FEATURE OF IMMOBILIZATION?

The control individuals (group 4) were at rest in bed for only a short time; whether increased resorption is a consequence of longer immobilization is another question. In one experiment directed at this question, the right hind limbs of adult dogs were immobilized in a plaster cast for 3 to 12 weeks. Bone from the left (or control) and the right immobilized limbs was compared at intervals during the experiment (ref. 9). Bone resorption did not alter for 6 weeks, but subsequently rose dramatically and then showed a decline (fig. 8.4). Bone formation showed an increase after 6 weeks and continued to rise at 12 weeks.

The pattern in this type of immobilization suggests that bone resorption is a later response; the data also indicate that a level of resorption is reached and is not exceeded. The rise in bone formation, which continues after resorption has ceased to increase, may be the result of stress. As resorption continues, causing loss of bone mass, the stress per unit volume of bone rises. Such a mechanism may be responsible also for the failure of resorption to show a continued rise. In hemostatic bone disorders such as osteoporosis, a similar sequence of events appears to occur; after considerable loss of bone, a point is reached at which resorption returns to normal and the remaining skeletal tissue is retained. Formation of bone is frequently elevated at this stage; in osteoporotic individuals with a long history of the disorder, elevated formation follows increased resorption, in somewhat the same way that it follows in the immobilized limbs of dogs.

WHAT IS THE PHYSIOLOGIC MECHANISM OF BONE LOSS?

The experimental study of immobilization osteopenia in dogs provided an opportunity for investigating the mechanism of development. Since parathyroid and thyroid glands are known to influence bone turnover (refs. 10, 11), the question is posed: Is the loss of bone mediated through the parathyroids, the thyroids, or both? The experiment consisted simply of comparing the bone loss in immobilized limbs in intact, parathyroidectomized, and thyroparathyroidectomized dogs. Table 8.1 shows the results of the comparison of the control and immobilized limbs. It is evident that removal of the parathyroids, the thyroids, or both, prevents bone loss (figs. 8.5 and 8.6). The parathyroid glands are responsible for bone resorption, although other physiologic mechanisms also play a role (ref. 12). It is not surprising, therefore, that removal of these glands stops the bone loss. The prevention of bone loss by the absence of the thyroids is less expected. Recent data, however, suggests that thyroxine levels have a profound effect on bone metabolism, and hypothyroid states result in a noticeable lack of cell response (ref. 13). A normal response may be elicited by the administration of thyroxine (ref. 14).

From this work, it is evident that cellular metabolism must be normal and there must be circulating parathyroid hormone. Since bone loss may be local, occurring only in the immobilized extremity, we attempted to find a local change in the blood of the immobilized limb. The pH, carbon dioxide pressure (pCO_2), and oxygen pressure (pO_2) were measured in the control and the experimental side of each animal at death; the blood leaving via the tibial vein in the immobilized limb was compared with the blood coming from the control side in each animal (fig. 8.7). It is evident that the depressed pH and pO_2, accompanied by the elevated pCO_2, occur only in the intact animals, which also are the only group in which osteopenia develops in the immobilized limb. The measurements, therefore, appear to reflect the bone loss rather than the immobilization. The factor responsible for the abnormal cell activity in disuse has not been found; again, possibly the piezoelectric effect should be checked.

WHAT FACTORS WILL PREVENT THE DEVELOPMENT OF DISUSE OSTEOPENIA?

A rather pessimistic view may be realistic in considering the prevention of disuse osteopenia in intact animals or man. In the presence of the parathyroids, calcitonin or any substance causing hypocalcemia will, in all probability, result merely in compensatory hyperparathyroidism. The depression of resorption by hormones has been demonstrated in persons with senile and post-menopausal osteoporosis (ref. 15), and bone loss has been prevented in aging women who have been given estrogen (ref. 16). It may well be, therefore, that patients with osteopenia of disuse also can be treated successfully with hormones.

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Table 8.1 A comparison percentage of bone formation and resorption in immobilized and control limbs (tibia)

	Resorption		*Formation*	
Animal	Control	Immobilized	Control	Immobilized
Intact	0.72	5.1	1.77	3.30
Parathyroidectomized	0.66	0.76*	2.56	3.76
Throidectomized	0.33	1.00*	0.25	0.35*
Thyroparathyroidectomized	0.28	0.06*	0.68	0.66*

*Significantly different from the value in the intact animal.

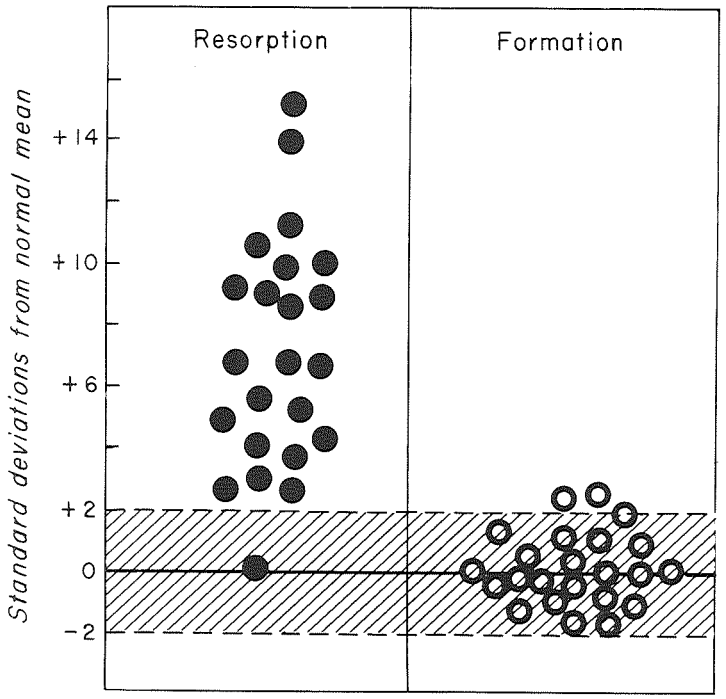

Figure 8.1 Bone turnover in osteoporosis. The normal mean and ± 2 SD of age-matched normals are shown in the hatched zone. The abnormality is in an elevated resorption level.

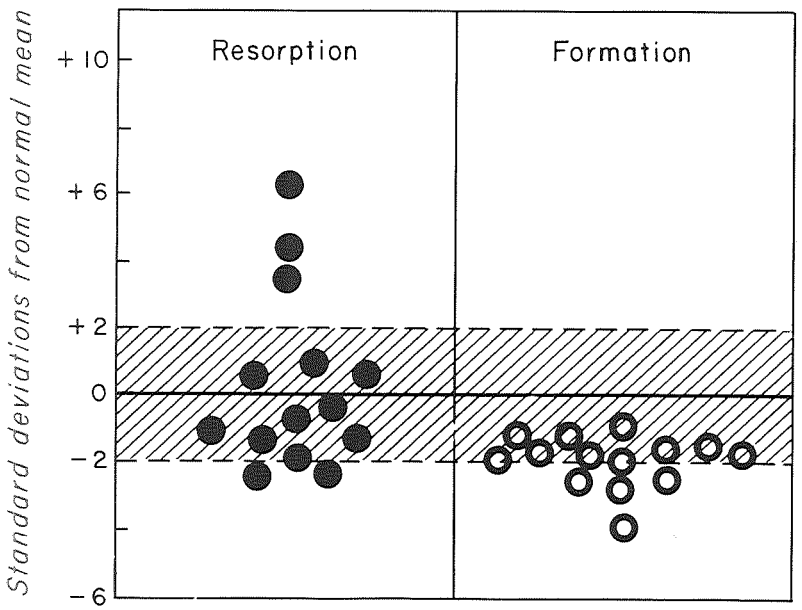

Figure 8.2 Bone turnover in persons at rest in bed from 4 to 17 days. The normal mean and ± 2 SD are shown in the hatched zone. Bone formation is low.

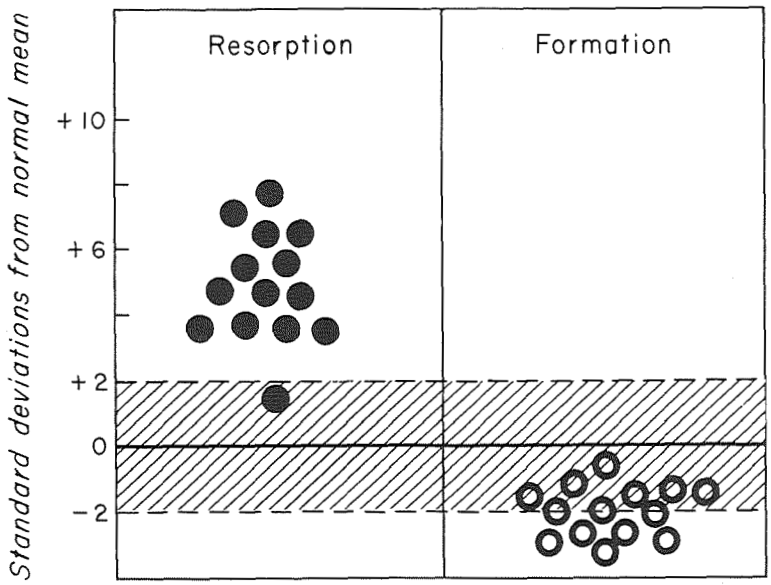

Figure 8.3 Bone turnover in osteoporosis and persons at rest in bed. The normal mean and ± 2 SD are shown in the hatched area. Resorption is elevated and formation is depressed.

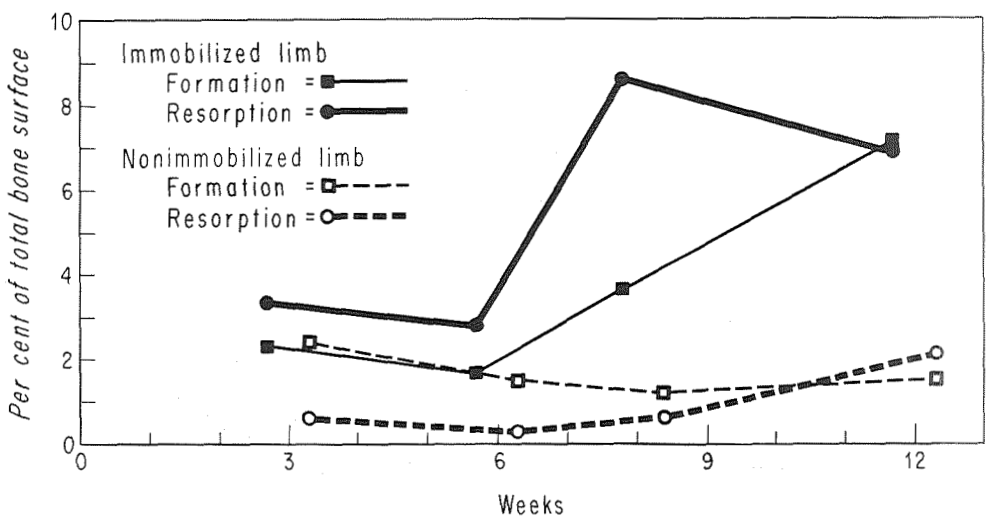

Figure 8.4 Bone resorption and formation in the control (nonimmobilized) and immobilized limbs of intact dogs after 3 to 12 weeks.

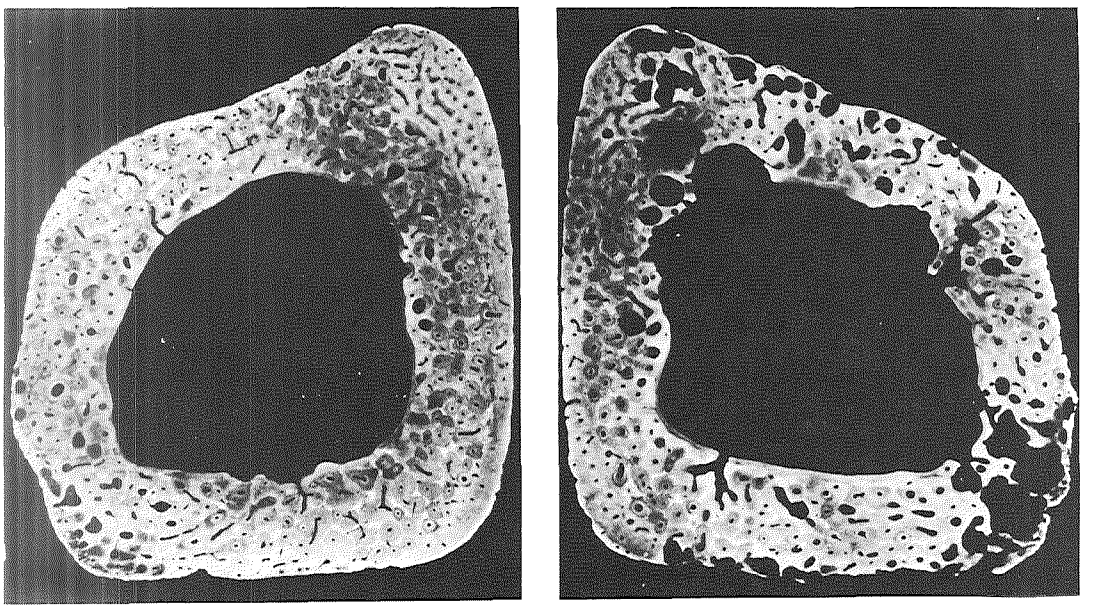

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Figure 8.5 Microradiographs of left and right metatarsals of an intact dog in which the right hind limb had been immobilized for 12 weeks. Resorption increased and consequently the number of holes in the cortical bone on the immobilized side increased (X 10). (From Burkhart, J. M.; and Jowsey, Jenifer,: Endocrinology, vol. 81, 1967, pp. 1053–1062, by permission of J. B. Lippincott Company.)

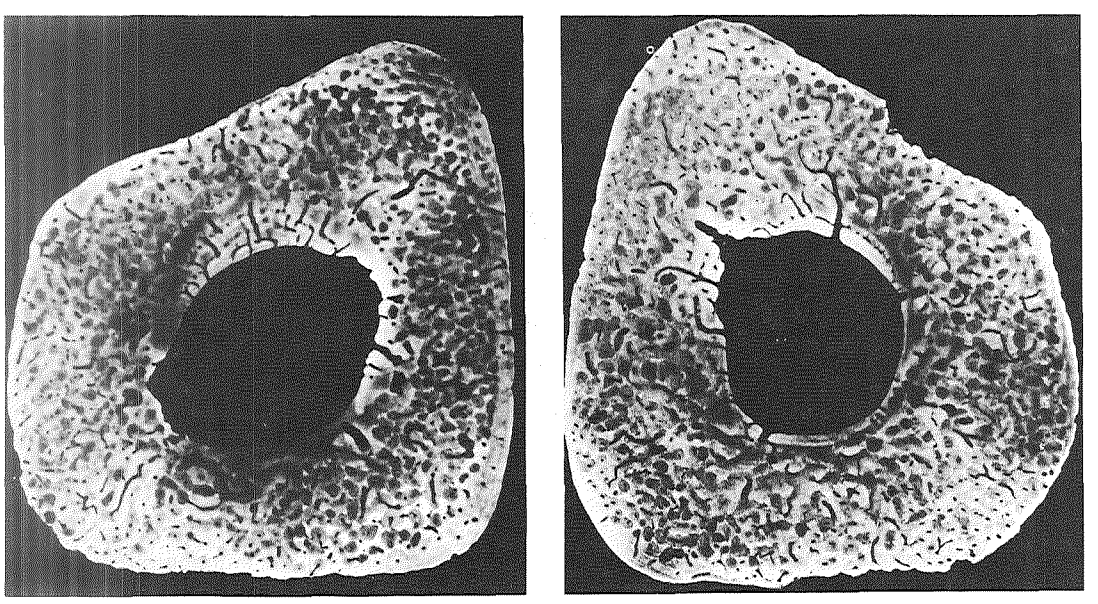

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Figure 8.6 Microradiographs of the left and right metatarsals of a parathyroidectomized dog in which the right hind limb had been immobilized for 12 weeks. No difference is evident between the bone from the left and right limbs (X 10). (From Burkhart, J. M.; and Jowsey, Jenifer: Endocrinology, vol. 81, 1967, pp. 1053–1062, by permission of J. B. Lippincott Company.)

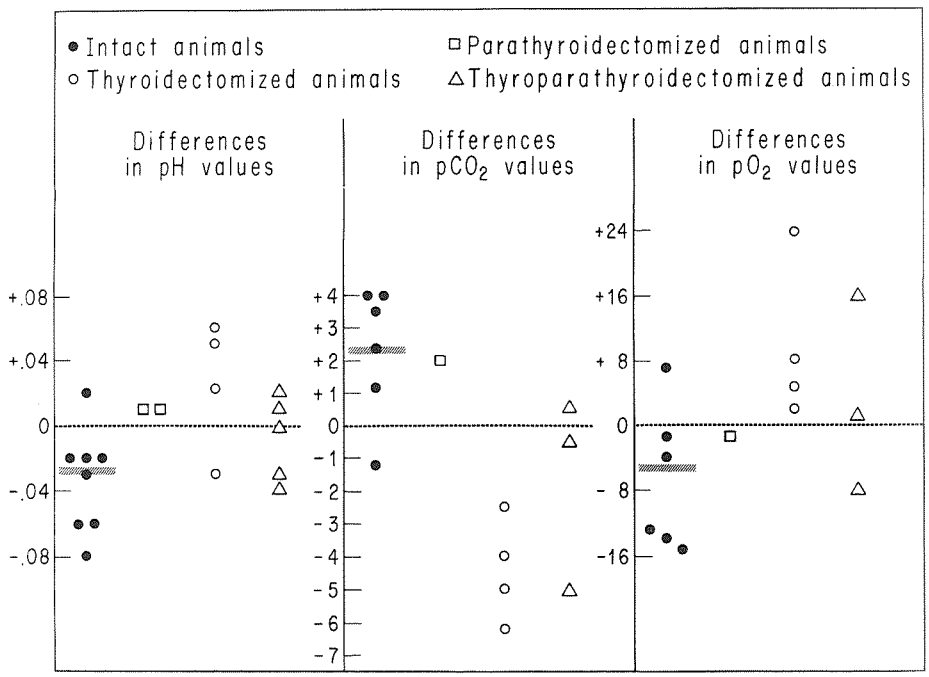

Figure 8.7 Comparison of blood measurements from immobilized and nonimmobilized limbs. (From Burkhart, J. M.; and Jowsey, Jenifer: Endocrinology, vol. 81, 1967, pp. 1053–1062, by permission of J. B. Lippincott Company.)

9 ESTIMATION OF TOTAL SKELETAL MASS IN MAN BY RADIOISOTOPE DILUTION*

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INTRODUCTION

The skeleton is an unusual organ in that it consists of a small cellular component of osteocytes living within a large extracellular mass or tissue of mineral plus protein matrix. It is heavy yet relatively water free as compared with most body organs, and it contains major fractions of the body's calcium, phosphorus, sodium, chloride, and nitrogen.

Unfortunately, most of these major elemental components of bone do not equilibrate fully with their radioisotopic tracers; therefore, the size of the skeleton cannot be measured with direct isotopic dilution techniques as can many components of the body. The mass of the skeleton may be inferred, however, by its nonparticipation in isotopic dilution studies: it is a major component in the body compartments of extracellular tissue (ECT) and in the fat-free body (FFB) or its water-free portion, the fat-free solids(FFS). With its relative acellularity, the skeleton is essentially potassium poor and thus has a low ratio of K to FFS (meq/kg). This is to be contrasted with the metabolically active tissues of the body cell mass (BCM), such as muscle or viscera, where the predominance of cells with their high intracellular concentrations of potassium give a high K/FFS ratio.

The importance of the two contrasting ratios in these two different systems was noted by Moore (ref. 1), who found that the overall K/FFS ratio fell in chronically ill and wasted patients. When analyzed radiologically, the skeletons appeared to change—but slowly; it was postulated that they were becoming more prominent in the overall K/FFS compositional ratio, whose decreasing numerator of potassium or exchangeable K (K_e) reflected the depletion of the BCM, especially muscle. Thus, an attempt to predict skeletal weight using the compositional terms of K_e and FFS was made from the observed values in isotope studies and calculated values obtained from regressions based on weight, sex, and age. The predicted values were then combined with Allen's data (ref. 2), and an equation devised that related skeletal size to K_e and the K_e/FFS ratio.

This section outlines the derivation of the equation and the supporting body composition regressions that use weight, age, and sex parameters to predict the compositional values needed. Additional data (ref. 3), from a patient with antemortem isotope dilution studies and postmortem skeletal dissection with chemical analysis, are presented for both the verification of this technique and for discussion of actual measurements of skeletal size in man.

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BODY COMPOSITION RELATIONSHIPS AND PREDICTIVE REGRESSIONS

In body composition studies, there are many ways to partition the body components into chemical elements, solid or aqueous phases, or anatomic categories. Physicians are most concerned with the concentrations or quantities within the anatomic areas, whereas most isotope dilutional techniques measure total quantities of body components, which usually cross several anatomic boundaries. There are, however, many interrelationships of anatomic and compositional categories that can be defined and correlated either among themselves or to externally measured parameters, such as weight.

Accordingly, the body may be partitioned into fat and the FFB, the latter comprising ECT and the cellular tissues of the BCM:

$$\text{Body weight (Bwt)} = \text{BCM} + \text{ECT} + \text{fat} \tag{1}$$

$$\text{FFB} = \text{BCM} + \text{ECT} \tag{2}$$

The BCM is the energy-exchanging, work-performing core of the body and encompasses primarily muscle, viscera, and brain. As it is intracellular, the BCM also relates to the volume of intracellular water (ICW) and to exchangeable potassium (K_e), of which 98 percent exists within cells. In contrast, extracellular fluid (ECF) is the largest aqueous component and the skeleton the largest solid component of extracellular tissue. Thus, it is apparent that the measurements of total body water (TBW), and its partitioning within (ICW) and without (ECF) cells; body potassium (K_e); and body fat are important investigative tasks.

The validity and accuracy of the isotope dilutional techniques using tritiated water (THO) for TBW, radiobromide (^{82}Br) for chloride and hence ECF, and ^{42}K for K_e after 4, 24, and 40 hr of equilibration time, respectively, have been presented and reviewed critically (refs. 1, 4). ICW is defined as TBW minus ECF, and regressions for prediction of the latter two quantities have been created from best-fit correlations (linear or exponential) between body weight, sex, and age and observed values in man (ref. 1). In young healthy males, TBW and ICW are predicted by these equations:

$$\frac{\text{TBW}}{\text{Bwt}}\,100 = 79.45 - 0.24(\text{Bwt}) - 0.15(\text{age}) \qquad \text{Male} \tag{3}$$

$$\frac{\text{ICW}}{\text{TBW}}\,100 = 62.3 - 0.16(\text{age}) \qquad \text{Male} \tag{4}$$

Body weight is the principal determinant in equation (3), but it appears as $(\text{Bwt})^2$ because heavier people have proportionately more fat than water content.

The estimation of body fat is important in these derivations. It is assumed that the body fat is anhydrous and that fat-free tissues of the body have an overall water content of 73 percent. The formula of Pace and Rathbun (ref. 5) is then used.

$$\text{FFB} = \frac{\text{TBW}}{0.732} \tag{5}$$

This coefficient of hydration (73 percent) in normal man is based on histochemical analyses, which range from 71 to 74 percent.

This value is quite constant in normal subjects, but corrections are necessary in the presence of abnormal hydration, as is often found in chronic illness. A nomogram for this purpose has been constructed by Moore et al. (ref. 1, p. 16) using the measured relationships of ICW to TBW and the observed ECF value to that predicted on the basis of age, weight, and sex. This correction can be of great importance in the estimation of the FFS of a subject.

With equation (5) it is possible to calculate total body fat and its contribution to total body solids, the residual being the FFS:

$$\text{Fat} = \text{Bwt} - \text{FFB} \tag{6}$$

$$\text{FFS} = \text{FFB} - \text{TBW} \tag{7}$$

By definition, the ECF can be found as the difference between TBW and ICW,

$$\text{ECW} = \text{TBW} - \text{ICW} \tag{8}$$

and extracellular potassium (K_{ec}) can be calculated from

$$K_{ec} = \text{ECW} \cdot (K)_{ec} \tag{9}$$

where $(K)_{ec}$ is assumed to be 4 meq/liter. It follows then that the total body potassium, as approximated within 4 percent by K_e, may be calculated by this relationship.

$$K_e = (K)_{ic} \cdot \text{ICW} + K_{ec} \tag{10}$$

Here intracellular potassium $(K)_{ic}$ concentration is taken as 150 meq/liter, a normal value found by tissue analysis and compositional studies in normals. The stability of this concentration gives a consistency to correlations between TBW, ICW, and K_e such that regressions like the one shown in figure 9.1 can be obtained. The predictive equations have been constructed from these tight correlations in the observed data from man and, hence, enable the investigator to make a good estimation of body composition when isotopes are not available.

DERIVATION OF PREDICTIVE REGRESSION FOR SKELETAL MASS

In an attempt to define skeletal mass as a major component of the FFB or the extracellular fat-free solids (FFS_{ec}), the preceding equations were applied to Allen's data (ref. 2) for estimates of dry bone mineral weight as predicted from bone width, body weight and density, and total body water. A factor of 1.5, from the bone composition study of Forbes et al. (ref. 6), was used to convert Allen's bone mineral (m) to dry, fat-free whole bone (mineral plus organic matrix). The bone (B) to fat-free body ratio (B/FFB) was found to be 10.3 percent with a small range of variation. Additional support for the dry, fat-free skeleton, approximately 10 percent of the FFB, was constructed from the literature data of Mitchell et al. (ref. 7) and Forbes and Lewis (ref. 8), where values of 8.7 and 11.6 percent, respectively, were calculated.

In a series of 40 ill patients undergoing body composition studies, a skeletal weight equal to 10.3 percent of the FFB, estimated to be present during good health, was calculated. As mentioned

earlier, the finding of a decreasing K_e/FFS ratio implied that the skeleton was becoming more prominent in the denominator as muscle wasting reduced the K_e of the numerator. Also, it was felt that minimal skeletal depletion had occurred, and that these estimates for bone weight, based on predicted FFB values, should give a reasonable first approximation. As body wasting proceeded, the observed K_e/FFS ratio dropped and the B/FFB ratio increased from 11 to 15 percent. A regression of statistical significance ($P < 0.01$) for fat-free dry bone (B) was created as follows:

$$B = \frac{FFS - 0.5}{1.32 + 0.005(K_e/FFS)} \tag{11}$$

This equation was used to predict the bone mass in the subject with premortem compositional studies and postmortem skeletal dissection. The predicted weight was 0.7 kg above the measured 2.8 kg and the B/FFB ratio was revised to 8.5 percent. A recalculation of the bone weights in the group of 40 patients then yielded this new regression:

$$B = \frac{FFS - 0.61}{1.61 + 0.0062(K_e/FFS)} \tag{12}$$

This equation can be plotted as a series of regressions between K_e and the K_e/FFS ratio (fig. 9.2). Thus, with this nomogram (or equation (12)) plus equations (3) through (10), a prediction for skeletal weight (dry, fat-free bone of mineral plus matrix) can be made from body weight, sex, and age.

A METHOD OF SKELETAL PROCESSING AND ANALYSIS

There are few data in the literature concerning actual skeletal weight in humans of known age, weight, body composition, or other major body parameters; further, there is no standardizing way to clean, dry, or define the skeletal mass. Thus, the processing and analysis of the skeleton is important.

Specifically, the cadaver used in this study was sealed in plastic after postmortem examination and then frozen at –10° C until dissection. After the subject was thawed to room temperature, a gross, sharp anatomic dissection was made of all tissues and organs. Small amounts of periosteal tissue remained, as did marrow, and the bones were sawed longitudinally to expose all marrow cavities. The skeleton was then cleaned in a dermestid beetle colony at high humidity for several months, as described by Russell (ref. 10). At this point, the bones were totally clean, yet even the fine spicules of the marrow cavities appeared intact. The bones were dried at 90° C to constant weight and the skeleton was crushed or split into small cubes for reduction to powder in a commercial hammer mill grinder.

Nitrogen content was measured by macroKjeldahl digestion and titration techniques, and fat content was determined by Soxhlet extraction with ether. The bone powder was dry ashed at 350° to 400° C in a muffle oven, and electrolyte composition was measured from the dry-ash material dissolved in acid.

The results are summarized in table 9.1 in terms of both the wet skeleton before and after beetle cleaning, and the dry, fat-free skeleton. The first four subjects are from published data. Note that the increased weight (as % Bwt) and increased water content in the presence of decreased

ash, calcium, and phosphorus content probably signifies an increased residue of soft tissue on this skeleton. This finding is also implied by the loss of 5.5 kg of weight during beetle cleaning. The resulting clean wet skeleton, however, has a very low fat content (less than 2 percent) and a low water content (12.2 percent), which reflect meticulous cleaning out of marrow and periosteal tissues by the beetles rather than dehydration as high humidity is maintained in the colony.

It would appear that this method gives results for the entire skeleton that are comparable to the most carefully hand-performed microdissections as reported by Baker et al. (ref. 11).

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Table 9.1 Skeletal mass and composition

Study	Sex	Body Wt, kg	% Bwt	% Water	% Fat	Skeleton % Protein	% Ash	% Ca	% P	Conditions
Mitchell et al. (ref. 7)	M	70.6	14.84	31.8	17.2	18.9	28.9	11.0	4.8	Wet skeleton
Forbes et al. (ref. 6)	M	53.8	17.58	28.2	25.0	19.7	26.6	10.7	4.6	Wet skeleton
Forbes et al. (ref. 9)	M	73.5	14.95	30.2	22.0	19.7	27.2	10.2	4.8	Wet skeleton
	M	62.0	16.68	39.5	10.1	20.8	29.2	11.5	5.2	Wet skeleton
Moore et al. (ref. 3)	F	43.4	20.3	40.3	18.2	19.4	21.2	7.0	3.2	Wet skeleton and soft tissues
	F	43.4	7.6	12.2	1.8	31.3	55.7	18.8	8.6	Wet skeleton without soft tissues after beetling
	F	43.4	6.5	0	0	36.4	64.7	21.9	10.0	Dry fat-free skeleton

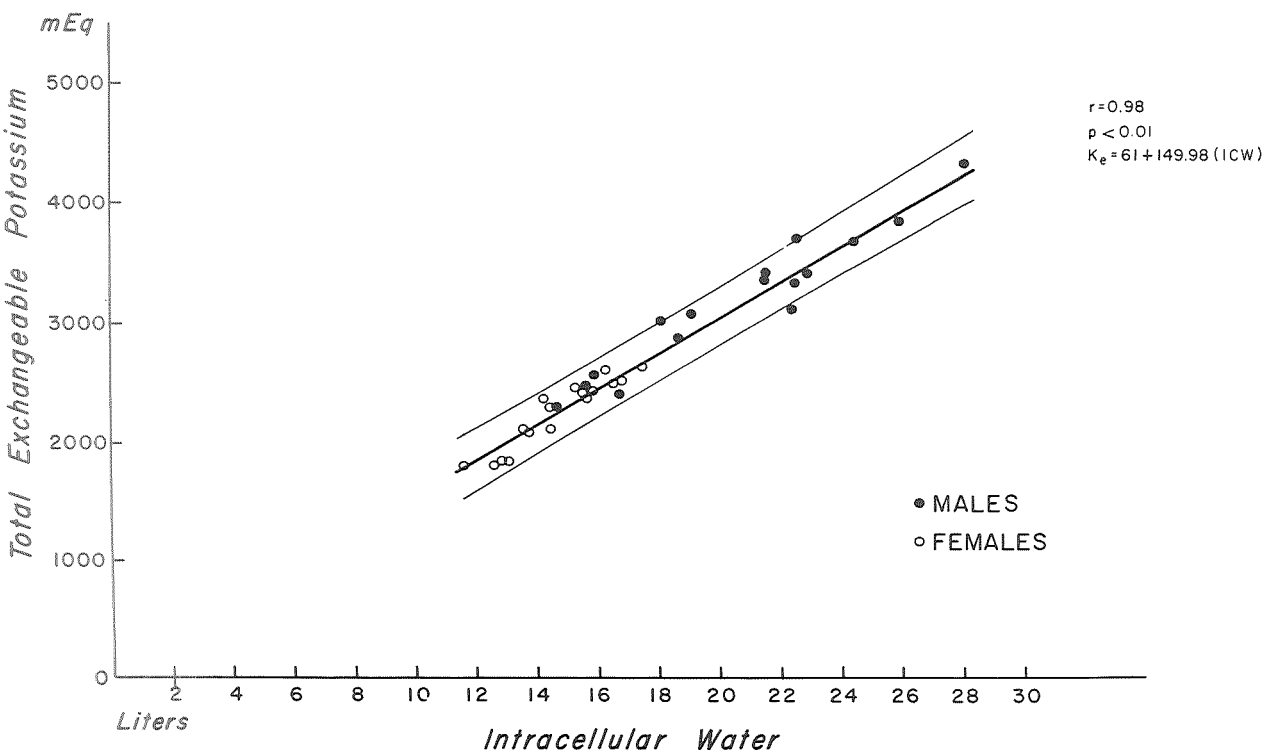

Figure 9.1 This regression of K_e on ICW (TBW - ECF) demonstrates the constancy of intracellular potassium (K_{ic}) concentration near 150 meq/liter ICW and forms the basis of the predictive regression of $K_e = 61 + 150 \cdot ICW$. From Moore et al. (ref. 1).

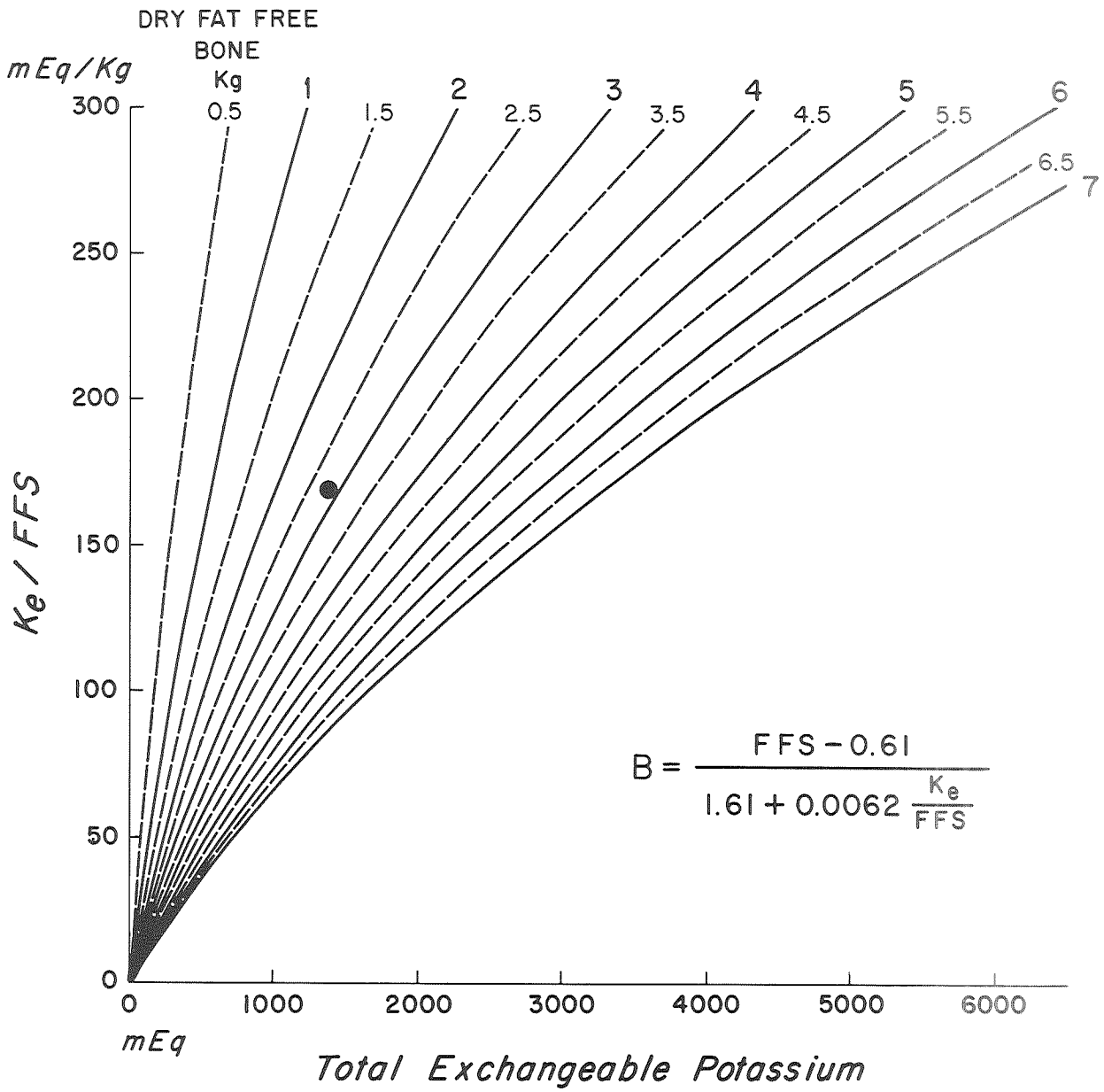

Figure 9.2 This nomogram predicts the dry, fat-free skeletal weight from regressions based on exchangeable potassium (K_e) and the ratio of K_e to fat-free solids (K_e/FFS). K_e is a measure of the body cell mass (BCM) and the ratio of bone to the fat-free body (B/FFB) is estimated as 8.5 percent. From Moore et al. (ref. 3).

10 DISUSE ATROPHY IN MACACA MULATTA AND ITS IMPLICATIONS FOR EXTENDED SPACEFLIGHT

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INTRODUCTION

Osteoporosis is a clinical syndrome with many causes. Most commonly it is associated with aging and accompanying hormonal changes. It appears after trauma, in endocrine and metabolic disorders, in vitamin deficiencies, after the administration of therapeutic agents, and in patients immobilized for extended periods of time, as with major fractures or paraplegia.

Disuse atrophy of bone is a local form of osteoporosis in which total bone substance is reduced due to the lack of musculoskeletal activity and/or weight-bearing. The clinical manifestations of disuse atrophy are somatic pains, especially backache, predisposition to vertebral collapse, and deformities resulting from structural alterations of weakened bones. Pathologically, there is a loss of internal lamellae of cortical bone. The trabeculae of cancellous bone become attenuated and finally disappear. Serum calcium, phosphorus, and alkaline phosphatase levels remain normal. The increased turnover of bone mineral results in hypercalcuria, negative skeletal balance, and acute bone atrophy (refs. 1–8).

The advent of manned space and lunar exploration has emphasized interest in those physiological processes of the body that are partially influenced by gravity. One of the complications of prolonged exposure of living systems to the hypogravic state may result from the wasting away of bony elements, and the failure of their restitution by the cellular process of regeneration.

The recent American and Soviet manned spaceflights demonstrated that astronauts can perform critical tasks and remain safely in space for up to 14 days. Nevertheless, postflight X-ray photometric examinations of selected bones of crew members of Gemini 4, 5, and 7 revealed a 12 to 15 percent decrease in the optical density of bony tissue. Similar changes in bone density were also noted in the Soviet dogs, Ugolek and Veterok, after their 22-day flight on Kosmos 110 (ref. 9).

A number of investigators believe that, in prolonged spaceflight, bone demineralization must be taken into account in assessing an astronaut's capability, and may present a hazard to an astronaut's health and cause serious physiologic disorders. Clinical experience with persistent hypercalcuria and hypercalcemia show increased incidence of bone fractures, deposition of calcium in joints, hypercalcuria, negative skeletal balance, and increased incidence of urinary tract calculi. Speculation that there will be bone demineralization in zero gravity is well supported by studies of bed rest and water immersion (ref. 10). Assuming that the resulting bone structure and strength constitute natural adaptation to the zero gravity environment, such adaptation would not necessarily be a hazard per se, so long as the astronaut remains in this environment. However, the

acceleration maneuvers of spaceflight and the rapid changes of the force environment when the astronaut returns from zero gravity to the earth's gravitational field make it mandatory that any changes in man's resistance to mechanical loads and the time constants involved in such changes be quantitatively understood.

The mechanisms operative in producing these changes in bone structure, the time constants involved, the reversibility of these effects, and corrective measures to counteract these adverse effects are not well understood. To provide a partial answer, a program was initiated to investigate the response of the Rhesus monkey to prolonged plaster of paris immobilization. Changes in bone structure as well as quantitative changes of bone strength in situ were observed (ref. 11). As a means of evaluating the practical and operational significance of these changes, some of the primates were exposed after immobilization to impact loads for which injury probability for normal controls was known.

These primates were restrained in a zoometrically designed seat and exposed to longitudinal (+Gz) transient acceleration in experiments designed to produce vertebral fracture of the type seen in man during longitudinal spinal impact. Some of these immobilized and impacted animals were allowed to recover for 7 months under normal conditions so that delayed manifestations of the impact trauma and recovery could be observed. This section summarizes the basic structural changes of bone and changes in spinal impact tolerance resulting from prolonged immobilization.

MATERIALS AND METHODS

Forty male clinically screened Rhesus monkeys (Macaca Mulatta), ranging in weight from 7 to 10 kg, were surveyed radiographically to demonstrate maturity by epiphyseal closure. The 20 control and 20 experimental animals were housed in an air-conditioned, windowless room illuminated with flourescent light 12 hr per day. Temperature of the room was kept at 78° ± 2° F. Throughout the entire experimental period, the animals were fed a standard diet of monkey chow from a single feedlot. Control and experimental primates selected were placed in metabolic cages for 7 days. Baseline anterior-posterior and lateral whole-body radiographs were taken of the axial skeleton on day 1 of the conditioning period. The radiographic data taken during this period were used as baseline data. After 7 days of conditioning, the control as well as the experimental monkeys were anesthetized with pentobarbital (1 cc/5 lb) and radiographed. The control animals were returned to the metabolic cages, and the animals selected for immobilization were prepared for plaster cast immobilization. Under anesthesia, the animals were wrapped in several layers of cotton gauze; the bony areas and promentia were padded with felt. Quick setting plaster of paris was used to construct a full body cast (fig. 10.1). The experimental animals were hand fed (required by the arm immobilization) twice daily; food intake was weighed and recorded. Monkey chow was dropped into the food trays of the controls. Water (distilled) was made available at regular intervals 16 times daily. At 2-week intervals, control and experimental primates were anesthetized, removed from their restraints, taken, weighed, radiographed, and examined for complications.

The 40 animals of this series of experiments were used for the three pilot experiments outlined below.

Experiment 1: Immobilization, necropsy. At the end of 60 days of immobilization, 12 animals (6 experimental, 6 controls) were killed with an overdose of pentobarbital. A complete necropsy was performed, and representative soft tissue samples of all organ systems prepared. Fresh, undecalcified, unembedded, undehydrated segmental sections were made of the left tibia and femur, calcaneus, and fourth lumbar vertebra. The bony sections of the control and immobilized animals were studied using macrophotographic techniques. (Results concerning the left tibia, femur, and calcaneous are not discussed here.)

Experiment 2: Immobilization, longitudinal spinal impact, immediate necropsy. Following 60 days of immobilization, 14 animals (7 experimental, 7 controls) were subjected to longitudinal spinal impact at predetermined acceleration levels. The control and immobilized animals were anesthesized, radiographed, and restrained on the impact vehicle as shown in figure 10.2. Immediately following impact exposure, these animals were killed with an overdose of pentobarbital. A complete necropsy was performed and representative soft tissue samples of all organ systems prepared for histopathological examination. The entire vertebral column was grossly dissected to study the macroscopic morphologic aspects of fracture patterns observed on radiographs of the cancellous and cortical bone.

Experiment 3: Immobilization, longitudinal spinal impact, necropsy delayed 7 months. Following 60 days of immobilization, 14 animals (7 experimental, 7 control) primates were subjected to longitudinal spinal impact. Immediately thereafter, the condition of these animals was ascertained by physical examination including neurologic evaluation and anterior-posterior and lateral whole-spine radiographs. Immediate postexposure radiographs confirmed markedly disparallel vertebral borders, loss of vertebral body height, and narrowing of intervertebral space. These animals seemed to be in good physical condition and without detectable severe neurologic deficit. Following impact exposure, the controls and immobilized primates were returned to their postimmobilization cages. Spinal radiographs were taken at monthly intervals. At the end of 7 months, these animals were killed with an overdose of pentobarbital. The entire vertebral column was grossly dissected and a detailed postmortem examination conducted with meticulous dissection of vertebrae, cartilaginous end plates, intervertebral disk structure, spinal cord, and paraspinous muscles and ligaments.

For investigation of impact acceleration effects, the Vertical Deceleration Tower at the Aerospace Medical Research Laboratory was modified as reported earlier (ref. 12). Immobilized and control primates were restrained in a zoometrically designed drop seat; the vehicle was raised to the predetermined drop height and released. The vehicle was allowed to free fall, guided by two steel cables. The energy absorbing brake was aluminum honeycomb, which provided a repeatable and reproducible rectangular acceleration time history. All acceleration time histories were recorded on high-capacity piezoelectric accelerometers. The acceleration levels and patterns were selected to produce no spinal injury detectable by radiographic analysis in the control animals directly after impact. The acceleration patterns were approximately rectangular in shape with an average acceleration value of 80 to 90 G and a duration of 10 to 17 msec.

To demonstrate bone remodeling dynamics at selected skeletal sites, 250 mg/kg of tetracycline or its derivative was administered intravenously weekly 4 weeks prior to the immobilization period and every 10 days during the immobilization period to all the primates in this experiment. The results of these tests are incomplete and will be reported at a later date.

PROCESSING OF MATERIAL

Bones of interest were grossly dissected and stripped of all soft tissue. Sagittal and transverse plane segmental sections were prepared using a bandsaw. The trabecular structure and marrow cavity were stripped of hematopoetic tissue and fatty tissue with a high-velocity stream of water. The specimens were identified, blotted, and dried in air.

Macrophotographs were taken and analyzed in detail for evidence of atrophy. Samples from specific regions of the controls were compared with corresponding regions of the immobilized bone. In vivo and postmortem radiographs were studied for comparison of trabecular architecture and cortical thickness.

Particular attention was given to the architectural changes of the femora and lumbar vertebrae of the immobilized and control monkeys. The gross mechanical properties of normal and immobilized vertebral bodies were investigated and reported elsewhere (ref. 11).

RESULTS

Experiment 1

Comparison of the macrophotographs of the vertebral bodies of the experimental and control animals revealed a marked difference in the vertebral bodies' architecture, which must be expected to result not only in modification of its static strength but in an alteration of its dynamic properties.

The normal vertebral body consists of cancellous bone covered by a thin surface layer of compact bone. The cortex is perforated by numerous orifices that channel the nutrient blood vessels to the vertebra. A sagittal section through the centrum of a vertebra reveals that the bony fibers of the cancellous tissue are arranged vertically and horizontally in line with the longitudinal axis of the vertebral column. The interior of the vertebral body is interrupted by vascular connective tissue and canals for veins, which converge toward a large, irregular aperture at the posterior portion of the vertebral body. The trabecular structure of the vertebral body presents a beautiful arrangement of lattice networks. The individual trabeculae are surrounded by hematopoetic tissue. The dynamic hydraulic pressures created within the vertebral body are a function of the "prestress" in the spinal column, systolic and diastolic pressure, and dynamic loading. Each individual body acts as a viscoelastically damped, load-carrying member. The combination of trabecular structure surrounded by a fluid medium provides optimal stress response and exhibits a complicated rate-dependent behavior, including instantaneous elasticity, delayed elasticity, and viscous flow. The degree of damping is a function of the geometric three-dimensional configuration, the physical properties of the viscous medium, the trabecular configuration, and surrounding ligaments and muscle.

Compression testing of fresh vertebral segments revealed at least a twofold and as high as a threefold difference in strength of the vertebral bodies with respect to axial compressive loads (ref. 11).

These tests were performed at low strain rates. It should be emphasized that the mechanical behavior of biologic tissue differs significantly under conditions of static, rapid, and impact loading, and that the present state of knowledge concerning the dynamic behavior of biologic material is still embryonic.

Macrophotographs of normal and immobilized dry bone segments showed a reduction in size and number of trabeculae, accompanied by a decrease in plate size, orientation, and porosity. Typical examples are given in figures 10.3 and 10.4. Note the increased irregularity and number of perforations below the lamina terminalis. The regions adjacent to the cartilaginous end plates and just below the lamina terminalis show a decrease in density, yet they remain fairly regular (fig. 10.5). Increased resorption of cortical bone was observed on the macrophotographs at points of muscle and tendinous attachment, which resulted in the formation of numerous resorption channels (fig. 10.6).

Experiment 2

The control and immobilized animals were anesthesized, radiographed, and both animals were similarly restrained on the impact vehicles (fig. 10.2) and impacted. Measurement of thoracic and lumbar vertebrae indicated that the G level required to produce an average 20 percent loss of vertebral body height in the immobilized primate was approximately 30 percent less than in the control animal. In both cases, necropsy showed that the mechanism of energy dissipation within the vertebral bodies was to drive the viscous hematopoetic tissue out of the paravertebral sinuses and under the surrounding ligamentous structure. Fractures were not seen on the radiographs of the control animals, a fact confirmed during necropsy examination.

Radiographs of the experimental animals showed no indication of vertebral body fracture. Necropsy confirmed vertebral body collapse in the immobilized animals, and showed protrusion of intervertebral disk substance into the adjacent vertebral body through minute stellate tears in the central portion of the adjacent cartilaginous end plate. This initial prolapse was not demonstrable radiographically. This pathology was seen in five of the seven immobilized animals. Central cartilaginous end plate tearing was not found in the control animals.

Experiment 3

Following 60 days of immobilization, the control and immobilized animals were anesthesized and subjected to longitudinal spinal impact at similar acceleration time histories as under experiment 2. Immediately following impact exposure, a routine anterior-posterior and lateral whole-body and spinal radiograph was taken and the animals were returned to normal cages (identical to control cages). Initial radiographs showed no indication of major vertebral body trauma. On a monthly basis for a period of 7 months, routine anterior-posterior and lateral spinal radiographs of immobilized and control animals were taken. At the end of 3 months, lateral radiographs showed a multiple discontinuity in the contour of the cartilaginous plate in five of the seven immobilized primates. Subsequent serial radiographs revealed a gradual development of disk herniation proceeding

in the longitudinal direction of the vertebral column. Radiographs taken at the end of 7 months stenciled the bony barriers surrounding the semisolid nucleus pulposus.

The spinal radiographs of the control primates were negative directly after impact and at each control time interval thereafter. The seven controls and seven immobilized primates were killed with an overdose of pentobarbital. Serial sagittal sections of selected vertebrae were prepared and photographed. Figure 10.7 is a sagittal section of T_6–T_7 excised from an immobilized animal. The bony barrier surrounding the herniated material (Schmorl's node) is visible. Vertebral collapse is evident along with disorientation of the trajectorial lines within the vertebral body. The central depression, or "fish," vertebrae within the vertebral body are also present.

DISCUSSION

Osteoporosis is defined as a generalized process resulting in less absolute skeletal mass than in a similar "normal" skeleton. Because of insufficient data, however, there is no precise standard or conventional numerical figure for normal or abnormal bone.

The most important practical determinant in disuse osteoporosis appears to be alterations of mechanical stress caused by decreased axial loading of bone through muscular and gravitational interaction. The osteoporosis develops and may be a generalized or local condition of bone rarefaction affecting trabecular and cortical bone.

The cellular components of bone comprise osteoblasts for the formation of bone, osteocytes for the maintenance of bone as a living tissue, and osteoclasts for the resorption of bone. The healthy mature skeletal system exhibits bone resorption and formation at characteristic and relatively constant rates, with variations that are a function of aging, physical activity levels, mineral nutrition, hormonal balance, systemic or local disease, and numerous other factors.

Bone formation consists of two processes, the formation of an organic protein matrix (principally protein, collagen, and mucopolysaccharides) and the deposition in this matrix of bone mineral, a complex microcrystalline compound of calcium and phosphate (hydroxyapatite) with a small amount of calcium carbonate. Bone is a polyphasic material, apatite characterized by a high elastic stiffness and compressive strength and low tensile strength. Collagen has a low elasticity but high tensile strength. These two materials and most likely a third (acting as a cement) combine to form a composite structure and account for the physical and mechanical properties of bone (ref. 13).

The influence of mechanical stress on the skeletal system has been well recognized. As early as Galileo, investigators have studied the problem of the load-carrying capacity of structural elements in the living system and the mathematical analysis of internal forces and deformations induced by applied loads.

The role of mechanical stress in determining bony architecture through influence on the bone cells has been a subject of long debate. The trajectorial theory of bone structure, as developed by Culmann in 1865, von Meyer in 1867 (refs. 14–16), Bardeleben in 1874 (ref. 17), and Wolff in 1892 (ref. 18), postulated that trabeculae were modeled along paths of compressive or tensile force.

Wolff's classical statement was that: "Every change in the form and function of bones, or of their function alone, is followed by certain definite changes in their internal architecture and equally definite secondary alteration in their external conformation, in accordance with mathematical laws" (ref. 18).

In Laws of Bone Architecture, Koch concluded that cancellous and compact bone are so composed as to produce maximum strength with a minimum of material, and that in form and structure bones are designed to resist compressive loads (ref. 19).

These concepts have been challenged by numerous investigators. However, certain facts concerning the structure of bone cannot be disputed: (1) the structure of bones is related to the mechanical stress to which they are subjected; and (2) a relationship exists between bone architecture and physical dynamics of remodeling, and between the geometry and cellular dynamics (ref. 20).

In recent years there has been a growing interest in the role of stress-induced electrical potentials in bone. Yasuda and Fukada were first to demonstrate that electrical potentials were developed when bone was stressed. These observations were as follows: (1) when excised bone is subjected to stress, it temporarily changes its shape and produces a dc electrical potential; (2) when living bone is subjected to a dc electric current, new bone formation can be stimulated at the negative electrode; and (3) when bone is axially compressed in vivo, new bone formation is stimulated (refs. 21–23).

Additional evidence reported by Becker and Bassett substantiated the fact that mechanical stress evokes an electrical response from bone. The deformation potentials are considered to be either or both (1) piezoelectric potentials, or (2) a change in pn junction potentials (refs. 24, 25).

Becker and other investigators have obtained confirming evidence that such potentials have the ability to orient the tropocollagen molecule (a precursor of the collagen fiber) in solution and to induce bone formation in vivo around the negative electrode, i.e., in areas of compression stress (refs. 26, 27).

The controlling influence exerted by mechanical stress is obvious in the osteoporosis that develops following immobilization or disuse. On prolonged exposure to reduced static forces on the skeleton due to altered average muscle tension and to reduced dynamic forces due to inactivity, the electrical signals generated by bone will be altered, and bone mineral loss will occur that will not subside until a new characteristic minimum effective strain equilibrium is achieved within the bone-cell-stress system of the axial and appendicular skeleton for the particular altered force pattern imposed on the bone. Under hypogravic or zero gravity environments the same general argument would apply, although it is obvious that the ratio of average static load on the bone to the dynamic load would differ from the immobilization and/or disuse condition.

The use of plaster of paris whole-body casts on experimental animals has several disadvantages, because in addition to work reduction, range of motion is virtually eliminated. Chronic, pathologic,

and functional disturbances include constipation and decubitus ulcers. One might argue that the data taken from such studies could be considered as the upper limit to the atrophy that may be expected under nonimmobilized agravic conditions. However, such consideration can only be applicable to the generalized, average condition. It is obvious that stress distributions over the skeleton will be quite different for the immobilization and for various agravic conditions. Because of differences in growth and development time, extreme caution must be exercised in extrapolating to man the time factors studied in this experimental series on the Macaca Mulatta. These studies will be supplemented by future test series to equivalent time periods in man. Bone remodeling dynamics in primates is probably similar to that in man; however, remodeling rates and their magnitude must be compared with reservation.

CONCLUSIONS

The development of manned spaceflight has been characteristically a program of incremental progression and will eventually mature into interplanetary capabilities. As mission durations and their complexity increase beyond Apollo, manned orbiting laboratories and the lunar proving ground will provide opportunity to observe the physiologic and psychologic effects of weightlessness on the musculoskeletal system and provide biomedical information needed for sustaining man on extended missions.

A considerably more detailed program is required for thorough evaluation of the consequences of the data reported here on the structural alterations of bone. The implications of these findings for the space program are evident. If man is placed in a reduced gravitational environment for prolonged periods, it is highly probably that the mechanical, circulatory, and metabolic behavior of the musculoskeletal system will differ significantly from those under gravitational conditions. Persistent altered static conditions on bony elements will result in transformation and adaptive changes in the trabeculae of cancellous bone, and these changes will not subside until a new mean characteristic equilibrium is achieved in the bone-cell/stress-cell system. It remains to be determined whether these adaptive alterations are so severe as to interfere with an astronaut's acceleration tolerance or his ability to adequately adjust to the earth's gravitational fields without a longer readjustment period.

The presently used biodynamic injury criteria for sustained acceleration, impact, and vibration are based on laboratory experiments and field data with human and animal subjects having their musculoskeletal system under "normal" conditions. Extended periods of physical confinement and zero gravity will probably alter bone strength, muscle mass, and the resulting overall mechanical strength of the musculoskeletal system to such a degree that injury levels for acceleration stress indices are needed that will predict physiologic condition in the hypogravic environment.

Several possibilities, such as the provision of artificial gravity, pharmacologic therapy, and physical exercise, are under investigation for preventing the undesirable effects of immobilization. The requirements for maintenance of muscle strength and skeletal mass still remain to be determined.

Meanwhile, the need for quantitative data describing the altered anatomy of bone in the hypogravic environment, and the corresponding changes and time functions associated with the transition from one gravitational state to another, remain. The same basic knowledge is also needed in support of clinical medicine to define bone fracture potential following bed rest or other prolonged disuse of parts of the skeletal system.

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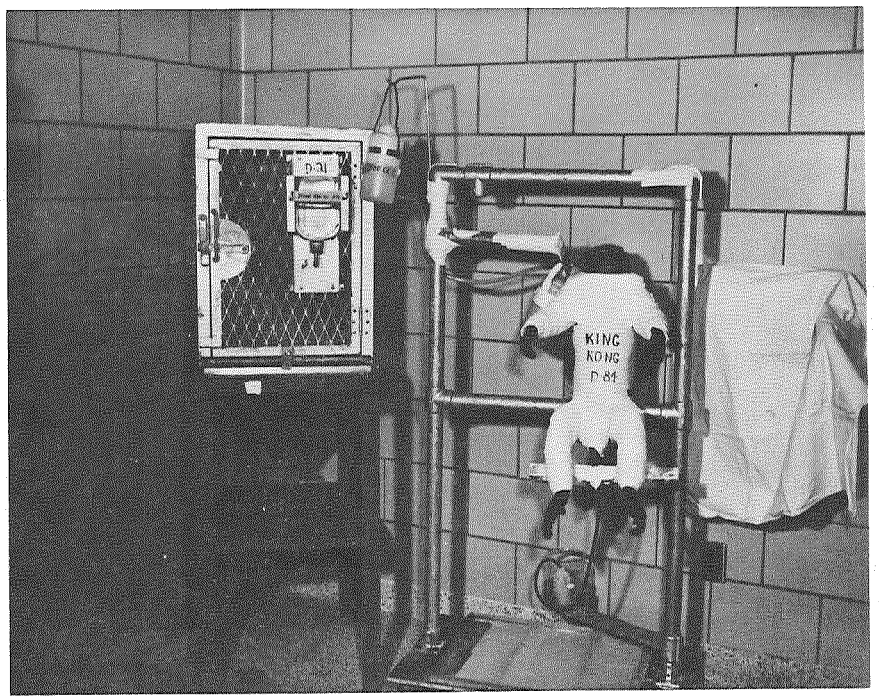


Figure 10.1 Plaster cast immobilization of experimental Macaca Mulatta and control

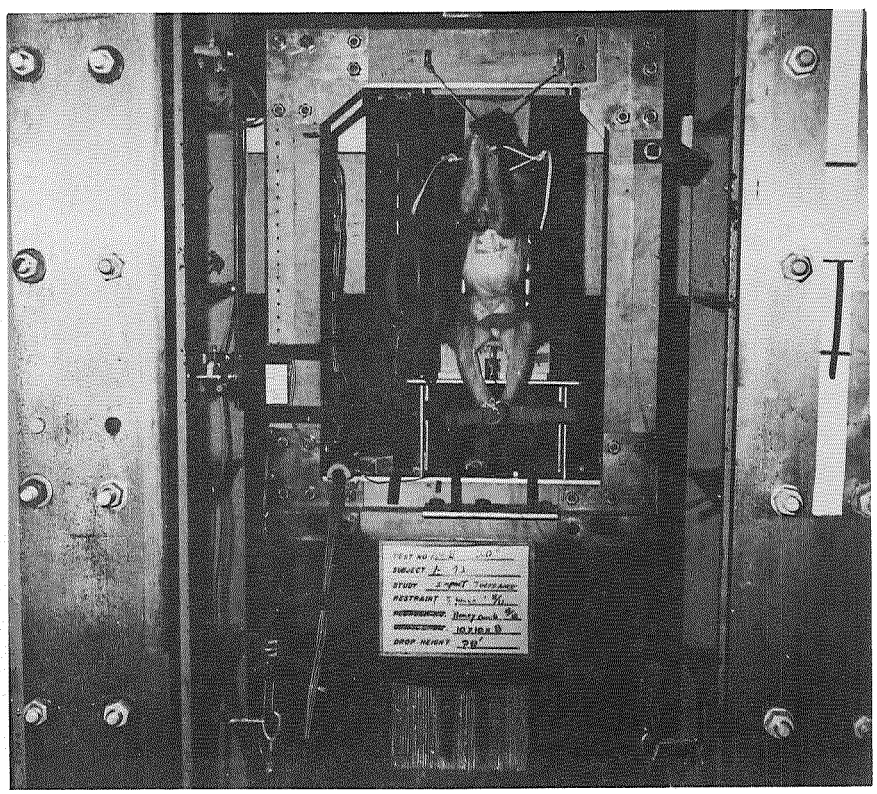


Figure 10.2 Anesthesized restrained primate on impact vehicle (the immobilized primate faces to the front, the control to the rear)

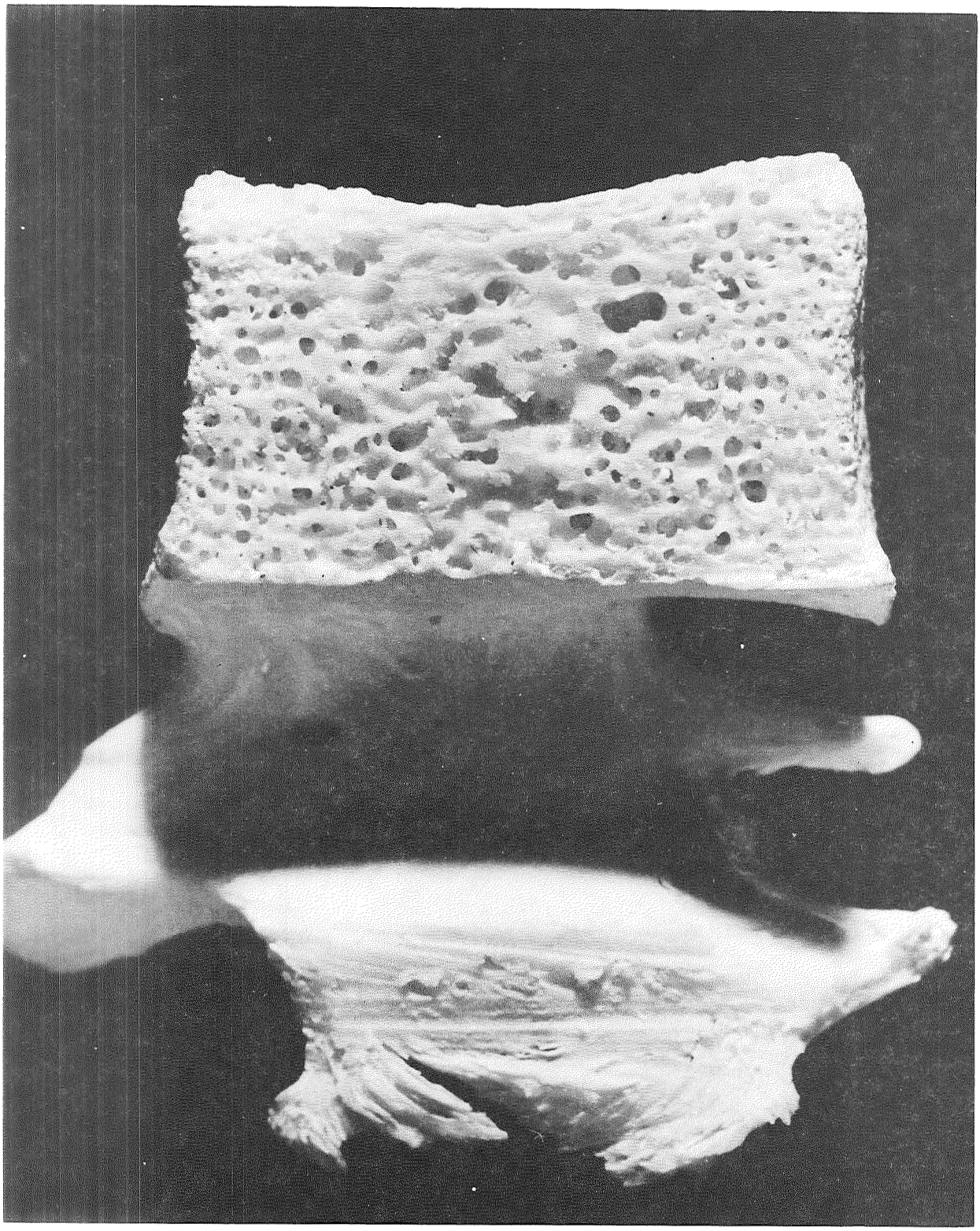


Figure 10.3 Normal vertebral body

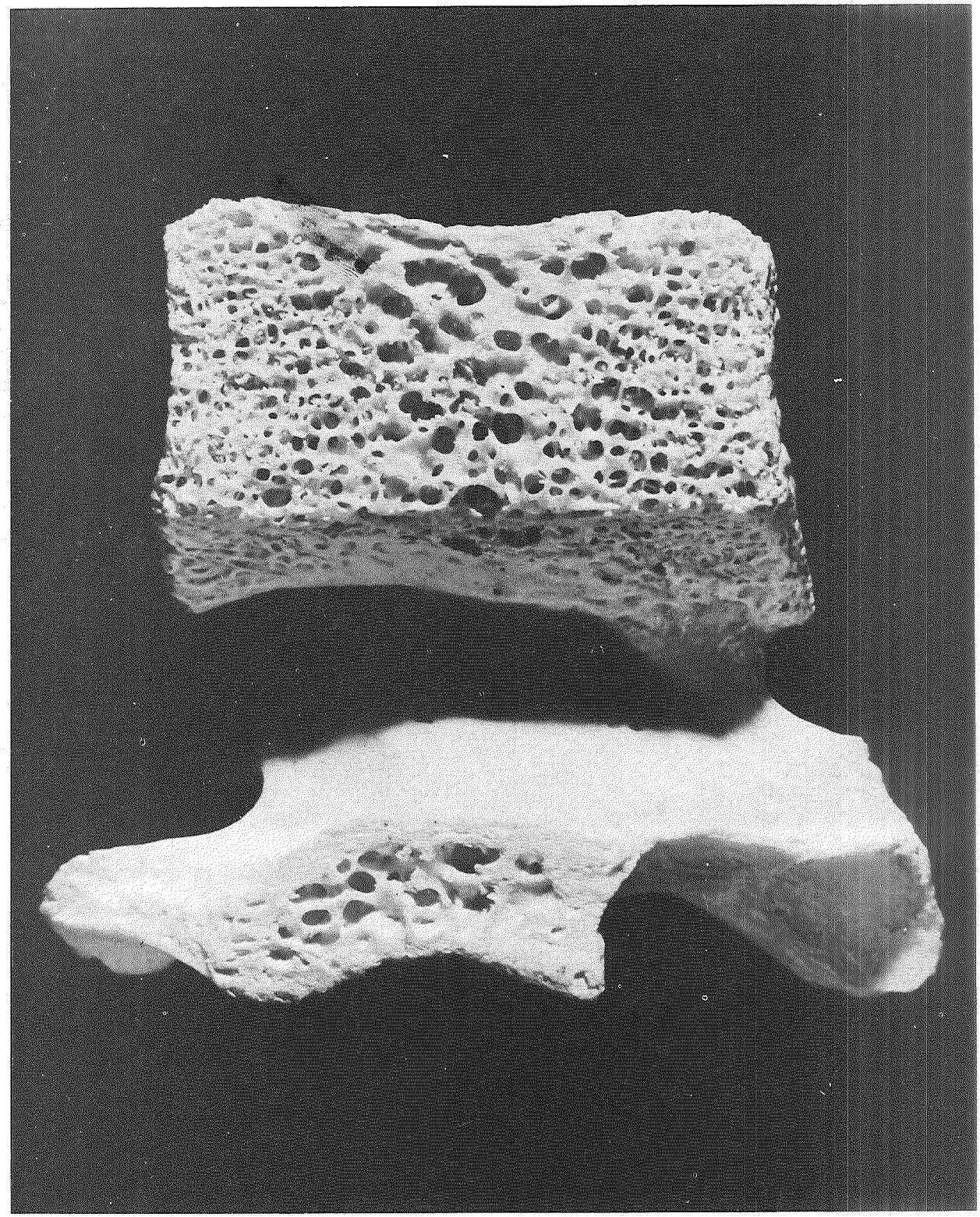


Figure 10.4 Changes in cortical bone of vertebral body as a result of 60 days of immobilization

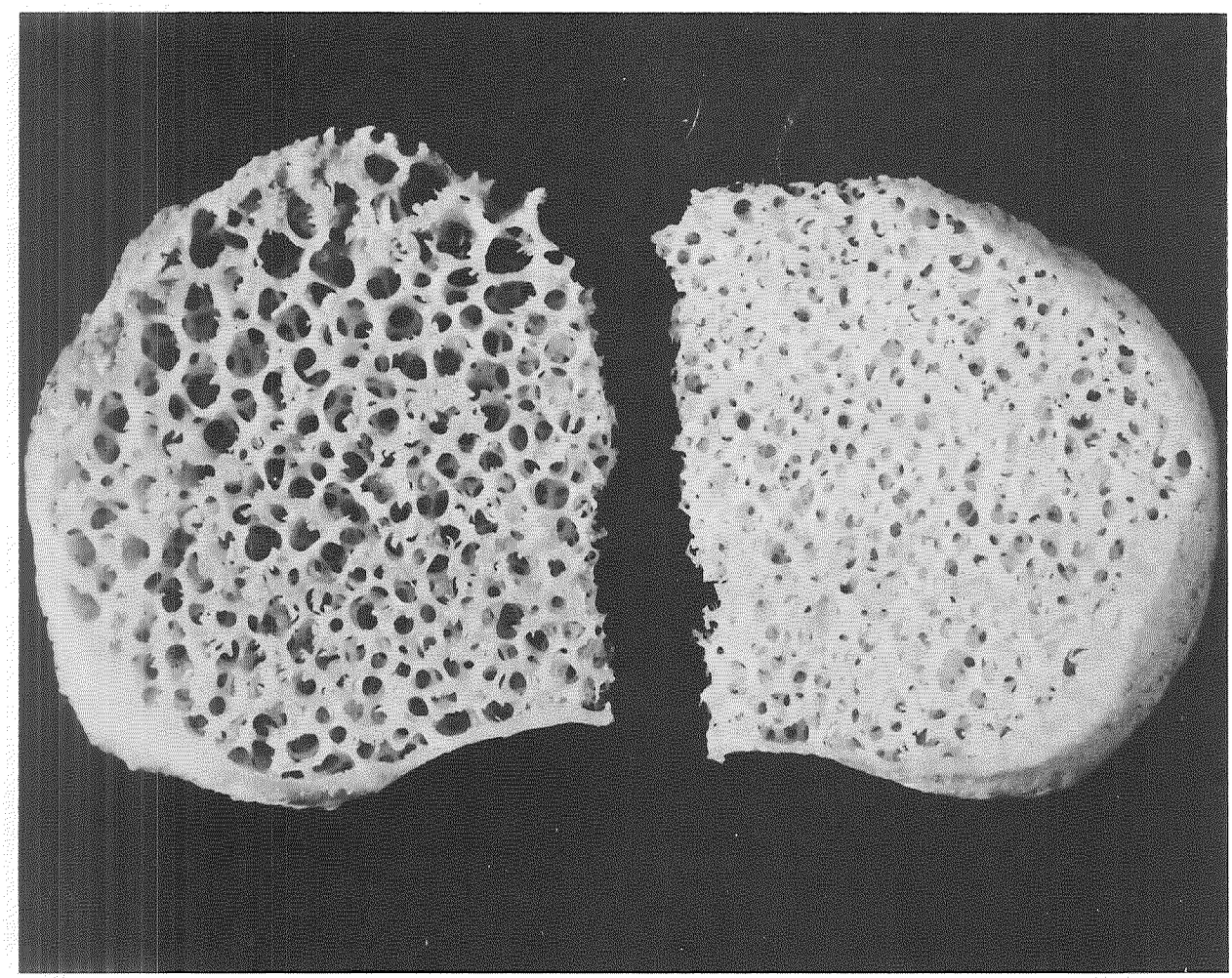
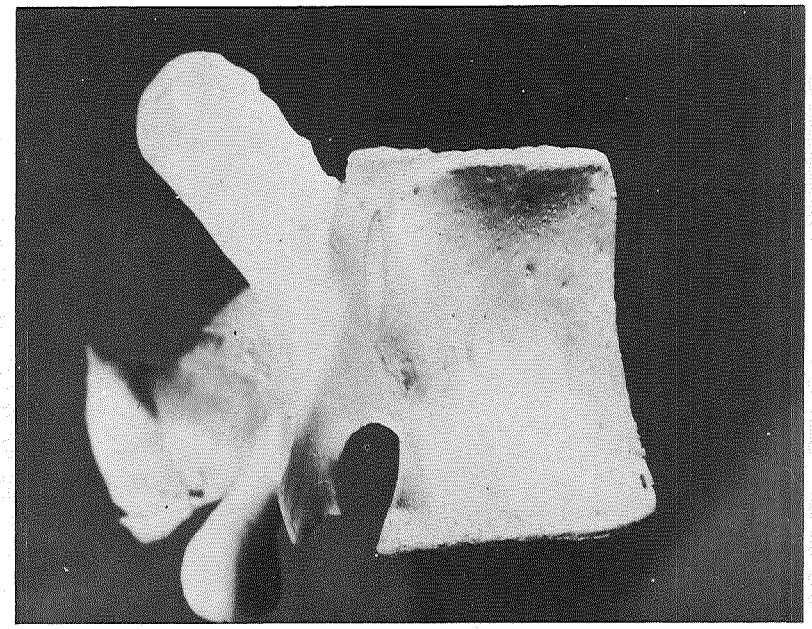


Figure **10.5** Subterminal plate cross section; left, immobilized primate; right, control primate



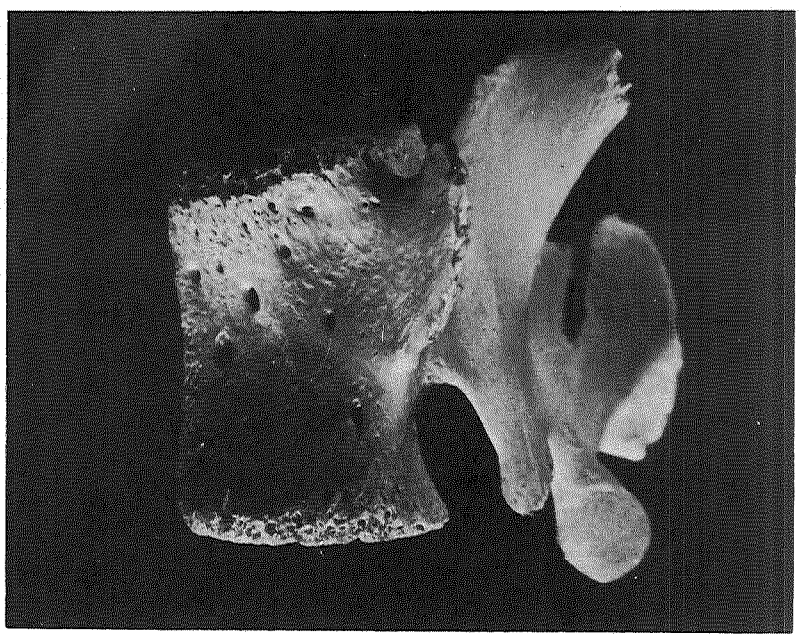


Figure 10.6 Sagittal section of L_4; left, control primate; right, immobilized primate

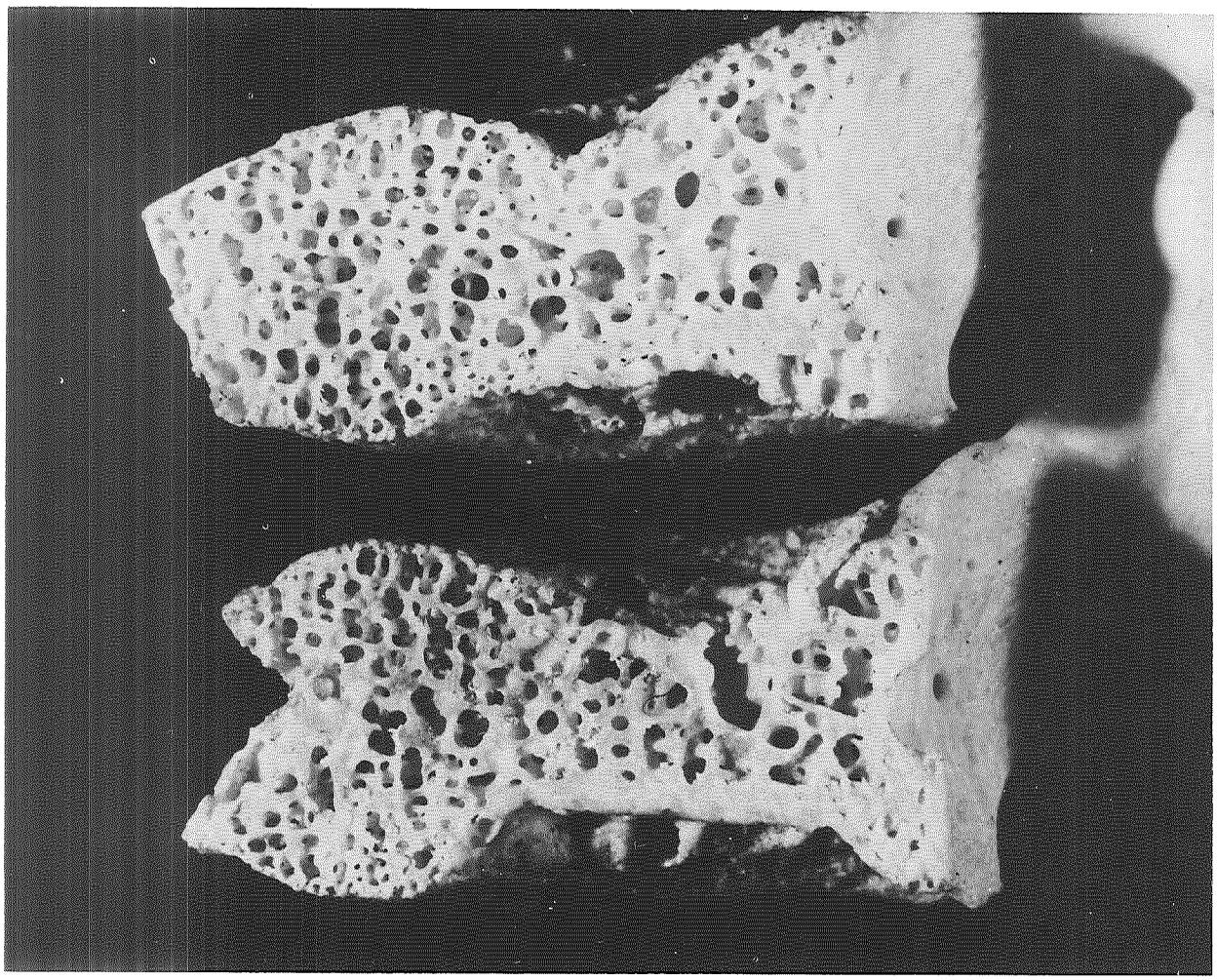


Figure 10.7 Vertebral body collapse and disk prolapse as a result of 60 days of immobilization, longitudinal spinal impact (85 G for 14 msec), and necropsy after 8 months

11 NONDESTRUCTIVE MEASUREMENT OF SOME PHYSICAL PROPERTIES OF BONE

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INTRODUCTION

Our investigations have emphasized the development of nondestructive methods for precise determination of the functional characteristics of bone. These include such physical properties as density, breaking strength, and modulus of elasticity.

Since bone is viscoelastic (refs. 1–3), the modulus of elasticity is not a purely linear relationship and has come to be expressed as a tangent of that stress/strain line (refs. 3, 4). Even with these limitations, the modulus of elasticity of bone affords invaluable information as to its behavior when external stresses are applied to it. The modulus has been correlated with the breaking stress of standardized specimens of human femurs by Sedlin and Hirsch (ref. 4) and in intact femurs by Mather (ref. 5). Thus, it might be possible to accurately predict the breaking strength of bone by means of *nondestructive* measurement of its modulus of elasticity.

The ability to determine functional physical properties of bone would be an invaluable aid in the study of changes in the skeleton in disease as well as in the altered environment of spaceflight. To this end, advantage is taken of the characteristics of ultrasonic energy transmission through cortical bone. Ultrasonic energy is mechanical energy; when transmitted through a material, it applies stresses to the particles of the material, and this mechanical energy is propagated by the elastic behavior of those particles. The velocity of an ultrasonic wave through a material depends on the material's modulus of elasticity and mass density:

$$\text{velocity} = \sqrt{\text{modulus of elasticity/density}}$$

If the density of the bone is known and the velocity of ultrasound through it is measured, the modulus may then be calculated. This formulation is derived for purely elastic materials, whereas bone is viscoelastic. Therefore, one should probably not expect identical values of the modulus of elasticity to be determined ultrasonically and directly.

There have been several studies of ultrasonic velocity in bone, some of which are based, at least implicitly, on this relationship. In 1958, Anast et al. (ref. 6) and Siegel et al. (ref. 7) reported on the measurements of longitudinal wave velocity of 20 kHz ultrasound across fracture sites in humans and experimental animals. In 1965, Horn and Robinson (ref. 8) evaluated the findings of Anast et al. and Siegel et al., and noted that there was no clear distinction between the sound delay in fresh fracture, a solidly united fracture, and an ununited fracture. They proposed the use of shear waves for more valuable information.

Selle and Jurist in 1966 (ref. 9) used the relationship of the resonant frequency of sound in the ulna and ulnar length to its density. In a study of 94 patients, these results showed a clear distinction between normal and osteoporotic patients. In 1966, Rich et al. (ref. 10) studied the correlation between the transmission time of 3 MHz ultrasound and the amount of calcium present in bovine bone samples and the forelimb of the rabbit. In 1967, Floriani et al. (ref. 11) reported on the relationship of the modulus of elasticity determined by ultrasonic velocity compared to the modulus of elasticity determined by static loading in the intact normal guinea pig femur.

Tests were performed on normal and pathologic specimens of human tibial cortical bone to develop and document the relationship of destructive and nondestructive testing, and to investigate the correlation, ultrasonic velocity measurements, mass density measurements, and mechanical loading.

MATERIALS AND METHODS

Standardized Specimens of Human Femoral and Tibial Diaphyseal Cortex

Fifty-one specimens were obtained immediately following lower extremity amputations in four patients. All specimens were constantly maintained in refrigerated lactated Ringer's solution, except during actual tests when they were held at room temperature (23° ± 1° C) in an atmosphere of 100 percent humidity. The complete testing was accomplished in four days, at most, after excision of the specimen. The specimens are grouped as follows:

Group A: Eight specimens of tibial cortex from a 16-year-old male who suffered acute vascular insufficiency secondary to a fracture of the midshaft of the femur. Specimens were obtained at amputation from sites well above the extent of avascular skin demarkation.

Group B: Sixteen specimens of tibial cortex from a 30-year-old male undergoing amputation for a fibrosarcoma localized to the femur.

Group C: Seventeen specimens of tibial cortex from a 58-year-old female with long-term diabetes mellitus and acute and chronic vascular insufficiency.

Group D: Ten specimens of tibial cortex from a 55-year-old female with a nonunion of the femur. The patient had not borne weight on the extremity for three years and had radiographic evidence of disuse osteoporosis.

The twenty-four specimens of groups A and B are considered normal because of age and the fact that there was no coexisting disease state that might affect the physical properties of the bones in the sites sampled. The specimens from each patient are grouped for convenience of presentation of the data. The number of values is not sufficient to establish a range of values for any normal or pathological process.

The specimens were cut from the full-thickness diaphyseal cortex. They were then shaped by hand machining under cold physiologic solution into lengths of 3 to 5 cm and into regular rectangular cross-sectional dimensions. Care was taken to cut the specimens along the longitudinal axis

of the osteonal pattern in order to avoid "cross-grain" differences (refs. 3, 12, 13). In an attempt to limit the shear component in bending tests, the specimens were shaped so that the length/depth ratio was 12 to 1 or greater (ref. 14).

Testing Methods

Ultrasonic Velocity Measurements The ultrasonic test system uses a Hewlett-Packard pulse generator, which emits 100-μsec, 100-V pulses (see fig. 11.1). These emissions serve two simultaneous functions: (1) to trigger the sweep of the oscilloscope, and (2) to drive a lead zirconate-titanate crystal transducer, which vibrates at its resonant frequency of 100,000 cps (100 kHz). This pulsed 100-kHz ultrasound is passed longitudinally through the test specimen and sensed by an identical crystal transducer at the opposite end. The sensing tranducer converts the mechanical sound energy to electrical energy, which is conveyed to the oscilloscope. This electrical impulse causes interruption of the path of the trigger sweep and allows direct determination of the transit time of the ultrasonic wave through the specimen. This transit time, divided by the length of the specimen, gives the average velocity of ultrasound in meters per second.

Mass Density Measurements Each specimen is weighed on an Ainsworth Type 21N analytical balance. The specimens are then weighed while completely submerged in distilled water. The apparent loss of weight in water, due to the displacement of an equivalent mass of water, is divided into the original weight of the specimen. This result is multiplied by the density of water at the testing temperature to give the mass density of the specimen under investigation.

Static Loading Tests All specimens were treated as center-loaded end-supported beams and tested in a 100 percent humidity chamber (see fig. 11.2). The testing apparatus provides constant loading at a rate of 0.12 kg/sec. Strain is measured by BLH Type SR-4 strain gauges. The strain gauge, bonded to the inferior aspect of the specimen by Eastman 910 cyanoacrylate, measures tensile strain as the specimen deforms in response to the stress. This strain is recorded through a Tektronix oscilloscope.

RESULTS

Table 11.1 presents the result of the ultrasonic velocity measuremenrs, density determinations, calculation of the modulus of elasticity nondestructively by ultrasonics, and determination of the modulus by static loading tests. Each measurement parameter of groups C and D is statistically significantly different from those of the healthy specimens in Groups A and B. The groups, however, represent multiple samples from individual patients, and their number is far too small to provide a basis for characterizing healthy or pathological bone.

It is also well documented that, in addition to pathologic conditions, the modulus of elasticity of bone varies according to its anatomic location (refs. 3, 4, 15). Although a mean value is presented with a standard deviation or range of values, *it is not the purpose of this investigation to establish any absolute or range of values for an anatomic location or pathologic condition.* The aim is to investigate the relationship of the physical properties of bone, as they vary, and attempt to find a constant correlation.

CORRELATIONS AND DISCUSSION

The use of ultrasonics in the nondestructive determination of the physical properties of a heterogeneous material such as bone is based on theoretical considerations. Thus, the critical step in the development of these nondestructive methods is the correlation of results with those obtained by standard destructive mechanical testing.

In all cases, the Pearson product moment correlation is used. The slope of the plotted result is calculated using regression by the least-squares method.

Velocity/Density

Figure 11.3 shows that the normal specimens exhibited a velocity/density correlation that is distributed rather evenly around the mean values. As the density of the 27 "abnormal" specimens (groups C and D) falls, there is a corresponding drop in the transmission velocity of ultrasound. This relationship correlates in a highly significant manner beyond the 0.001 level ($r = 0.87$) for the latter group.

Velocity/Es (Modulus Determined Mechanically)

The direct linear correlation (fig. 11.4) between these important parameters of all 51 specimens is also significant beyond the 0.001 level ($r = 0.86$).

Es/Ev

The various parameters of velocity, density, and mechanical modulus (Es) are intimately and directly related to the ultrasonic modulus (Ev): therefore, the Es/Ev correlation may be examined. In figure 11.5 each of the 51 values of Es is plotted against the Ev for the same specimen. The correlation is highly significant, again well beyond the 0.001 level ($r = 0.91$). The relationship calculated by the method of least squares is $Es = 0.1348 + 0.7564\ Ev$.

This constant relationship between the modulus of elasticity of bone determined destructively and nondestructively has significance in the study of the physical properties of bone. Sedlin and Hirsch have established the relationship between the modulus of elasticity of standardized samples of human bone and their ultimate breaking stress (ref. 4).

Since the correlation is of significance, the regression by least-squares formulation ($Es = 0.135 + 0.756\ Ev$) allows conversion of the ultrasonic modulus (Ev) to that determined mechanically (Es). This value may then be used in the calculation of breaking stress.

Furthermore, since the Ev is a function of the velocity of ultrasound in the bone, it follows, by a similar correlation, that both the modulus of elasticity *and the breaking strength of the bone* are directly related to that velocity.

This type of correlation is significant since it allows accurate nondestructive determination of the precise physical characteristics of bone. It opens a new area in our investigation of the structural and physical properties of bone by permitting the determination of these parameters without destroying or altering the specimen.

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Table 11.1 Results of ultrasonic testing, density measurement, and static loading tests expressed as mean values

Group	*Velocity, m/sec*	*Density, mg/mm^3*	*Ev, X 10^4 newton/mm^2*	*Es, X 10^4 newton/mm^2*
A&B	3526 (100)*	1.97 (.03)	2.44 (.13)	1.97 (.12)
C	3147 (81)	1.85 (.05)	1.84 (.12)	1.63 (.16)
D	2868 (52)	1.66 (.05)	1.37 (.21)	1.09 (.27)

*1 SD.

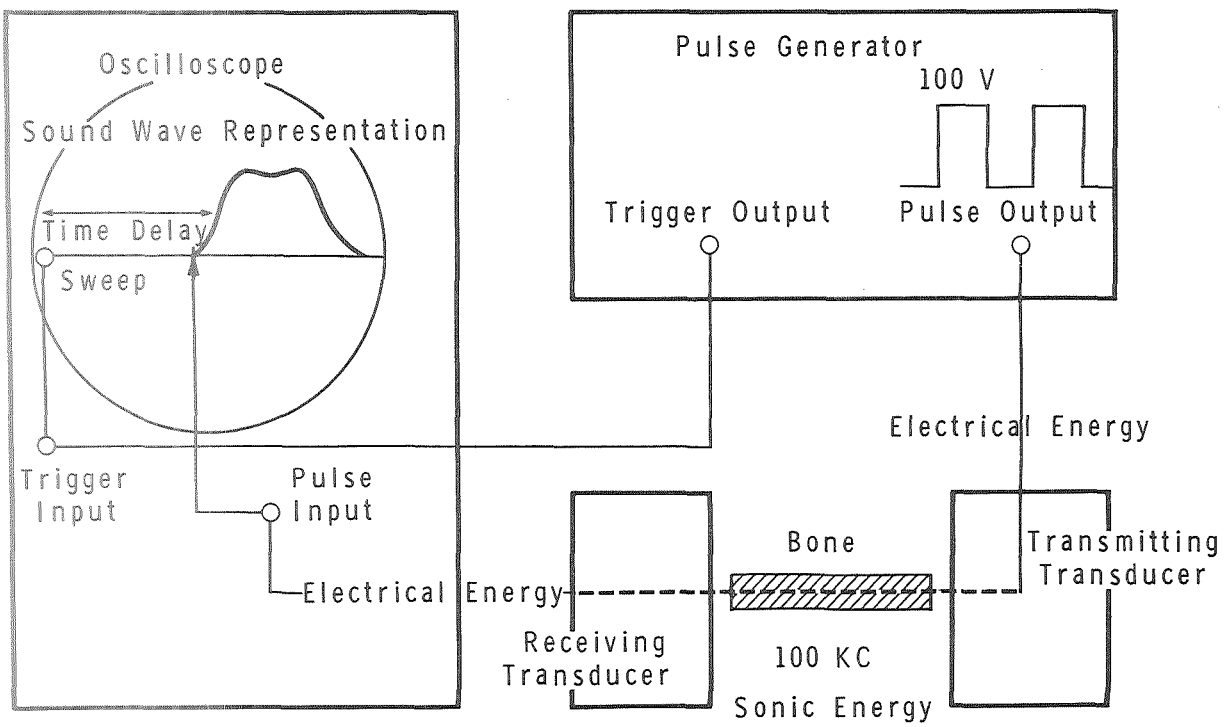

Figure 11.1 Schematic of ultrasonic testing system

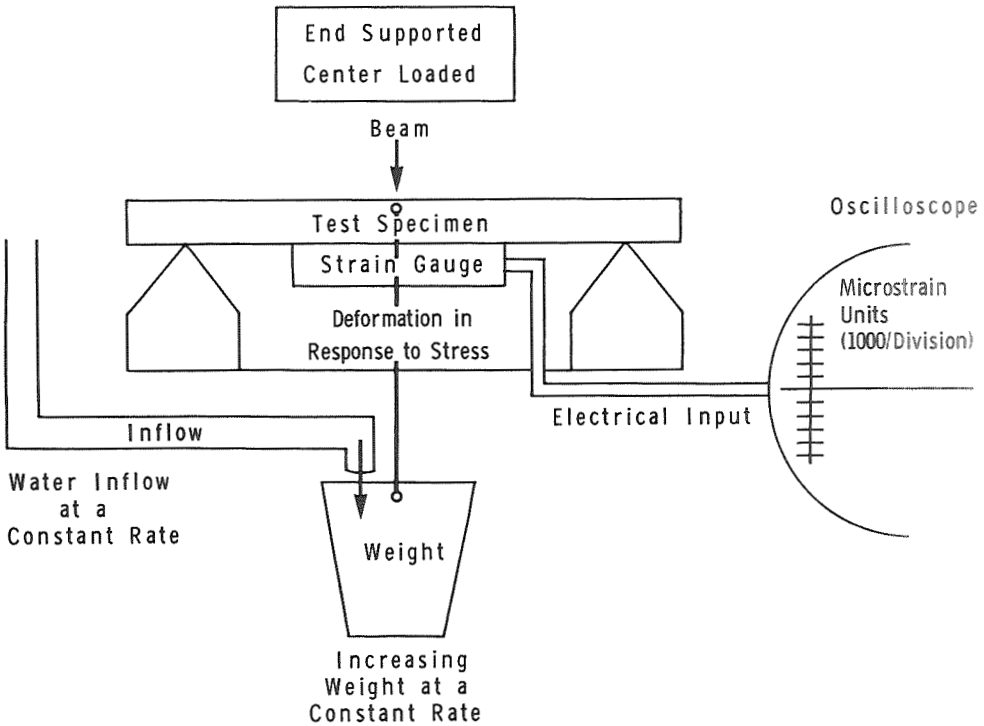

Figure 11.2 Schematic of mechanical static loading tests

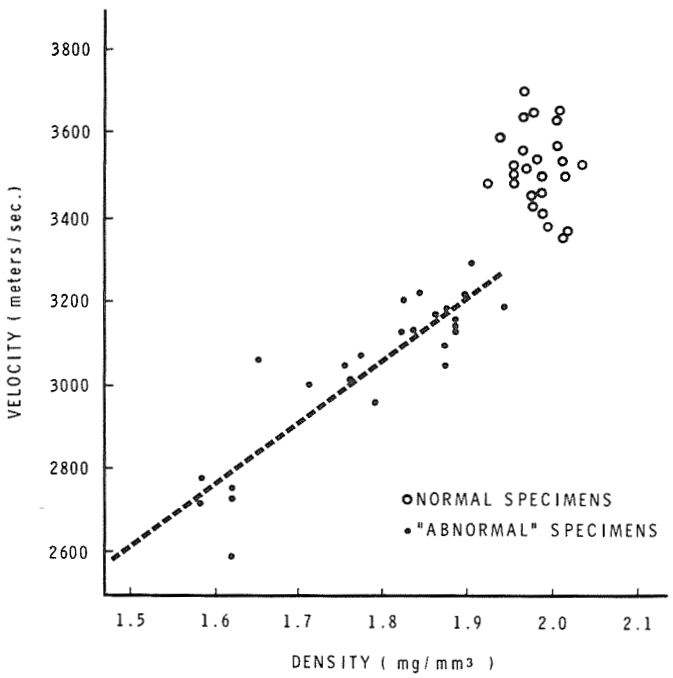

Figure 11.3 Correlation of ultrasonic velocity and mass density; regression line is drawn only for the 27 "abnormal" specimens

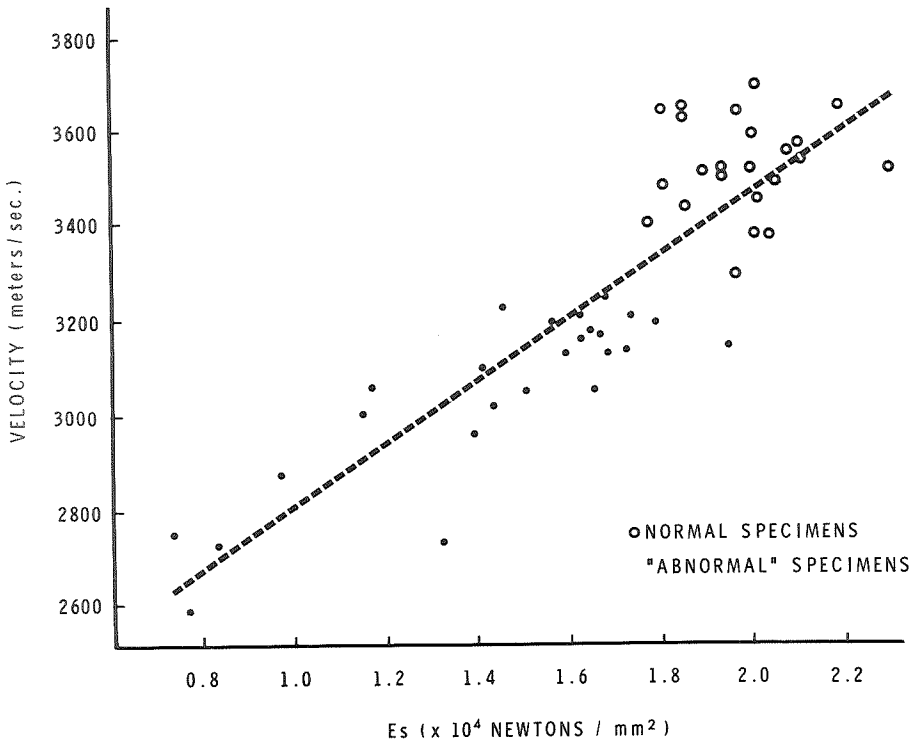

Figure 11.4 Correlation of ultrasonic velocity and Es (modulus of elasticity determined by static loading)

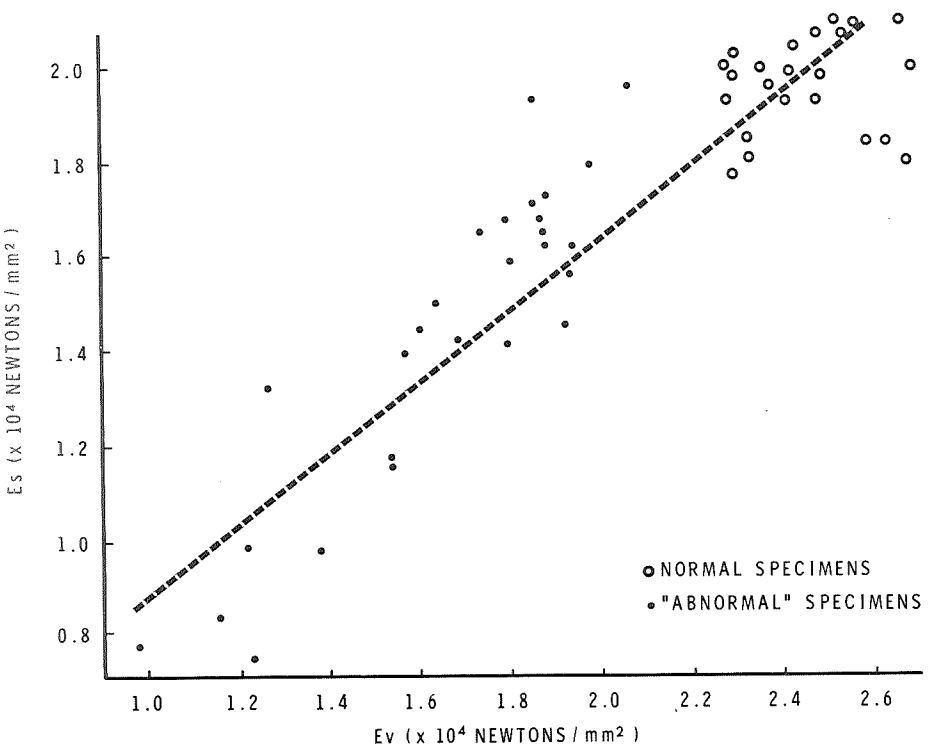

Figure 11.5 Correlation of Es (modulus of elasticity determined by static loading) and Ev (modulus of elasticity determined by ultrasonic velocity measurement)

12 DISCUSSION

Dr. Nordin: I want to leave this final thought with you. I would suggest that X-ray densitometry, though interesting, instructive, and valuable, is probably not the ideal way to look at the effect of immobilization in spaceflight. The fasting urinary calcium level could be a substitute for full balance studies.

Dr. Lutwak: Dr. Nordin suggested the use of calcium:creatinine (c/r) ratios in random urine specimens to evaluate daily calcium excretion. He is now examining these ratios at different times of the day; many people have developed random c/r ratios as indices for total excretion. The suggestion that the overnight value (fasting value) represents basal excretion may help those who cannot obtain 24-hr collections. Unfortunately, we have another factor in space studies: the upset of the normal diurnal patterns. Meals are usually not eaten at the usual times nor in the usual sequence, the sleep-wake pattern is disordered, and other influences affect the diurnal metabolic cycles. Examination of creatinine excretion values at different periods through the 24 hr shows diurnal variation. This variation pattern may be disturbed in spaceflight too. The c/r ratios may be of value, however, as a tool for examining the metabolic effects of immobilization in ground-based studies. Although the ratios may be a very good approximation, they are no substitute for the complete balance study. Did you find any relationship between the serum calcium and phosphorus abnormalities and the duration of paraplegia?

Dr. Nordin: That's a very good question, but I have not tested that.

Dr. Lutwak: Recently we observed a herd of bulls that had been receiving very high calcium intakes (65.90 g/day) for many years (ref. 1). We calculated this to be equivalent to a man ingesting about 5 g calcium/day. After 7 years of continuous calcium intake, the bulls had not developed hypercalcemia but rather hypocalcemia; we suggest this is due to secondary hypercalcitonism resulting from increased calcium absorption. May not such a phenomenon play a part in the lower calcium excretion you report?

Dr. Nordin: That explanation does not fit in with the phosphate data. Calcitonin is calciuric and phosphaturic. The situation of low tubular resorption of calcium but high tubular resorption of phosphorus fits in better for parathyroid shutoff than for calcitonin response. I think this is an overreaction of the parathyroids, the counterpart of secondary hyperparathyroidism that goes on to a state of mild hypercalcemia. If stimulated long enough, the parathyroids produce a state of chronic mild hypercalcemia. One wonders what the stimulus is. Similarly, if you suppress the parathyroids, calcium settles down to a low level; but we won't know the answer until we measure parathyroid levels. I think this is chronic parathyroid shutdown.

Dr. Wunder: Before you gave up on the idea of relating the size of one bone to the total skeleton, did you attempt to use heteroxic analysis, the type that Brody (ref. 2) and Huxley (ref. 3) used in the 1930s? They plotted the logarithm of the size of the bone against the logarithm of the total body size. Linear analysis assumes that a linear relationship is best; but in most cases, when one compares one structure or function with another, a double logarithmic relationship is better. I wonder if that would have been a better way of predicting total bone mass?

Dr. Nordin: I have no idea; it didn't occur to me to try that technique. We will have to have a look at that, but I think we've got a very high predictive value from linear correlations. The weakness of our study was that we didn't think of measuring the volume of these skeletons until it was too late. We have now got to go back to another 20 skeletons and measure their volumes.

Dr. Donaldson: I was interested in your urine and serum phosphorus data. We have found very similar results in terms of urine and serum calcium but not phosphorus; we will present some of these data later. I have a question dealing with the validity of carrying out your predictions in this way. Are you assuming that all bone loss is distributed equally in the skeleton? What if some bones are more affected than others; might this explain the differences between densitometry and balance studies?

Dr. Nordin: Well, there are two different questions here. As far as prediction is concerned, we can only say that in the 20 skeletons that we've studied, this was the best fit to the data that we could get, and it appeared to be a remarkable uniformity. We did 40 different measurements, and we got all of the R values between all of them. The correlations between individual bones were remarkably high. If you plot femur weights against metacarpal weights, they will correlate; or one bone against another will also correlate. If you go through Trotter's data, you will find this is also the case (ref. 4). As far as aging changes are concerned, all the bones seem to be involved; there are high correlations internally. Now I'm sure there are disease situations where this does not apply. It certainly does not apply, of course, in conditions like rheumatoid arthritis or local disease of any kind. I have the strong impression that, for the aging situation and the bed rest-weightlessness situations, the whole skeleton is involved, although different bones lose at different rates. This seems to me to be a function of their surface to volume ratio. But the whole skeleton seems to be involved.

Dr. Donaldson: Then you wouldn't assume that there is any relationship between weight-bearing and the bones involved?

Dr. Nordin: I do see some evidence of this. The femur does appear to be much less involved than the metacarpals and the trabecular bones. As far as the aging pattern is concerned, a trabecular bone seems to lose about 50 percent of its mass with age, whereas the femur only loses about 5 percent. This seems to be a constant pattern. As long as you have that pattern imprinted on your computer program, the predictive value of the femur and of the metacarpal is taken into account in those equations.

Dr. Gatts: You say "inactivity" in your title, yet you use the term "immobility." I wonder if you're talking about nonweight-bearing rather than immobility and if this isn't a fairly important distinction.

Dr. Jurist: Were those X-rays you showed from paraplegics?

Dr. Nordin: The X-rays I showed were taken from cases of tuberculous joint disease in which the limbs had been placed in plaster. And the urinary calcium data that I showed were taken from 10 cases of paraplegia following car accidents. That would be immobility, but also inactivity.

Dr. Gatts: There is an important distinction between these two conditions. Nonweight-bearing is the important element here, not immobility.

Dr. Johnston: What volume did you use in your corrections to get the best correlations with gamma absorption? Was this from scanning or from X-ray measurement?

Dr. Nordin: The gamma-ray data that I showed you are direct mass measurements, that is, ash per centimer, exactly like John Cameron's; there was no correction for depth. Now there were certain other measurements that I call porosity measurements, in which we divided by the bone depth as measured on the lateral X-ray at the wrist. But so far as the predictive value for estimating skeletal weight is concerned, the best correlations are obtained from the ash per centimeter values. The weight or mass of a skeleton correlates best with the mass of something else. Bone weight is neither volume nor porosity but the combination of the two. The measurements that predicted most accurately are the mass measurements that were done on the peripheral bones, so that gamma-ray scanning (expressed as mass, or ash per centimeter) is better in this respect than a volume or a porosity measurement.

Dr. Johnston: I don't understand how you get around the problem of the small person with a normal skeletal mass compared to the large person with decreased mass? How do you take this factor into account?

Dr. Nordin: When, for example, you scan the phalanx, the radius, or the ulna by the gamma-ray method, you get the same result whether you are dealing with a small person with normal bones or a large person with porotic bones. Now that applies to the whole skeleton also. It correlates with the weight of the skeleton. It does not matter if you have a large person who is porotic or a little person who is normal; the weight of the skeleton would be the same, and the mass per centimeter by gamma-ray scanning would be the same.

Dr. Johnston: So you need to know whether the person tested is large or small?

Dr. Nordin: That's why I say we haven't yet got a reference standard. It is a great shame we didn't make measurements of skeletal volume, because this is what one requires for this particular technique.

Dr. Cameron: In regard to the neutron activation data you presented, was there a calculation of the accuracy or was there actually some experimental data?

Dr. Nordin: Chamberlin has experimental data on reproducibility using human cadavers (ref. 5).

Dr. Barzel: Is there any difference between the right and left hand? Is the dominant hand more, or less, calcified?

Dr. Nordin: Insofar as we've looked at this, there doesn't seem to be any difference. Stanley Garn (personal communication) has also found no difference.

Dr. Lutwak: I would like to pursue Dr. Donaldson's question about the differences among bones. In a recently completed study with Dr. Lennart Krook, adult dogs placed on calcium deficient diets gradually became osteoporotic. The animals were sacrificed at different periods during the course of the study, and all the bones were analyzed for density and ash weight. We found that, in dynamic situations where change is occurring, as one might expect in a hypogravic or hypodynamic situation, alveolar bone showed a loss of density first. Subsequently, we observed vertebral changes and, finally, changes in the long bones. When one studies skeletons obtained from cadavers, one is looking at a steady-state situation. In a hypogravic condition, studying a single bone, or even two or three bones, will not really present a true picture of events. As you suggested before, the urinary calcium is a much better index for overall skeletal calcium metabolism.

Dr. Nordin: I agree about the urinary calcium. But as far as the other point is concerned, I think there is a fallacy here. I think this is simply a matter of the rate at which demineralization goes on in the different bones. I have yet to see convincing evidence that the process is not happening everywhere; although for purely anatomical surface-to-volume ratio reasons, the rates differ in different bones. It's easier to detect in trabecular bones, which lose calcium proportionally ten times faster than cortical bones. When you do a number of measurements in this way, the relationships between the changes in the different bones are taken into account in these equations.

Dr. Lind: What was the age range at death of these skeletons? Isn't this important?

Dr. Nordin: This was a random collection of skeletons that differed from each other in respect to size and age. I assume that these twenty were representative of a larger cross section of the population. If it so happened that a localized bone tumor, or something similar, was included, the prediction wouldn't work. But this is an empirical concept which assumes that aging bones lose tissue in a fairly regular manner, though at different rates in different bones. There is a general pattern, just as Trotter showed (ref. 4). There is a clear pattern for young and old, male and female, Negroes and whites.

Dr. Lind: But these skeletons presumably came from the older age group, so that presumably you've built in a bias.

Dr. Nordin: I think that'a a perfectly fair comment. It would be nice to know the ages of those skeletons, but when you try to go back and find out who these skeletons came from, a great wall of silence descends.

Dr. Cameron: I'd like to take a few more minutes to discuss a new idea we are working on, which I think shows some promise. We have developed an improved bone mineral unit that reads directly in grams per centimeter of bone mineral as well as the width of the bone without additional calculation. Norland Associates of Ft. Atkinson, Wisconsin, is making a unit based on this idea, and they have a prototype available. This should be a great step forward in making this technique generally available.

The photon source of choice in general is ^{125}I with an energy of 27 keV, which is similar to the ordinary diagnostic X-ray energy; ^{241}Am, with an energy of 60 keV, is not as suitable for most bones, but is useful for very heavy bones such as the femur. Its long half-life (450 yr) makes it attractive, as it does not have to be replaced.

I'd like to emphasize in closing that the investigator should not limit himself to only one technique for the study of bones but should use as many techniques as he has available. A combination of techniques is best.

Dr. Barzel: I wanted to ask whether you actually used other methods to quantitate bone loss?

Dr. Cameron: No, in Wisconsin we have been short of clinical investigators interested in calcium metabolism. Almost all of our studies have been in cooperation with people elsewhere. The data that I showed were collected by physicists, and other studies were not made.

Dr. Vogt: Have you looked at the effect of changing tissue volume on the density changes measured with your monochromatic source?

Dr. Cameron: We have doubled the "tissue thickness" by increasing the water or increasing the "superstuff" and the bone mineral stays within 1 to 2 percent, that is, within our measurement accuracy. Recently we have been using a cast or mold of the hand and a single point measurement. We can use a large number of photons at this point—100,000 or even 1 million photons. With this technique we can now get reproducibility with a standard deviation of 1 percent. We are now using two photons (iodine and americiam) with this single point technique, so that we can determine also the soft-tissue component. We can see approximately 2 percent change in soft tissue with this technique.

Dr. Vogt: So what you are saying then is that increasing the tissue fluid has a small effect?

Dr. Cameron: It isn't measurable by the single photon technique, and theoretically it should not be.

Dr. Vogt: Should it be measurable with the polychromatic source?

Dr. Cameron: No, the scatter is going to increase slightly and also the absorption, and you're going to have some additional hardening of the beam; so there will be some change with a polychromatic source, but it will not permit you to measure the change of tissue fluid.

Dr. Barzel: In one of your diagrams you showed us a photon beam running through the bone and mentioned that this gives you the measurement of mass per centimeter. Do you take multiple paths through this centimeter, or is it just a single path?

Dr. Cameron: As the photon beam moves across the bone, it measures at each point the total amount of bone mineral in grams per square centimeter. If you integrate this across the scan distance in centimeters, you get bone mineral mass in grams per centimeter length of bone.

Dr. Nordin: I think Dr. Cameron wasn't quite clear in his reply to Dr. Vogt. Since he has used "superstuff," he has already corrected for the soft-tissue baseline and adding more "superstuff" doesn't make any added difference.

Dr. Cameron: That's right. Under these circumstances there would be no difference. Does Dr. Mack's group immerse the bones in water?

Dr. Mack: We have done so, but we do not do so routinely.

Dr. Abendschein: The assumptions that have been presented in the ultrasonic technique are open to some question. The resonant frequency measurements assure the velocity in human bone to be 2,800 m/sec. In support of this, the work cited is that of Clayton Rich (ref. 6), who measured bovine cortical bone, which has a different architecture (refs. 7 and 8). The basic system is that of a doubly suspended vibrating rod, and, of course, the ulna is complicated by four joints rather than two. You speak of a 4 percent error, and yet merely tightening the forearm muscles changes the resonant frequency by 5 percent, and holding on to the middle of the forearm changes the resonant frequency by 25 percent.

Dr. Cameron: That's correct, but the technique still appears useful in the diagnosis of osteoporosis and further work needs to be done.

Dr. Abendschein: My point is that reproducibility from person to person and from laboratory to laboratory, using this technique, is going to have to be tightly controlled if any meaningful results are to be obtained.

Dr. Cameron: That 4 percent value is with an experienced subject; the error may be as high as 10 percent with inexperienced subjects. The simplicity of the technique encourages us to evaluate it further.

Dr. Jurist: I would like to ask Dr. Jowsey for a definition of the term osteopenia. I assume she is using the terms osteopenia and osteoporosis interchangeably.

Dr. Jowsey: Well, I try not to. By osteopenia I mean bone loss; by osteoporosis I mean the disease that has generalized bone loss associated with it. The person with immobilization osteopenia does not have the disease of osteoporosis, and only part of the skeleton may be affected. It's not a new term but one that avoids the connotations and associations given to the clinical disease of osteoporosis.

Dr. Jurist: Was the dog you treated with calcitonin a normal dog, or did he have diffuse osteoporosis or osteopenia?

Dr. Jowsey: The dogs have osteopenia due to immobilization by casting.

Dr. Jurist: Did the calcitonin treatment increase the density of the bone?

Dr. Jowsey: The calcitonin did not increase the bone density nor even prevent the osteopenia of disuse.

Dr. Jurist: Do you see a visible difference in the density of the heads of the femur between the normal and the osteopenic dog? The head of the femur, which is a fairly static part of the skeleton normally, is different in density than the osteopenic one. How long was this in the cast?

Dr. Jowsey: This dog was casted for 12 weeks.

Dr. Jurist: This raises the question of whether your patient with osteoporosis showed any visible change when treated with calcitonin.

Dr. Jowsey: None whatsoever. We wouldn't expect it.

Dr. Lutwak: What was the calcium content of the diets you used for dogs, rabbits, and humans treated with calcitonin?

Dr. Jowsey: The humans were maintained on their habital diet, which was a little over 1 g/day. The dogs and cats were fed on normal animal chow, which is usually rather high in calcium.

Dr. Lutwak: I would expect that you would not find any effect with calcitonin in any of these, because you were getting maximum calcitonin response from the dietary calcium. No matter how much additional calcitonin they were given, there would be no greater effect on the bone. You might, however, see a pharmacological effect on the blood, that is, on the calcium concentration.

Dr. Jowsey: The experiment demonstrated that, in the presence of a clearly defined effect on the serum calcium and phosphate values, increased resorption, caused by immobilization, was not prevented by calcitonin. Your point is answered by the control group, which developed osteopenia even though, as you suggest, they may be getting a maximum calcitonin response.

Dr. Barzel: Have you given thyroid replacement to some hypothyroid dogs and cats to see if the effect of casting will reappear?

Dr. Jowsey: No, we haven't done that experiment.

Dr. Howard: Is there any information about blood flow changes in bones with immobilization?

Dr. Jurist: Dr. Robert Ray and associates have recently written on the subject of blood flow in bone (ref. 9). No changes were observed in blood volume, or rate of flow, or in tissue volume in legs of rabbits immobilized in plaster casts, when the opposite unimmobilized legs served as controls and both legs were monitored by ^{131}I-tagged serum albumin. Such kinetic studies on skin, muscle cortex, and marrow in animals should be repeated in human beings.

Dr. Nordin: What made you assume that the difference in O_2 and CO_2 are related to bone loss rather than to muscle or some other tissue?

Dr. Jowsey: Because we found it only in the animals that developed osteoporosis, and we didn't find it in the animals that had their legs in casts but did not get osteoporosis.

Dr. Nordin: I might argue that they got osteoporosis *because* they had the blood changes. The data could suggest that the low blood pH was responsible for the osteoporosis.

Dr. Jowsey: I think that may be true, but there are no data to justify drawing such a conclusion. At this point I would rather present the facts.

Dr. Nordin: That applies to all correlations. You wanted to see whether the osteoporosis was related to blood pH and found that it was. Then you proceeded to say that the osteoporosis was the cause of the fallen pH and not the result. I would just be careful how you present that point.

Dr. Jowsey: We found these changes only in the animals that got osteoporosis.

Dr. Schmid: In those two patients that you presented with osteoporosis, was your conclusion that they did have accelerated bone formation after calcitonin?

Dr. Jowsey: No. One patient showed a decrease, and I think this was explained by the fact that she was in bed more than normal because of a throat infection. The other patient showed no significant change in bone formation.

Dr. Schmid: I was looking at your figures for osteoid width and bone formation.

Dr. Jowsey: These are two different parameters. The width of osteoid is a measure of the degree of calcification of bone. In both patients we showed this slight, but just significant, increase in osteoid width, which would suggest a slightly lower rate of mineralization. Slightly lower serum calcium levels could explain this. It cannot be described morphologically as osteomalacia.

Dr. Jurist: In a discussion of the reduction in bone mass from inactivity, water immersion, or weightlessness, the question of the relationship to human osteoporosis always arises. The easiest way to present the subject of osteoporosis is to diagram the broad problem of rarefying diseases of the human skeleton. The term *osteoporosis* simply means "porous bones," with a decrease in cortical and trabecular bone mass without any change in the external volume of the skeleton because of substitution of fibrous tissue, fat, and water for viable bone tissue.

Figure 12.1 illustrates the cycles of those cells responsible for maintenance of bone tissue equilibrium. The squares enclose the names of various disorders of bone that may alter an enzyme system or arc of the cycle of bone cells. Two arcs of the cycle of bone cells are disturbed in osteoporosis: the arc of bone resorption and the arc associated with cell proliferation necessary for bone reformation. The difference between physiologic reduction in bone density, or physiologic osteoporosis, and irreversible loss of bone, or pathologic osteoporosis, is only beginning to come to light. Osteoporosis occurs in Canadian geese during the period of recovery from moulting, in deer in the winter season when antlers begin to regrow, and in domestic hens in heavy lay; but a satisfactory experimental laboratory animal with the disorder is yet to be found. Cats, rats, and mice, when fed on all-meat diets supplemented with vitamin D during the period of rapid growth or during lactation, appear to develop a physiologic and reversible form of osteoporosis. In such instances, the skeleton becomes rarefied, and the lesion superficially resembles that seen in idiopathic or senile osteoporosis in human beings. It is not pathological osteoporosis, insofar as the disorder is typically physiologic and reversible by treatment with dietary measures.

In the human, osteoporosis is an irreversible, slowly progressive disorder that does not respond to any known form of medical treatment. In severe osteoporosis, as illustrated in figure 12.2, the process of bone remodeling is disturbed in both compacta and spongiosa, and both phases of bone remodeling are deranged. Bone resorption continues at normal, or possibly even slightly increased, rates, while the process of bone formation or repair is relatively slow. Metabolic activity of the cycle of bone cells must be abnormal, but the cause is not known and constitutes one of the major challenges of modern medical biology. Fortunately, in human beings all the other ten rarefying diseases of bone combined are uncommon compared to osteoporosis.

Osteoporosis occurs typically in the thoracic and lumbar segments of the axial skeleton and reduces cortical and spongy bone mass simultaneously in ribs, vertebrae, pelvis, and the necks of the femurs. The reduction in mass of spongiosa is associated with a steady decline in thickness of compact cortical bone. The loss of compacta is critical and causes mechanical failure, or the spontaneous collapse of bone structure recognizable as a pathological fracture. In this respect,

osteoporosis has the same effect as other disorders, such as osteitis fibrosa cystica, osteomalacia, multiple myeloma, or malignancy, which in early stages are difficult to diagnose in ordinary clinical roentgenograms. Scurvy, renal osteodystrophy, and osteogenesis imperfecta in middle-aged individuals also produce spontaneous fractures and can deceive an inexperienced clinician. A thorough hospital investigation is necessary to establish the diagnosis of osteoporosis, because only by exclusion of other diseases of known etiology is it possible to be absolutely certain that any one patient has the disorder.

One of the most important and trenchant questions about osteoporosis in man is whether it is basically a physiologic or specific pathological entity. Well-established data demonstrate that long-lived mammals, human beings in particular, display a time-dependent slow process of atrophy, or failure of retention of bone mass, which occurs in proportion to age and reduction in muscle mass. Decline in both bone and muscle mass is rapid at about the age of 50. The sample of the U.S. population that is most available for study of physiologic osteoporosis is found in the anatomical dissecting rooms of medical schools. Bones of white males are denser than white females; Negro females have denser bones than Caucasian females. In physiologic osteoporosis, all of the bones of the skeleton decline gradually in density and, to some extent, all are affected by the process, *but there are no spontaneous vertebral body compression fractures.*

In physiologic osteoporosis, the bones of the axial and appendicular skeleton are reduced in density in definite, though unequal, proportions, and are subject to fracture only from significant external forces, usually accidental. In pathologic osteoporosis, the bones of the axial skeleton are always much more severely affected than the bones of the appendicular skeleton, and multiple fractures occur spontaneously, or without any significant external force. Histologically, some cases show areas of excessive osteolysis and an abnormally large number of empty osteocyte lacunae in deposits in trabecular and haversian bone. The bone tissue is enveloped in fibrous and lipoid connective tissue. The cortex becomes porous, light in weight, hard in substance, and very brittle. Microradiographs show fully calcified new and old lamellar bone, but many of the vascular channels contain plugs of amorphous calcium deposits. Bone accretion, as determined by tracer studies, is frequently normal but may be either low or high, depending on the age of the patient and the stage of the disease. All of the usual laboratory analyses of serum and urine for calcium, phosphorus, and alkaline phosphatase are surprisingly normal, even in patients completely disabled with multiple fractures.

Many writers assume that physiologic and pathologic osteoporosis are similar in every respect, except that the reduction in bone mass is quantitatively greater. Whether or not this assumption is valid, the task at present is to make a determined search for characteristic clinical, morphological, and biochemical differences between the two conditions.

One of the most curious facts about osteoporosis is that the disease occurs most frequently in normal, healthy, Caucasian females, who appear only occasionally at autopsy in U.S. hospitals. Approximately 1 percent of the males and less than 5 percent of the females in a series of over 200 autopsies have pathologic osteoporosis with spontaneous fractures of the vertebral bodies of the dorsal and lumbar spine. As a rule, only patients with collapsed thoracic vertebral bodies,

ballooning of the lumbar intervertebral discs, and thickening of the vertical trabecular structures can be positively identified as instances of pathologic osteoporosis. Differing from living patients with osteoporosis, 95 percent of autopsy populations have little or no abnormal reduction in bone mass and no pathological fractures of ballooned discs; however, other conditions, such as spondylosis, metastatic bone disease, and single traumatic lesions, are relatively common. There is no point at which osteoporotic bones can be distinguished from nonosteoporotic bones by measurements of bone density or bone calcium. Thus, bone density, as determined by total bone calcium, is only one index and not a typical feature of either the incidence or the development of pathologic osteoporosis. Measurements of bone mass in cross sections of rib show lower values in some patients *without* than in others *with* severe osteoporosis. The question remains, therefore: why is the incidence of pathologic osteoporosis higher in relatively healthy subjects observed in old people's homes than in autopsy populations or hospital populations with severe debilitating diseases? In homes for the aged, pathologic osteoporosis occurs in one-fourth of the healthy females and one-tenth of all the healthy males of average age 75. Long life and advanced age are factors in the progress of the disorder, but aging per se is not critical, because most people develop spondylosis, not collapsed vertebrae, with the progress of time.

Sex, race, and aging, as already mentioned, are underlying factors but not the cause of pathologic osteoporosis. White males with severe, debilitating diseases, such as emphysema, chronic alcoholism, and various disorders of the gastrointestinal tract, show a lower incidence of osteoporosis than healthy females; and the reduction in bone density is relatively slight, more similar to physiologic osteoporosis and not comparable to that of pathologic osteoporosis. In a hospital population of subjects with severe pathologic osteoporosis, the rate of females to males is 7 to 1. In the typical female with osteoporosis, the decline in bone density is not gradual but precipitous, occurring over a period of 2 to 3 years and thereafter progressing relatively slowly, characterized only by sporadic episodes of disability from spontaneous fractures. Severe pain from acute fractures persists for a period of about 3 to 4 weeks and disappears when the fracture is healed. The density of the healed collapsed vertebra becomes higher than that of the uncollapsed, adjacent, rarefied vertebral bodies.

An ancillary but associated question about the difference between pathologic and physiologic osteoporosis: is a fracture-factor of unknown character present in aged individuals without osteoporosis of the dorsal or lumbar spine? Fractures of forearm, shoulder, and neck of the femur occur more frequently in aged than in young individuals, irrespective of the occurrence of osteoporosis. In most instances, however, fractures are not spontaneous but the result of accidents secondary to unsteady gait, chronic arthritis, etc. It is reasonable to suppose that a combination of physiological decrease in bone density and increased susceptibility to accidents accounts for the rise in incidence of fractures of the long bones in the aged. The epidemiology of fractures in adult individuals suggests the existence of an endogenous fragility factor, exclusive of aging or osteoporosis, that is predictable and constant for human populations and for special sites of predilection in the human skeleton. Perhaps microflaws or microfractures in the interstitial lamellae of the aged in special sites, such as the neck of the femur, are the basis of an endogenous fragility factor, but neither the causation nor the relationship to vertebral collapse in patients with pathologic osteoporosis is clear. In the United States, the epidemiology of fractures has been

investigated chiefly in the Negro population, because, for some unknown reason, possibly genetic, the 10 percent of the American population that is derived from African Negro ancestry infrequently shows pathologic osteoporosis and rarely presents fractures of the hip. In other populations of the world, the relationship between race and pathologic osteoporosis is apparent but not sufficiently clear cut or well studied in family trees to suggest an inbred metabolic or genetic disorder. A study of thousands of Bantu manual laborers demonstrates that the osteoporosis in the South African Negro population is simply the natural consequences of chronic ascorbic acid deficiency.

In U.S. patients with severe osteoporosis, as noted above, the levels of serum calcium, phosphorus, and alkaline phosphatase are generally normal. Mineral, vitamin, and general nutritional status are usually the same as in normal individuals. Metabolic balance studies on young subjects with rapidly progressive osteoporosis and multiple fractures reveal daily losses of calcium, phosphorus, and nitrogen in the urine and feces in excess of the amounts in the dietary intake. However, aged individuals with slowly progressive osteoporosis are usually not in negative calcium, phosphorus, and nitrogen balance. Kinetic studies with ^{47}Ca are extremely difficult to evaluate in patients in the early or acute stages of osteoporosis, because the method reflects the activity of less than 0.1 percent of the available bone mass. The 99.9 percent of the unavailable or non-exchangeable bone mass cannot be measured over a sufficiently long period of time by present day isotope dilution methods. In general, the patient with severe osteoporosis displays a normal or low rate of bone accretion associated with high or normal rates of bone resorption, but the latter is measurable only with the use of certain mathematical assumptions that are of debatable validity.

While the etiology is not clear in the majority of patients with severe osteoporosis, especially Caucasian females in the United States, some interesting exceptions can be found in every hospital. For example, patients with Cushing's syndrome, either endogenous or exogenous hypercortisonism, develop severe osteoporosis. Hypercortisonism often produces osteoporosis in young children and aged individuals. During the period of rapid growth and during periods of advanced age, when skeletal metabolism is highly active, depression of osteoblastic activity by hypercortisonism appears to produce irreparable damage to the skeleton. The effects on the skeleton are the same in endogenous hypercortisonism from pituitary and adrenocortical glands and from exogenous hypercortisonism caused by large doses of adrenal steroids for treatment of nephrotic syndrome, lupus erythematosus, exfoliative dermatitis, psoriasis, pemphigus, and rheumatoid arthritis. Hypercortisonism inhibits proliferation of mesenchymal cells, including osteoblasts, and prevents absorption of large amounts of calcium from the gastrointestinal tract. Removal of adrenocortical gland tumors in young individuals is followed by repair of bone structure by appositional new bone formation, but recovery of the original mass of rarefied tissue in nongrowing adults has not been observed radiographically. Neither has it been possible to prove that middle-aged individuals regain bone mass lost from either physiologic or pathologic osteoporosis after treatment with high calcium diets for periods as long as 20 years.

In cases of severe pathologic osteoporosis, the spongiosa is not only resorbed but also remodeled, reconstructed, or translocated to reinforce the vertical trabecular bone. Long-term roentgenographic observations by the writer on patients with severe osteoporoiss reveal progressive

accentuation of vertical trabecular markings. The absence of reinforced vertical trabecular bone markings should draw attention to the possibility of rarefying bone disease other than osteoporosis. Observations on roentgenograms of osteoporotic and nonosteoporotic autopsy subjects with either metastatic bone disease, multiple myeloma, hyperparathyroidism, or osteomalacia suggest that osteoporosis produces a special form of reduction in bone density. Indeed, some patients with physiologic osteoporosis and osteoarthritis have lower spongiosa density than that found in some patients with severe osteoporosis. In the latter, remodeling of spongiosa and the hypertrophy of vertical vertebral trabeculae maintain sufficient bone mass for mechanical support, with the minimal amount of bone as a tissue that is necessary to sustain a maximal function of the vertebral body as a weight-bearing organ. Such observations suggest that survey works on bone mass in random autopsy populations do not provide reliable information about osteoporosis. Random autopsy populations only provide information about a heterogeneous mixture of normal and abnormal forms of bone diseases. Measurements on bone density in an autopsy population of an intensively studied group of matched cases of Caucasion females, with and without a diagnosis proven by long-term clinical observations that excludes all other forms of rarefying bone disease will provide further knowledge of the nature of pathological osteoporosis.

Some of the difficulties in present research on osteoporosis come from retention of ideas that are handed down as truisms but could be false. Osteoporosis is believed to be the end result of a number of different disorders and not a single specific abnormality of the skeleton. The fact is, however, that all the disorders of endocrine and nutritional metabolism that are believed to produce osteoporosis are relatively uncommon and may be only superimposed on osteoporosis in individuals in which the disorder could be expected to occur from some other cause, i.e., genetic. One out of four women and one out of ten men can be expected to develop osteoporosis in the seventh decade of life.

The view that osteoporosis is caused by an abnormality in calcium-homeostasis lacks solid evidence to support it. Osteoporosis could be the cause, not the result, of an abnormality in calcium-homeostasis, and could develop without an unbalance between bone resorption and bone formation. Osteoporosis could be a slowly developed disorder of the *relatively unavailable bone mass*, not the exchangeable or *available bone mass* and, therefore, not easily detectable by present-day techniques of kinetic analysis of bone format and bone resorption rates. It is unusual to look at a section of bone in an aged human being and not find large amounts of necrotic bone. The amount of osteonecrosis is slightly greater in osteoporotic than in nonosteoporotic individuals. Unfortunately, there is no method for measurement of levels of bone cell viability in aged osteoporotic individuals, and further investigations are necessary along these lines.

The assumption that an increase in rate of bone resorption produces osteoporosis in the aged is questionable. Biopsy specimens show insufficient osteoclastic activity to account for the attenuation of trabecular bone, and it is necessary to postulate that osteolysis is the mechanism of bone destruction. Our view is that it is more reasonable to suppose that osteonecrosis and failure to replace lost bone by cytodifferentiation of new mesenchymal cells is more consistent with the bone change in severe pathological osteoporosis.

Dr. Jurist: We have been interested in this idea introduced by Dr. Zollinger for many years. I have not been able to get my colleagues at the Atomic Energy Commission to try this on a series of women with osteoporosis, but I would like to. I am not fearful of the relatively large error in this method because, when we do a red count on a patient in the clinic, we are not worried about minor errors. If a patient has a red count of 4 million instead of 5 million, we worry about it and do something about it. The magnitude of the changes in bone structure that you saw in these patients that have collapsed fractures is about 30 percent of the total bone mass. I would be very grateful if I could measure bone mass with a 10 percent or even a 15 percent error. I think Dr. Zollinger's approach is an exciting one. With more studies of this kind, this equation might be sharpened up further.

Dr. Nordin: Well, I was just wondering why you didn't think my method of measuring total skeletal mass was equally as exciting.

Dr. Jurist: Well, I do. I certainly do.

Dr. Whedon: How did your measured final determinations of skeletal mass compare with your calculations by means of body composition?

Dr. Zollinger: The original equation predicted for this subject a skeleton of 3.5 kg: it measured out just between 2.8 and 2.9 kg. Because of this high prediction, the data from the patients in Dr. Moore's series were recalculated; and the skeleton, as a function of the fat-free body, was shifted down from 10.3 percent to 8.5 percent of the weight of the fat-free body. Then a new nomogram and predictive equation were created.

The acquisition of a normal human with premortem composition studies and consent for postmortem total body analysis is rather difficult. Additionally, about 150 man-hours are needed to dissect out all tissue components, after which they are reduced to homogeneous powders by passing frozen cubes of tissue, plus dry ice, through a stainless steel hammer mill.

Dr. Whedon: How about animal studies?

Dr. Zollinger: This work has been done only in man so far, as our interests are predominately in its clinical applications.

Dr. Barzel: I want to congratulate Dr. Kazerian. This work is of great importance and has given us information that we, as physicians, have never been able to obtain. He has described a way of measuring actual loss of bone strength and a technique to quantify this loss.

Dr. Jurist: How long were these animals immobilized, and what was their diet?

Dr. Kazarian: They were immobilized for 56 days and were fed regular monkey chow.

Dr. Lutwak: Will you describe in more detail the technique you used with tetracycline for bone labeling?

Dr. Kazarian: The tetracycline was administered in multiple doses during the month before the experiment. We used tetracycline and chlortetracycline and assumed that the haversian systems that were labeled were the same that underwent resorption.

Dr. Jurist: I would like to raise a question about the specimens from the patients with osteoporosis. Do you interpret the values that you've obtained by all these different parameters to indicate that there was a change in the quality of the bony material, as well as mass, in patients with osteoporosis?

Dr. Abendschein: These were specimens of cortex from a tibia with disuse osteopenia. Certainly, the modulus of elasticity and its density were of a much lower magnitude than in the normal bone.

Dr. Jurist: What about the ultrasound studies?

Dr. Abendschein: The velocity of ultrasound in the diseased bones was 2,800 m/sec, over 600 m/sec or 30 percent lower than in the normal bone. We have data for bone graft specimens from guinea pigs that show the same type of change in fractures and bone graft healing. We found the velocity in normal femurs to be around 3,800 m/sec, healed fractures about 3,400 m/sec, and the velocity in a pseudoarthrosis approaching that of soft tissue at 1,900 m/sec.

Dr. Cameron: This agrees very well with Jurist's data showing that osteoporotic women, when compared to their age-matched pairs, showed about 25 percent lower frequency. This is basically the same measurement as velocity of sound; both are a function of Young's modulus. It may be that the transmission of ultrasonic pulses is a simpler and more precise measure, but it is more extensive than measuring the resonant frequency, which requires a simple oscillator system with a frequency meter.

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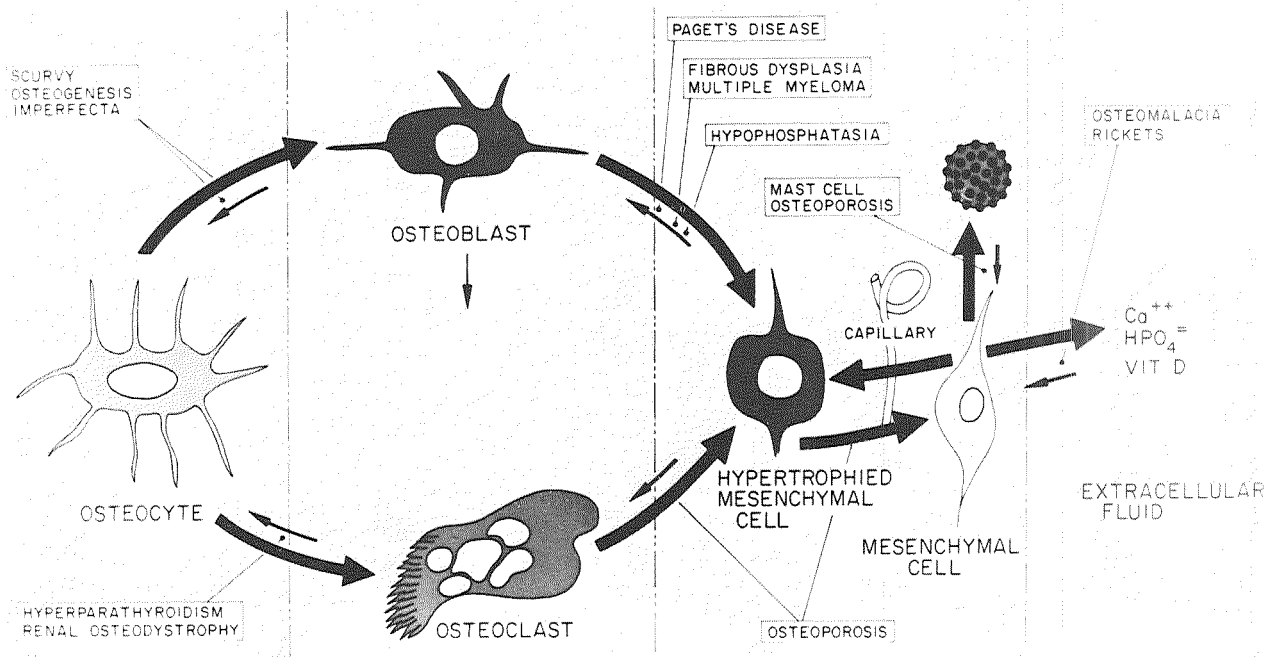

Figure 12.1 Diagrammatic representation of the pathogenesis of osteoporosis and 10 other rarefying diseases of bone classified according tothe presumptive effects on the cycle of bone cells. Throughout life, bone tissue equilibrium is maintained by a cycle of bone cells. The cycle consists of mesenchymal cells, osteoblasts, osteocytes, and osteoclasts. The number of cells of each classification in the total bone cell population is controlled by equilibria indicated by the arrows. The length of the arrow illustrates the direction of cell specialization, and the proportion of each cell form in the total population of bone cells in the disease process. In pathologic osteoporosis the cell population of mesenchymal cells and mast cells increases, while the population of osteoblasts and osteocytes decreases. The eventual effect is relative increase in soft tissue at the expense of the hard tissue components of the bone tissue, particularly in the vertebral bodies of the dorsal and lumbar areas of the spinal column.

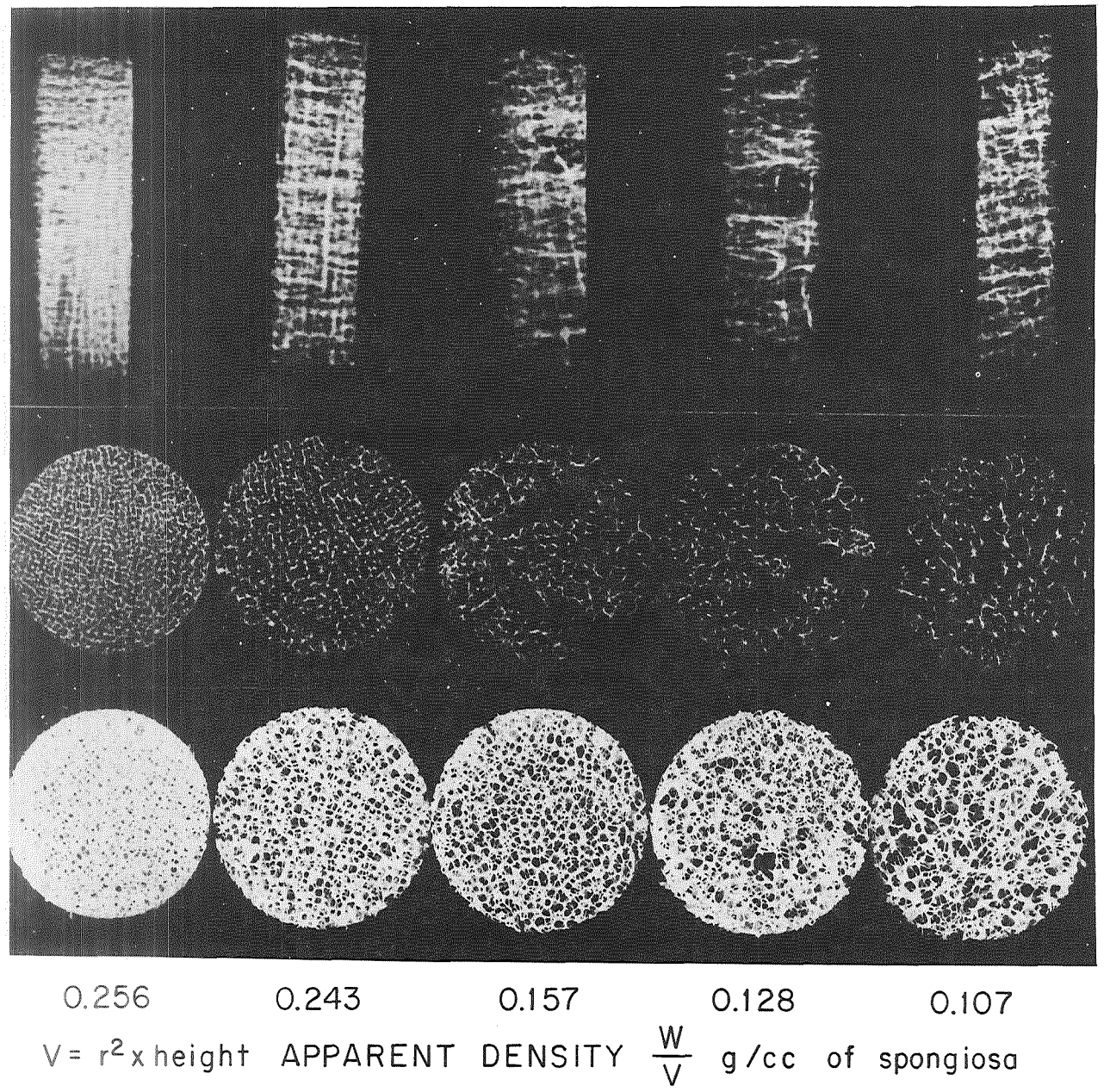

Figure 12.2 Coin-shaped segments of the first lumbar vertebra obtained from five autopsy subjects, showing five levels of reduction of bone mass. Roentgenogram of the segments: (Top) end views showing progressive increase in diameter and mass of the vertical bone trabeculae; (middle) vertical view showing the attenuation of the horizontal trabecular structure and increase in *diameter* of the vertical bone trabeculae; (bottom) photograph of the fat-free dry trabecular segments showing the progressive increase in volume of *spaces* occupied by soft parts. Note that when the apparent density falls below 0.243, the attenuation of the structure is associated with reinforcement of the vertical trabecular bone. Thus, bone remodeling, removal, and reconstruction occur when osteoporosis develops.

Keynote Paper

13 EFFECTS OF BED REST ON THE OXYGEN TRANSPORT SYSTEM*

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INTRODUCTION

A symposium on the abuse of rest in the treatment of disease was held in 1944 (ref. 1) and marked an awakened interest in the clinical side effects of bed rest and immobilization. Classical studies on the physiological effects of bed rest were published during the late 1940s. They have been followed by a multitude of investigations covering clinical aspects of bed rest, and lately, physiological aspects as they relate to space travel. Bed rest is also of considerable basic physiological interest, since it represents the low extreme of a continuous scale of patterns of physical activity and is associated with important changes in the oxygen transport system of the body.

The first modern study of physiological effects of bed rest was conducted by Cuthbertson in 1929 (ref. 2). He reported on significant metabolic effects, increased nitrogen and calcium excretion. Important studies covering both circulatory and metabolic aspects of bed rest were published from 1945 to 1949 by Taylor, Keys, and collaborators (refs. 3, 4) and by Deitrick, Whedon, and Schorr (refs. 5, 6). These two groups originally described some of the salient features of the circulatory changes induced by prolonged bed rest.

Taylor and coworkers (refs. 3, 4) studied a group of six healthy 20- to 33-year-old men before and after a 3- to 4-week period of bed rest. Basal heart rate showed a continuous increase during bed rest, but there was no change in resting (supine) cardiac output and stroke volume, as measured by the acetylene rebreathing technique. They found a marked increase, 40 bpm, in heart rate during treadmill exercise at a standard submaximal level and a decrease in maximal oxygen uptake (measured in two subjects only). The cardiovascular response to tilting at 68° was impaired after bed rest, as judged by higher heart rates and lower blood pressures. Total heart volume decreased by 17 percent. There was an average plasma volume loss of 15.5 percent and a total blood volume loss of 9.3 percent. Deitrick et al. (ref. 5) immobilized their four 20- to 29-year-old male subjects for 6 to 7 weeks in bivalved plaster casts extending from the umbilicus to the toes. An increase in resting heart rate and deterioration in cardiovascular response to head-up tilting and to submaximal exercise were clearly

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demonstrated also in this series. Plasma volume and total blood volume were significantly lowered after 3 weeks of bed rest, but approached control levels after 6 weeks.

Current data on circulatory effects of bed rest are discussed against the background of a recent study (ref. 7) from our laboratory dealing with adaptive changes in oxygen transport and body composition after bed rest and after training. In this study, maximal oxygen uptake was used as the index of maximal cardiovascular function (refs. 8, 9).

Five 19- to 21-year-old college students were selected for the study from a larger group of volunteers. Three of them had participated only to a minimal degree in college sports activities and had maximal oxygen uptakes between 33 and 45 ml/(kg·min). Two engaged in competitive sports, one as a runner and one as a semiprofessional football player; they had maximal oxygen uptakes of 61 and 47 ml/(kg·min), respectively.

EXPERIMENTAL DESIGN AND PROCEDURE

The investigation was divided into three phases: (1) a short control period, (2) a 3-week bed rest period, and (3) a 2-month physical training period. Identical sets of studies were performed at the end of each period. An attempt was made to collect comprehensive information on how large, induced changes in the level of physical activity modify the various steps in the transfer of oxygen from ambient air to the tissues. Results from the training phase will be discussed only briefly.

The duration of bed rest was 20 days. The subjects were confined to two air-conditioned hospital rooms and were attended by nursing and service personnel around the clock. They were granted bathroom privileges and spent approximately 10 min daily out of bed, but they were never allowed to support their body weight in the standing position. The subjects were permitted to move freely in bed and to sit up in bed for eating or reading. Only trivial psychological difficulties were experienced during the bed rest period.

Food intake consisted of a regular mixed hospital diet with approximately 40 percent of total calories from fat, 15 percent from protein, and 45 percent from carbohydrate. The total number of calories offered to each subject ranged from 2,470 to 3,380. The food was served on individual trays, and an attempt was made to regulate the caloric intake individually to prevent any change in total body weight.

The degree of immobilization was evaluated objectively from multiple direct measurements of oxygen uptake. Samples were collected in each subject over ten to fifteen 10-min periods, covering the entire range of activity during bed rest. Mean oxygen uptake was 30 to 35 percent above BMR (range 0.22 to 0.48 liter/min). These studies were supplemented with 24-hr heart rate monitoring using portable tape recorders during the 9th to 11th (mean 9.4) day of bed rest. R-R interval frequency histograms and cumulative heart rate frequency distributions were derived using portable tape recorders, a system for rapid playback, and a small digital computer for electronic measurements of all R-R intervals. The results appear in figure 13.1. Less than 5 percent of all heart beats were at a rate higher than 100 bpm. The 50th percentile of the group mean distribution corresponds to a rate of 71 bpm. Individual variation was large.

Calcium metabolism studies provided additional data relevant to the assessment of the degree of immobilization. Total urinary output of calcium increased from a mean of 17.3 meq/24 hr to 20.8 meq after six days' bed rest. The difference was significant ($p < 0.05$). A further increase to 22.9 meq ($p < 0.05$) was seen between the 6th and 18th days. Intake of calcium did not change significantly.

Basal heart rate was computed from 7- to 8-hr continuous tape recordings of the electrocardiogram during sleep. Two recordings were made: (1) between the 9th and 11th day, and (2) between the 14th and 19th day of the bed rest period. The R-R interval corresponding to the 95th percentile was computed from a cumulative frequency distribution based on electronic measurement of all R-R intervals in the recording, using a total of 20,000 to 25,000 beats. The mean basal heart rate was 47.3 bpm at the midpoint of the bed rest period and increased to 51.3 bpm near the end of the bed rest phase. This change corresponds to an average daily increase of 0.4 bpm/day. The difference was statistically significant ($p < 0.05$).

Even extremely rigid regimens for bed rest, such as the one used by Deitrick et al. (ref. 5) employing extensive casts, have not completely eliminated periods of increased activity, e.g., for testing. Lack of objective, quantitative data on the degree of immobilization frequently makes it difficult to compare the results from different series. The validity of the indices used in the present study has not been fully ascertained. Analysis of similar data from studies on comparable experimental groups by Taylor et al. (ref. 4), and Birkhead et al. (refs. 10, 11) and Issekutz et al. (ref. 12) shows a general agreement both with respect to indices and results. Measurements of calcium excretion, basal heart rate, and oxygen uptake are all easily performed. The equipment for analysis of 24-hr heart rate data is more complex. It may be argued that changes in heart rate related to sensory stimuli and mental activity are not differentiated from those resulting from physical movement. Nevertheless, it is likely that heart rate data of this type provide at least a crude quantitative index of the overall level of activity during bed rest.

RESULTS

Maximal Oxygen Uptake

Changes in maximal oxygen uptake, determined during treadmill exercise, are presented in figure 13.2. The 20 days of bed rest resulted in a reduction in mean maximal oxygen uptake from 3.39 to 2.43 liters/min or 28 percent ($p < 0.01$); the range was 20 to 46 percent. The relative magnitude of the decrease was the same in trained and sedentary subjects, but the two subgroups differed markedly during recovery. The three sedentary subjects exceeded their control values after 8 to 12 days of training. The two previously trained subjects did not reach that level until the 29th and 43rd days of the training period.

Direct measurements of maximal oxygen uptake before and after a period of bed rest unmodified by any exercise program are available in only a limited number of subjects. Taylor et al. (ref. 4) found reductions of 22 and 13 percent in two subjects after 3 weeks. Birkhead et al. (ref. 10) reported a residual decrease of 13 percent in four subjects after 6 weeks of bed rest followed by 18 days of retraining. Another group of four subjects studied by the latter authors (ref. 11) showed a 7 percent decrease in maximal oxygen uptake after 30 days of combining bed

rest with rest in a chair for 8 hr daily. Contrary to these reports and our own data, Chase et al. (ref. 13) found no changes in their four bed-rest subjects after 15 days. The results of studies combining bed rest with various exercise programs are highly variable (refs. 11, 13, 14).

Pulmonary Measurements

In normal subjects, pulmonary function is a limiting factor with respect to maximal oxygen uptake only at high altitude. There is no evidence from our data that a postbed rest condition is an exception to this rule.

Bed rest did not cause any significant change in total lung capacity, forced vital capacity, or 1-sec forced expiratory volumes. Neither the slopes nor the intercepts of diffusing capacity plotted against cardiac output, or of pulmonary capillary blood volume similarly plotted, were significantly changed from control values by bed rest.

Ventilatory volumes at rest and during submaximal exercise showed only minor variations with bed rest. The breathing pattern during upright submaximal exercise was slightly modified. Respiratory rate was higher than at the control phase; as a result, tidal volume was lower. Mean ventilatory volume during maximal work was 129 liter/min at the control phase and 99 liter/min after bed rest. Corresponding respiratory rates were 43 and 50 breaths/min. Tidal volumes were 2.9 liters (50% of vital capacity) at the control phase and 2.1 liters (34% of vital capacity) after bed rest. The ventilatory coefficient (ventilatory volume/oxygen uptake) during maximal exercise did not change. The decrease in ventilatory volume was directly related to the changes in maximal oxygen uptake.

Blood Volume

Blood volume decreased significantly from 5.065 to 4.700 liters ($p < 0.05$). The fall in plasma volume (from 2.791 to 2.565 liters) was slightly more pronounced than the fall in red cell mass (2.274 to 2.132 liters) and was statistically significant ($p < 0.05$). These findings are in agreement with Deitrick et al. (ref. 5) who showed that total blood volume decreases significantly during the initial phase of bed rest up to 3 weeks, and then tends to return to control level; this has been the typical course in later studies also (ref. 15).

Body Composition and Skeletal Muscle Morphology

Total body weight did not change significantly. Lean body mass (calculated from specific gravity) decreased during bed rest from a mean of 66.3 to 65.3 kg ($p < 0.05$). All subjects except one showed a decrease in total body water during bed rest, but the magnitudes of the changes were small. The changes were primarily due to a decrease in the calculated intracellular fraction. None of the changes was significant, and it is unlikely that they were of major functional importance.

There were no gross changes in ultrastructural morphology of voluntary muscle (quadriceps). Average blood vessel diameter, number of blood vessels per cross-sectional area, capillary basement membrane thickness, and distribution of blood vessel size showed no significant variation between subjects or as the result of bed rest.

Circulatory Measurements

From the above studies it is clear that changes affecting the links of the oxygen transport system proximal and distal to the cardiovascular system cannot account for the large decrease in maximal oxygen uptake after bed rest.

Circulatory findings during maximal work are summarized in table 13.1. It is evident that the decrease in maximal oxygen uptake was primarily due to a decrease in stroke volume and cardiac output; maximal heart rate and arteriovenous oxygen (A-V O_2) difference did not change significantly. The average decline in maximal cardiac output was 5.2 liters/min, or 26 percent. The stroke volume was similarly reduced in all subjects. The average decrease corresponded to 29 percent of the control stroke volume. Results from exercise at submaximal levels, both in the upright and in the supine position, demonstrated changes consistent with those during maximal exercise.

Cardiac Output Cardiac output was measured with the dye-dilution technique. Data are presented in figure 13.3. Mean values were approximately 2 liter/min lower after bed rest at all submaximal levels of oxygen uptake. Maximal cardiac output was 20.0 liter/min before and 14.8 liter/min after bed rest.

Heart Rate It is evident from figure 13.4 that mean heart rates were higher after bed rest at all levels of exercise and at rest, in the supine as well as in the upright position. The difference during supine exercise at 600 kpm/min was 25 bpm, i.e., 129 before and 154 after bed rest ($p < 0.05$). Corresponding figures for treadmill exercise at an oxygen uptake of 1.5 liter/min were 35, 129, and 164 bpm. Maximal heart rate was 193 before and 197 bpm after bed rest.

Stroke Volume Bed rest produced a marked decrease in stroke volume as shown in figure 13.5. Mean stroke volume at rest in the supine position was 103 ml at the control phase and 86 ml after bed rest. A similar decrease was also seen at rest in the sitting position, from a mean of 79 ml before bed rest to 60 ml after.

Mean values during supine exercise at 300 and 600 kpm/min were 113 and 116 ml before bed rest. Significantly lower values, 92 and 89 ml, were seen after bed rest ($p < 0.05$).

Stroke volume remained the same through three levels of submaximal treadmill exercise in the control study before bed rest. Mean values at oxygen uptakes of 1.3, 2.0, and 2.6 liter/min were 101, 104, and 103 ml, respectively, and were about the same as the resting values in the supine position. A decrease of approximately 30 ml occurred with bed rest. Mean values at oxygen uptakes of 1.2, 1.8, and 2.0 liter/min were 72, 68, and 74 ml. The reduction was significant ($p < 0.02$). The stroke volume during maximal work ranged from 88 to 138 ml at the control study, the mean being 104 ml. There was a significant ($p < 0.02$) decrease with bed rest to a mean of 74 ml (range 45 to 95 ml).

Arteriovenous Oxygen Difference Arteriovenous oxygen (A-V O_2) differences were calculated from measurements of oxygen uptake and cardiac output. Mean A-V O_2 difference was consistently higher after bed rest. Data are presented in figure 13.6. Mean A-V O_2 difference at rest in the supine position at the control phase was 4.2 ml/100 ml and was 4.6 after bed rest. Corresponding figures at rest in the sitting position were 5.8 and 7.8 ml/100 ml.

Mean A-V O_2 differences during supine exercise at 300 and 600 kpm/min were 9.2 and 11.5 ml/100 ml, respectively, at the control phase. At both loads, A-V O_2 differences after bed rest were wider: 10.3 and 13.5 ml/100 ml, respectively.

Mean A-V O_2 differences during upright submaximal work at the control phase were 11.4, 13.0, and 13.8 ml/100 ml at oxygen uptakes of 1.3, 2.0, and 2.6 liter/min. Corresponding figures after bed rest were 12.7, 13.3, and 14.7 ml/100 ml at oxygen uptakes of 1.2, 1.4, and 2.0 liter/min; that is, for any given oxygen uptake, the mean A-V O_2 difference was higher after bed rest than during the control study.

Mean A-V O_2 difference during maximal treadmill exercise during the control phase was 16.2 ml/100 ml. The mean value after bed rest was 16.5, and individual changes ranged from −1.8 to +1.7 ml/100 ml.

Blood Pressure There were no significant changes in mean arterial blood pressure at rest in the supine or in the sitting position. The mean blood pressure during supine exercise at 300 and 600 kpm/min showed a small but significant decrease ($p < 0.05$). There was no significant difference between exercise pressures before and after bed rest during submaximal treadmill exercise. However, two subjects fainted during upright exercise after bed rest and showed a progressive fall in mean blood pressure with increasing work loads.

The mean pressure during maximal treadmill work in the control study was 113 mm Hg. There was a significant ($p < 0.01$) fall to 90 mm Hg after bed rest.

Peripheral Resistance As shown in figure 13.7, bed rest produced no major change in total peripheral resistance at rest in the supine position. Mean values were 1,098 in the control phase and 1,212 dyne cm sec^{-5} after bed rest. Total peripheral resistance at rest in the sitting position showed a moderate increase with bed rest (1,362 to 1,639 dyne cm sec^{-5}). Measurements during supine exercise at 300 and 600 kpm/min did not show any significant changes as the result of bed rest but the level was slightly higher. The relation between total peripheral resistance and oxygen uptake during submaximal treadmill exercise also changed very little with bed rest. Values during maximal work were 449 at the control study and 520 dyne cm sec^{-5} after bed rest.

Heart Volume Individual data for total heart volume measured from biplane chest films are presented in figure 13.8. A decrease in heart size was observed in all subjects. The mean control value was 860 ml, and the mean value after bed rest 770 ml ($p < 0.01$). The decrease of 90 ml corresponds to 11 percent of the initial volume. Both absolute and relative changes were largest in the two previously trained subjects.

The three untrained subjects showed a rapid return of total heart volume to the control value during the training period. All had exceeded their control values (after 13 to 15 days of training). In contrast, the two previously trained subjects had barely returned to their control values at the completion of the training program.

COMMENTS

Attempts to explain the deterioration in circulatory performance in the upright position after bed rest have centered around failure of cardiovascular reflexes and depletion of intravascular volume.

There is no evidence that prolonged bed rest results in an impairment of mechanisms controlling resistance vessels. In our series, the A-V O_2 difference during submaximal exercise was higher after bed rest for any given level of oxygen uptake both in the upright and in the supine position. The A-V O_2 difference during maximal treadmill work was approximately the same after bed rest as in the control study (16.5 and 16.2 ml/100 ml, respectively). There was no significant change in total peripheral resistance, but values tended to be slightly higher at the end of immobilization. The view that prolonged immobilization does not affect sympathetic outflow is supported by other data, e.g., the heart rate response after bed rest among our subjects. The maximal heart rate was unchanged, and the relation between heart rate during exercise and relative work load was the same as in the control study.

The decrease in blood volume, taken as an isolated phenomenon, cannot explain a poor adaptation to upright position. A dissociation between changes in blood volume and response to tilt was evident already in the study of Deitrick et al. (ref. 5), with a tendency to normalization of the blood volume toward the end of 6 weeks without any improvement in heart rate and blood pressure response to tilting. Further evidence against intravascular volume's being the sole factor has been provided by Stevens et al. (ref. 16). Normalization of the blood volume at the end of a period of prolonged bed rest with 9-α-fluorohydrocortisone or venous occlusive cuffs failed to improve orthostatic tolerance. Similarly, McCally et al. (ref. 17) prevented blood volume changes by ADH administration during a short-term immersion experiment, but this did not influence the response to tilting.

Experimentally induced, acute changes in plasma volume unrelated to immobilization seem to influence maximal oxygen uptake during upright exercise only when a very large amount of plasma has been lost. Saltin (ref. 18) found no change in maximal oxygen uptake after dehydration with a loss of up to 25 percent of the plasma volume.

Impaired control of capacitance vessels, due to lack of gravitational stimuli during bed rest resulting in peripheral venous pooling and a decrease in central blood volume and right ventricular filling pressure, has been proposed as the main mechanism behind the orthostatic intolerance after bed rest (ref. 7). There is some support for this view, mainly from experiments evaluating various countermeasures during and after bed rest.

Whedon et al. (ref. 6) reported that use of an oscillating bed largely prevented both metabolic and circulatory changes. Stevens et al. (ref. 19) were able to prevent partially changes in response to tilting and to upright exercise by using a long-term lower body negative pressure during bed rest. However, the protective effect, with respect to exercise tolerance, was reflected only in the results of a Balke test. Measurements of maximal oxygen uptake showed large individual variability and no significant difference. Eight hours daily of quiet sitting added to bed rest resulted in a maintained tilt tolerance in three of four subjects in a series studied by Birkhead et al. (ref. 10). However,

Miller et al. (ref. 14) found that use of an antigravity suit during tilt tests after bed rest modified the response but did not abolish orthostatic intolerance.

Vogt and Johnson (ref. 20) suggested that changes in body fluid compartments may result in an excessively rapid shift from intravascular to extravascular compartments when the subject is exposed to upright position following prolonged bed rest. This would result in a rapid fall in intravascular volume after a change to upright position and would not necessarily be reflected in blood volume measurements made under basal conditions.

There is little doubt that a portion of the decrease in maximal oxygen uptake, observed in our series, must be attributed to orthostatic intolerance secondary to fluid extravasation and/or impaired control of capacitance vessels. These factors, however, do not appear to offer a complete explanation, since there was marked reduction in stroke volume during supine as well as during upright exercise. In fact, the relative decrease during supine exercise at 600 kpm/min (24 percent) was almost as large as that during upright exercise.

The findings during supine submaximal exercise were similar to those reported in the three subjects studied by Birkhead et al. (ref. 10), but the changes were somewhat less marked in our subjects. Mean values for cardiac output, heart rate, and stroke volume from both studies are presented in table 13.2.

Two of our subjects experienced fainting episodes during the treadmill test after bed rest. After appropriate rest, however, they were able to complete the test. Table 13.3 compares circulatory data during supine submaximal and upright maximal exercise in the two subjects who fainted during treadmill exercise after bed rest, with corresponding data in the three subjects who did not faint; the reduction in maximal oxygen uptake, cardiac output, and stroke volume was approximately twice as large in those who fainted. However, it is evident that differences between the two groups, in stroke volume and cardiac output of the same relative magnitude, were present also during supine exercise, i.e., preceding the fainting episodes and at a time when mean arterial pressure was similar in both groups and unchanged compared with the control study. These data strongly suggest that both the fainting episodes and the decrease in maximal oxygen uptake were related to a restricted stroke volume and cardiac output.

Gravity-induced fluid shifts resulting in a decreased circulating blood volume cannot explain the low stroke volume in the supine position. Pooling of blood in the large capacitance veins with inadequate return of blood to the right ventricle after bed rest cannot be excluded, but the contrast afforded by studies on patients with postural hypotension is of interest. In these patients, there is evidence of lack of control of venous tone, low stroke volume, and decreased cardiac output in the upright position. In the supine position, on the other hand, such patients show normal cardiac output and relatively high stroke volume during exercise (ref. 21). To state this finding in another way, if one eliminates venous pooling in patients with postural hypotension, circulatory adaptation to muscular exercise becomes normal with respect to stroke volume and cardiac output. Such is not the case in normal subjects after bed rest.

The findings in the present study suggest that bed rest impairs circulatory adaptation to muscular exercise by an unidentified myocardial effect. The relation between total heart volume and stroke volume during supine exercise is consistent with an effect on the myocardium. Total heart volume declined significantly during the period of immobilization, the average decrease being 90 ml or 10 percent of the control value. Holmgren and coworkers (ref. 22) found a linear relation between heart volume and stroke volume during supine exercise at submaximal levels. In the present study, the same general relation was found during the control phase, but at the end of the period of immobilization the decrease in stroke volume was relatively larger than the decline in heart volume in each of the five subjects.

Moderately heavy exercise in the supine position during bed rest has, in two series (refs. 11, 13), prevented any decline in maximal oxygen uptake measured during upright exercise. The effect on orthostatic intolerance was variable. It seems reasonable to postulate that the decrease in physical performance capacity after prolonged bed rest is mediated by at least two mechanisms: (1) an orthostatic intolerance secondary to uncompensated changes in the magnitude or distribution of the intravascular volume, and (2) a separate depressive effect on myocardial function, probably related to inactivity.

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Table 13.1 Maximal treadmill exercise. Mean circulatory data before and after bed rest

Maximal Exercise	*Before*	*After*	*Change, %*
Oxygen uptake, liter/min	3.30	2.43	−26.4
			−17.8 −42.1
Cardiac output, liter/min	20.0	14.8	−26.0
			−4.9 −47.4
Heart rate, bpm	193	197	+2.1
			±0 +5.9
Stroke volume, ml	104	74	−28.8
			−5.4 −48.8
A-V O_2 diff, vol %	16.5	16.4	−0.6
			+1.7 −1.8

Source: Reference 7.

Table 13.2 Mean cardiac output, heart rate, and stroke volume at rest and during supine exercise before and after bed rest

		Cardiac Output		*Heart Rate*		*Stroke Volume*	
Supine Position		Control	After Bed Rest	Control	After Bed Rest	Control	After Bed Rest
Rest	A	5.6	5.8	72	94	78	56
	B	6.4	5.8	63	68	103	86
300 kpm/min	A	11.9	10.3	98	116	119	89
	B	10.9	9.6	100	107	113	92
600 kpm/min	A	17.0	12.6	128	154	132	82
	B	14.4	12.4	129	154	116	88

A = Birkhead et al. (ref. 10), n = 3.

B = Present series, n = 5.

Source: Reference 7.

Table 13.3 Circulatory data in the two subjects who fainted (F) and the three subjects who did not (N) faint during upright exercise after bed rest*

		Supine Exercise			*Maximal Treadmill Exercise*		
		Before Bed Rest	After Bed Rest	Change, %	Before Bed Rest	After Bed Rest	Change, %
Oxygen uptake, liter/min	F	1.68	1.59	−2	2.51	1.57	−37
	N	1.40	1.42	+1	3.82	3.01	−22
Cardiac output, liter/min	F	15.0	10.7	−29	16.7	9.8	−41
	N	13.6	12.0	−13	22.3	18.1	−19
Stroke volume, ml	F	103	61	−37	88	50	−43
	N	127	98	−23	115	90	−22
Mean arterial blood pressure, mm Hg	F	106	106	±0	98	72	−26
	N	100	100	±0	132	102	−23

*The work load during supine exercise was 600 kpm/min on both occasions in all subjects except BB of group N, who was studied only at 300 kpm/min both before and after bed rest.

Source: Reference 7.

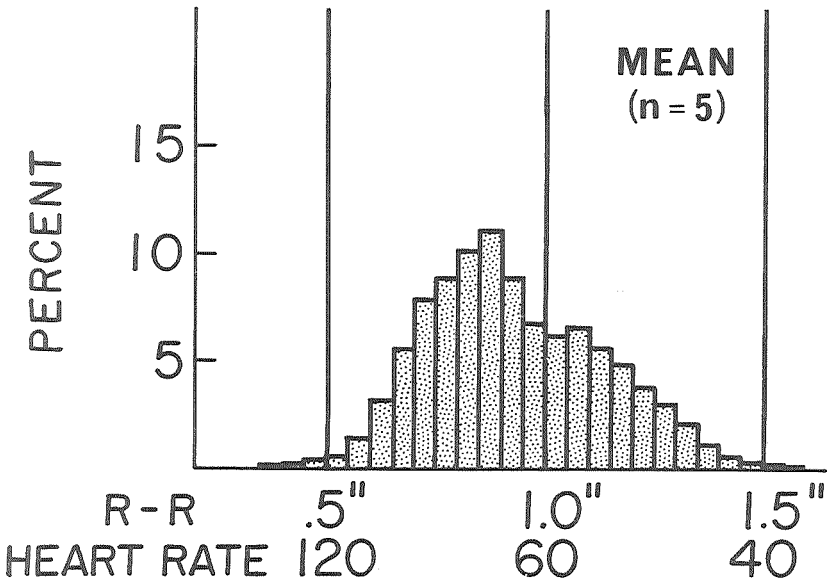

Figure 13.1 Twenty-four-hour heart rate monitoring during bed rest. The panel to the left gives the relative number of beats at different R-R intervals. The panel to the right displays the data as a cumulative percentage distribution, i.e., percent of all heart beats during the 24-hr period above a given rate. The distribution curve indicates that less than 5 percent of all beats were at a rate above 100 and that the median corresponds to a heart rate of 71. Source: Reference 7

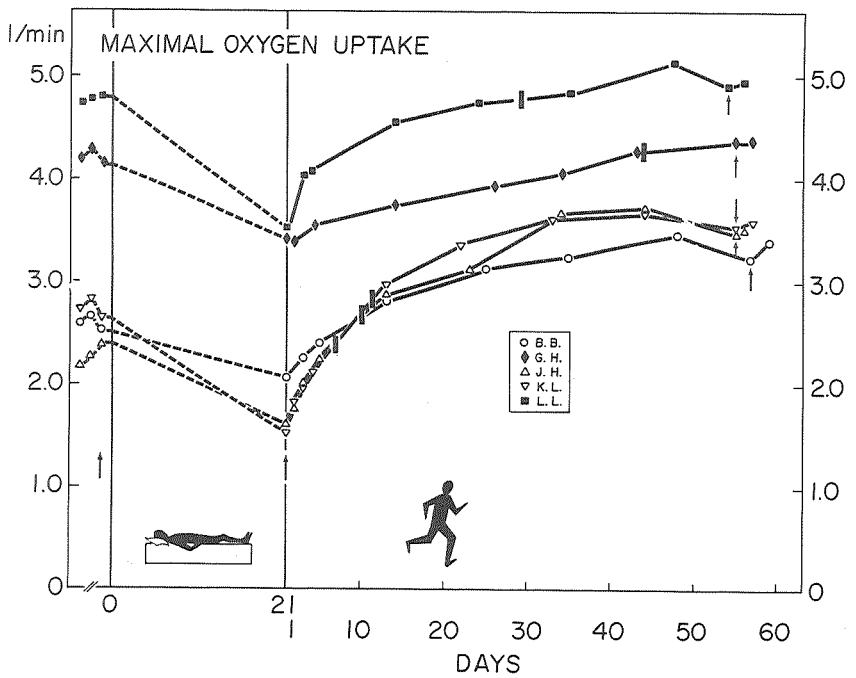

Figure 13.2 Changes in maximal oxygen uptake with bed rest and training. Individual data before and after bed rest and at various intervals during training. Arrows indicate circulatory studies. Heavy bars mark the time during the training period at which the maximal oxygen uptake had returned to the control value before bed rest. Source: Reference 7

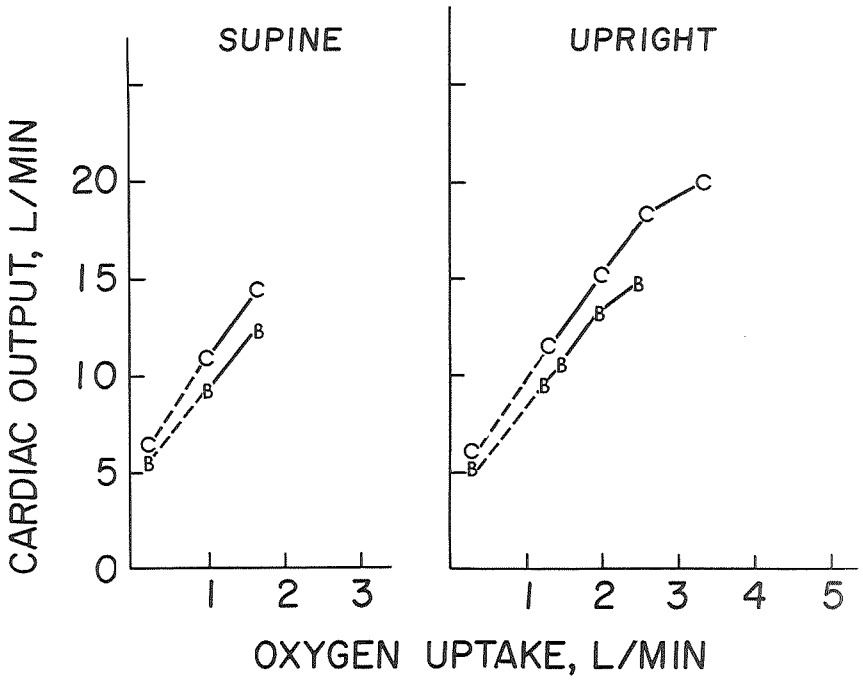

Figure 13.3 Cardiac output in relation to oxygen uptake. Mean values at the control study (C) and after bed rest (B). Source: Reference 7

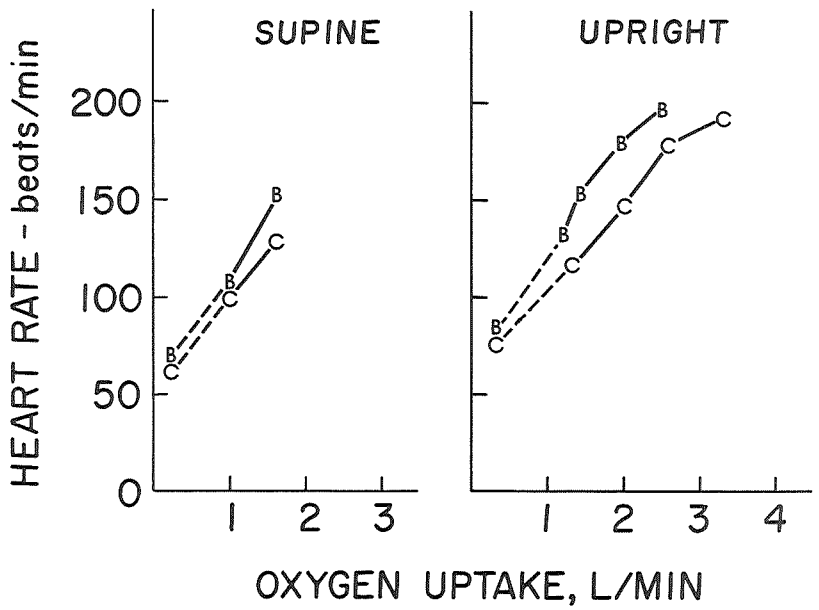

Figure 13.4 Heart rate in relation to oxygen uptake. Mean values at the control study (C) and after bed rest (B). Source: Reference 7

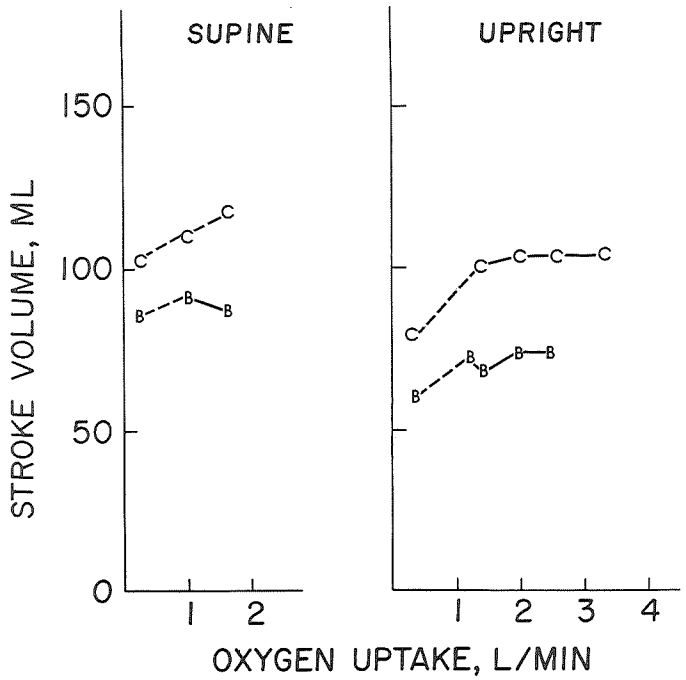

Figure 13.5 Stroke volume in relation to oxygen uptake. Mean values at the control study (C) and after bed rest (B). Source: Reference 7

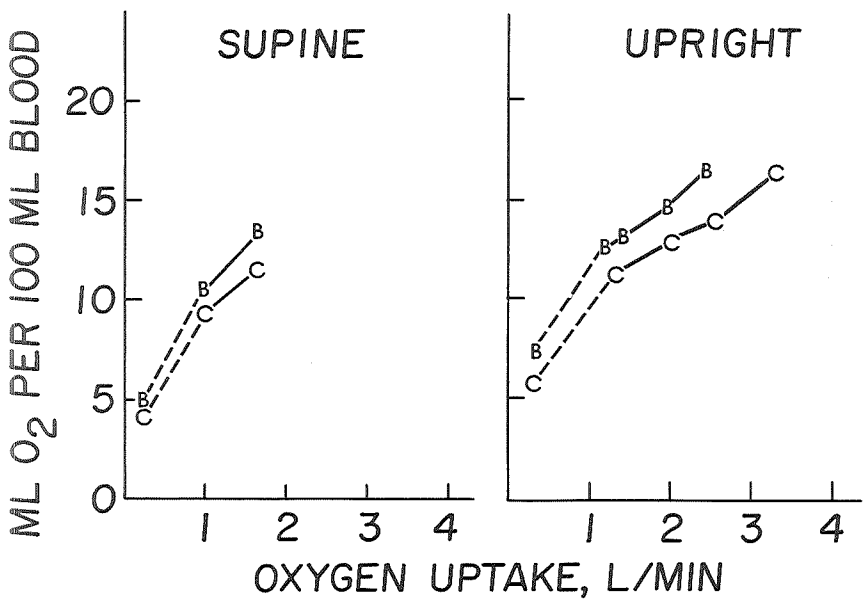

Figure 13.6 Arteriovenous oxygen difference in relation to oxygen uptake. Mean values at the control study (C) and after bed rest (B). Source: Reference 7

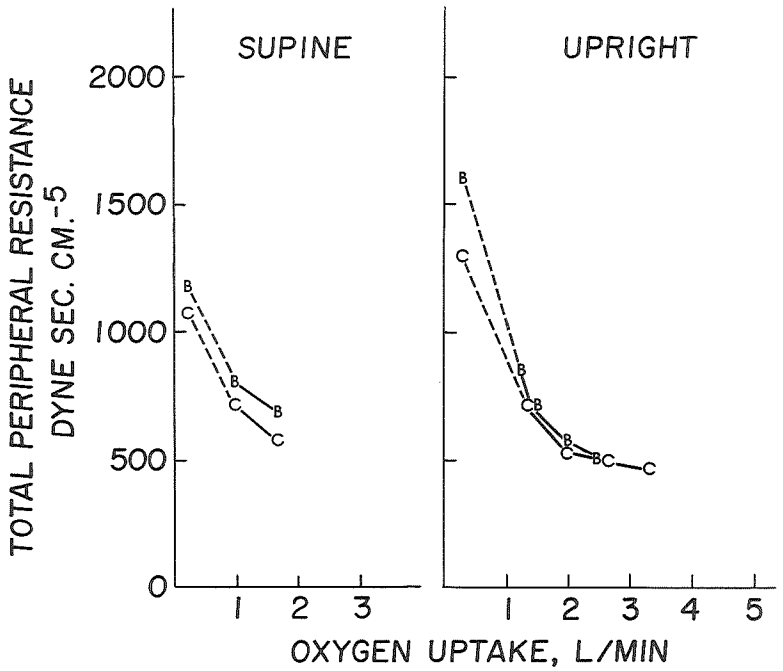

Figure 13.7 Total peripheral resistance in relation to oxygen uptake. Mean values at the control study (C) and after bed rest (B). Source: Reference 7

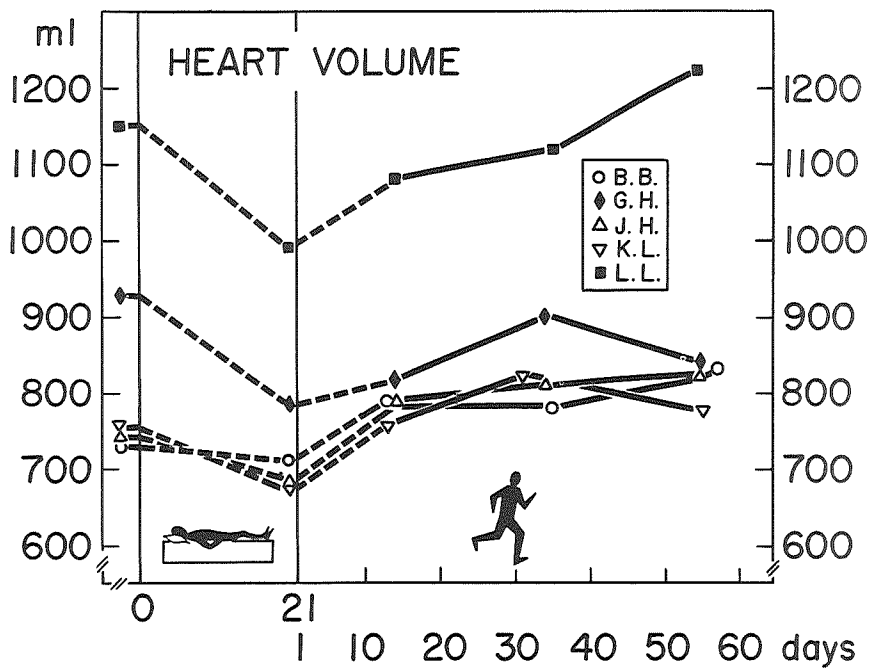

Figure 13.8 Changes in heart volume with bed rest and training. Individual data before and after bed rest and at different intervals during training. Source: Reference 7

14 HEMODYNAMIC AND BODY FLUID ALTERATIONS INDUCED BY BEDREST

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INTRODUCTION

Throughout the eons of time man has shown remarkable adaptability to virtually every type of environmental change. However, in each of these changes at least one factor has remained constant—the presence of gravity. As a result of the ever-constant presence of a 1 G environment, man has evolved the necessary anatomic structures and physiologic mechanisms to permit him to live and function effectively. Man's adaptation to the 1 G environment has been the subject of extensive study and review (ref. 1).

The phenomenon of deadaptation to gravity has also been of concern for many years (refs. 1–13). Initially, this interest stemmed from the changes seen as a result of prolonged recumbency and/or immobilization. With the advent of manned spaceflight, the problem has taken on a new dimension (refs. 7, 14). Whereas the changes resulting from prolonged bed rest or immobilization could be reduced by early ambulation, true weightlessness cannot be avoided in the course of prolonged spaceflight. Thus, it is imperative that the mechanisms involved in deadaptation to earth's gravity be delineated.

Studies were undertaken to develop an understanding of deadaptation physiology. Some aspects of these studies were completed 3 years ago and are available in detail as a NASA technical report (ref. 15). Other information is complete but only recently reported (ref. 16). In addition, preliminary information on current 28-day bed rest studies will be reviewed.

MATERIALS AND METHODS

In the studies to be described, all subjects were healthy male volunteers, aged 21-35, from the Federal Correctional Institution, Lompoc, California. In all studies, the conditions of bed rest were constant. Subjects were required to maintain all body components in the horizontal plane 24-hr per day throughout the period of bed rest. The only exception was the elevation of the forearms with the elbow in contact with the bed for reading.

Meals and all excretory activities were conducted in this position. Within the horizontal plane, the subjects were allowed complete freedom of position assumed, i.e., supine, prone, or lateral. One standard hospital pillow was permitted for head support.

In all studies, patients were maintained on metabolic diets containing 2.5 g sodium and 1.0 g calcium. Caloric content was based on the subject's predicted needs. All subjects were weighed daily on a metabolic balance.

There have been three types of studies and they will be described in order of performance. In all cases subjects were carefully screened to exclude disease, and were subjected to a noninstrumented 70° foot-down passive tilt prior to entry into the study to exclude those with autonomic insufficiency. An English saddle was used for all tilts. Statistical analysis was performed by a paired Student's t test. Significance was considered to be present if $p < 0.05$.

FOURTEEN-DAY BED REST STUDY

This study of 20 subjects consisted of a 7-day ambulatory period followed by a 14-day bed rest period. Subjects were placed on a metabolic formula diet 48 hr prior to entry into the study and were maintained on this diet throughout the study. All excretions were analyzed for calculation of electrolyte balance data. RISA ^{131}I plasma volume was determined after the 7:30 a.m. urine closeout sample and prior to breakfast on days 1 and 7 of ambulation and days 1, 2, 6, 12, and 15 of bed rest. On day 7 of ambulation and day 15 of bed rest, the volunteer underwent right heart catheterization. Baseline recordings of EKG, heart rate, and brachial and pulmonary artery pressure were obtained. Pressures were measured by means of Statham P23D transducers zeroed to the phlebostatic axis in both supine and tilted positions. Cardiac output was measured by the indicator dilution technique utilizing indocyanine green dye (ref. 17). All data were recorded on an oscillographic recorder. Oxygen consumption was determined in conjunction with the cardiac output measurements.

Following supine resting measurements, the subject was placed in the 70° tilt posture, which was maintained for 20 min or until presyncope. During the tilt, the above electrical and hemodynamic measurements were monitored continuously, with the exception of cardiac output. The latter was determined at 5, 10, and 18 min of tilt. Following recovery from tilt, the subject performed 50-watt supine bicycle exercise. During the fifth minute of exercise, cardiac output, oxygen consumption, heart rate and pressures were measured again. Following the subject's recovery from exercise, 3 mg of tyramine were injected into the pulmonary artery with continuous pressure monitoring for 5 min. Depletion of norepinephrine stores or malfunctioning adrenergic receptors resulting from bed rest would be expected to give a postbed rest response at variance from the prerecumbency result.

Five of the 20 subjects were required to perform a controlled Valsalva manuever prior to tilting during the catheterization study pre- and postrecumbency. These were performed by having the subject make a forced expiration into a Flack tester obtained from NASA's Bioinstrumentation Section. This is a whistle-like device with a spring-loaded plunger providing 40 mm Hg resistance to expiration. The forced expiration was maintained for 10 sec and pressures, EKG, and heart rate were monitored continuously.

An additional aspect of the study was the evaluation of the effects of plasma volume maintenance on changes in tilt and exercise tolerance induced by bed rest. Thus, 10 of the 20 subjects received 0.2 mg 9-α-fluorohydrocortisone (9-α) daily during the bed rest phase.

TEN-DAY BED REST STUDY

Eight subjects were studied under metabolic conditions identical to those described for the 14-day study. The study consisted of two 10-day ambulant periods alternated with two 10-day bed rest periods. During one of the bed rest periods, 0.2 mg 9-α was given b.i.d. During the other bed rest period, the subject received an identical placebo.

RISA ^{125}I plasma volume was determined prior to, and at the termination of, each bed rest period on the day of the prebed rest and postbed rest tilt and exercise studies. Sodium and potassium excretion, fluid balance, and body weights were determined daily. Serum sodium and potassium were determined on the days of plasma volume studies. Seventy-degree passive tilt was performed on the last day of ambulation, after 10 days of bed rest, and after 1, 2, and 10 days of resuming normal ambulation. The duration of the tilt was 20 min or until development of syncope or presyncope. There was no vascular instrumentation during tilt. Blood pressure was determined every 30 sec during tilt and heart rate was continuously monitored by an EKG-linked tachometer. Following recovery from tilt, supine bicycle exercise was performed at 50, 75, and 100 watts for 6 min. Recovery was allowed between exercise periods. Average heart rate was determined at 3 and 6 min after exercise and continuously during recovery.

TWENTY-EIGHT-DAY BED REST STUDY

This study is currently in progress and all data presented should be considered preliminary. Although 14 subjects have completed the study to date, some data from the initial six subjects are being reevaluated and are not considered here.

In contrast to the earlier studies, subjects were fed a full food metabolic diet for one week prior to and throughout the study. The diet was selected to fit the subject's desires and needs and was identical from day to day. Excretions were handled in a manner similar to earlier studies.

The study consisted of a 2-week ambulant period, a 4-week bed rest period, and a 2-week recovery period. Tilt and exercise studies were similar to those noted for the 10-day study. In addition, alterations in limb volume during 70° tilt have been monitored by impedance plethysmography. Tilt and exercise studies were performed on day 14 of the ambulant control period, at the termination of bed rest, and after 2, 7, and 14 days of ambulant recovery. Oxygen consumption is measured during the fifth minute of each exercise level in all studies.

The primary purpose of this study is the assessment of fluid compartment alterations induced by bed rest. The various compartments were measured by modification of prior techniques as follows: plasma volume by RISA ^{125}I (ref. 18); red cell mass by ^{51}Cr (ref. 19); extracellular fluid volume by ^{82}Br (ref. 20); total body water by $^{3}H_2O$ (ref. 21). All volume studies were performed in the fasting state after morning urine closeout. The dose of isotope delivered was determined gravimetrically rather than volumetrically. The combined study was performed on days 1 and 9 of the ambulant control period; on days 2, 14, and 28 of bed rest; and on days 7 and 14 of the ambulant recovery period. In addition, the last seven subjects had a special-purpose RISA ^{125}I plasma volume performed at the time of the pre- and postrecumbency tilt.

The format for this study was as follows: following injection of the isotope, samples are drawn at 10-min intervals for 30 minutes; the subject is tilted to 70° for 20 min; immediately on tilt-down and at 5-min intervals thereafter additional samples are drawn. Pre-tilt plasma volume is determined by extrapolation of the least-squares regression line of the three pre-tilt plasma samples to zero time (T_o). Post-tilt plasma volume is determined as follows: the pre-tilt regression line is extrapolated to the time of tilt-down (T_d); the post-tilt least-squares regression line is also determined and extrapolated to time of T_d; plasma volume is then determined by multiplying the ratio of the regression line intercept points at T_d by the pre-tilt plasma volume.

This study also was concerned with the determination of aldosterone excretion at selected points during all phases of the study. In analysis of results, the weekly mean was determined as well as the response on tilt days before and immediately after bed rest.

RESULTS

Hemodynamic Results

Fourteen-Day Study The tilt tolerance of each subject during the pre- and postrecumbency tilt is indicated in table 14.1. Every subject had tolerated a noninstrumented tilt prior to entry into the study. However, there was a 50 percent incidence of vasodepressor syncope before and after bed rest when the subject was studied with cardiac catheter and arterial needle in place. Treatment with 9-α did not influence tilt tolerance.

Any increase in the incidence of vasodepressor syncope induced by bed rest alone might have been masked by the high incidence of syncope resulting from vascular instrumentation. However, the hemodynamic measurements represent more sensitive indicators of bed rest-induced alteration. Comparison of results obtained from study of the 9-α and control groups showed no significant intergroup differences in hemodynamic response to either tilt or exercise. Thus, the hemodynamic results of all subjects could be treated as if they were derived from a single homogeneous group.

These results are illustrated in figure 14.1. In the determination of each mean result, the data of an individual subject were used only if both prerecumbency and postrecumbency results for the particular measurement were available at the time interval under study.

The fall in cardiac index during tilt prior to bed rest was statistically insignificant at all time intervals. However, the decrement was larger and statistically significant after 5 and 10 min of postrecumbency tilting. Central blood volume showed a statistically significant decrease at 5 and 10 min of tilt before and after bed rest. However, there was evidence of a larger continuing fall at 10 min of postrecumbency tilt. In subjects who tolerated 18 min of tilt, neither cardiac index nor central blood volume differed significantly from the supine value. Thus, these subjects presented a much more stable cardiovascular response to tilt.

Highly significant decreases in stroke volume with associated significant increases in heart rate were noted at all time intervals studied pre- and postrecumbency. The magnitude of change was much greater after bed rest.

Peripheral vascular resistance showed a significant increase at 5 and 10 min of tilt before bed rest and a larger increment after bed rest. Although the mean increase after 18 min of tilt was large before and after bed rest, the considerable spread in values and the small number of paired observations precluded demonstration of a statistically significant change. There was a consistent increase in pulmonary vascular resistance during the postrecumbency tilt. However, this change was significant only at 5 min of tilt.

An interesting phenomenon was noted in virtually all subjects during the 70° tilts. This consisted of oscillations in blood pressure and heart rate at a rate of 0 to 6 times per minute, clearly slower than respiration. Figure 14.2 illustrates these "vasomotor waves." They are present only in the brachial artery pressure tracings and are not reflected in the pulmonary artery pressure. Changes in heart rate (not shown) are reciprocal, rising as pressure falls and vice versa. Independent respiratory variations are apparent. In an attempt to evaluate the relationship of these waveforms to tilt intolerance, the subjects were divided into stable and hypotensive groups. Those with syncope or presyncope during prerecumbency tilting made up the prerecumbency hypotensive group, while those with syncope or presyncope during the postrecumbency tilt made up the postrecumbency hypotensive group. Figure 14.3 shows the frequency and amplitude of the wave activity in the two groups. In the stable group the waves occurred at a mean rate of 1 to 2/min in the supine position, rising to a mean rate of 3 to 4/min during tilt. They remained at this frequency throughout tilt. In the hypotensive group in the supine position, wave frequency was 0 to 1/min, rising to 1 to 4/min during the initial minute of tilt and then showing a statistically significant fall to the supine frequency during the terminal 2 min before the hypotensive episode. There was no significant difference when comparing the pre- and postrecumbency results within the two groups. In terms of waveform amplitude, variations in the supine position were not significantly affected by bed rest. The absence of waveforms in the prerecumbency hypotensive group was significantly different from the stable group. On assuming the tilt before bed rest, the hypotensive subjects developed larger magnitude waveforms than did the stable group. However, waveforms increased in amplitude significantly in the stable group after bed rest, and were similar in height to those seen in the hypotensive group during both tilts. The striking and important difference between the stable and hypotensive group lay in the decrease in height of the waveforms in the hypotensive group within the final 2 min *before the hypotensive episode.* This decrease in height was highly significantly different from results obtained in the stable group, and the difference was present both before and after bed rest.

Figure 14.4 illustrates the Valsalva response of a representative volunteer before and after bed rest. If anything, there is a greater overshoot during phase 4 postrecumbency, in contrast to the response of a patient with autonomic insufficiency, as shown in figure 14.5, which indicates the typical absence of an overshoot during phase 4.

The brachial artery systolic pressure response to tyramine injection is noted in table 14.2. It is apparent that response to tyramine did not differ significantly following bed rest, when the control and 9-α groups were looked at separately, or when the data were pooled.

Mean hemodynamic response to 50-watt supine exercise is shown in figure 14.6. The notable alterations following bed rest are the significantly smaller increments in cardiac index and stroke volume as a result of exercise. In fact, stroke volume during postrecumbent exercise did not differ statistically from the resting value. These changes were associated with a significantly greater rise in heart rate during postrecumbent exercise. Although the data are not shown here, there was no change in oxygen consumption at 50-, 75-, or 100-watt exercise after bed rest compared with results obtained before bed rest.

Ten-Day Study Vasodepressor syncope occurred in only one subject, and that followed placebo bed rest. Figure 14.7 illustrates the mean heart rate response at various tilt intervals before and after bed rest with and without treatment with 9-α. Heart rate response was significantly faster at all times following placebo bed rest, while a significant increase was seen only at 15 min of tilt after 9-α bed rest. The pre-bed rest tilt responses observed prior to the two bed rest periods showed no significant difference. However, after placebo bed rest the heart rate response was significantly faster while supine and at 10 min of tilt, as well as terminally, when compared to the 9-α bed rest period. Similar data were obtained for the second postbed rest tilt (after 24 hr). Tilt responses obtained at later intervals were similar to prebed rest responses.

Figure 14.8 illustrates the heart rate response to 100-watt exercise. Heart rate rose to significantly higher levels at both 3 and 6 min of exercise after placebo bed rest, but the difference was significant only after 6 min of exercise following 9-α bed rest. During exercise, the postplacebo bed rest rates were always higher than the post-9-α bed rest rates. However, the difference was not regularly significant. The significant difference following the two periods lay in the recovery from exercise. The placebo rates were significantly higher than 9-α postbed rest rates at 1, 5, and 10 min of recovery. The 9-α recovery response was similar to that seen before bed rest.

Twenty-Eight-Day Study To date, 4 of the 14 subjects developed vasodepressor syncope on the first postbed rest tilt. One of those subjects also experienced a similar episode on the final tilt. The hemodynamic results from this study are shown in figure 14.9. Basically, the results are similar to those from earlier studies with greater rises in heart rate in response to both 70° tilt and 100-watt exercise. Oxygen consumption at rest and during 100-watt exercise was unchanged after bed rest.

The consistently lesser rise in lower limb volume seen during 70° tilt following bed rest is particularly interesting.

Balance and Fluid Volume Results

Fourteen-Day Study The average 24-hr sodium and water balance during ambulation and bed rest is shown in figure 14.10. The average daily water balance was 200 to 300 ml less during bed rest than during ambulation. The two groups did not differ significantly and, in both groups, water loss was obligatory. However, free water clearance did become less negative late in bed rest. The negative sodium balance similarly was highly significant in both groups, amounting to approximately 17 meq/24 hr.

Figure 14.11 shows the analysis of mean sequential sodium and water balance. Sodium losses were most profound during the initial few days of bed rest; however, balance never returned to

ambulant levels during the 2 weeks of bed rest. Although the initial sodium loss was much less profound in the 9-α group, the mean daily loss (fig. 14.10) did not differ significantly from the control group.

Figure 14.12 shows the alterations in plasma volume and calculated red cell volume in the placebo and 9-α groups at various stages of study. Plasma volume decreased significantly from ambulant values during both bed rest intervals in the control group. No significant plasma volume change was seen in the 9-α group at any interval of study. Derived red cell volume was unaltered during study.

Ten-Day Study The metabolic alterations induced by 10 days of bed rest with and without 9-α are shown in figure 14.13. Following placebo bed rest, there was a slight weight loss in contrast to 9-α bed rest where a slight gain occurred. The difference between terminal 9-α and placebo bed rest weight was 1.39 kg and was statistically significant. Placebo bed rest resulted in a significantly less positive water balance when compared to ambulation or 9-α bed rest. Further, there was a small but significantly greater average sodium excretion during placebo bed rest, and significant sodium retention during 9-α bed rest.

Table 14.3 shows the mean plasma volume results before and after placebo and 9-α bed rest. It is apparent that a significant decrease in plasma volume occurred during placebo bed rest, while no significant change occurred during 9-α bed rest. The prerecumbency values for the two periods were virtually identical, while the 9-α postrecumbency value was approximately 350 ml greater than after placebo bed rest.

Twenty-Eight-Day Study Figure 14.14 encompasses the study results to date, including mean daily sodium excretion, mean daily water balance, mean daily weight, aldosterone excretion, and alterations in the various fluid compartments. The data were subjected to statistical analysis only where it seemed appropriate at this point in the study. The data so analyzed are identified by 95 percent confidence limits. As in our earlier studies, there was an increase in sodium excretion and a less positive water balance during bed rest. In addition, a distinct retention of sodium and water was observed throughout the first week of ambulant recovery from bed rest. Sodium retention was less prominent during the second week. There was an associated mean weight loss of 0.3 kg during bed rest, which returned to prerecumbency levels during the recovery period.

When all results during a given week of study were pooled, aldosterone excretion was unaltered during any phase of bed rest. The interesting finding was that during the first week of ambulant recovery there was an increase over control values in aldosterone, which approached significance. Comparison of the last control day and the first ambulant recovery day, both tilt-study days, showed aldosterone excretion to be significantly higher ($p < 0.0025$) on the first post-bed rest day.

Consideration of the fluid compartment alterations indicates that the most obvious changes occurred in plasma volume. When compared with control values, significant decreases were seen at

each bed rest phase, while significant increases were seen during the recovery phase. Although significant, the decrease in plasma volume during bed rest amounted to only 150 ml, with a rise above this level of 300 ml after bed rest.

Extracellular fluid volume was significantly lower on days 2 and 28 of bed rest. Considering the entire bed rest period, the decrease amounted to a minimum of 110 ml and a maximum of 330 ml. The postrecumbency overshoot was also observed in the extracellular fluid volume, showing a 550-ml rise over the last day of bed rest during the first ambulant week. This increase was to a level significantly higher than control values during the first week of the ambulant recovery period ($p < 0.01$).

Studies to date have revealed no significant decrease in total body water during bed rest. There does appear to be an overshoot, amounting to approximately 1.0 liter, following resumption of ambulation. However, this difference has not achieved statistical significance ($p < 0.15$).

Red cell mass shows a significant decrease at all bed rest intervals with a leveling off following bed rest. Interestingly, the decreases during bed rest (shown by the dotted line in fig. 14.14) could be totally accounted for by adjusting results to the total volume of red cells withdrawn to that point. This would assume no manufacture of red cells, or an increased rate of destruction paralleling the replacement rate. The failure of red cell mass to show further decrease with phlebotomy following bed rest suggests a phenomenon of one type or the other. This results in the upswing noted in the corrected values.

Table 14.4 shows the alteration in plasma volume induced by 70° tilt before and after bed rest. The actual decrease in plasma volume was 100 ml greater before than after bed rest, and the difference was not statistically significant. Another interesting observation was that the post-tilt regression line intercepted the pre-tilt regression line (a point at which plasma volume was presumably back to pre-tilt levels) more quickly after bed rest than it had before bed rest.

Composite Plasma Volume Results The availability of complete plasma volume data on 26 control subjects and 18 subjects treated with 9-α permitted comparison of the changes seen in the various studies. Figure 14.15 shows the results of this analysis. To make data numerically comparable, all results were normalized to an ambulant value of 3.0 liters. A consistent drop in plasma volume during bed rest of 150 to 300 ml is readily apparent. Clearly, the daily dose of 0.2 mg 9-α slows, but does not prevent, the plasma volume fall, while a daily dose of 0.4 mg prevents a decrease in plasma volume—at least during the period of the 10 days studied.

DISCUSSION

The high incidence of vasodepressor syncope both before and after bed rest in the 14-day study was undoubtedly related to vascular instrumentation; similar effects have been noted by others (refs. 22–24). In the absence of vascular instrumentation, vasodepressor syncope during tilt occurs quite infrequently, even after 28 days of bed rest. This is somewhat surprising considering the stress of a 70° passive tilt. In the usual case, it has been possible to terminate the tilt before total loss of consciousness. However, two cases have been observed in which sinus arrest without ventricular activity lasted for several seconds. Another case showed what appeared to be

a sympathetic crisis after tilt-down with severe vasoconstriction and marked shivering lasting almost 15 min. Thus, the problem of postrecumbency orthostasis manifested by fainting cannot be totally ignored. When it does occur, the results may be quite serious. The sequence of studies described was done in an attempt to define the bed rest-induced changes in cardiovascular homeostasis that result in the increased incidence of syncope noted in our studies and others (refs. 5, 9, 13, 25).

The 14-day study was designed to give an overview of the hemodynamic and related metabolic changes induced by bed rest. It also was of interest to determine whether maintenance of plasma volume by a small chronic dose of 9-α would prevent postrecumbency orthostasis. Subsequent studies were designed to follow up on the changes noted in the 14-day study.

A number of possible mechanisms for production of postrecumbency orthostasis have been suggested in prior literature. Keys referred to the situation as "cardiovascular deconditioning" (ref. 6). This term has been used extensively in recent years, probably without sufficient justification. The presence of a functional or hyperfunctional neurovascular system was suggested in each of our studies by the greater increases in heart rate observed following bed rest. This was further confirmed in the 14-day study by an equal or greater increase in peripheral vascular resistance, unchanged responsiveness to tyramine, and an equal or greater sympathetic response to Valsalva maneuver. However, none of the studies described excludes the possibility of altered myocardial function. In fact, the failure of stroke volume to show the expected incremental increases with exercise after bed rest could be explained on this basis. A similar response to post-bed rest exercise was recently reported (ref. 26).

Another potential explanation for postrecumbency orthostasis is the decrease in plasma volume during bed rest, which has been noted by others (refs. 9,11) and presumably occurs as a result of decreased stimulation of the central low-pressure baroreceptors during recumbency. This is said to cause inhibition of ADH and aldosterone with resulting sodium and water diuresis (refs. 27–30). However, the degree of decrease in plasma volume has not shown good correlation with the occurrence of vasodepressor syncope (ref. 9). In all our studies there has been a definite decrease in plasma volume during bed rest as a result of sodium and water diuresis. However, this obligatory loss of water does not appear to be due to inhibition of aldosterone secretion, which was unchanged as measured by urinary excretion in the 28-day study. Another reason for the diuresis must be sought. Stahl has suggested that the saluresis and diuresis may result from an increase in effective renal perfusion pressure and renal blood flow, with an increase in GFR and filtered solute load, as well as from augmented countercurrent exchanger flow (ref. 31). An alternative explanation lies in the effect of the unidentified "third factor," which is part of the efferent limb of the volume control system. It is said to act at the nephron level to reset glomerulotubular balance (ref. 32).

The magnitude of plasma volume decrease during bed rest is variable; from our experience, however, it probably does not exceed 200 to 300 ml. When this is coupled with the 200-ml decrease in red cell volume, the total decrease in intravascular volume during bed rest amounts to 500 ml. This is conceivably enough to increase the likelihood of syncope in the faint-prone subject, particularly when another 500 ml of plasma water is filtered out of the vascular

compartment during 70° tilt. The total decrease of 1000 ml in circulating blood volume during the postbed rest 70° tilt, rather than the 500-ml prerecumbency decrease, probably represents the only real change in effective vascular filling pressure following bed rest. Certainly, our plethysmographic results are consistent in their indication that lower limb volume increase during tilt is not greater after bed rest than before—if anything, it is less. Since the degree of plasma loss or extravasation is the same both before and after bed rest, the plethysmographic results suggest that the veins are less passively compliant after bed rest. Thus, an increased degree of venous pooling after bed rest with a further fall in effective circulating blood volume seems quite unlikely. Dow has shown a similar venoconstrictor response to hypovolemia (ref. 17).

Like other investigators (ref. 13), we were concerned about the possibility that fluid loss was occurring not only from the plasma compartment but also from other compartments. This would result in an increase in extravasation of plasma water during postrecumbency tilting, a fact previously postulated by us as well as others (refs. 3, 7, 13, 33). One earlier study showed a large decrease in both extracellular fluid (^{35}S) and total body water during bed rest (ref. 33), which we expected would be confirmed by our results. We have studied our subjects' extracellular fluid volume with both ^{35}S and ^{82}Br. However, we have found the former method to be full of pitfalls even when performed by complex methodology (ref. 34). The ^{82}Br studies show a total decrease in extracellular fluid volume (ECV) of approximately 300 ml. Due to a difference in the space measured and considering experimental variation, this decrease probably represents no more than the measured plasma volume decrease. We have found no decrease in total body water. In our experience, a detectable decrease by this method would have to exceed 500 ml. The failure to demonstrate extravascular dehydration by isotopic methods would seem to be confirmed by the failure to show any evidence of greater transudation of plasma water during the postrecumbency tilt. Extravascular dehydration would lower tissue pressure and would thus increase filtration rate (ref. 35).

The question should be raised why we cannot account for the large decrease in water balance during bed rest by alterations in body fluid volumes. The likely answer may lie in the fact that, with inactivity, there is a smaller insensible loss of fluid. The fluid previously lost by sweating and respiration would then be eliminated by the kidneys.

Thus, at this point, we would postulate that orthostasis and predisposition to syncope after bed rest occurs as a result of a decrease in circulating blood volume, an increase in vascular reactivity with increased resistance to blood flow, and, possibly, as a result of alterations in myocardial function. The failure to augment stroke volume during postrecumbency exercise could easily be explained by altered ventricular function. Many of the results, such as the increase in peripheral vascular resistance, the greater response to Valsalva maneuver, and the lesser increase in lower limb volume following bed rest, suggest a hyperactive neurovascular system. Thus, the postbed rest response might be due to a situation similar to the denervated organ (refs. 36, 37), with resulting increased responsiveness to catecholamines. On the other hand, a resetting of baroreceptor response could produce the same effect, similar to the situation seen in hypertension (ref. 38). In either case, the result may be marked vasoconstriction with diminished blood flow. The denervated organ effect seems less likely in view of the unchanged responsiveness to tyramine after bed rest.

The behavior of the vasomotor waves in the hypotensive subjects in the 14-day study suggests that maximal vasoconstriction is indeed present just prior to syncope, both before and after bed rest. That is, the waveforms tend to become reduced in frequency and amplitude. A similar finding has been noted in Raynaud's disease (ref. 39). It is postulated that the markedly reduced venous return results in some critical lower limit of central vascular filling, which signals the imminent occurrence of failure of blood flow. The low-pressure baroreceptors, sensing this impending catastrophe, would inhibit all sympathetic stimulation in an attempt to reduce peripheral vascular resistance and maintain blood flow. The sudden increase in capacity of the poorly filled vascular system would result in a dramatic drop in blood pressure. The elimination of beta adrenergic activity associated with a high level of parasympathetic activity would result in marked cardiac slowing or standstill. Also, it is conceivable that the decrease in ventricular volume induced by inadequate venous return and increased sympathetic tone results in a depressor reflex initiated by intracardiac receptors as postulated by Epstein et al. (ref. 40). This is similar to the mechanism previously proposed by Sharpey-Schafer (ref. 41).

The same hyperresponsiveness of the neurovascular system, or resetting of baroreceptors, probably accounts for the increase in aldosterone output on resuming the upright posture. This results in the rise seen in plasma volume, extracellular volume, and total body water above prebed rest levels.

Finally, the results of 9-α therapy require comment. Since prevention of plasma volume depletion by a daily dose of 0.4 mg 9-α reduced the cardioacceleration seen as a result of tilt and exercise and increased heart rate recovery from postrecumbency exercise, it appears that plasma volume alteration plays a major role in postrecumbency orthostasis. It is also possible that 9-α has some effect in preventing neurovascular hyperreactivity.

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Table 14.1 Seventy-degree tilt tolerance (minutes)

Control Group			*9-α-Fluorohydrocortisone Group*		
Subject	Before Bed rest	After Bed rest	Subject	Before Bed rest	After Bed rest
1	13.0	18.0	2	9.5	20.0
3	10.0	12.5	5	10.5	20.0
4	20.0	2.5	7	20.0	20.0
6	20.0	19.5	9	12.5	20.0
8	20.0	20.0	11	15.5	11.5
10	8.5	20.0	14	20.0	20.0
12	6.0	8.0	16	20.0	14.0
13	20.0	20.0	17	20.0	13.0
15	8.0	20.0	18	20.0	16.0
19	20.0	20.0	20	4.0	6.0
Vasodepressor syncope incidence	50%	50%		50%	50%
Average tolerance (min)	14.5	16.1		15.2	16.2

Table 14.2 Tyramine stimulation test maximum rise in arterial systolic pressure (mm Hg)

Control Group			*9-α-Fluorohydrocortisone Group*		
Subject	Prerecumbency	Postrecumbency	Subject	Prerecumbency	Postrecumbency
3	17	36	2	7	13
4	17	16	5	13	5
6	19	19	7	39	16
8	13	10	9	13	6
10	19	19	11	16	19
12	14	10	14	14	23
13	6	6	16	15	19
15	16	17	17	25	11
19	10	5	20	24	14
Mean	15	15		18	14
			p*	0.5	0.8
		Combined Group			
Prerecumbency mean		16.5	Postrecumbency mean		14.5
			p**		0.5

* Comparison with control group data

** Comparison with prerecumbency data

Table 14.3 Plasma volume alteration induced by 10 days of bed rest with and without 9-α-fluorohydrocortisone

		Placebo	*Drug*	*Difference*	*p*
Prerecumbency	Mean	3114	3085	−29	<0.4
	SE(95%)	±179	±118		
Postrecumbency	Mean	2827	3175	+348	<0.025
	SE(95%)	+206	+242		
Difference		−287	+ 90		
		<0.001	<0.2		

Table 14.4 RISA^{125}I plasma volume alteration induced by 70° tilt before and after 28 days of bed rest

		Pretilt	*Posttilt*	△	*p*
Prebed rest	Mean	2804	2273	531	<0.0005
	SE(95%)	±69	±34	±53	
Postbed rest	Mean	2752	2326	426	<0.0005
	SE(95%)	±71	±102	±38	
				<0.1	

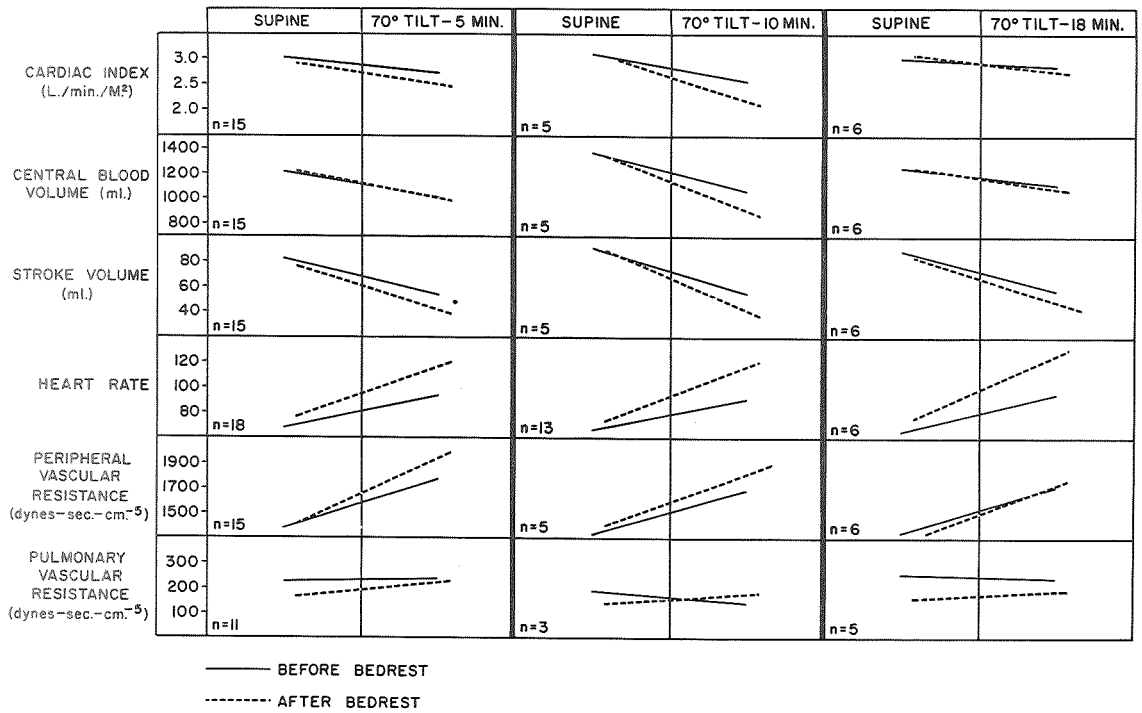

Figure 14.1 The hemodynamic response to 70° passive foot-down tilt before and after 14-days of bed rest

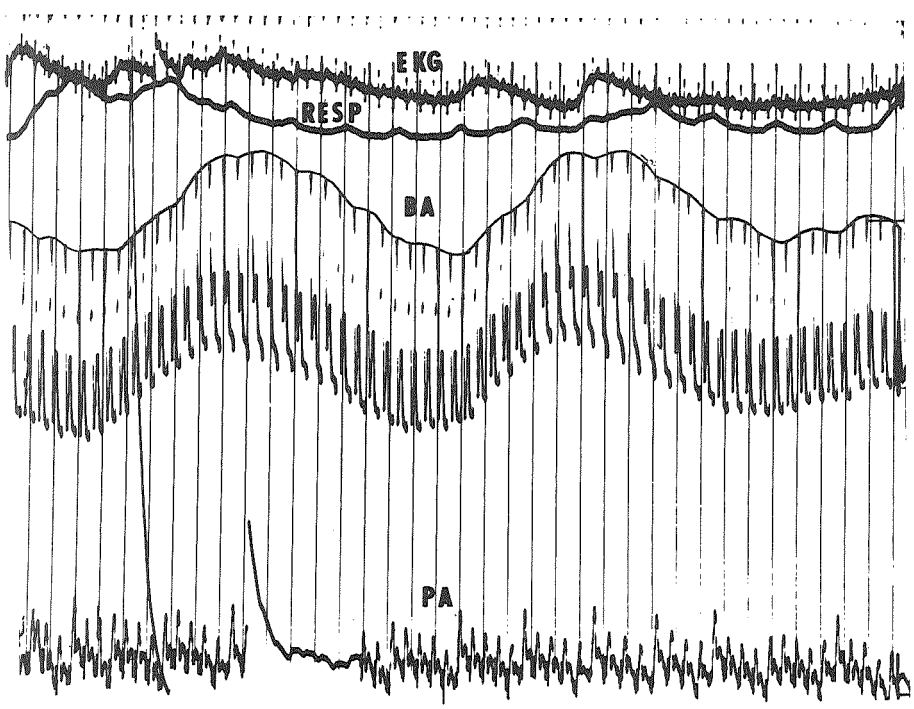

Figure 14.2 Vasomotor waves are present in the brachial artery pressure tracing (BA), but are not seen in the pulmonary artery pressure tracing (PA). Waves are unrelated to respiration (RESP)

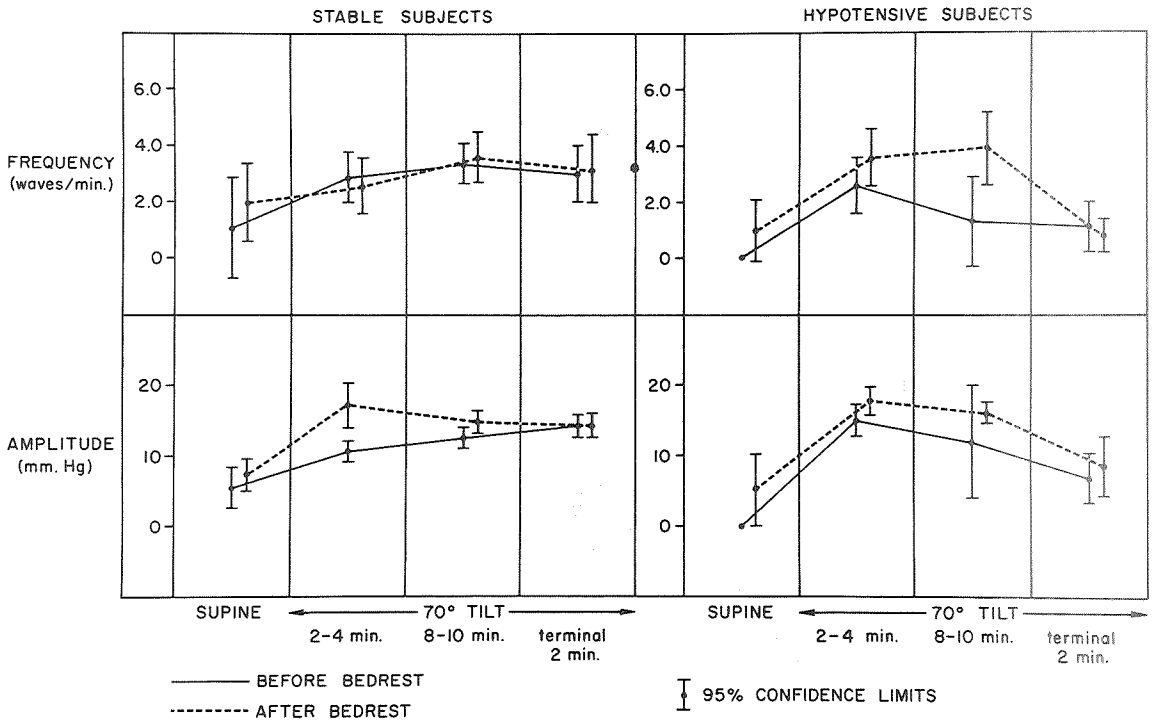

Figure 14.3 Summary of brachial artery vasomotor wave frequency and amplitude at rest and after various intervals of 70° tilt in stable and orthostatic intolerant subjects before and after 14 days of bed rest

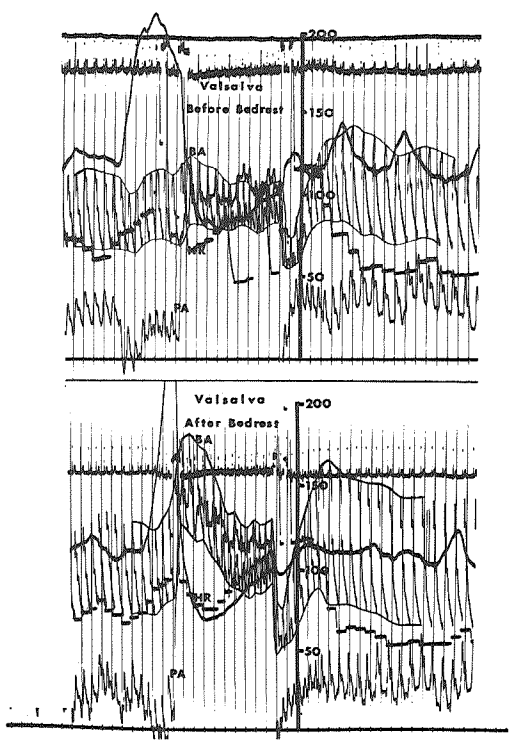

Figure 14.4 Valsalva response before and after 14 days of bed rest

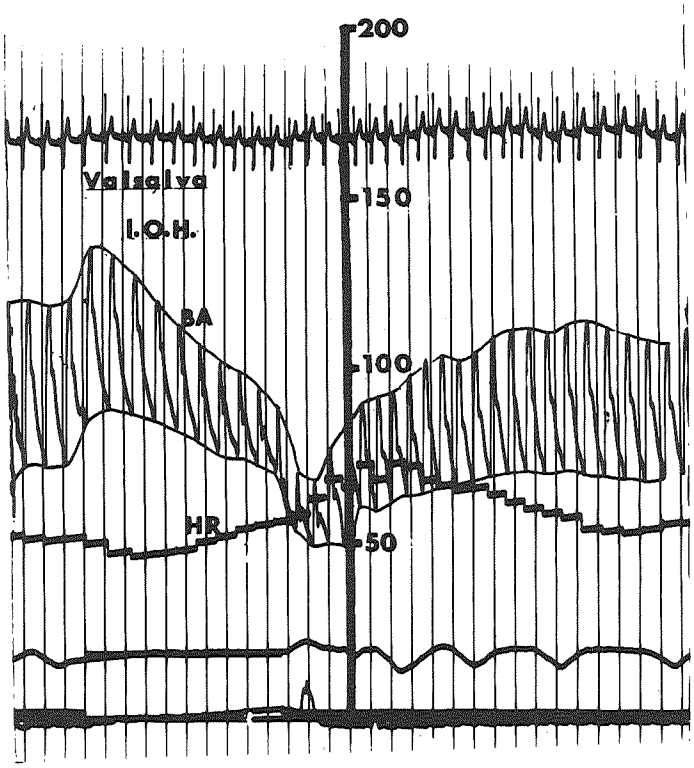

Figure 14.5 Valsalva response of a patient with idiopathic orthostatic hypotension (IOH)

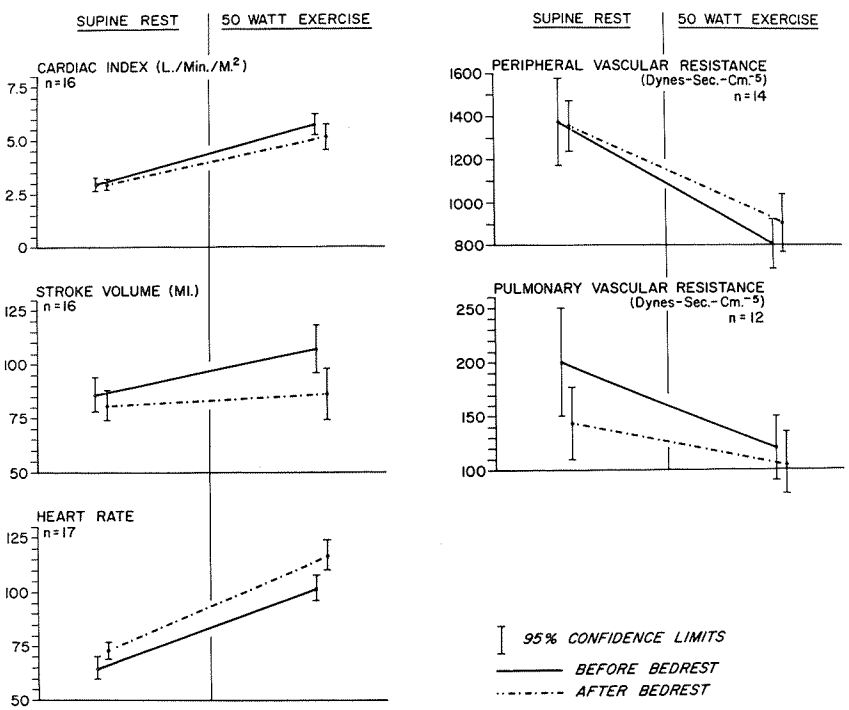

Figure 14.6 Hemodynamic response to 50-watt supine bicycle exercise before and after 14 days of bed rest

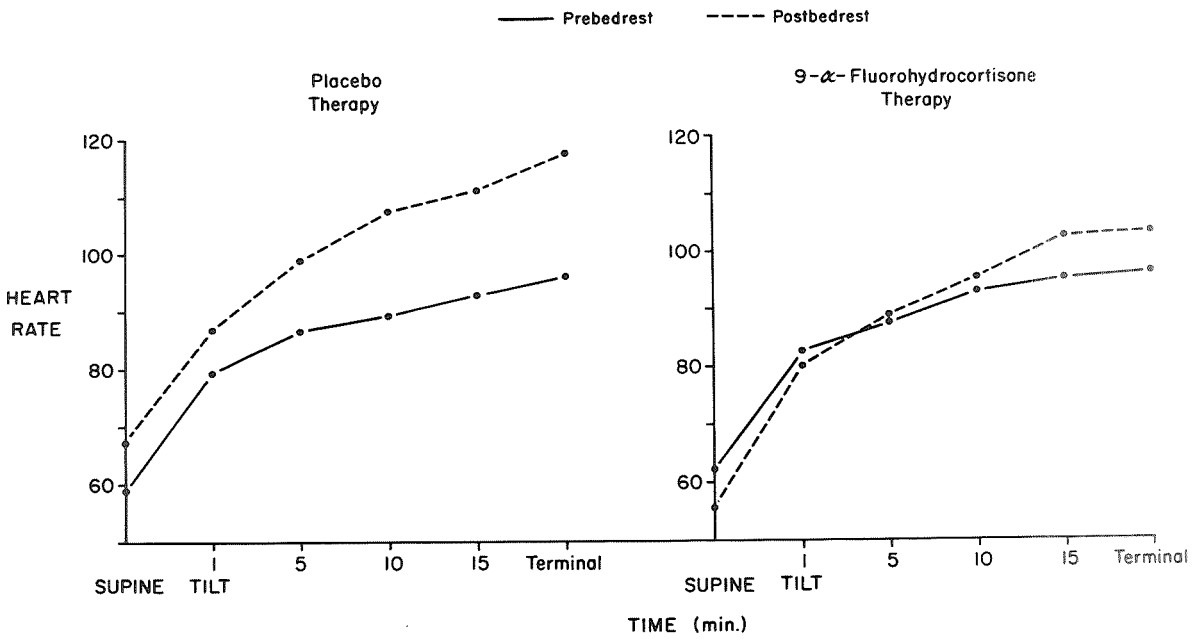

Figure 14.7 Heart rate response to 70° tilt before and after 10 days bed rest on placebo and 9-α therapy

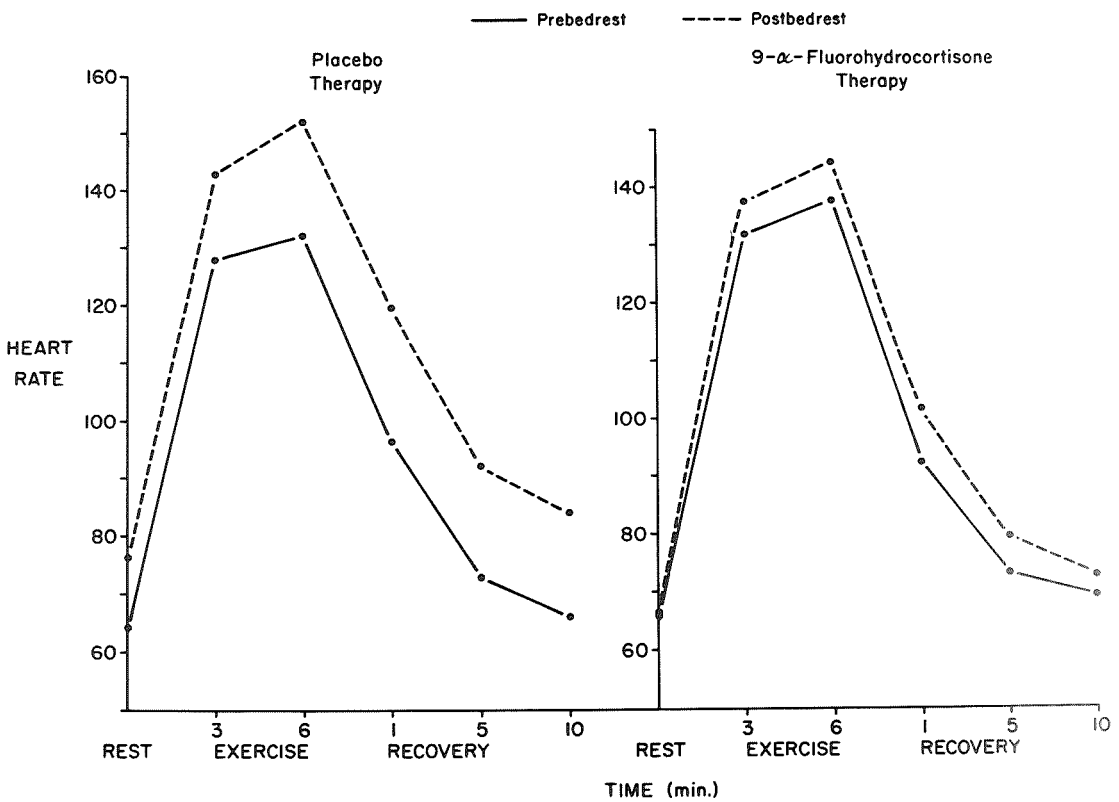

Figure 14.8 Heart rate response to 100-watt exercise before and after 10 days of bed rest on placebo and 9-α therapy

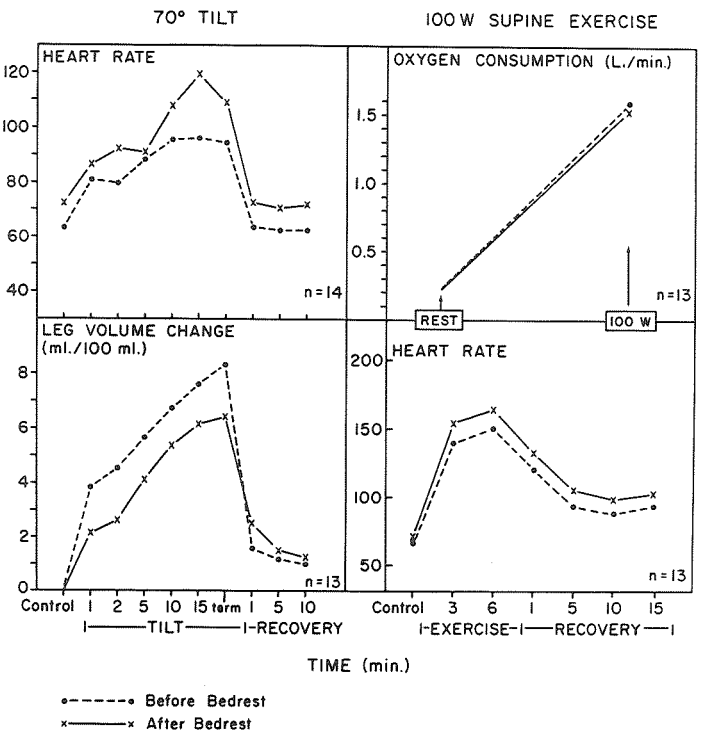

Figure 14.9 Hemodynamic response to 70° tilt and 100-watt supine exercise before and after 28 days of bed rest

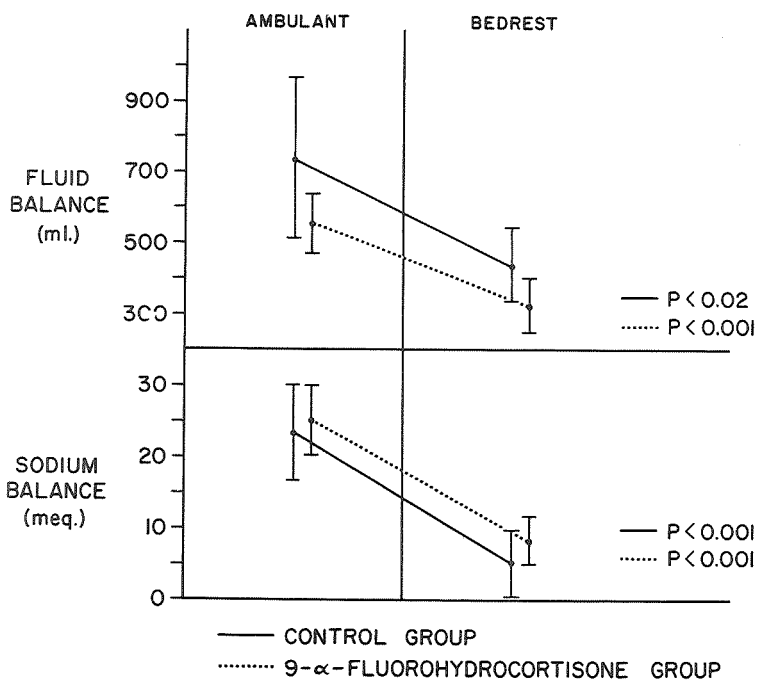

Figure 14.10 Average 24-hr sodium and water balance during the ambulatory and 14-day bed rest phases of the control and 9-α groups

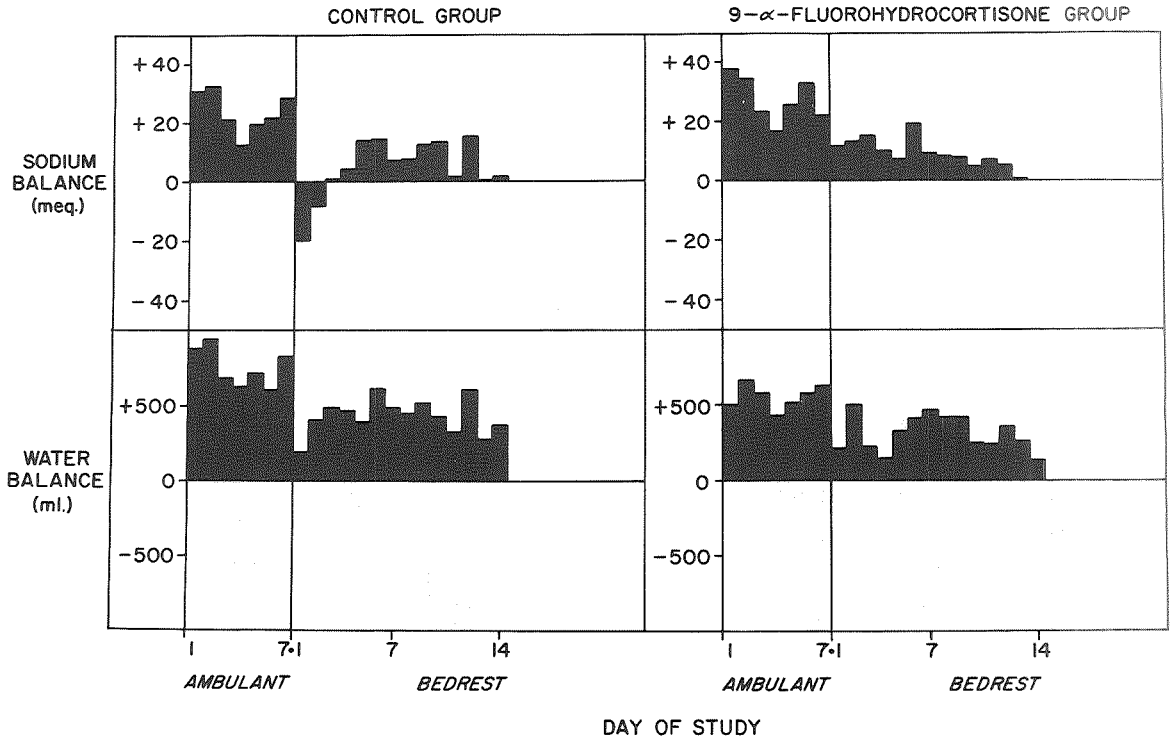

Figure 14.11 Mean day-to-day sodium and water balance of the control and 9-α groups

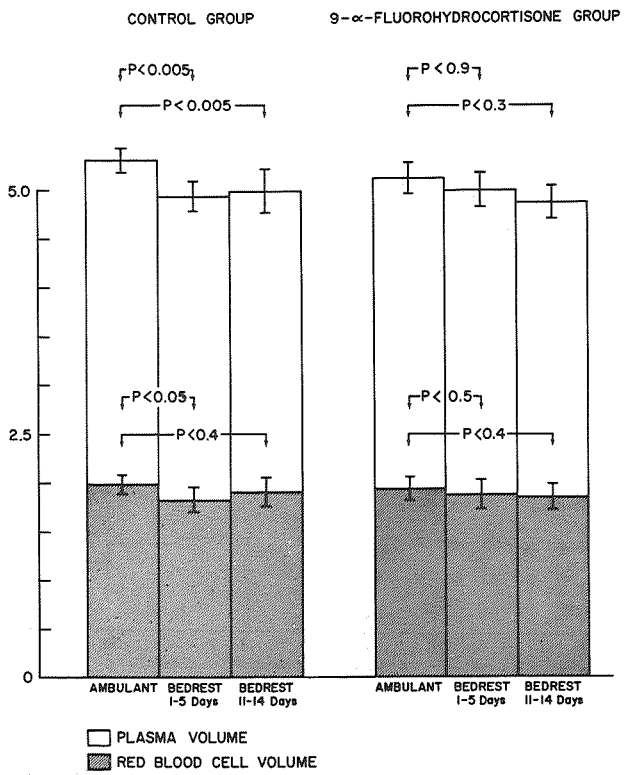

Figure 14.12 Plasma and red cell alterations resulting from 14 days of bed rest with and without 9-α therapy

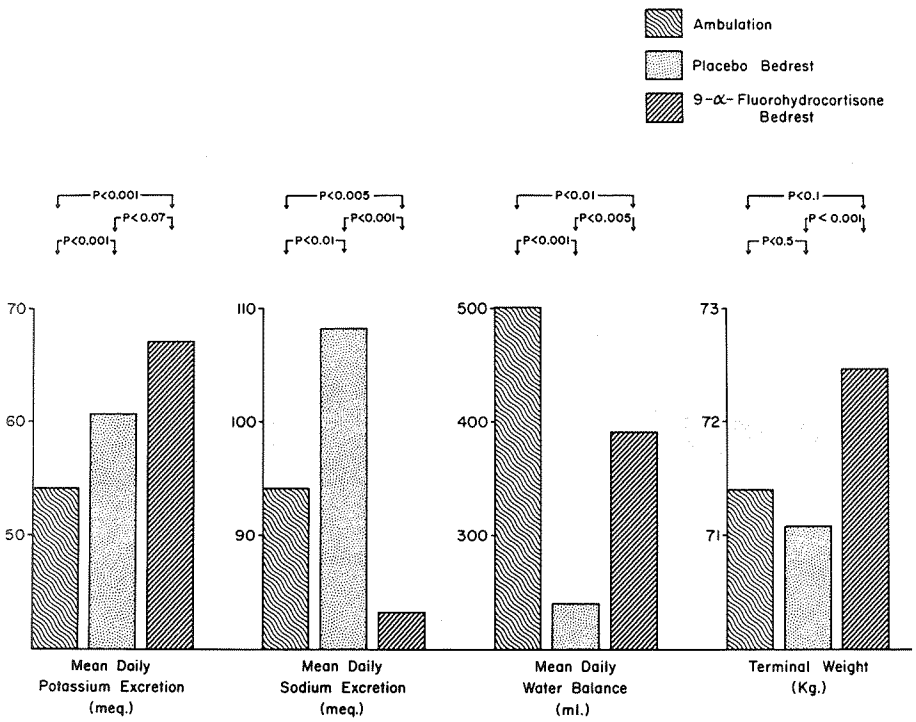

Figure 14.13 Metabolic alterations induced by 10 days of bed rest with and without 9-α therapy

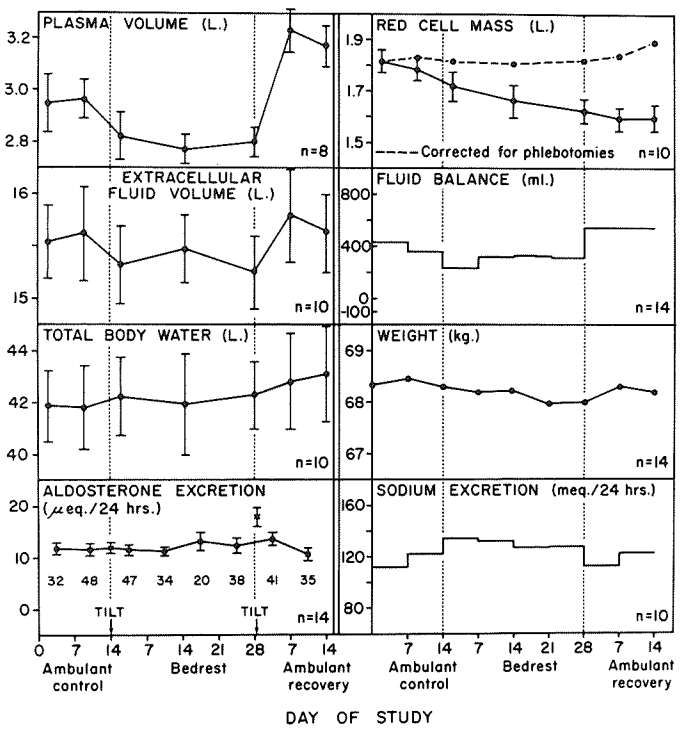

Figure 14.14 Alterations in body fluid compartments, aldosterone excretion, sodium and water balance, and body weight induced by 28 days of bed rest

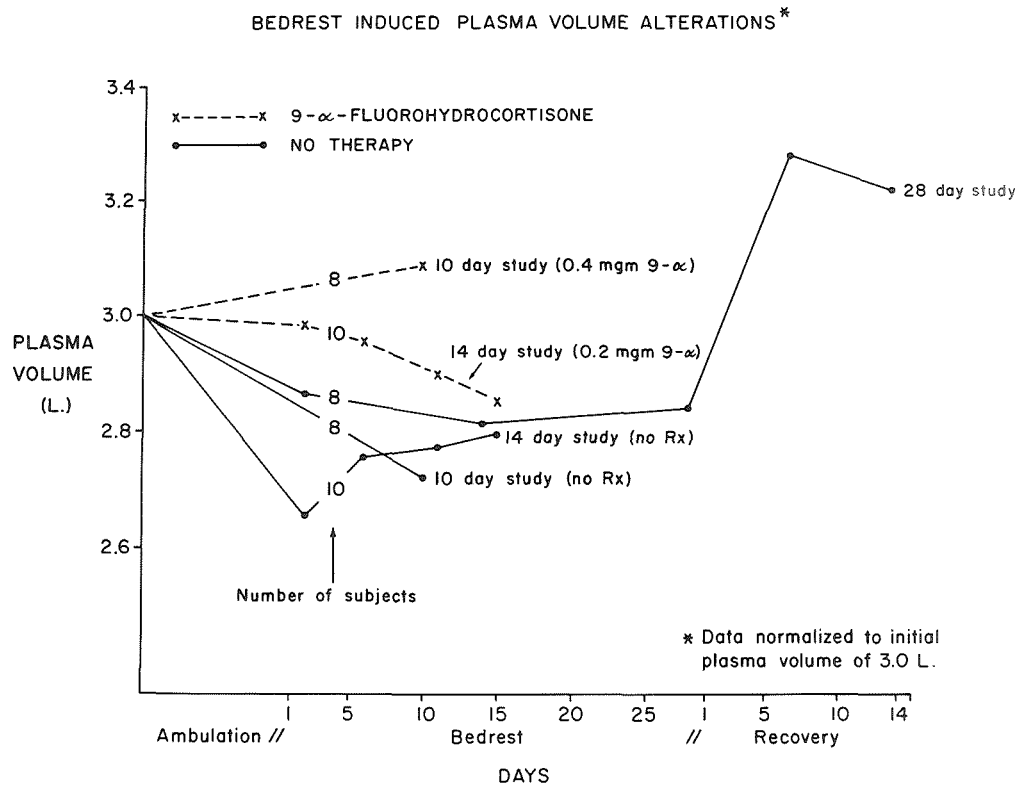

Figure 14.15 Plasma volume alterations induced by bed rest of various durations with and without 9-α therapy

15 EFFECTS OF BED REST ON FOREARM VASCULAR RESPONSES TO TYRAMINE AND NOREPINEPHRINE

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INTRODUCTION

Patients or normal human subjects who have been in bed for more than several days may complain of blurred vision, dizziness, or feeling faint when they first attempt to stand (refs. 1–3). When they are tilted from the recumbent to the erect position, blood pressure falls and heart rate increases excessively in comparison to their usual response to this maneuver (refs. 1–4). Such responses suggest failure of peripheral vascular compensatory mechanisms. These might be similar to mechanisms that fail in other conditions associated with postural hypotension (refs. 5–7). Thus, there could be a defect either in the ability of vascular smooth muscle to respond to constrictor stimuli or in the complex of biosynthesis, metabolism, or release of norepinephrine at adrenergic nerve terminals. Under appropriate circumstances, responses to norepinephrine may indicate disturbances in the reactivity of vascular smooth muscle. In contrast, responses to tyramine may indicate disturbances in peripheral adrenergic nerve function. To test for these functional disturbances, we observed forearm vascular responses to brachial artery infusions of tyramine and norepinephrine after 2 weeks of bed rest and after control periods of normal vigorous daily activity.

METHODS

Four healthy male students, 22 to 26 years old, served as subjects and were admitted to the Clinical Research Unit of the University of Pittsburgh Health Center for 1 month. Each selected a diet on the first day; thereafter the daily intake of carbohydrate, protein, lipid, and sodium was the same. They drank water ad libitum.

The study was subdivided into three periods. An initial control period consisted of 8 days of unrestricted activity. The second period consisted of 12 days of bed rest. The third or recovery period consisted of 6 more days of activity and began when the subjects got out of bed. While active, the subjects walked regularly and engaged in competitive sports such as tennis or basketball. Their normal sleep schedules were not disturbed. During the 12 days of bed rest they remained recumbent continuously, even when using a bedpan. They were allowed to move their limbs and roll about but were told not to raise their heads higher than a pillow.

Experiments were conducted on the last day of each period. Care was taken to conduct separate sessions under the same conditions. Tests on each individual were done at the same time of day, in a darkened room, with the temperature maintained at 82° F. Subjects reclined comfortably on a soft pad.

A water plethysmograph was used to measure blood flow and venous distensibility in the right forearm (refs. 8–10). Determinations were made by inflating a cuff around the upper arm proximal to the plethysmograph. Initially, this maneuver trapped blood in the forearm so that arm volume increases (measured by water displacement) were proportional to blood flow into the forearm. Forearm blood flow was expressed in ml/100 ml forearm/min. Venous distensibility was determined from the increase in arm volume produced by leaving the cuff inflated to a pressure that eventually caused a 30-mm Hg increase in transmural venous pressure. Forearm vascular resistance, expressed in arbitrary units, was obtained by dividing mean arterial pressure by forearm blood flow. Venous tone, expressed in arbitrary units, was obtained by dividing the 30-mm Hg increase in transmural venous pressure by the resulting change in forearm volume. This change in forearm volume always was measured with reference to the stable and reproducible arm volume that results when appreciable water covers the arm (refs. 8–10). During observations, a pressure cuff at the wrist was inflated to 200 mm Hg to exclude blood flow to and from the hand. Transmural venous pressure was measured from a vein on the superficial aspect of that part of the forearm within the plethysmograph, using a polyethylene catheter and strain gauge pressure transducer. Arterial blood pressure was measured through a catheter in the brachial artery using a strain gauge pressure transducer. Heart rate was counted from the undamped record of arterial blood pressure. Measurements were recorded using a photographic oscillograph.

After the equipment was prepared and calibrated, arterial blood pressure, heart rate, forearm blood flow, and venous distensibility were allowed to stabilize. When the values stabilized, usually after 35 to 40 min, two sets of basal observations were made. Tabulated basal values were the averages of these two determinations. After basal observations were completed, the wrist cuff was deflated for 10 min and then reinflated for 1 min before drugs were infused. Infusions were administered through the catheter in the brachial artery. The doses of tyramine (9, 18, 36 μg base/min) were administered first. They were infused consecutively in ascending order. Each dose lasted 7 min; observations were made only during the final 5 min. The infusions of tyramine were stopped and the wrist cuff deflated. Twenty-five minutes later the wrist cuff was reinflated, and 1 min after that, infusions of norepinephrine (0.0375, 0.075, 0.15 μg base/min) were started and the observations were repeated. Fresh solutions were prepared for each experiment by diluting tyramine hydrochloride and L-norepinephrine bitartrate in 5 percent glucose and water. The solutions were infused at rates of 0.191, 0.382, and 0.764 ml/min; 5 percent glucose and water infused at these rates have not been associated with detectable changes in forearm blood flow or venous tone (refs. 8, 9).

Each subject voided all urine into special portable receptacles during the last 3 days of each period (control, bed rest, or recovery). These urine samples were refrigerated and pooled for individual 24-hr collections. Aliquots (60 cc) were adjusted to pH 3.5, treated with sodium metabisulfite (0.5 mg/ml), centrifuged, and frozen at −20° C. After thawing, catecholamines were isolated by absorption on alumina and elution with acetic acid (ref. 11). Total catecholamines, norepinephrine, and epinephrine were measured fluorometrically using a trihydroxyindole procedure modified for the "Technicon Autoanalyser" (refs. 12–14). Analysis of 10 aliquots of a single 24-hr urine sample gave standard deviations of 1.7 μg/24 hr for norepinephrine, 1.6 μg/24 hr for total catecholamines, and 0.7 μg/24 hr for epinephrine. Values were not corrected for recovery,

which averaged 83 ± 5.4 percent (mean ± SD) when known quantities of norepinephrine were added to 10 samples of urine. Similarly, no correction was made for recovery of epinephrine, which averaged 75 ± 4.0 percent.

Analysis of variance, the Duncan multiple range test, and a parallel line bioassay were used for statistical comparisons (refs. 15, 16).

RESULTS

Basal Values before Infusions of Tyramine or Norepinephrine

Control and recovery sessions were similar with respect to all basal variables, except mean arterial pressure and forearm vascular resistance, which were higher in the control session (fig. 15.1). Bed rest and control sessions were similar with respect to heart rate and venous tone; basal mean arterial pressure and forearm vascular resistance were lower in the bed rest session, while forearm blood flow was higher. Bed rest and recovery sessions were similar with respect to all basal variables.

Values During Infusions of Tyramine or Norepinephrine

Infusions of tyramine or norepinephrine into the brachial artery were not associated with changes in mean arterial blood pressure or heart rate. Thus, infusions did not appear to produce systemic effects or activate circulatory reflexes. (See tables 15.1–15.3 and fig. 15.2.) There were dose-response regressions for forearm vascular resistance and venous tone; levels increased significantly as doses of either tyramine or norepinephrine increased. Sessions were compared statistically, using a parallel line bioassay (ref. 16).

Control vs. Recovery Sessions Control and recovery sessions were similar with respect to the doses of tyramine or norepinephrine required to produce given levels of forearm vascular resistance. Also, these sessions were similar with respect to the doses of tyramine or norepinephrine needed to produce given levels of venous tone.

Control vs. Bed Rest Sessions Significantly more tyramine and norepinephrine were required in the bed rest than in the control session to produce given levels of forearm vascular resistance. There were no significant differences between the two sessions with respect to the doses of tyramine or norepinephrine needed to achieve given levels of venous tone.

Recovery vs. Bed Rest Sessions Significantly more tyramine was required in the bed rest session to achieve a given forearm vascular resistance than in the recovery session. There were no differences between the two sessions with respect to the doses of norepinephrine needed to produce a given level of resistance. Also, significantly more tyramine was required in the bed rest session to achieve a given venous tone. There were no differences between the two sessions with respect to the doses of norepinephrine needed to produce a given level of venous tone.

Urinary Catecholamines

Catecholamines in the urine were lowest during bed rest and highest in the recovery period (tables 15.4 and 15.5). Bed rest levels of total catecholamines and norepinephrine were significantly lower than recovery levels but not significantly lower than control levels. Bed rest levels of epinephrine were significantly lower than either control or recovery levels. In the recovery period, average levels of total catecholamine, norepinephrine, and epinephrine were higher than in the control period; however, only total catecholamines were significantly higher.

DISCUSSION

In these experiments, forearm vascular resistance and venous tone increased appreciably during infusions of tyramine and norepinephrine into the brachial artery. Thus, the caliber of resistance vessels decreased while mean arterial pressure remained stable; also, the caliber of capacitance vessels decreased at a uniform level of transmural venous pressure (30 mm Hg). Reductions is vascular lumen at constant distending pressures reflected increased contractile tone in vascular smooth muscle. Systemic effects of the infusions were not observed and circulatory reflexes did not appear to be activated; thus, the increases in vascular tone were produced only by the actions of the drugs on forearm blood vessels.

Control, bed rest, and recovery sessions were compared in terms of the relative amount of vasoconstrictor drug needed to produce given levels of forearm vascular resistance or venous tone (refs. 16, 17). These comparisons strongly suggest that the vasoconstrictor action of tyramine was diminished after 12 days of bed rest. This conclusion is supported by the fact that significantly more tyramine was required in the bed rest than in the other two sessions to produce a given level of forearm vascular resistance, and by observations on venous tone, which indicate that the requirements for tyramine were significantly greater in the bed rest than in the recovery session. It is noteworthy that tyramine appeared to be more effective in the control session, less effective after bed rest, and more effective again after recovery. This pattern of change suggests even more strongly that bed rest attenuated the vasoconstrictor action of tyramine.

The action of norepinephrine on forearm blood vessels may not have been different in the three sessions, since virtually the same dose was required in all sessions to produce given levels of venous tone. Similarly, bed rest and recovery sessions did not differ with respect to the dose of norepinephrine required to give a certain forearm vascular resistance, while significantly more norepinephrine was needed in the bed rest than in the control session to achieve a given resistance. However, basal forearm vascular resistance before infusions was significantly lower in the bed rest than in the control sessions; thus, lower resistance levels during infusions could have resulted from differing initial basal levels rather than from an altered effect of norepinephrine.

The recovery session did not differ from the bed rest session with respect to basal levels of mean arterial pressure, forearm vascular resistance, venous tone, and heart rate. Thus, differences between these sessions cannot be attributed to differences in basal hemodynamic levels. Significantly more tyramine was needed after bed rest to produce a given effect, whereas the effects of norepinephrine in the two sessions were not different. Changes in vessel responsiveness to constrictor stimuli may not account for the attenuated effect of tyramine after bed rest because the action of norepinephrine was not attenuated. Also, changes in the pattern of drug distribution within the forearm probably do not account for attenuation. The effects of norepinephrine in bed rest and recovery sessions were comparable and not different as might be expected if distribution to parts of the forearm differed in the two sessions.

The vasoconstrictor effect of tyramine may have been attenuated after bed rest because of some functional change in peripheral sympathetic nerves. These nerves are distributed in the walls of blood vessels at varying distances from smooth muscle cells (ref. 18). Frewin and Whelan

observed that brachial artery infusions of tyramine were without effect on the sympathetically denervated human forearm. They concluded that the drug in the doses employed had no significant direct action on vessels and that its effect was produced solely by release of a substance from nerve endings (ref. 19). Their doses of tyramine were nearly identical to doses employed in the present study. Tyramine acts on vesicular granules in terminal adrenergic nerves to release norepinephrine (refs. 20, 21). Also, the vasoconstriction produced by tyramine and the quantity of norepinephrine appearing in venous effluent are correlated closely (ref. 22). Thus, vasoconstrictor responses to tyramine in the present experiments probably indicate the extent to which tyramine released endogenous norepinephrine from adrenergic nerves. In particular, venoconstrictor responses to tyramine may reflect the extent of norepinephrine release: in the human forearm, veins appear to have few beta or dilator receptors (ref. 8), and veins are not greatly influenced by metabolites from nearby tissues (refs. 23–25). Therefore, adrenergic stimuli may produce only venoconstriction. Also, in these experiments, tyramine probably caused the activation of alpha or constrictor receptors predominantly in resistance vessels. Frewin and Whelan observed a small vasodilator response to tyramine only after blockade of alpha receptors (ref. 19). In the present study, it seems unlikely that the endogenous norepinephrine released by tyramine had sufficient dilator activity to offset its vasoconstrictor activity.

We have concluded that a diminished vasoconstrictor effect of tyramine accurately reflects decreased release of norepinephrine by this amine. This may have resulted from: (1) a change in the fate of tyramine, (2) a change in the neuronal content of norepinephrine, or (3) a functional change in the capacity of the neuron to synthesize norepinephrine.

It is unlikely that bed rest produced nerve cell membrane changes that altered the fate of tyramine by inhibiting transport into the neuron. Such changes, which would impede access to endogenous norepinephrine and limit release, also would impede uptake of administered norepinephrine. In these experiments, the action of norepinephrine was comparable in recovery and bed rest sessions and not augmented after bed rest as would be expected if uptake were depressed (refs. 17, 26).

It is possible that the metabolism of tyramine within peripheral sympathetic nerves was enhanced during bed rest. Less tyramine would be available to release endogenous norepinephrine if this were the case. The enzyme that utilizes tyramine as a substrate and accounts primarily for its metabolic degradation is monoamine oxidase (ref. 27). Currently, there is no information that suggests that bed rest changes the content or activity of monoamine oxidase in peripheral sympathetic nerves. Moreover, it is difficult to account for orthostatic intolerance after bed rest on the basis of increased monoamine oxidase. Changes in this enzyme have not been associated consistently with altered release of norepinephrine or altered vascular responses during sympathetic nerve stimulation (refs. 27–29).

Vascular effects of tyramine may be diminished significantly at a time when the concentration of norepinephrine in specimens of arterial wall is not diminished (ref. 30). Also, after treatment with reserpine, recovery of responses to nerve stimulation and tyramine correlate very well (ref. 31); however, responses to these same stimuli correlate very poorly with the total tissue content of

norepinephrine (refs. 31–33). Thus, the action of tyramine probably depends on only a small fraction of the total norepinephrine present in sympathetic nerves (ref. 31). The integrity and replenishment of this fraction are determined by intact granular uptake mechanisms (refs. 27, 32, 34) and very likely by other factors in the functional capacity for norepinephrine biosynthesis within the nerve (refs. 35–46). Depression of either mechanism (uptake into granules or biosynthesis) during bed rest might account for a diminished vasoconstrictor effect of tyramine.

It is attractive to consider that bed rest attenuated the effect of tyramine because of a functional change in sympathetic nerves that depressed the capacity for norepinephrine biosynthesis. The lower urinary levels of catecholamine during bed rest undoubtedly reflect a decrease in sympathetic activity compared to control and recovery periods (refs. 36, 47, 48). The increase in norepinephrine biosynthesis produced by sympathetic nerve stimulation implies that biosynthesis declines in the absence of stimulation (refs. 35–46). The marked reduction of turnover of norepinephrine in adrenergic tissues during hibernation agrees with this idea (ref. 49). It seems reasonable that in the four subjects reported here norepinephrine biosynthesis was depressed during bed rest and that this functional change may have contributed to the diminished action of tyramine. The precise mechanisms for these results remain to be elucidated.

In these experiments, each subject responded to tilting after bed rest with decreases in arterial blood pressure and increases in heart rate that were greater than were observed during tilting in control or recovery periods (unpublished results). The poor tolerance to tilting and diminished response to tyramine after bed rest may have resulted, in part, from a functional change within peripheral sympathetic nerves.

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Table 15.1 Values during brachial artery infusions of tyramine and norepinephrine*

	Tyramine									*Norepinephrine*								
	9 μg/min			18 μg/min			36 μg/min			0.0375 μg/min			0.075 μg/min			0.15 μg/min		
	C	BR	R	C	BR	R	C	BR	R	C	BR	R	C	BR	R	C	BR	R
A. Forearm Vascular Resistance (units)																		
MW	17.8	14.9	16.5	17.8	19.8	20.5	13.7	18.4	28.1	21.1	12.8	21.9	16.5	15.9	23.8	19.8	16.9	34.3
RC	15.4	11.9	13.1	16.0	10.9	16.5	18.4	12.9	23.7	21.8	10.1	22.2	26.8	12.5	24.5	26.8	19.0	27.3
JD	23.7	9.6	11.5	28.6	11.8	15.8	30.7	11.8	19.4	29.6	16.2	16.5	47.8	19.8	20.0	57.3	25.3	31.7
TW	34.4	15.3	36.8	39.1	22.0	46.7	47.8	26.8	46.7	35.2	39.5	39.5	50.6	43.7	44.1	62.3	59.3	53.6
Mean	22.8	12.9	19.5	25.4	16.1	24.9	27.6	17.5	29.5	26.9	19.6	25.0	35.4	23.0	28.1	41.6	30.1	36.7
SEM	4.23	1.34	5.87	5.35	2.80	7.35	7.61	3.43	6.01	3.36	6.73	5.00	8.25	7.07	5.42	10.68	9.89	5.81
B. Venous Tone (units)																		
MW	6.0	5.3	5.7	6.1	5.4	6.7	6.4	6.3	8.4	5.6	5.2	5.4	6.2	6.0	6.0	6.4	5.8	7.4
RC	4.6	4.6	4.4	4.8	4.5	4.7	5.0	4.5	5.7	4.6	4.2	4.7	5.5	4.1	5.2	5.8	4.8	7.3
JD	5.1	4.9	4.4	5.8	5.9	4.8	5.9	5.6	6.4	5.2	8.2	4.5	6.1	8.7	4.8	8.3	10.4	6.0
TW	5.5	5.4	6.3	6.5	5.2	8.5	8.5	7.1	12.1	6.3	6.5	6.0	7.7	6.5	7.5	9.1	11.0	11.5
Mean	5.3	5.0	5.2	5.8	5.2	6.2	6.4	5.9	8.2	5.4	6.0	5.2	6.4	6.3	5.9	7.4	8.0	8.0
SEM	0.30	0.18	0.48	0.36	0.29	0.90	0.74	0.55	1.44	0.36	0.86	0.34	0.47	0.94	0.60	0.78	1.58	1.19

*C = initial control session; BR = bed rest session; R = recovery session, SEM = standard error of the mean.

Table 15.2 Analysis of variance of data in table 15.1*

		Tyramine				*Norepinephrine*			
		FVR		VT		FVR		VT	
Source of Variation	df	MS	F**	MS	F**	MS	F**	MS	F**
Doses	8								
Sessions (C vs BR vs R)	2	357.63	10.20††	3.78	4.40†	324.48	5.58†	0.66	–
Regression	1	250.26	7.14†	16.17	18.80††	902.82	15.52††	31.28	17.67††
Parallelism	2	18.85	–	2.62	3.05	9.10	–	0.57	–
Quadratic	1	1.90	–	0.55	–	10.88	–	1.87	1.06
Difference of quadratic	2	0.42	–	0.13	–	11.58	–	0.40	–
Subjects	3	721.62	20.59††	10.32	12.00††	1433.17	24.64††	13.71	7.75††
Error	24	35.05		0.86		58.16		1.77	
Total	35								

*FVR, *forearm vascular resistance;* VT, *venous tone.*

**All F values were calculated with the mean square for error as divisor. Significant F values for regression and nonsignificant F values for parallelism indicate that the chief requirements for a parallel line bioassay are satisifed (ref. 16).

†$p \leq 0.05$
††$p \leq 0.01$

Table 15.3 Relative potency of tyramine and norepinephrine in control, bed rest, and recovery sessions*

Comparisons	*Tyramine*				*Norepinephrine*			
	FVR		VT		FVR		VT	
Control vs. bed rest	8.151	14,180.0** 2.327	1.473	3.617 0.752	3.233	16.753** 1.515	0.792	1.632 0.332
Recovery vs. bed rest	7.052	7,680.7** 2.096	3.094	8.708** 1.312	1.905	6.415 0.920	0.773	1.584 0.320
Recovery vs. control	0.865	3.854 0.127	1.744	4.669 0.901	0.589	1.227 0.190	0.975	2.115 0.442

*Relative potency (upper and lower 95 percent confidence limits) indicates the ratio (and limit for the ratio) of a dose of tyramine (or norepinephrine) that gives a certain forearm vascular resistance (FVR) or venous tone (VT) in one session to the dose that gives the same level in another session. In the bed rest session for example, a dose of tyramine 8.151 times larger than in the control session would be required to give a certain level of FVR. Comparisons were made in the same way for VT during tyramine and FVR or VT during norepinephrine. Doses required to give a certain level of FVR or VT in one session were considered significantly different from levels in the other session if a ratio of 1 was not included within the 95 percent confidence interval (ref. 16).

**Indicates differences significant at $p < 0.05$.

Table 15.4 Urinary catecholamines*

Subject	*Norepinephrine, µg/24 hr*			*Epineprhine, µg/24 hr*			*Total Catecholamine, µg/24 hr*		
	Control	Bed Rest	Recovery	Control	Bed Rest	Recovery	Control	Bed Rest	Recovery
MW_3	39.6	32.3	62.3	6.4	4.2	6.2	46.0	36.4	68.4
MW_2	47.8	34.7	139.4	7.0	12.5	17.4	54.8	47.2	156.8
MW_1	33.8	14.6	30.9	24.8	8.6	6.9	58.6	23.2	37.8
RC_3	29.4	24.6	27.3	5.7	9.7	3.7	35.1	34.3	31.0
RC_2	(24.6)	24.3	70.0	(1.8)	0.5	18.9	(26.4)	24.8	88.8
RC_1	22.7	29.8	16.0	6.8	1.0	5.5	29.5	30.8	21.5
JD_3	21.9	10.2	18.7	5.2	5.8	12.9	27.1	16.0	31.6
JD_2	16.1	19.1	34.1	4.9	3.7	18.8	21.0	22.9	52.9
JD_1	26.6	11.3	20.7	16.6	6.8	11.0	43.2	18.1	31.6
TW_3	31.6	23.4	65.3	11.3	2.4	16.8	42.8	25.9	82.1
TW_2	27.0	25.0	44.8	5.4	2.7	13.9	32.4	27.7	58.7
TW_1	59.3	42.0	27.3	22.3	5.4	9.0	81.6	47.4	36.3
Mean	31.7	24.3	46.4	9.8	5.3	11.8	41.5	29.6	58.1
SEM	3.5	2.8	10.0	2.1	1.0	1.6	4.9	2.9	10.9

*Subscripts 3, 2, 1 for subjects indicate data for 24-hr urine samples collected 3, 2 and 1 day before the end of a particular period. Values in parentheses for RC_2 are computed "missing entries" (ref. 15).

**Dotted lines are above pairs of mean values that differ statistically ($p < 0.05$). Solid lines are above pairs of mean values that are not different (ref. 15).

Table 15.5 Analysis of variance of data in table 15.4*

		Norephinephrine		*Epinephrine*		*Catecholamine*	
Source of Variation	df	MS	F	MS	F	MS	F
Sessions	2	1521.47	4.83	132.93	6.70**	2469.32	10.08**
Days	2	648.39	1.37	24.65	1.19	600.23	1.05
Subjects	3	1330.07	6.27†	37.35	2.57	1546.04	5.70**
Sessions X days	4	887.51	4.19**	128.44	8.83†	1604.72	5.92**
Sessions X subjects	6	315.12	1.49	19.85	1.36	244.90	—
Days X subjects	6	474.75	2.24	20.79	1.43	569.31	2.10
Error (sessions X days X subjects)	(11)	212.13		14.55		271.09	
Total	(34)						

*F was calculated by using the appropriate first-order interaction in the case of sessions and days. In all other cases F was expressed in terms of the second-order interaction. The degrees of freedom for error and total in parentheses were reduced by 1 to adjust for the "missing entry" (ref. 15).

**$p < 0.05$

†$p < 0.01$

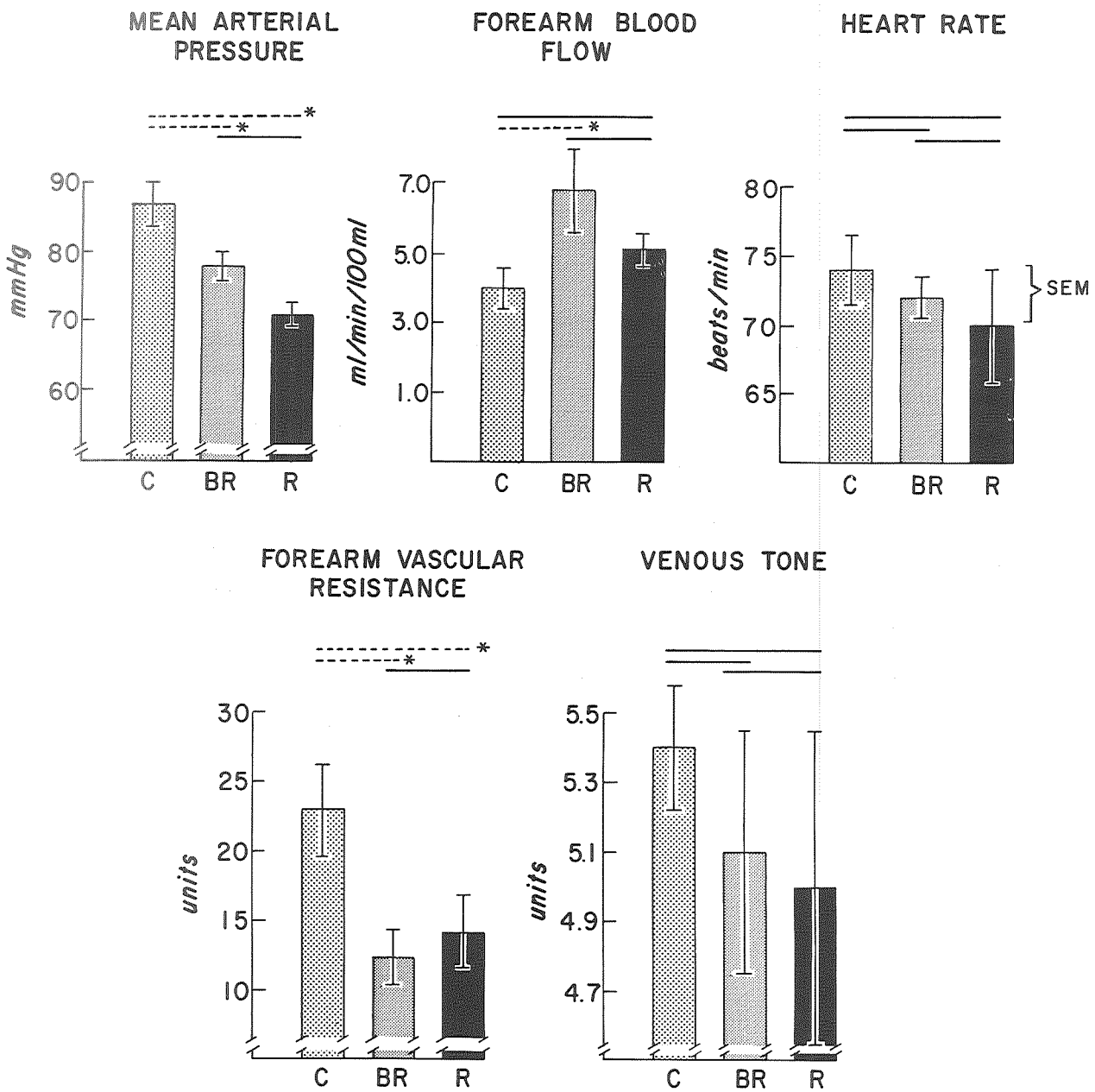

Figure 15.1 Basal values before infusions of tyramine or norepinephrine. C refers to data from the initial session after 8 days of normal vigorous activity. BR refers to data from the second session, after 12 days at rest in bed; R refers to data from the third session, 6 days after resuming normal vigorous activity. Each bar is the average of observations on four subjects (± one standard error of the mean). Dotted lines are above two averages that differ statistically ($p < 0.05$); solid lines are above two averages that are not different (ref. 15).

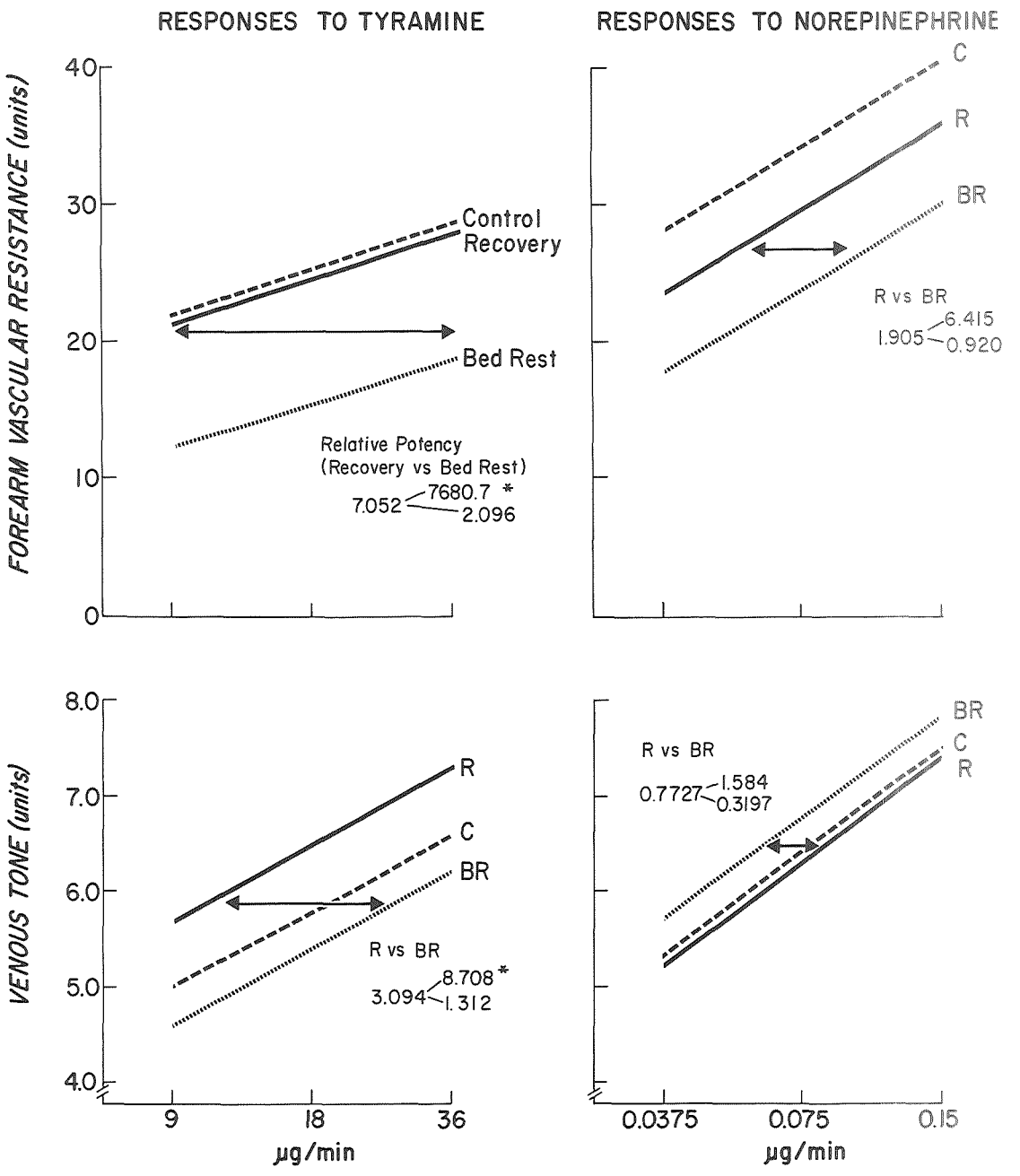

Figure 15.2 Forearm vascular resistance and venous tone during infusions of tyramine and norepinephrine. (See legend to figure 15.1 and footnote to table 15.3.). The dose-response curves in each panel were drawn with a regression coefficient calculated by using data from all three sessions (C, BR, and R) after analysis of variance indicated a significant F value for regression and nonsignificant deviation from parallelism (table 15.2). The three sessions were compared for levels of forearm vascular resistance or venous tone during infusions using a parallel line bioassay (ref. 16). For example, the dose of tyramine needed to produce a given level of forearm vascular resistance after bed rest was significantly larger than the dose required in the recovery session. The values for relative potency are to compare bed rest and recovery sessions. Asterisks indicate that two sessions differed significantly in the dose of drug needed to produce given levels of resistance or venous tone.

16 THE EFFECT OF TOTAL BODY EXERCISE ON THE METABOLIC, HEMATOLOGIC, AND CARDIOVASCULAR CONSEQUENCES OF PROLONGED BED REST

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INTRODUCTION

Physical exercise conditioning has received the greatest attention as a means of preventing adverse physiological effects of prolonged exposure to weightlessness and/or bed rest. It was thought that the changes associated with physical exercise conditioning in normal ambulatory individuals would have a salutory effect in individuals exposed to prolonged bed rest. However, a number of isotonic and isometric exercise regimens have been studied and found to have no significant effect on such factors as blood volume or the degree of orthostatic intolerance induced by bed rest, although their effects in preventing musculoskeletal deconditioning have been more encouraging (refs. 1–6).

The first phase of a study of exercise effects in the prevention of the physiological changes induced by prolonged bed rest has been completed; the preliminary data are the subject of this section. Because all analyses have not been completed, metabolic and certain special assays, such as renin, renin substrate, and ADH, are not reported. Some of the hematologic data were reported in Section 14, and it is believed that interpretation of the data is not justified at this early stage.

MATERIALS, METHODS, AND RESULTS

A total body ergometer (TBE) has been developed that simulates zero gravity while permitting exercise under conditions of normal stress to the fully ambulatory musculoskeletal system, i.e., along the long axis of the body (ref. 7). The TBE consists of a horizontal bar that moves in tracks and is connected by cables to coiled springs and weighted inertial wheels (fig. 16.1). The effort required to move the bar can be varied by 10-lb increments of spring load and/or inertial wheels. Strain gauges measure force at the bar parallel and perpendicular to the direction of travel. Force, velocity, and power levels were obtained by a computer and recorded on a strip chart as a change in force-time profile, with force giving a square waveform and the velocity a sinusoidal curve. The subject's feet were secured in rubber foot restraints and the leg and machine weight counterbalanced to near zero load by coiled springs. The subject lies on a padded couch capable of bidirectional low inertial movement.

Eight experimental subjects, ages 18 to 22, were selected by careful medical, dental, and psychological screening from among 56 young airmen volunteers who had just completed basic training at Lackland AFB. The subjects were housed in the metabolic ward and fed a metabolic control diet consisting of 3200 cal/day composed of 139 g protein, 528 g carbohydrate, and 58 g fat.

STUDY PHASES

The experimental period covered 16 weeks comprising 5 weeks of control, 5 weeks of bed rest, and 6 weeks of recovery. Tests during the period were scheduled as shown on figure 16.2. During the control and recovery phases, all eight subjects exercised on the TBE for three 20-min periods daily, for a total energy expenditure of 600 kcal/day. During the bed rest phase, all subjects were kept at absolute recumbency, allowed to sit on a bedside commode for a bowel movement daily, and discouraged from excessive activity in bed. The exercise group comprised four subjects who continued on supine exercise on the TBE at 600 kcal/day. These subjects were lifted from the TBE throughout the bed rest period. The other four subjects made up the no-exercise group during the bed rest phase. All subjects were exercised to exhaustion on the TBE once during the fourth control week and the second and fifth weeks of recovery.

Psychomotor Testing

Psychomotor testing was accomplished weekly during the control and post-bed rest periods using a battery of five tests:

1. Mercury simple reaction-time test, designed to measure neuromuscular coordination in terms of mean reaction time, for ten trials of handmovement to depress a level in response to a light.
2. Mercury hand-steadiness test, designed to measure hand steadiness as subject inserts a stylus in each of nine various sized holes for 15 sec; scored by total number times the stylus touched the edges.
3. Rater complex reaction-time test, designed to test speed and accuracy of discriminations over a 20-min period in which four different figures are displayed randomly on a screen and the subject is required to depress a button corresponding to the figure shown; scored as total number of correct responses.
4. Multidimensional pursuit task, designed to measure sustained neuromuscular coordination and attention over a 20-min period in which the subject uses a simulated cockpit, with stick and rudder pedals, to maintain a "target" orientation despite programmed shifts; scored as length of "time on target."
5. Neptune test, designed to measure the ability for differential response to various rapidly occurring stimuli by means of a complexly programmed apparatus with which the subject performs various monitoring, tracking, memory, and information-processing tasks, often simultaneously; scored in terms of response times, time on target, and/or number of correct responses per stimulus over the 20-min period.

Sleep EEG were recorded on five subjects (two in the nonexercise group, three in the exercise group) every other week during the study. Sleep survey questionnaires were done daily.

Psychological tests, including the IPA 8 Parallel-Form Anxiety Battery, Multiple Affect Adjective Check List (MAACL), and the Measurement of Depression (SDS) were done twice weekly (on Sundays and midweek) throughout the study period. The Harrower Multiple Choice Rorschach (MCR) test was administered once in each phase of the study. Subjects participated in group therapy once throughout the period.

Orthostatic Stress Testing

Orthostatic stress testing, by application of lower body negative pressure (LBNP), was carried

out during control weeks 1, 3, and 5; 4 days prior to reambulation in the fifth week of bed rest; and in weeks 1, 2, 4, and 6 of recovery. The LBNP testing protocol consisted of a 30-min period to obtain baseline data while the subject was in the negative pressure box with the rubber diaphragm in place around the abdomen, a 20-min period of –40 mm Hg negative pressure, and a 15-min recovery period. A single bipolar chest lead ECG was monitored throughout the procedure. Blood pressure (BP) was obtained each minute by standard cuff and auscultation. Forearm mercury-in-rubber strain gauge plethysmography, using the occlusion cuff technique, was carried out in all three LBNP testing periods. Plasma renin and renin substrate* response to LBNP was determined on venous blood drawn during the baseline period and again during the last minute of negative pressure. Antidiuretic hormone (ADH)** response was also determined during these same periods.

Maximal treadmill exercise testing was carried out weekly during the control period, at 24 hr after start of ambulation, and during weeks 2, 4, and 6 of the recovery period. An orthogonal lead system was used to monitor the ECG throughout exercise and in the postexercise period. The subjects walked at 3.3 mph with a 1 percent rise in treadmill grade per minute. Oxygen consumption were determined by Douglas bag collection, and BP by cuff and auscultation.

Strength of triceps, biceps, quadriceps, gastrocnemius, and dorsiflexion of the foot was measured weekly using the Cybex† muscle-testing device. Limb circumference of these same muscle groups was measured weekly with a weighted circumferential tape.

The two groups were slightly different in terms of heart rate (HR), both at rest and in response to LBNP on initial testing (fig. 16.3). The HR response showed a distinct decline by the fifth week of control period, and the difference between the two groups remained (fig. 16.4). Blood pressure response was not changed by the period of exercise training. When the group means for the fifth week of control were compared with those obtained at the end of 5 weeks of bed rest (fig. 16.5), the percentage change in HR from baseline during LBNP showed a mean increase of 147 percent for the no-exercise group and 234 percent for the exercise group. One-week postbed rest responses (fig. 16.6), compared with the fifth week of control, showed both groups to have returned essentially to prebed rest levels. There was no difference between the exercise and no-exercise subjects in terms of ΔHR response to LBNP at the end of 5 weeks of bed rest.

The two groups showed essentially the same response of the cardiovascular system (measured in terms of HR) to orthostasis (2-min seated posture and 5-min quiet standing). The baseline recumbent HR for the exercise group was ~50/min and for the no-exercise group, ~72/min. At 5-min quiet standing, the no-exercise group showed an increase in HR of 158 percent compared with 172 percent increase for the exercise group.

Renin, renin substrate, ADH, and catecholamine analyses were incomplete at the time this section was prepared.

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*Renin studies were performed by Dr. A. F. Fascola, Lilly Laboratories for Clinical Research, Indianapolis, Indiana.
**ADH assays were performed by Dr. F. D. Nash, Indiana Univ. Medical Center, Indianapolis, Indiana.
†Cybex, a trade name of Technicon Cybex, Inc., Ardsley, N.Y.

Work Tolerance Data

The comparison of the physiological responses to maximum exercise on the TBE with maximum treadmill exercise (TBE/TM) showed a very consistent ratio throughout the 16-week study period. Mean ratios were ventilation (liter/min), 85.9 percent; $\dot{V}_{O_2}$ (cc/kg/min), 88.7 percent; maximum HR, 96.5 percent; and RQ, 92.2 percent. Correlations between mechanical work expenditure on the TBE and physiological effects have not been completed.

Orthogonal lead ECG showed no significant change in any of the parameters, either within a given time in the same individual or between groups.

For the no-exercise group, maximal treadmill testing demonstrated approximately a 20 percent decrease in $\dot{V}_{O_2}$ at 24 hr postbed rest (fig. 16.7) compared with the control phases. At 2 weeks of postbed rest, $\dot{V}_{O_2}$ of the same group continued its 20 percent reduction; by 4 weeks of postbed rest, the $\dot{V}_{O_2}$ showed a 6 percent mean decrement, and it returned to control levels by the sixth week postbed rest. The exercise group showed only 4.5 percent decrease in $\dot{V}_{O_2}$ at 24 hr postbed rest, and it returned to control levels by 2 weeks of postbed rest. Average maximum HR increased 11 bpm in the no-exercise group at 24 hr postbed rest, with all other phases comparable to control values. The exercise group showed no change in maximum HR between phases. The total time on the treadmill (fig. 16.8) required to reach maximum exercise decreased 45 percent in the no-exercise group at 24 hr postbed rest, 32 percent at 2 weeks postbed rest, and 14 percent at 4 weeks postbed rest, with return to control levels by 6 weeks. In the exercise group, a 12 percent decrease in total time at 24 hr postbed rest was seen; however, one of the four subjects' total time decreased by approximately 50 percent. When only the remaining three subjects are considered, there was no change in total time.

At 4 weeks in the control phase, and at 2 and 4 weeks of the postbed rest phase, maximal tests were performed on both the TBE and treadmill. These test results show distinct differences in physiologic responses (figs. 16.9, 16.10). In response to maximal exercise on the TBE, the no-exercise group showed no change in $\dot{V}_{O_2}$ from the fourth week of the control phase to 2 weeks postbed rest and showed a slight increase (10 percent) by the fourth week postbed rest. The exercise group showed a 12 percent increase in $\dot{V}_{O_2}$ between the fourth control week and 2 weeks postbed rest. In both groups there was also an increase in minute ventilation, RQ, and maximum HR between the control period and 2 weeks postbed rest.

Psychobiologic Studies

Sleep survey questionnaires revealed an increase in subjective dreaming during the bed rest period for no-exercise subjects, who also experienced increased REM sleep. EEG results also revealed a significant increase in stages 3 and 4 of deep sleep for both groups, especially for the no-exercise subjects. The psychological test data grouped for each test showed no significant difference between the exercise and no-exercise groups for the different phases. There were trends suggesting that the no-exercise subjects were experiencing increased feelings of anxiety, depression, and hostility, but not to a significant degree. However, an evaluation of the MAACL longitudinally (control, bed rest, and recovery), using the first two administrations as baseline estimates, reflected significant psychological changes, which showed a marked increase during the bed rest period. During the control phase and throughout the study, dream material dealt mainly with future plans

and expectations. As bed rest approached and began, the subjects' dreams were concerned with control, dependence and independence, disruption of the study by subjects and others, death, escape, and fear of physical degeneration. As bed rest ended, dream themes dealt with the fear that the physical degeneration and loss of strength would persist. Of the four psychomotor tests, only the Mercury measurements of simple reaction time and hand steadiness were meaningful. Both measures showed postbed rest decrement, with no difference between the exercise and no-exercise groups. The weekly results of the other three tests showed great variance within groups during the control and postbed rest phases.

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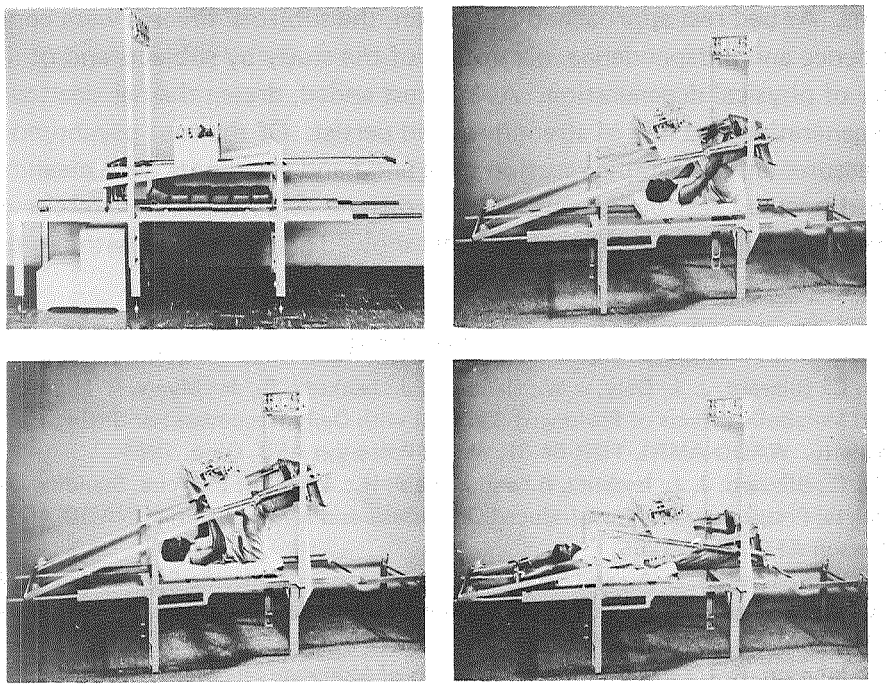

Figure 16.1 Total body ergometer (TBE) shown in various phases of exercise cycle

Control | Bed Rest | Recovery

Exercise (4)

Exercise (8) | No exercise (4) | Exercise (8)

CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC	CBC
Chem[1]	-	Chem[2]	-	Chem[5]	-	-	Chem[5]	-	Chem[2]	Chem[1]	Chem[2]	Chem[5]	Chem[1]	-	Chem[2]
TM	TM	TM	TM	TM	-	-	-	-	-	TM	TM	-	TM	-	TM
LBNP	-	LBNP	-	LBNP	-	-	-	-	LBNP	LBNP	LBNP	-	LBNP	-	LBNP
-	-	1,2,3,4	1,3	1,2,3,4	1,3	1,3	1,3	1,3	1,2,3,4	1,2,3,4	1,2,3,4	1,3	1,2,3,4	-	1,3
-	-	TBW	-	TBW	-	TBW	-	TBW	-	TBW	-	TBW	-	-	TBW
Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle	Muscle
-	-	-	Rbc mass surv	-	-	Rbc mass surv	-	Rbc mass surv	-	Rbc mass surv	-	-	-	Rbc mass surv	-
-	-	-	PV	PV	PV	PV	-	-	PV	PV	-	-	-	PV	-
Week 1	2	3	4	5	1	2	3	4	5	1	2	3	4	5	6

CBC = WBC, differential, Rbc, MCV, MCH, MCHC Reticulocytes, Hb, Hct

Chem[1] = Ca, total and Inorg. P04, Na, K, $C0_2$ combining power, uric acid, BUN, Creatinine, Alk.phos., SGOT, CPK, total serum proteins and electrophoresis, lipoproteins, serum iron, FBS, plasma osmolality, plasma Hb, serum haptoglobin, Fibrinogen, immunoglobins, and transferrin.

Chem[2] = Same as Chem[1] plus: Total iron binding capacity

Chem[5] = Same as Chem[1] plus: Cholesterol, triglycerides, phospholipids, total lipids, total and direct bilirubin

TM = Treadmill testing

LBNP = lower body negative pressure stress testing
1 = plasma renin and renin substrate
2 = antidiuretic hormone
3 = parotid cortisol
4 = urinary catecholamines

TBW = Total body water - tritium dilution

Muscle = muscle group strength and girth

Rbc
mass = Red cell mass ^{51}Cr
surv = Red cell survival ^{51}Cr

PV = plasma volume, I^{125} albumin

Figure 16.2 Schedule of testing during experimental period

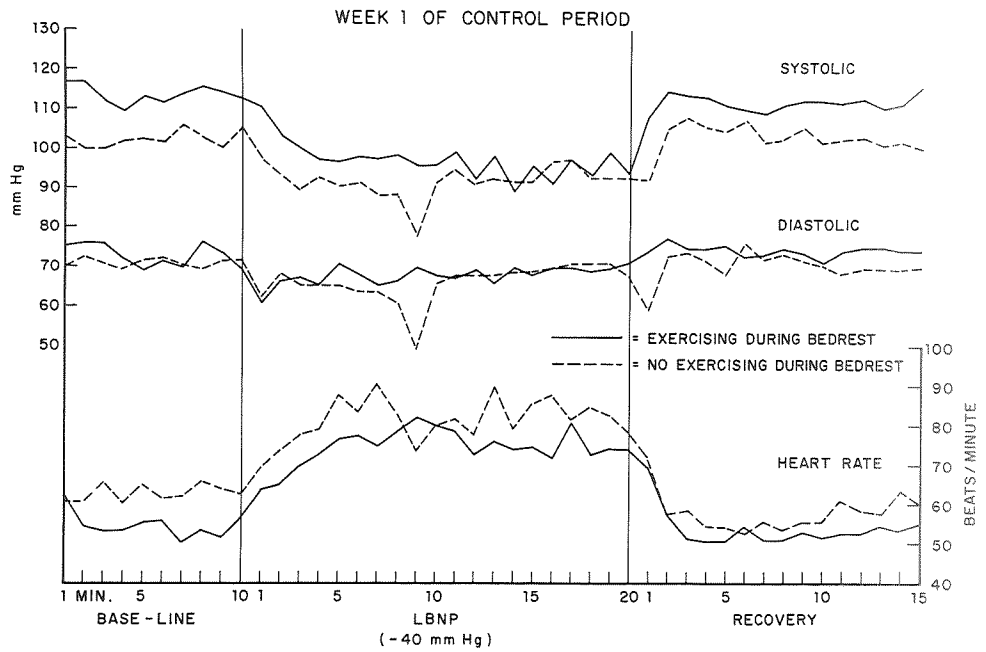

Figure 16.3 Blood pressure and heart rate response (group means) to lower body negative stress test during first control week

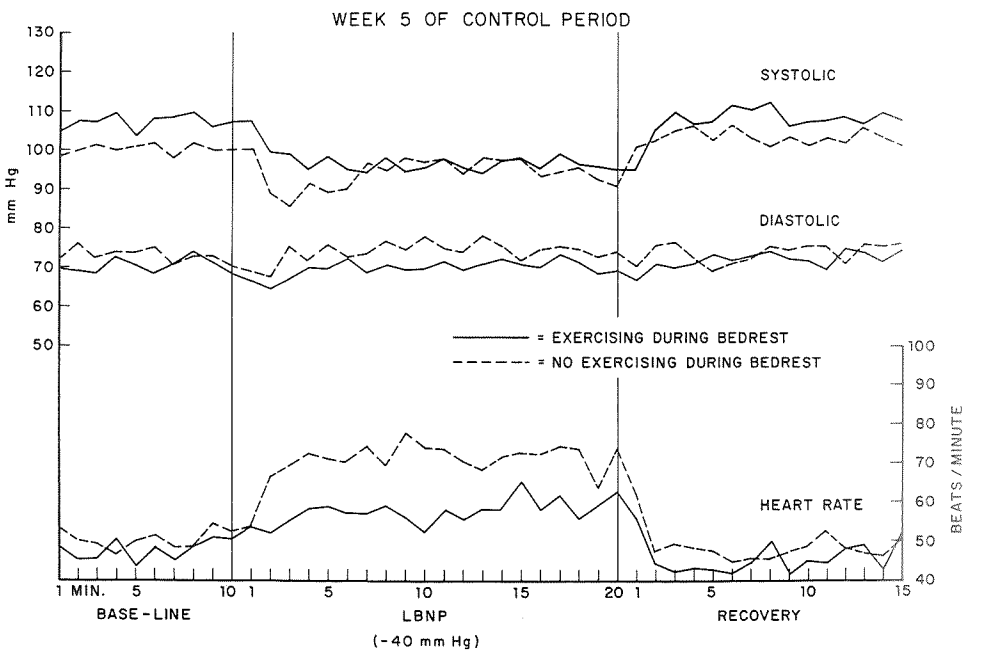

Figure 16.4 Lower body negative pressure during week 5 of control phase

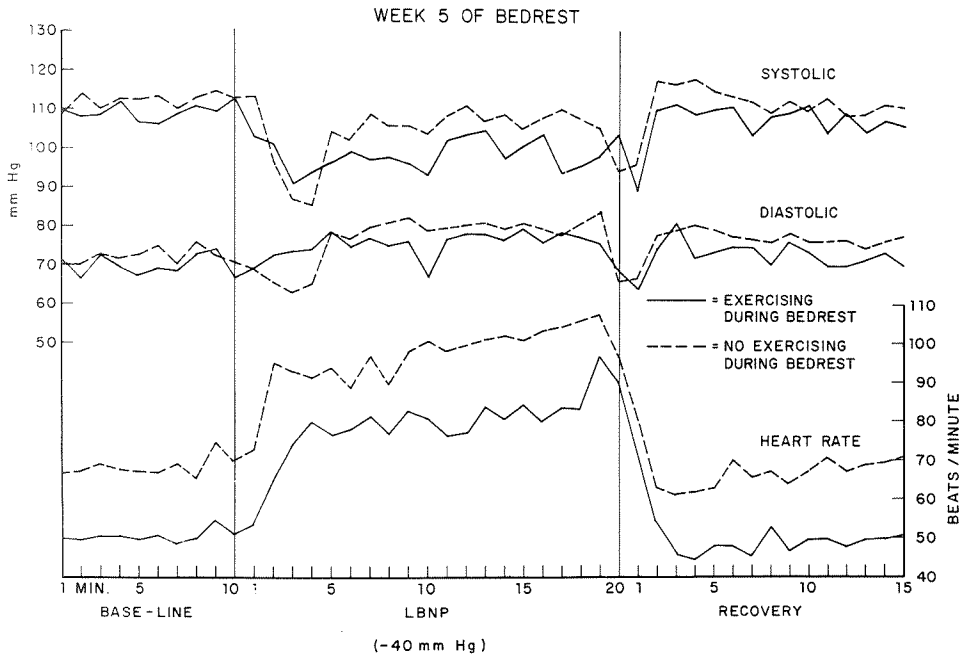

Figure 16.5 Lower body negative pressure during week 5 of bed rest phase

Figure 16.6 Lower body negative pressure at end of 1 week of recovery in postbed rest phase

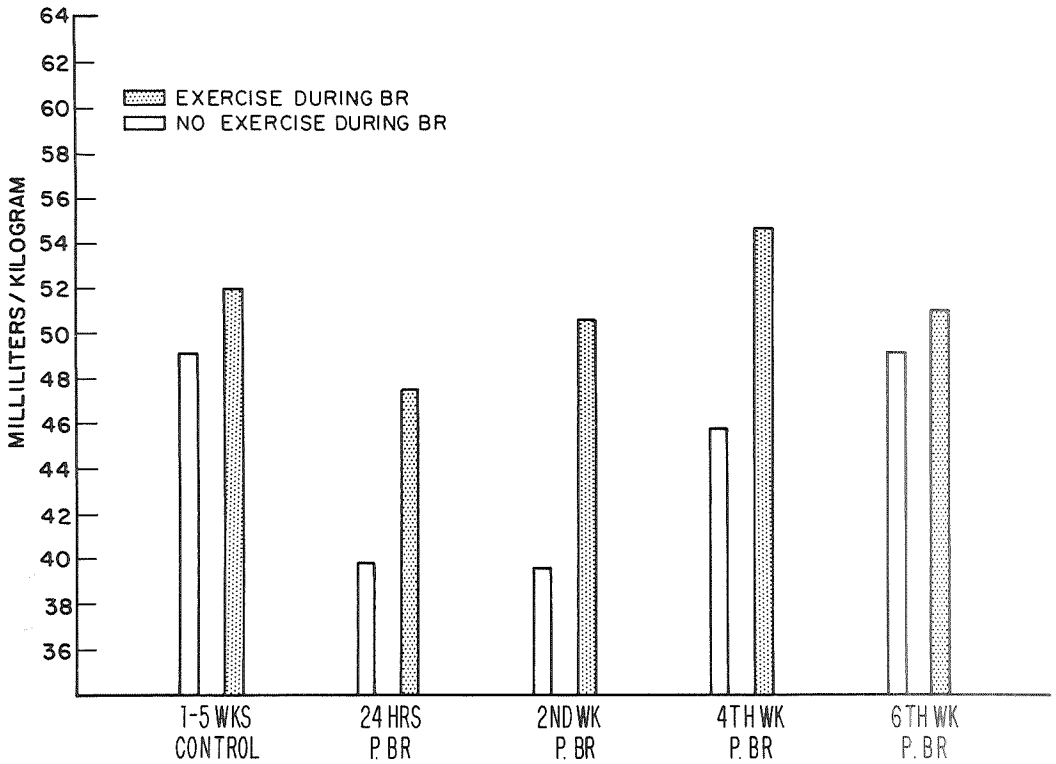

Figure 16.7 Mean maximal oxygen consumption ($\dot{V}_{O_2}$) following treadmill testing

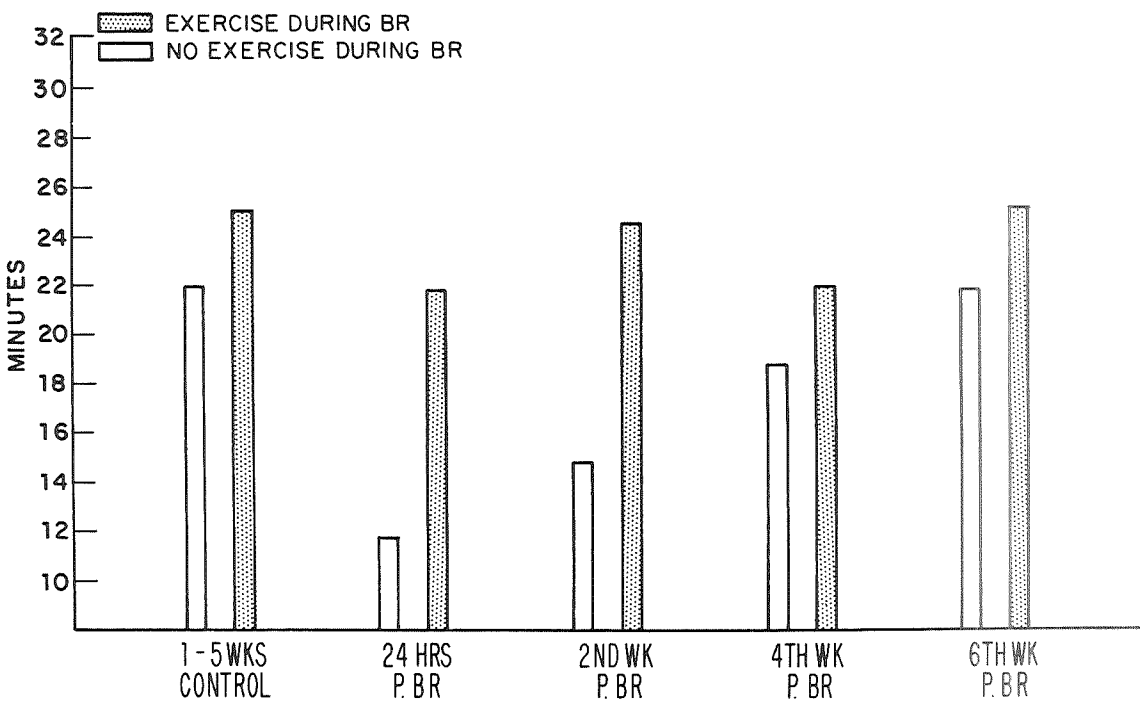

Figure 16.8 Total duration (means) of maximal exercise stress testing

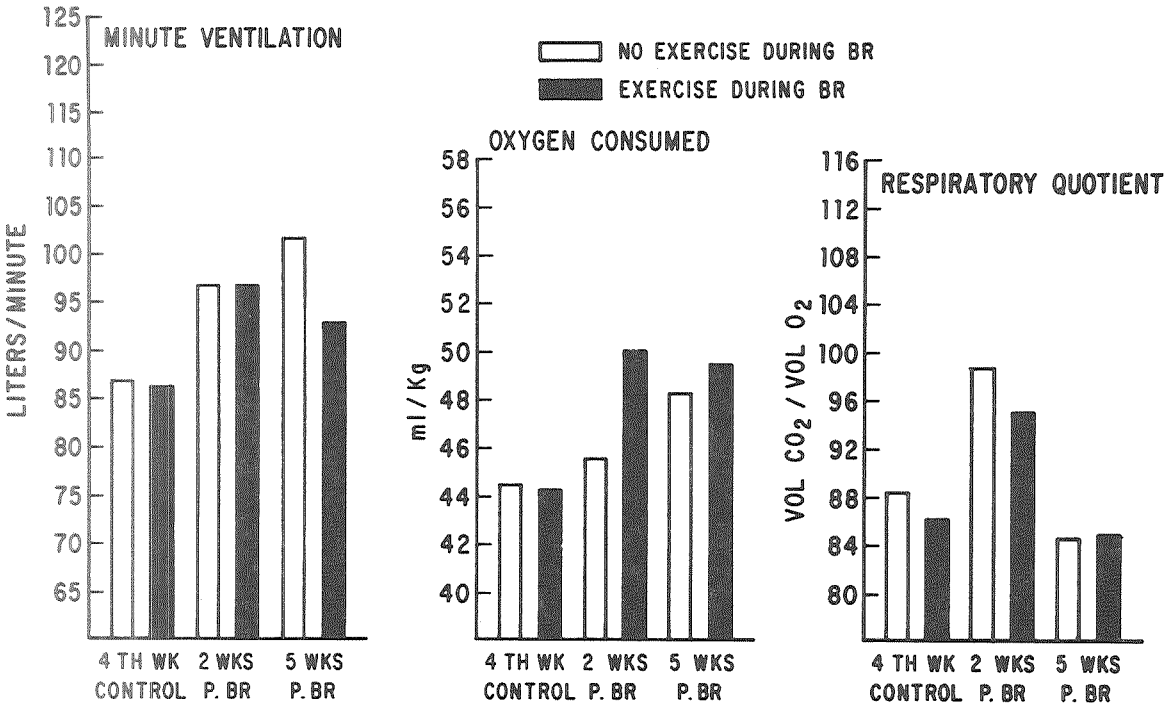

Figure 16.9 Maximal TBE exercise effects on minute ventilation, $\dot{V}_{O_2}$, and RQ

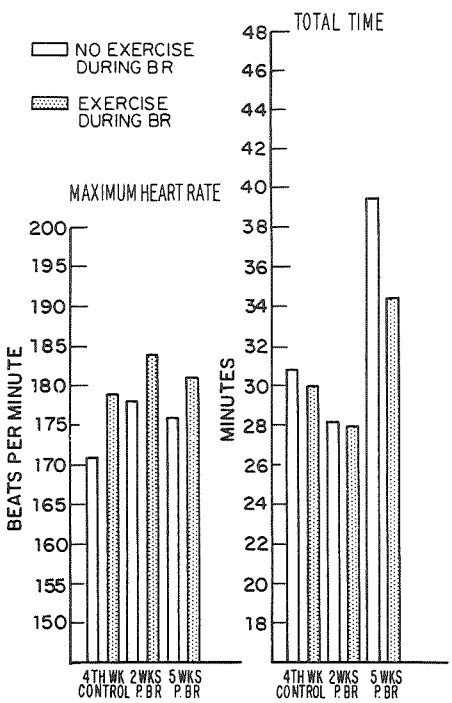

Figure 16.10 Maximal TBE exercise effects on maximum heart rate and total exercise duration in minutes

17 DISCUSSION

Unidentified Questioner: Dr. Hyatt, you keep referring to vasodepressor syncope. Is this the only kind you saw?

Dr. Hyatt: Anyone who has ever done any vascular studies during syncope has generally seen an increased systemic vascular resistance. It seems to me that the hemodynamic findings observed depend on when, in the course of syncope, the studies were made. We feel, as do others, that if you wait long enough with hypotension, typical vasodepressor syncope with a fall in resistance will occur.

Dr. Piemme: You show a decrease in plasma volume during the course of bed rest. The lesser circulation should share this loss of volume with the capacitance side of the circulation. Dr. Blomquist showed that the heart becomes smaller after bed rest. Yet, your measurements of central blood volume show no difference, and I don't understand that. How did you measure central blood volume?

Dr. Hyatt: Our central blood volume technique was essentially the standard one. Our injections were made into the pulmonary artery, and our withdrawals were made from the brachial artery. The catheter locations were identical for all the studies. I have no explanation for the lack of change in central blood volume.

Dr. Zollinger: As I understand your summary graph, Dr. Hyatt, the red cell mass fell about 250 cc from sampling losses. Shouldn't plasma volume have risen about 500 cc by the end of the test period? This would seem to be merely a restitution of the blood volume to its normal level in relation to body weight.

Dr. Hyatt: All things being the same, we would have expected that blood volume that was lost due to phlebotomy would have been restored during the experimental procedure by an increase in plasma volume.

Dr. Lind: A good deal has been said here about the use of the Whitney strain-gauge plethysmograph, most of it critical in nature. Let us put the record straight: any plethysmograph is only as good as the hands that operate it; the circumstances have to be taken into account. First, as a method to measure blood flow, the Whitney device is technically as accurate as any other method. Its ease of use and its flexibility make it the method of choice at present, except where the blood

flow of special areas is concerned, such as the whole hand (though finger blood flow can be measured successfully). Second, as a method to measure *whole* limb volume, the Whitney device is, of course, inappropriate, since it measures only the volume of a small segment of the limb. Nevertheless, this method can be used to give at least a qualitative assessment of limb volume, particularly if more than one gauge is used. Consider the calf. If only one gauge is placed on the calf, around the belly of the muscles, this can change in dimension not only in response to shifts of blood or fluids but also to shortening of the gastrocnemius and soleus muscles. These muscles tend to shorten on tilting, for example, and show that part of the calf increases in volume largely due to movement of the muscle. This is particularly true if a footboard is used on the tilting table. If a second gauge is placed at the lower border of these muscles, close to tendons, then shortening of the muscle will result in a *reduction* of the volume of the segment under that second gauge. If a third gauge is placed close to the ankle, little volume change will occur if the muscles shorten. So if three gauges are used and if every care is taken to try to ensure that the limb remains relaxed and changes in muscle tension are avoided, then it is feasible to draw qualitative conclusions about the limb volume. The picture is now one of a more complicated arrangement than simply slapping on one strain gauge (a feature that probably enticed everyone to use this gauge in the first place). The question is whether you wish to indulge in the additional effort or attempt the even trickier procedure of volumetric assessment. Whichever you do, don't find fault with an instrument simply because of misuse.

Dr. Henry: Is there any reason to suppose that the general arousal of the subjects was less during the period of recumbency because of their environmental situation, or as a result of the recumbency itself? In other words, do boredom and monotony play a role here? Isn't it well-recognized that the veins don't contract until you have lost quite a considerable percentage of the thoracic blood volume? This turns out to be about a 1,000 cc in the normal person. If you had a subject with a decreased blood volume, even if the veins of the lower extremities had the same capacity and compliance, they would hold a greater percentage of the total blood volume during the tilt, and central blood volume would be depleted.

Dr. Schmid: When we say bed rest, we mean the entire environment, including the absence of stimuli and the absence of gravitational challenge. Let me say a word about the plethysmograph we used. In making the measurements of venous volume changes during the infusions of tyramine, we put the arm under an appreciable volume of water in each session so that the geometrical configuration of that vein, and its distending pressure, were the same in all sessions regardless of the plasma volume. This is basic to the use of the water plethysmograph as a means of measuring venous response. So differences in geometry could not account for the differences in response.

Dr. Gauer: My paper tomorrow will show that this same response to the distensibility of the forearm occurs during long-term water immersion. If we immerse the man long enough (for 6 hr or more), this increased distensibility persists for several hours. Let me comment on the question of blood pooling in the legs during tilt after bed rest or immersion. As long as the system is more or less at rest, the capacitance vessels behave like passive containers. However, when a critical level of hypovolemia is reached, the sympathetic tone increases, and the peripheral capacitance vessels constrict. I would conclude from the observations of Dr. Hyatt, as well as others, that, after bed rest, the subject is nearer this critical point.

Dr. Schmid: Dr. Peter Newberry of the Royal Canadian Air Force has also demonstrated this phenomenon. He derived an equation to predict tolerance to tilt and acceleration. Under ordinary conditions, when one assumes a standing position, venous compliance does not determine tolerance; but under more stressful conditions, such as on a centrifuge, tolerance is largely determined by venous compliance.

Recently Epstein demonstrated that, when syncopal signs and symptoms begin during study with the lower body negative pressure chamber and if the stress is continued, a venoconstriction occurs at a time when there is dilatation of the peripheral resistance vessels. That was the basis for my comment that syncope may be a failure of resistance vessels rather than a failure of capacitance vessels.

Dr. Diamond: If there is decreased biosynthesis and release of norepinephrine during the bed rest period, how would you account for the tremendous tachycardia during head-up tilt?

Dr. Mitchell: We certainly know that deconditioned subjects have a normal or accentuated chronotropic response to tilt. The catecholamine may not be depleted all over the body to the same degree; therefore, the catecholamine stores in the myocardium may not be affected the same as those located in the peripheral vessels.

Dr. Piemme: There are at least two mechanisms that contribute to abnormal orthostatic response: the plasma volume loss, and an altered venous tone or compliance. Other groups have attempted to look at venous tone, but they were really measuring resting venous tones and no differences were found. But when one challenges the system, one finds that venous tone cannot compensate for the stress of an orthostatic response. A combination of plasma volume loss and a decrease in venous tone, then, could account for the orthostatic intolerance seen with bed rest; there is less blood in a larger compartment. There are two ways to approach the study of this problem. One can prevent the volume decrement with a drug such as 9-α-fluorohydrocortisone, as Dr. Hyatt and others have done; plasma volume remains normal but syncope still develops with tilting. One can simulate gravitational effects by the use of lower body negative pressure during bed rest to maintain a normal venous tone; with the use of this device, tilt-table tolerance remains normal. It seems to me that we don't have to postulate any mechanisms other than these two.

Dr. Whedon: I would like to cite our study published in 1948 in which we measured the circumference of the calf in the horizontal position before tilt, and immediately after return to the horizontal position at the end of the tilt, in the control phase, during bed rest, and in the recovery phase (ref. 1). We used a very wide steel tape in such a way that it was always under constant tension. We concluded from our measurements that there was a gradually increasing leg volume at the end of tilt during the course of the 6 or 7 weeks of bed rest. We felt that this represented an increase both in the volume of fluid outside the vessels in the leg and in vascular volume; therefore, we felt that this indicated a gradually diminishing venous tone in the legs as a result of bed rest that was brought out under the stress of tilting.

Dr. Schmid: I might postulate a mechanism for the increased extravascular edema. If, at the point where people begin to become intolerant to the tilt, there is a failure of resistance vessels as well as a failure of a compensatory venoconstriction, then capillary filtration pressure ought to be increased remarkably and capillary filtration should be enhanced. I should like also to bring up the possibility of an active vasodilating mechanism in the etiology of vasodepressive syncope. This system is difficult to study, but in at least two circumstances, there is indirect evidence that reflex vasodilation may play a part in fainting.

Dr. Henry: The classic theory that fainting is due to vasodilation was begun with Barcroft's studies and seemed to be a commonly accepted one.

Dr. Mitchell: I agree with Dr. Piemme's comments that we have demonstrated that both volume and vascular reactivity changes occur. However, I still am not convinced that there is no change in ventricular performance after bed rest.

Dr. Taylor: What I have to say relates to what we were asked to do in the beginning of this conference and that is to suggest further work to be done. Environmental temperature might be a potentially useful tool in the spaceflight situation. It seems to me that we have to "exercise" the autonomic nervous system and that the spacesuit provides a tool for doing this with a good deal of precision. One could look at the effects of vasoconstricting and vasodilating the peripheral blood vessels, using this device and circulating warm or cool water through the undergarment. You might be able to reverse some of the abnormal physiological effects such as catechol depletion. The exercise physiologist would like to see studies of more than just 2 min of work at submaximal levels. He would like to see what happens to prolonged work, and he would like very much to know the maximal oxygen intake. I think we must have reservations about what is really happening to work capacity in the deconditioning environment, and particularly in spaceflight. It might be useful to compare heart rates under standardized conditions, such as during sleep and immediately after awakening, and to follow the trends in these measurements during prolonged spaceflight.

Finally, I'd like to raise the question: just how important is it for astronauts to be in good physical condition prior to flight and then gradually lose physical fitness over the duration of the flight? As I understand it, the astronauts are not likely to be subjected to very strenuous activity (except for occasional extravehicular activity), and there is no evidence that a very fit subject will undergo deconditioning at a slower rate. Would an unfit subject lose calcium at a slower rate?

Dr. Birkhead: I sometimes wonder if we have learned a great deal about the effects of prolonged bed rest since the classic studies of Drs. Whedon and Taylor in the 1940s. I would like to discuss briefly some results of one of our bed rest studies in normal subjects carried out in the Division of Research of Lankenau Hospital about 2-½ yr ago. This work was supported by the Navy through the Naval Air Development Center at Johnsville, Pa. Table 17.1 is taken from this study, in which the subjects were at complete bed rest for 18 days. We chose to administer the same work load before bed rest and also 10 days after bed rest, after 7 days of retraining. Of course, we would have preferred to measure maximal oxygen consumption immediately after bed rest, but this was difficult to do. The oxygen uptake values on the tenth day after bed rest are very similar to those figures obtained by Dr. Blomquist. It is interesting to note the association between the heart rate,

the maximal oxygen consumption, and the work load. For the same work load, before and after bed rest, the heart rate is from 7 to 20 beats greater in three of four subjects. However, this is reflected in somewhat greater oxygen consumptions, so that the O_2 pulse is quite similar. Figure 17.1 shows the average changes in heart rates in the same four subjects at 5 and 30 min of exercises at 600 kgm/min during the prebed rest and postbed rest training periods. The training regimen consisted of a morning and an afternoon bicycle ergometer ride at 600 kgm/min for 30 min each; the gradual decrement in heart rate during these two training periods demonstrates the well-known training effect on heart rate. The slopes of the lines connecting the day-to-day average heart rates is clearly steeper during the prebed rest training period than the slope of the line in the postbed rest training period, indicating a less rapid postbed rest training effect, as measured by the heart rate response to exercise. Figure 17.2 compares the alterations in heart rate and arterial oxygen saturation during 5 min of $+G_x$ acceleration in two subjects, before and after 18 days of bed rest. This study was carried out in collaboration with the Aeromedical Acceleration Laboratory at Johnsville, Pa. The noticeable decrease in arterial oxygen saturation during acceleration was believed due to an alteration in the ventilation/perfusion ratio. The increased desaturation during acceleration after bed rest indicates that inactivity adversely affects tolerance to this type of acceleration. This should have some importance in manned spaceflight.

Dr. Blomquist: Evidence indicates there is no relation between the change in maximal oxygen uptake with bed rest and tilt-table tolerance. If we decrease the activity pattern of normal subjects who are not put to bed, we get a decrease in maximal oxygen uptake but no significant change in tolerance to tilt. Chase et al. (ref. 2) show clearly the dissociation between changes in maximal oxygen uptake and changes in response to tilt. They actually had some subjects who improved their maximal oxygen uptake during bed rest plus heavy supine exercise, but these subjects still showed an abnormal response to tilt.

Dr. Saltin: I think I agree with Dr. Taylor that you can reduce your plasma volume perhaps as much as ½ liter and increase it to more than 1 liter with only small changes in cardiac performance or maximal oxygen uptake. During exercise after bed rest, reduced venous tone can be a factor of possible importance. Those who believe it is the whole story have to demonstrate a reduced venous return in supine exercise also: to my knowledge there are no data on that point. It is hard to see how normal subjects in the supine position would have a reduced venous return during the exercise. Figure 17.3 shows the results of a study of plasma epinephrine and norepinephrine in well-trained and untrained Swedes. They were exercising at different maximal work loads. You can see that when we relate the norepinephrine level in arterial plasma to the relative work loads, they all fall on almost the same line all the way up to maximal exercise. If we recall the data from the 600-kg submaximal exercise studies in the supine position from the Dallas study that Blomquist reported on, that was almost maximal work for the subjects after bed rest. That means that they had a very high noradrenalin level in the arterial plasma, and I wondered if that shouldn't be enough to bring about venoconstriction.

Dr. Piemme: Sjöstrand (ref. 3) has presented data showing that there is nothing that will increase blood volume, and especially central blood volume, like exercise, even out of proportion to lean body mass. The subjects he measured who had the largest blood volume were cross-country skiers and tall, thin, long-distance runners. Another consequence of achieving fitness is an increase

in vagal tone associated with a very slow pulse rate. Because of the pulmonary blood volume, they have a capacity for a large stroke volume. With these men put to bed, they will get a decrease in blood volume for two reasons: the bed rest effect, and the decrease in fitness. The overall change cannot be attributed to bed rest alone. We are now talking about two different kinds of deconditioning, which is why many of us don't like the term "deconditioning." If you take a normal sedentary subject and put him to bed on an exercise program, he will tend to increase his blood volume as he becomes more fit, off-setting the bed rest effect; these effects are particularly difficult to sort out. How long does it take to develop an increase in blood volume as a result of exercise? When your subjects are put to bed, are they still on the up-limb of the increase in blood volume?

Dr. Lancaster: I can't answer that. We do have blood volume studies but, because of restrictions on the amount of the isotopes that can be administered safely, we measured blood volume only twice during the control period.

Dr. Lecocq: Drs. Sinclair and Clark in our laboratory have made some measurements in moderately conditioned subjects (new recruits in basic training program) and say that it takes 4 to 6 weeks to achieve a full blood volume effect.

Dr. Saltin: There is a lot of argument about what happens to blood volume during training. Many workers find a full effect in 4 to 6 weeks; but if you measure the total amount of hemoglobin, this peaks in a somewhat shorter time and shows a 5 to 10 percent rise within 2 weeks; using ^{51}Cr-tagged red cells, it is very rare to get a significant rise within the first month.

Dr. Vinograd: Have you noticed a difference in exercise response using resistive exercises as opposed to inertial exercises?

Dr. Lancaster: There is a difference but there is a lot of individual variation, so that we aren't able to answer that question completely.

Dr. Webb: I agree with Dr. Piemme's proposal that the essential problem here is the increased venous compliance or decreased venous tone (an inelegant term would be "flabby" vein). Many other conditions could aggravate the tilt intolerance: a reduced circulating blood volume; dehydration; lack of exercise; and anything that would produce body heat storage, which would cause cutaneous vasodilation and loss of central blood volume into the skin. The warmth does not have to be environmental in origin; it can be from exercise or fever.

Dr. Taylor has suggested that we "exercise" the autonomic nervous system of astronauts prior to reentry by alternating exposure to heat and cold. This would be very simple to achieve in a spacesuit with a water-cooling garment in it. But I think one should be very careful to ensure that the astronauts approach reentry without heat storage. They should be cool physically as well as emotionally.

Dr. Schmid: I'd like to bring a reference to everyone's attention by Dr. R. Gordon et al. (ref. 4). They (and others) demonstrated that, in rats, exercise and exposure to cold are very

potent stimuli of norepinephrine biosynthesis. Anything that will activate the sympathetic nervous system will stimulate biosynthesis. In the context of this discussion, this may improve the capacity to sustain a vasoconstrictor response.

Dr. Mitchell: I'd like to emphasize what Dr. Blomquist said earlier about the importance of separating the lack of gravity state from the lack of activity state. We recently studied a group of men who had been very physically inactive because of blindness. Although inactive, they remained upright, sitting and standing most of the day and thus were exposed to gravitational stress. They showed a marked reduction in maximal oxygen uptake and stroke volume. If there is a myocardial factor in the deconditioning of bed rest, it might be useful to raise the cardiac output to a very high level several times a day to keep the heart muscle working properly. This could be done by exercise or by heat stress.

Dr. Murray: I think exercise really would be a better way to raise the cardiac output each day. To achieve significant heat storage, you must raise the environmental heat to high levels, which is often very uncomfortable and inconvenient.

Dr. Whedon: I'd like to raise two questions that are related and might be of some practical interest to medical operations of NASA. Does orthostatic intolerance occur gradually or suddenly? Figure 17.4 presents data on tilt-table tests in the Cornell bed rest study that show the percentage of change in pulse rate and pulse pressure on tilting (ref. 1). Dr. Blomquist said that our study showed a progressive deterioration with time in bed, and I think that this is probably true of two subjects, the first and third man shown. But the second man reached maximal deterioration immediately, by the time of the first test a week after the start of bed rest; and the fourth man (who was such a "fainter" that we had to plot his data in terms of "minutes to faint" instead of in changes of pulse rate and pulse pressure) also deteriorated early. I suspect that if there is an inherently "weak" or unfit cardiovascular system, it will show deconditioning effects soon after exposure to bed rest or weightlessness; if the system is in better condition, deconditioning will take a little longer. The very bottom of figure 17.5 demonstrates that subject S.W. did very much better when we wrapped his lower legs all the way to the groin with tightly fitted ace bandages. This led me to think that elastic leotards might be a very simple protective device that could be used by the astronauts shortly before and during reentry, particularly if for any reason reentry could not be made with the astronauts in a horizontal position.

Dr. Taylor: For the short-term flights, this makes a great deal of sense. I'm not actually very impressed by the amount of disability that the astronauts show, and what disability there is seems to go away rather quickly. But, eventually, longer flights will be carried out, and we don't know what is going to happen when subjects remain weightless for months or longer.

Dr. Hyatt: I'd like to emphasize that we make sure we separate the orthostatic effects from the effects of exercise in our thinking of the response of bed rest and weightlessness.

Dr. Gauer: I want to mention the results of immersion experiments in two groups of trained and untrained subjects (Dr. Stegemann, Cologne, personal communication). After immersion, all of

the athletes faint when tilted, but the untrained subjects do very much better. This would demonstrate that muscular training does not improve orthostatic resistance.

Dr. Von Gierke: It looks to me as if bed rest challenges two control systems, one for physical exercise and the other for the gravity reception system. I see a lot of effort going into experimental designs to keep physical exercise constant throughout the series of experiments. But I haven't seen any experiments that try to introduce changes in gravity stress and keep it controlled over a long period of time. For example, I suggest a subject at bed rest be exposed for long periods of time to gravitational stress on a tilt table, or better yet on a centrifuge, to stimulate gravity receptors. At the same time, exercise conditions could be controlled from minimal to maximal work loads.

Dr. McCally: Dr. Whedon demonstrated that wrapping the legs after bed rest protects the subjects from orthostatic hypotension, and yet many investigators find there is no increase in leg volume on tilting after bed rest.

Dr. Hyatt: This isn't inconsistent at all because, if you put leotards on a subject, you squeeze out whatever blood is in the leg veins; it doesn't make much difference whether the legs have more or less blood. There is always a shift of 300 to 500 cc of blood out of the thorax to the legs when one is tilted or standing, and there doesn't seem to be any difference between bed rest and postbed rest conditions. When one dons leotards, he prevents this normal shift of blood, and that helps the subject just as if he had been given a transfusion of blood.

Dr. Gauer: Tomorrow I shall try to convince you that the normal control position for the circulation in Homo sapiens is the upright posture. With this philosophy, one comes to conclusions on the reaction of the circulation to a change in posture that differ considerably from conclusions usually obtained whereby Homo sapiens is treated as a horizontal animal.

Dr. Lind: I'd like to present some data on the physiological effects of static or isometric forearm contraction (ref. 5). With this method, one can study two aspects of muscle function: the maximum capacity of the muscles to exert tension, and the capacity of the muscles to maintain submaximal tension for a certain length of time until fatigue intervenes. This requires a good dynamometer and well-motivated subjects who are prepared to persevere to the point of fatigue. In addition, there are interesting cardiovascular responses that are quite different from those resulting from rhythmic exercise. For example, at the point of fatigue, isometric exercise results in only a modest rise of heart rate, seldom in excess of 120 bpm; systolic and diastolic pressures increase approximately in parallel, so that mean blood pressure rises dramatically to reach values commonly of 140 mm Hg. As you all know, at the point of fatigue in rhythmic exercise, the heart rate has reached maximal or near maximal levels, while mean blood pressure shows little change. The responses to isometric exercise occur at fatigue irrespective of the tension exerted; for example, they occur in about 5 min at a tension of 30 percent of the maximal voluntary contraction (MVC), or in about 1 min at 50 percent MVC. Furthermore, the responses are obtained both from small muscle groups such as finger movements and from large muscle groups as in the legs.

It seems to me that this might prove to be a simple but effective device to try in spaceflight. I don't know whether it would prevent deconditioning or would bring about conditioning of a previously deconditioned vascular system, but it should be worth studying. I think it would be useful before and after spaceflight as a provocative test of the cardiovascular system.

Dr. Mitchell: I think this is the poorest kind of stress that one can place on the left ventricle: a very high pressure load and no volume load. In isotonic-type exercise, mean blood pressure remains fairly constant, and cardiac output increases markedly. This gives the left ventricle an increased amount of volume work, which does not greatly increase myocardial oxygen consumption.

Dr. Lind: I agree with you that theoretically this may not be useful for the heart muscle, but whether it will be useful for a conditioning device remains to be established.

Dr. Mitchell: If there is deterioration of cardiac function as a part of the deconditioning syndrome, I do not believe that isometric exercises would be of much benefit in preventing this.

Dr. Lind: Most of the comments this morning have related to failure of the peripheral circulation in deconditioning, and isometric exercise certainly stimulates the local peripheral circulation. It also happens that you get an increase in cardiac output and a generalized vasoconstriction with static exercise.

Dr. Lancaster: There was a study done by Brannon at the Wilford Hall Hospital with isometric exercises during bed rest that showed maintenance of muscle strength but not maintenance of physical work capacity or orthostatic tolerance.

Dr. Lind: Most of the work procedures given to the astronauts are a mixture of isometric and isotonic work. To sort out the relative benefits of these two kinds of exercise, one should not mix the two but test each separately. This device also could be used to study muscular power and to try to evaluate some of the muscle fatigue effects that Dr. Dietlein was talking about yesterday.

Dr. Saltin: What happens to catecholamines during isometric exercise? In those cases where we found an increase in mean blood pressure, we had a perfect relationship between the increase in blood pressure and the noradrenalin in plasma.

Dr. Lind: We haven't looked at that yet. But we have some pretty good indirect evidence that catecholamines, if present, are not the primary driver of this response.

Dr. Mitchell: If we compare the Blomquist study and the Hyatt study, we see that Dr. Blomquist found a 365-cc decrease in blood volume and Dr. Hyatt a 500-cc fall, and there were relatively similar changes in plasma and red cell component volume. This certainly fits with earlier data. We seem to have general agreement also as to what happens to capacitance and resistance vessels. We are still in doubt, however, as to whether there is a myocardial component involved in the deconditioning of bed rest.

Dr. Taylor: Well, I don't know of any data in man, but in rats, vigorous exercise increases the heart weight to body weight ratio, and this happens rather promptly. If you then stop the exercise program, heart muscle undergoes a loss of mass. One interesting aspect of these animal experiments is that the time intervals are about right; these changes occur in 10 days or 2 weeks. These experiments can be criticized because body composition wasn't well controlled.

Dr. Wunder: The interpretation of those data on relative heart size changes in rats is rather difficult, because exercising animals tend to grow somewhat more slowly and a relatively smaller animal has a relatively larger heart. You must get experimental and control rats of the same size. When we exposed young mice for long periods of time to chronic centrifugation and permitted them to develop, we thought we had larger hearts due to the gravitational effect. But when we corrected for the effect of development on the heart size to body size ratio, there was no measurable change.

Dr. Taylor: I mentioned that the body composition has to be controlled, and I would presume that the ideal way of doing this would be relating heart size to lean body mass.

Dr. Piemme: I think the critical experiment is to carry out a prolonged bed rest study, obviating the volume and venous tone changes with lower body negative pressure and test maximal oxygen consumption with supine exercise, using sedentary subjects who aren't trained.

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Table 17.1 Effect of 18-day recumbent bed rest and retraining on exercise response

		Heart Rate, 600 kgm/min *		*Maximum Test Ride, 5 min* **					
		5 min	30 min	kgm/min	Heart Rate	O_2 Uptake, liter/min	O_2 Pulse, cc	RQ	Lactate, mg%
LB	(B)	136	155	1050	180	2.16	12.0	1.02	64.8
	(A)	167	164	1050	200	2.24	11.2	1.08	96.8
CD	(B)	120	123	1200	187	2.56	13.7	1.00	66.0
	(A)	152	150	1200	195	2.63	13.6	1.03	65.5
JG	(B)	108	119	1500	170	2.84	16.7	0.95	49.0
	(A)	118	132	1500	180	3.19	17.8	0.96	74.3
ES	(B)	119	134	1050	180	2.62	14.5	1.04	76.2
	(A)	133	161	1050	180	2.47	13.7	1.02	69.3

*Average of 2 days

**Postbed rest maximum test after 10-day ambulation, 7-day retraining.

(B) Before bed rest

(A) After bed rest

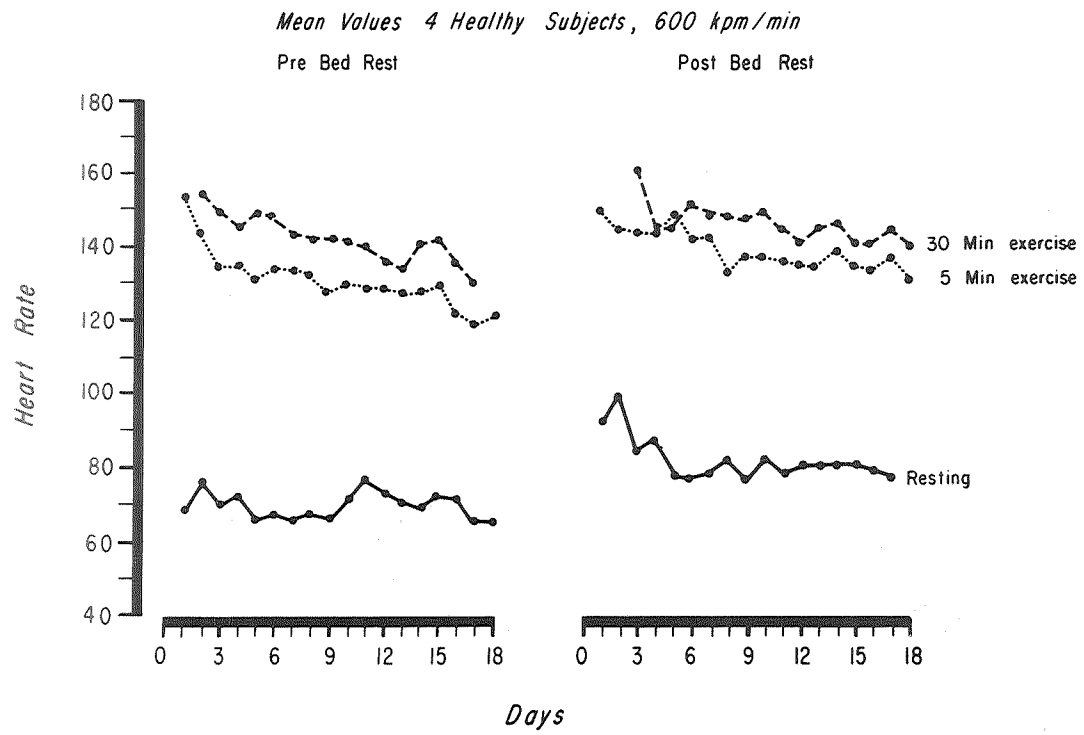

Figure 17.1 Effect of 18-day continuous recumbent bed rest on heart rate response to training load

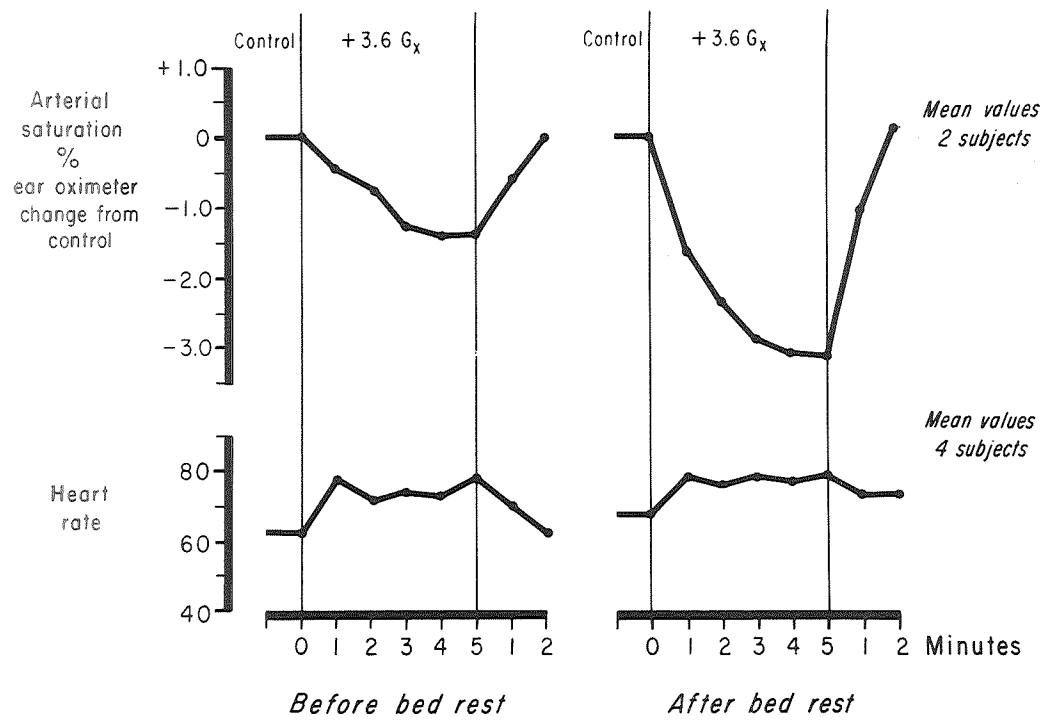

Figure 17.2 Arterial blood oxygen saturation and heart rate responses to forward acceleration after 18-day recumbent bed rest

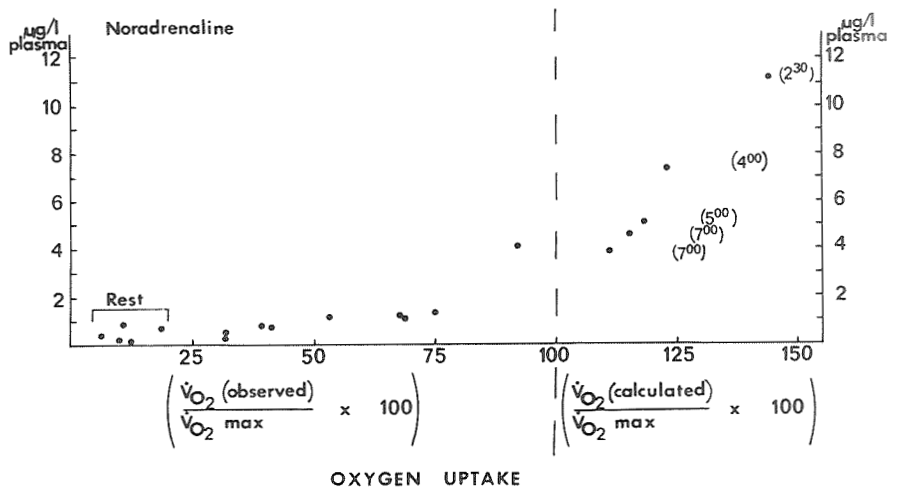

Figure 17.3 Changes in plasma noradrenaline in µg/liter of plasma during graded exercise to maximal work loads (*Circ. Res. In press*).

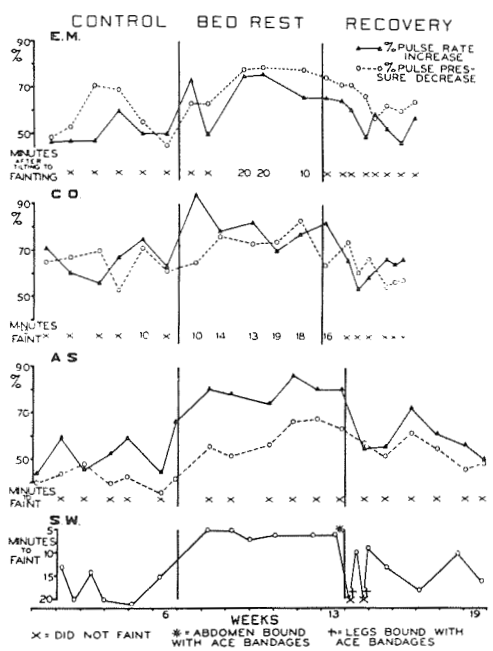

Figure 17.4 Effect of immobilization on the responses of pulse rate and pulse pressure to tilting to 65°, feet downward, for 20 min in four normal male subjects. The chart also shows for each test the number of minutes in the tilted position required for fainting to occur; an "x" indicates that on that test the subject remained in the tilted position for more than 20 min without fainting. For subject S.W., who fainted on nearly every test, the method of charting has been altered so that minutes required for fainting to occur are plotted on the ordinate line.

Keynote Paper

18 THE EFFECTS OF LONG-TERM BED REST ON MINERAL METABOLISM*

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INTRODUCTION

Many factors are involved in the maintenance of normal skeletal mass, including weight-bearing, muscular activity, innervation, skeletal blood flow, and hormonal influences. These factors have been examined in recent reviews (refs. 1-3) with some speculation regarding the relative importance of each. Decreased bone mass, as assessed by X-ray inspection, has been documented in studies of polio (ref. 3), paraplegia (ref. 4), and immobilization during the healing of fractures (ref. 5). Even in the face of some continued muscular activity, decreased muscular pull on bone results in decreased bone density, as has been described in muscular dystrophy (ref. 6). The effect may be local when a single limb is immobilized, or generalized in quadriplegics. This decreased bone density may appear within 2 to 3 months (refs. 3, 5, 7), when there is a constant negative calcium and phosphorus balance. Urinary calcium excretion may be 2 to 3 times the basal levels and may be proportional to the degree of immobilization (ref. 3), although in some polio studies the urine calcium excretion was distinctly disproportional to the amount of inactivity (ref. 8). Urinary tract stone formation may be a hazardous consequence of increased mineral excretion in urine.

The importance of weight-bearing and muscular activity may be appreciated from the decreased bone mass and negative mineral balance that result from immobilization of normal individuals with intact neural, vascular, and endocrine systems. When measured carefully from the beginning of muscular inactivity, mineral excretion increases progressively for 4 to 7 weeks, then reaches a fairly constant level. This was noted in the study of volunteers immobilized in half-casts for 23½ hr per day (ref. 9), and also in a more recent study in which the volunteers were permitted much more movement and the semi-Fowler position was used to simulate the position and activity of an astronaut (ref. 1). Both studies showed similar negative caicium balances. Assuming lengthy periods of decreased muscular activity during the weightlessness of future spaceflights and the impracticality of unrestricted fluid intake, a sustained negative mineral balance and its associated bony changes eventually could prove to be a serious threat to the skeleton and the urinary tract.

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*Conducted under NASA Grants T-58941 and T-81070.

A study was initiated at the U. S. Public Health Service Hospital, San Francisco, to determine the effect of long-term bed rest on mineral balance and bone density in normal individuals. Three healthy male volunteers 21 and 22 years of age were studied during 4 weeks of ambulation, 30 to 36 weeks at rest in bed, and another 4 weeks of ambulation after bed rest. They were maintained on a diet of 2100 Cal with 908 mg calcium and 1386 mg phosphorus throughout ambulation and bed rest. Hard candy supplements accounting for 100 Cal/day and negligible amounts of calcium and phosphorus were added when hunger or weight loss became a problem. The study subjects were permitted to move or roll over in bed, feed themselves, shave with electric shavers, and raise themselves up on one elbow, but were not permitted to sit up or dangle their legs. Urination and bowel movements were accomplished via urinal and bedpan with the subjects horizontal in bed.

METHODS

Urines were acidified daily with hydrochloric acid, pooled for 7 days; and aliquots were analyzed for calcium, phosphorus, sodium, potassium, magnesium, nitrogen, creatinine, and hydroxyproline. Pyrophosphate was done weekly on a fresh specimen. Stools were stored in epoxy-lined canisters at –22° C, and each collection, from an initial brilliant blue marker to the subsequent marker given 7 days later, was diluted with distilled water and glacial acetic acid, and homogenized on a paint mixer. Aliquots were ashed in a muffle furnace, then reconstituted in hydrochloric acid and analyzed for calcium, phosphorus, magnesium, sodium, potassium, and nitrogen.

Each week the mineral excretion in sweat was measured for a 48-hr period and the value extrapolated for the entire week. At the beginning of the collection, each subject was thoroughly washed with 0.1 percent acetic acid solution to remove all residual sweat mineral, then dressed in pajamas and socks and placed on bed linen that had been rinsed in acid and distilled water. After 48 hr without skin care or bathing, the entire body was again washed with acetic acid and the bath fluid saved for analysis. Bed linen, pajamas, and socks were soaked in acetic acid and subsequently wrung into a separate container. The bath and linen specimens were reduced in volume by boiling in the presence of acid and analyzed for calcium, sodium, potassium, and nitrogen.

Pooled aliquots of diets were analyzed after being homogenized in a blender at low speed for calcium, phosphorus, sodium, potassium, magnesium, and nitrogen.

Weights were checked daily on a metabolic scale. Plasma volume and red blood cell volume were measured by ^{131}I-labeled serum albumin and ^{51}Cr-tagged autologous cells, respectively. Bone densitometry was measured radiographically in a cooperative study with Dr. Pauline Mack, and by γ-ray densitometry in a cooperative study with Dr. John M. Vogel.

LABORATORY PROCEDURES

Calcium and magnesium in serum, urine, sweat, stool, and diet were determined by atomic absorption spectrophotometry. Standard autoanalyzer methods were used for measuring phosphorus in serum, urine, stool, and diet; sodium, potassium and nitrogen in serum, urine, sweat, stool, and diet; and creatinine in serum and urine. Hydroxyproline was determined by the method of Prockop and Udenfriend (ref. 10). Pyrophosphate content was determined on aliquots of fresh unacidified urine from 24-hr collections by a modification of the method of Fleisch et al. (ref. 11).

RESULTS

As anticipated from previous short-term studies (ref. 9), all three subjects excreted more calcium in their urine throughout bed rest than in the ambulatory periods before and after. The mean urinary calcium excretion reached a peak of 329 mg/day during the seventh week of bed rest, with a mean value throughout bed rest of 257 mg/day versus 201 mg/day during ambulation before and 167 mg/day after bed rest (fig. 18.1). Fecal excretion of calcium also increased during bed rest; the mean net calcium balance was –207 mg/day during bed rest versus –109 mg/day before and –78 mg/day after bed rest (table 18.1, fig. 18.2). Over the total period of bed rest, the mean total loss of calcium was approximately 4 percent of each subject's total skeletal calcium. Urinary phosphorus and phosphorus balance changed in the same direction as calcium balance, supporting the conclusion that bed rest led to a net loss of bone salt (fig. 18.3).

These changes in calcium and phosphorus metabolism correlated well with a decrease in calcaneus density, as measured by γ-ray densitometry. All three subjects lost significant amounts of bone mineral during bed rest and regained it during the next several months of ambulation. The bone mineral loss may have been responsible for some of the morbidity during reambulation, particularly foot and ankle pain, which was experienced by all three subjects.

Bed rest was not accompanied by significant alterations in serum calcium and phosphorus concentrations. Increased calciuria was not accompanied by crystalluria or renal calculus formation. Studies of magnesium, sodium, potassium, nitrogen, and fluid balance showed minor changes during bed rest.

The mechanism of the loss of bone mineral during bed rest was not determined. Increased excretion of hydroxyproline and pyrophosphate observed during this period would be compatible with some increase in parathyroid hormone activity, but decreased bone formation was also a very distinct possibility (figs. 18.5, 18.6). The mechanism of the loss of bone mineral during bed rest was not determined. Increased excretion of hydroxyproline and pyrophosphate observed during this period would be compatible with some increase in parathyroid hormone activity, but decreased bone formation was also a very distinct possibility (figs. 18.5, 18.6). Serum electrolyte and creatinine clearance changes, plus urine excretion patterns of sodium, potassium, and water are illustrated in figures 18.7 to 18.10.

An obvious question is raised by these results: Although these changes were not as marked as had been feared, can they be prevented? The second phase of the study is under way to answer this question, at least in part.

If bed rest affects bone by a lessening of muscular activity, exercise should be effective in preventing these bony changes. In support of this view, Whedon and coworkers (ref. 12) noted some amelioration of the negative calcium balance with an oscillating bed. Other workers, however (refs. 13, 14), failed to show improvement of the calcium balance with short periods of exercise.

The oral administration of supplemental phosphate may prove to be a more effective method of preventing calcium loss during bed rest. Many studies have shown that phosphate causes a marked

reduction in urinary calcium excretion (refs. 5, 15-20), and this effect does not appear to be accompanied by decrease in gastrointestinal calcium absorption, contrary to common medical belief (refs. 7, 20-25). In fact there may be little change in fecal calcium excretion and an increase in the net calcium balance.

The purpose of the second phase of the study is to compare the effectiveness of exercise with that of phosphate in modifying the changes of bed rest. The exercise program employed was developed and evaluated by Dr. Pauline Mack, involving 80 min/day of isometric and isotonic exercise using an Exer-Genie. The phosphate regimen being used is that employed by Hulley and Goldsmith (ref. 18) using potassium phosphate (Hyper-Phos-K) with 1336 mg/day phosphorus and 2672 mg/day potassium, effectively doubling the amounts of these minerals in the diet. A total of 2500 Cal containing 980 mg/day calcium was provided in the diet.

The results to date show that exercise of this type has not proved effective in preventing the negative mineral balance induced by bed rest. Phosphate appears to be effective in reducing urinary calcium excretion, but complete balance data are not yet available. No diarrhea or other side effects resulted from phosphate administration.

DISCUSSION

As mentioned above, the importance of weight-bearing and muscular activity in maintenance of normal skeletal volume is obvious from the changes that result from immobilization. Much less obvious is determining which of these two is more important. Clearly weight-bearing plays no role in the maintenance of normal homeostasis in many bones, such as the upper extremities and skull, which do not bear weight. However supine muscular exercise alone seems inadequate to compensate for the lack of weight-bearing in lower extremities. Supine exercise for 80 min/day clearly does not simulate all the factors involved in ambulation. The work of Issekutz et al. (ref. 22) suggests that 3 hr of quiet standing comes closer to simulating ambulation than 4 hr of bicycle exercise. Lamb (cited in ref. 1) showed that 2 hr of walking and back exercises failed to offset the changes from bed rest for the remaining 22 hr/day. However 3 hr of vigorous walking and weight lifting did have an effect, but no more than 3 hr of quiet standing. It may be that the compression forces of weight-bearing are more important than the tension forces of muscle pull in ameliorating the negative calcium balance of bed rest. Further, the weight-bearing forces may need to be operant 3 hr/day or more to be effective, judging from the results of Issekutz and Lamb. Authors who have commented on the ineffectiveness of weight-bearing, on the other hand, generally employed briefer periods. Plum and Dunning (ref. 13) used a tilt table raised to a near vertical position (85°), but only for ½ to 1 hr daily. Wyse and Pattee (ref. 14) started their patients in an upright position on the tilt table for 5 min twice daily and gradually increased the length of time, but the maximum was 1 hr and 50 min in one patient.

Since weight-bearing is impossible at zero gravity, an effective means of simulating this might need to be devised. In some work yet to be published, Dr. Donald Young at Ames Research Center immobilized monkeys in chairs and compressed their lower extremities in a rhythmic fashion to simulate weight-bearing. Compression forces were equal to one-half the body weight. Calcium

excretion data on one control monkey compared with one that underwent compression showed significantly less calcium loss in the latter. If additional data bear this out, it would be well worth considering a series of studies on humans.

CONCLUSION

Nine months of horizontal bed rest caused distinct os calcis dimineralization in three healthy young men, accompanied by a negative calcium balance that persisted until reambulation. Measures designed to prevent these changes of calcium balance and bone are presently under investigation.

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Table 18.1 The effect of prolonged bed rest on mean calcium balance (3 subjects).

Calcium (mg/day)	*Ambulation*	*Mean Bed Rest*	*Reambulation*
Intake	908	908	908
Output			
Urine	201	257	167
Stool	795	852	790
Sweat	21	20	29
Total	1017	1129	986
Balance	−109	−221	−78

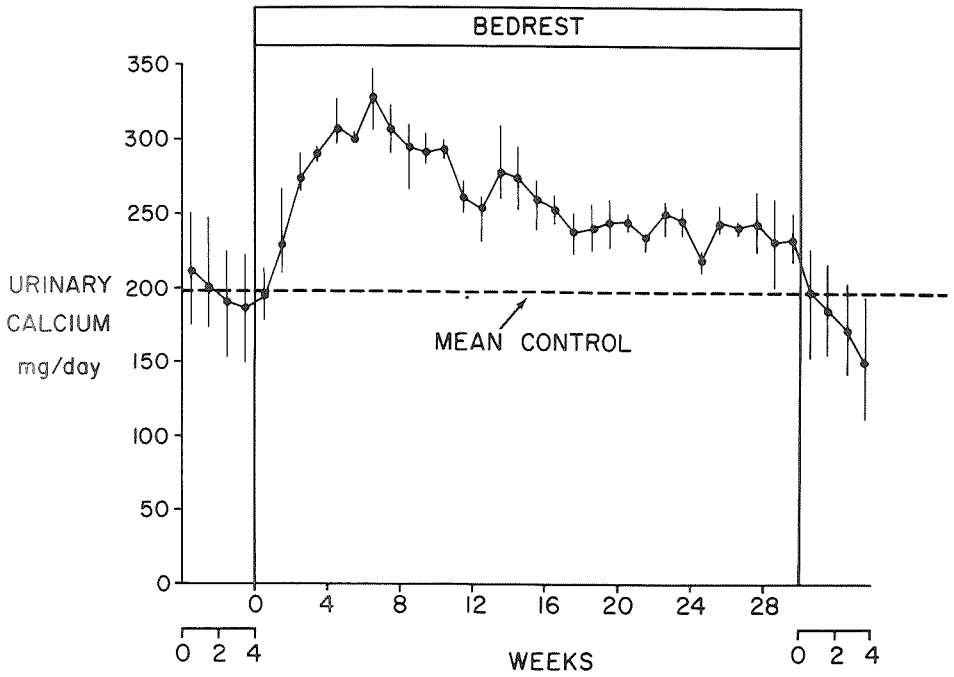

Figure 18.1 The effect of prolonged bed rest on mean urinary calcium excretion (3 subjects)

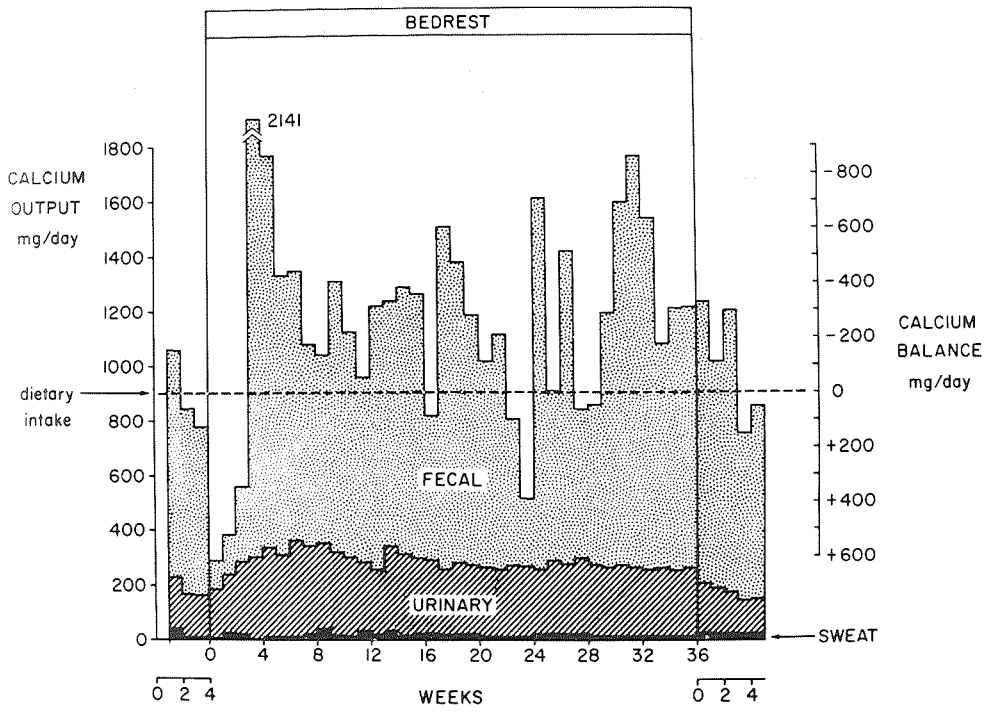

Figure 18.2 The effect of prolonged bed rest on calcium balance (RR)

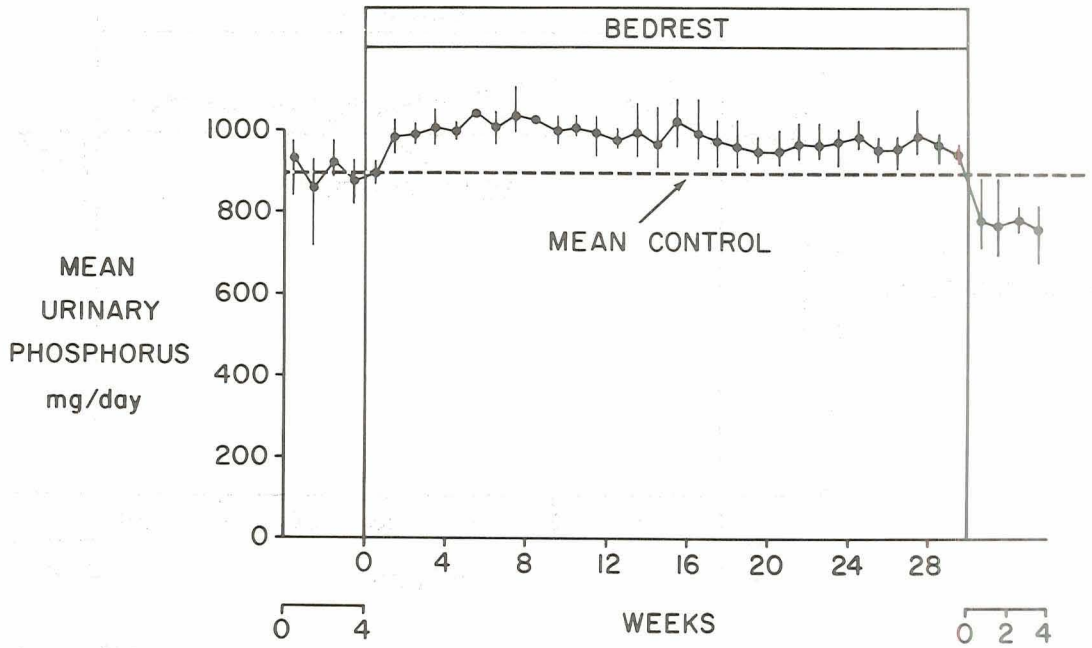

Figure 18.3 The effect of prolonged bed rest on urinary phosphorus excretion (3 subjects).

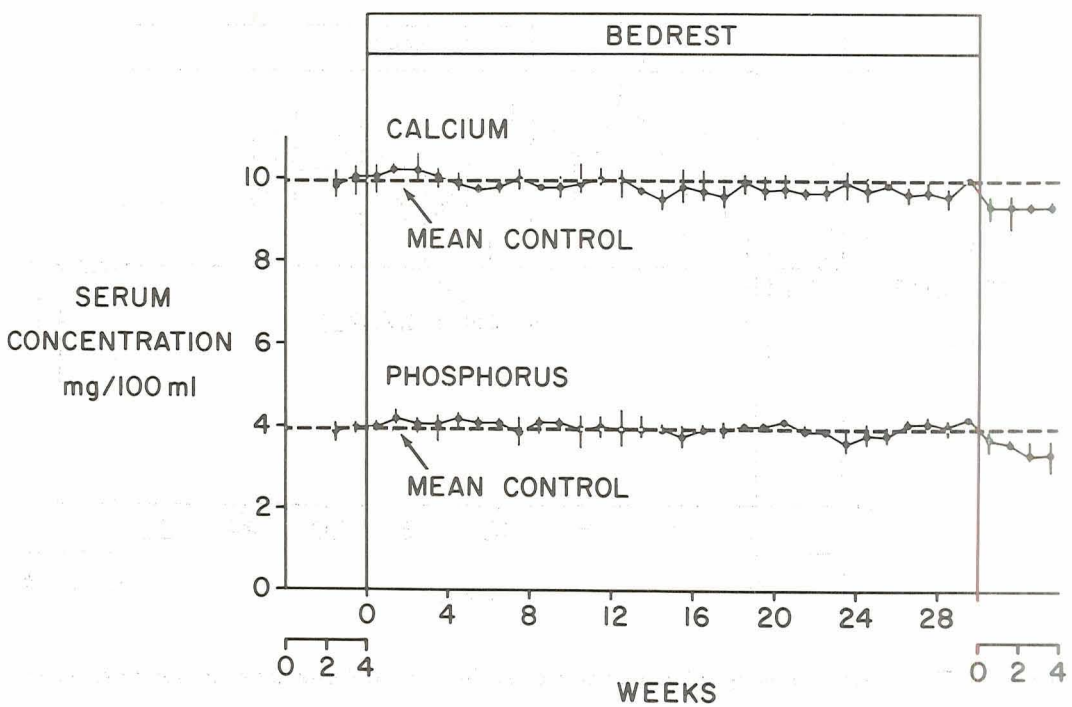

Figure 18.4 The effect of prolonged bed rest on mean serum calcium and phosphorus concentrations (3 subjects)

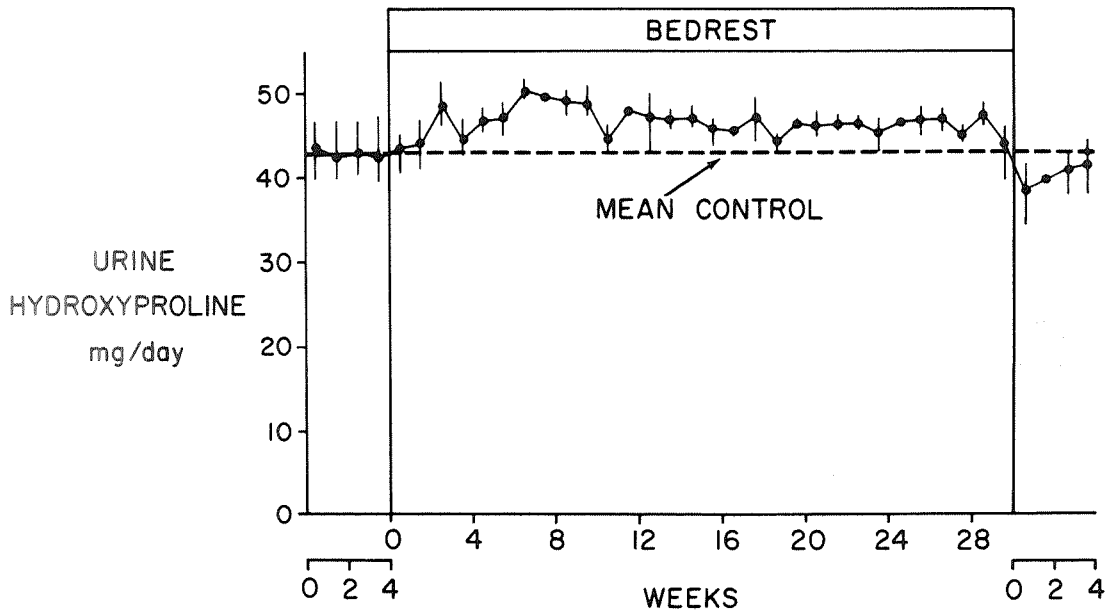

Figure 18.5 The effect of prolonged bed rest on mean urinary hydroxyproline excretion (3 subjects)

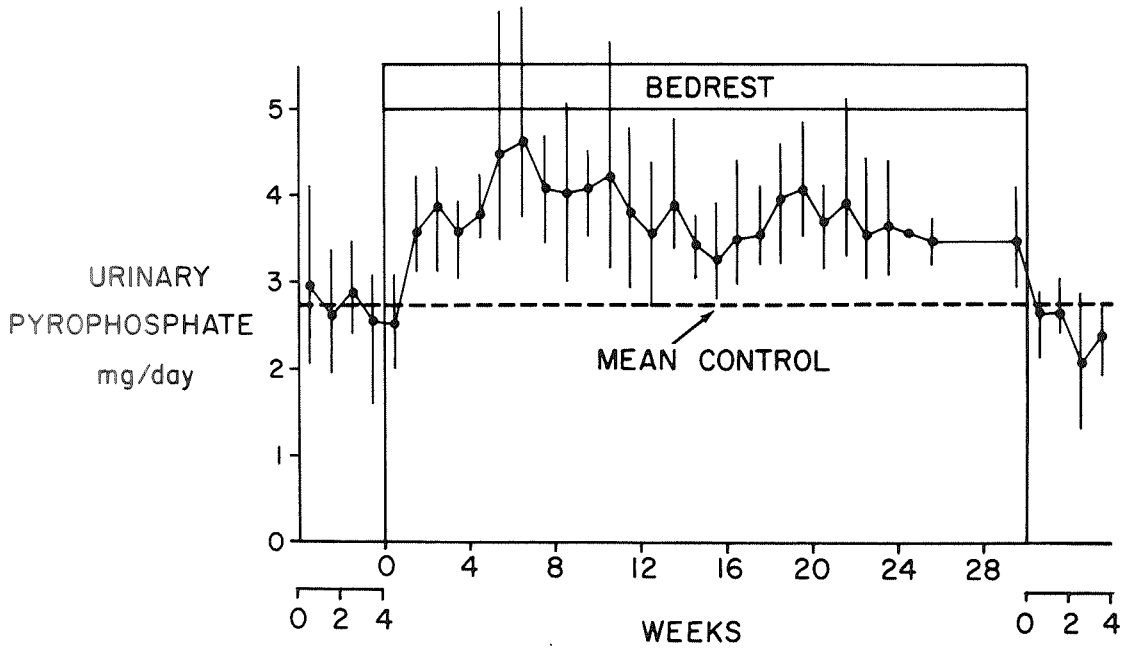

Figure 18.6 The effect of prolonged bed rest on mean urinary pyrophosphate excretion (3 subjects)

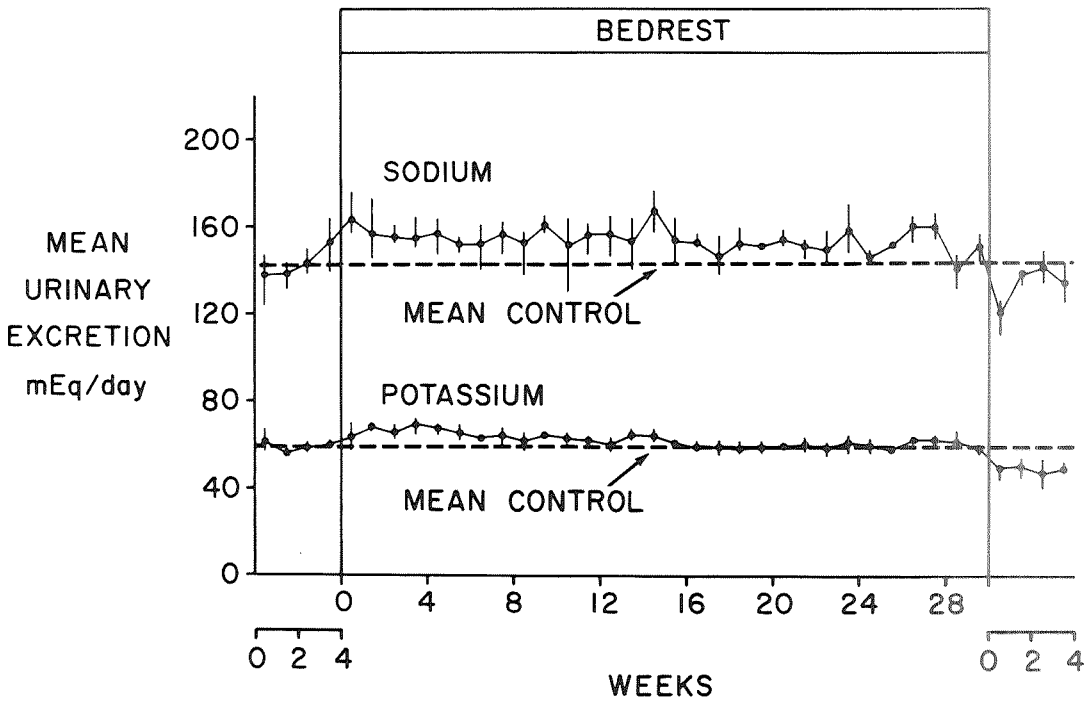

Figure 18.7 The effect of prolonged bed rest on serum calcium and phosphorus concentrations (3 subjects)

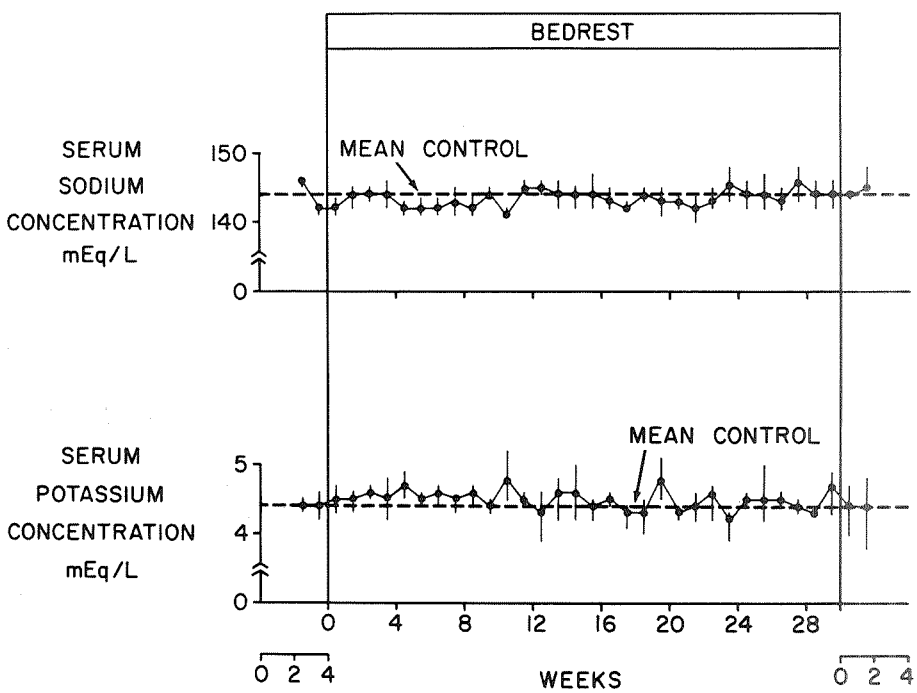

Figure 18.8 The effect of prolonged bed rest on mean serum sodium and potassium concentration (3 subjects)

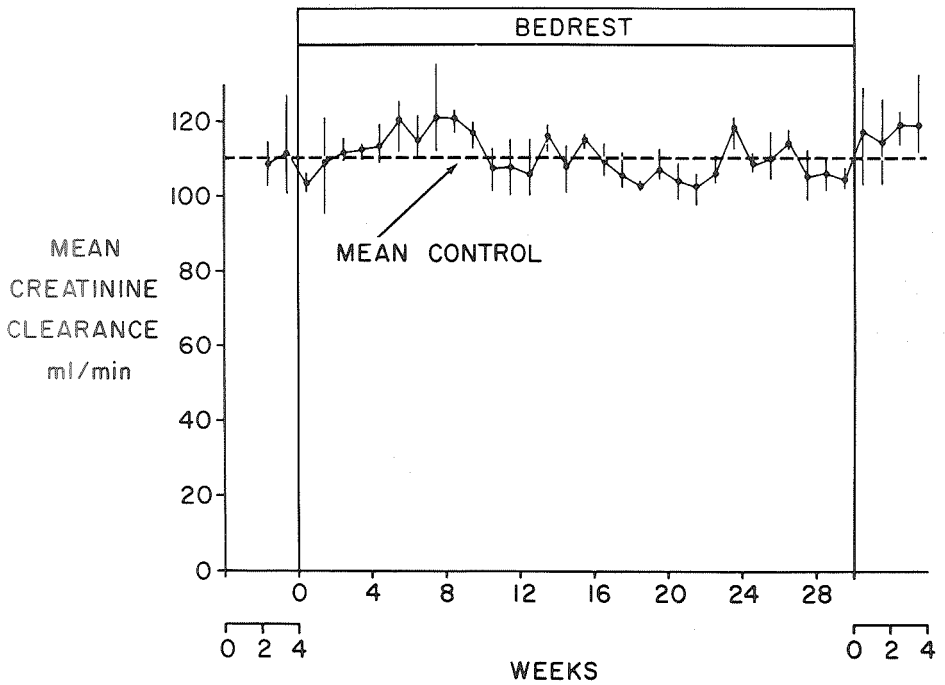

Figure 18.9 The effect of prolonged bed rest on mean creatinine clearance (3 subjects)

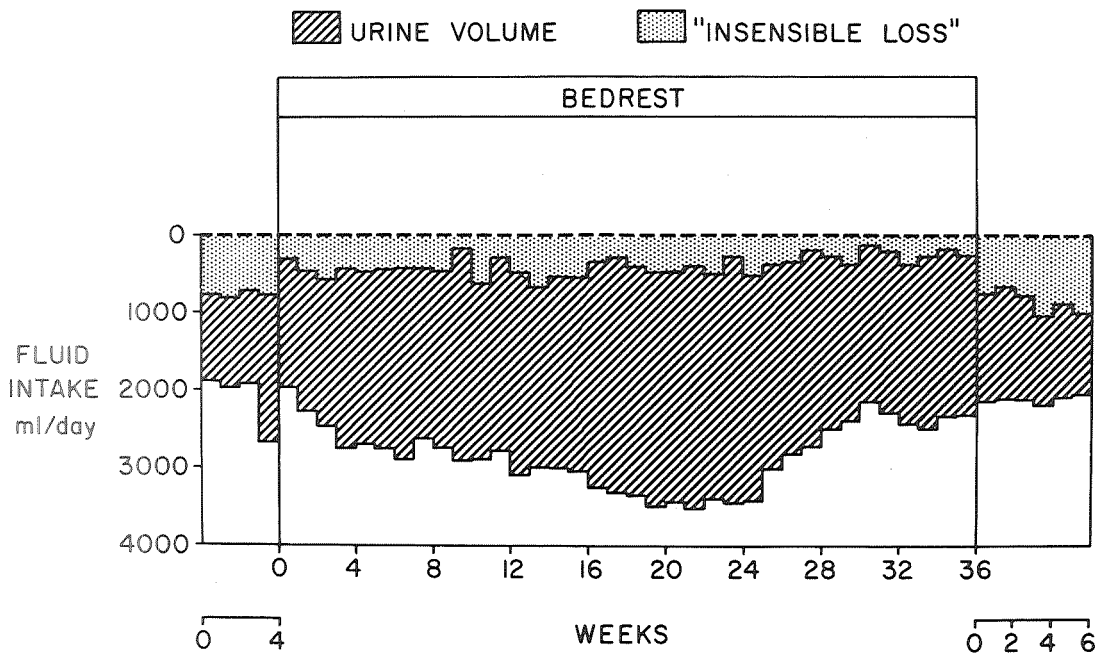

Figure 18.10 The effect of prolonged bed rest on fluid balance (RR)

19 CHANGES IN BONE MINERAL CONTENT OF THE OS CALCIS INDUCED BY PROLONGED BED REST*†

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INTRODUCTION

The progressive demineralization of bone of an immobilized limb or bedridden patient is well recognized. The factors leading to the demineralization are complex and their relative importance still is unknown. Muscular pull on the periosteum, alterations in blood flow in the active and inactive limb, and longitudinal gravity stress all play a role in assuring the integrity of the mineral content and architecture of bone. Periods of weightlessness of up to several months are projected for the space program, necessitating thorough evaluation of the effects of zero gravity on the mineral content of bone. For this purpose, absolute bed rest has been used as the best available simulator for studies of the possible effects of zero gravity on man.

The nuclear medicine program, in cooperation with the Metabolic Unit of the U. S. Public Health Service Hospital, San Francisco, has undertaken a study of the effects of prolonged (9 months) bed rest on bone and calcium metabolism and methods that might effectively alter the mineral loss known to result from bed rest. (Earlier studies have been limited to a maximum of 6 weeks.) The purpose of the study was to follow the bone mineral changes during three complete bone cycles. A controlled calcium balance study was also performed.

This section presents the changes in bone mineral content of the os calcis, estimated from changes in the absorption of a monoenergetic γ ray, namely the 27.5 keV emission of ^{125}I.

METHODS

Three subjects in their twenties were admitted to the study after careful examination to ascertain whether they were both physically and mentally capable of withstanding a 9-month period of bed rest. They were maintained on a rigidly controlled metabolic diet that contained 910 mg calcium per day. Because of instrumental design problems, the metabolic study had been under way 3 months before γ-ray densitometry was begun; however, these studies were performed on the three subjects at regular intervals from months 3 to 9 of bed rest and for 5, 7, and 8 months after reambulation.

The instrumentation was designed to permit scanning of the os calcis with the patient in the recumbent position. The scanning head consisted of a 3-mm X 2-in. NaI crystal scintillation detector with 3/32 collimation moving synchronously with a source holder containing 100 mc of ^{125}I with 3/32-in. collimation, 6 in. from, and in direct alinement with, the detector. The foot to be scanned is placed between this scanning mechanism in a dental rock mold fashioned for each patient (fig. 19.1). The mold is filled with water during the scan to provide a moderate degree of tissue

*Conducted under NASA Contract T–58941 and T–80173.

†Read by Dr. Stephen Hulley.

equivalency. This latter technique is important, since the soft tissue about the bone is not uniform and would add to the bone density values in a nonpredictable way. As shown in figure 19.2, the scanning head is suspended from a frame containing precision stepping motors whose function is programmed by a motor power supply and limit switches. This device permits a rectilinear scan in a vertical plane. Each horizontal row accumulates transmission data for each 1/64 in. in 256 increments before being stepped vertically 1/8 in. to begin a new row. Sixteen rows of data are accumulated in a 4096 multiparameter multichannel analyzer in a 16 X 256 array, and stored on magnetic tape and tally punch tape for later processing. Three methods of direct readout are provided: (1) a volumetric display, a planar representation of the 16 X 256 data points, each being intensity modulated by the magnitude of the data in that location (fig. 19.3); (2) an isometric display, a three-dimensional display in which each row is depicted in analog fashion much like a chart recorder and sequential rows lie one behind the other (fig. 19.4); and (3) digital printout on a high-speed Franklin printer.

Each row of data was calculated as shown in figure 19.5. The average count rate to the left and right of the bone, i.e., through tissue plus water, represents I° or 100 percent transmission. The transmission value for each 1/64 in. is ratioed to I°; the natural log is then computed for each data point and summed for the entire row. This figure is expressed as a positive value, as absorption; therefore, the higher the sum value, the denser the bone. No bone would give a value of $\ell n\ I^\circ/I^\circ$ or $\ell n\ I$ or 0.

RESULTS

Figure 19.6 shows bed rest data from the first subject (G.B.); the row location is indicated on the abscissa and the value for each row on the ordinate. Each curve represents the composite values for a given scan. Note the sequential progressive decrease in the absorbence value for each row. The figure also shows that there is an area of from ½ in. to about 1-¼ in. from the bottom of the heel where the rate of mineral loss and, therefore, decrease in absorbence is more rapid and pronounced. The areas higher on the bone appear to change more slowly. The subject R.R. showed a slower though just as great a change in the same time period (fig. 19.7). Again, the most significant change occurred in the ½- to 1-¼-in. area with minimal change in the areas higher on the bone.

On ambulation after 6-½ months, the first subject, G.B., showed a slow initial rate of remineralization; but by March of this year, he had returned to 90 percent of the previous March value (fig. 19.8). The curves increase sequentially. The solid curve represents the scan obtained just before ambulation (7/22/68), also shown in figure 19.6. This subject had the densest bones at the beginning of the study and lost the least mineral.

The subject, C.S., who had the next most dense bones, remained in bed the full 9 months and illustrates the same sequential increase in mineral, and the more rapid increase in the ½- to 1-¼-in. area (fig. 19.9). The area from 1-¼ in. up showed an early continued loss before a gain was recorded, suggesting that there is remodeling within this bone during reminieralization (fig. 19.10). The last subject, R.R., demonstrates the same pattern (fig. 19.9) on ambulation. He too regained rapidly in the lower part of the bone while the upper areas continued to lose before all areas demonstrated a net gain. He was the only subject who did not have a sharp rise in the second and third row, which usually represents a thicker cortex at the bottom of the heel.

CONCLUSIONS

These studies demonstrate that there is a significant loss of mineral content of the os calcis during 9 months of bed rest (6-½ months in one subject). There is a rapid regain after ambulation, which does not reach prebed rest values until 4 to 6 months have elapsed. The subject with the densest bones lost the least mineral. The areas with the greatest mineral loss showed the earliest and most rapid mineral gain. Areas higher on the bone were slowest to lose, lost the least, and, after ambulation, continued to lose for a short period before demonstrating a net gain. This pattern suggests remodeling of this bone. It is concluded that prolonged periods of bed rest—and therefore zero gravity conditions—can materially reduce the mineral content of the os calcis and place this bone at risk when ambulation or gravity conditions are reinstituted.

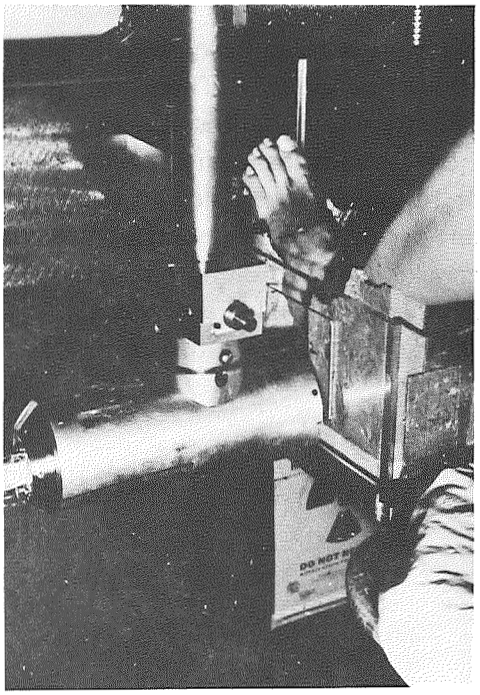

Figure 19.1 Instrumentation and positioning of subject for scan

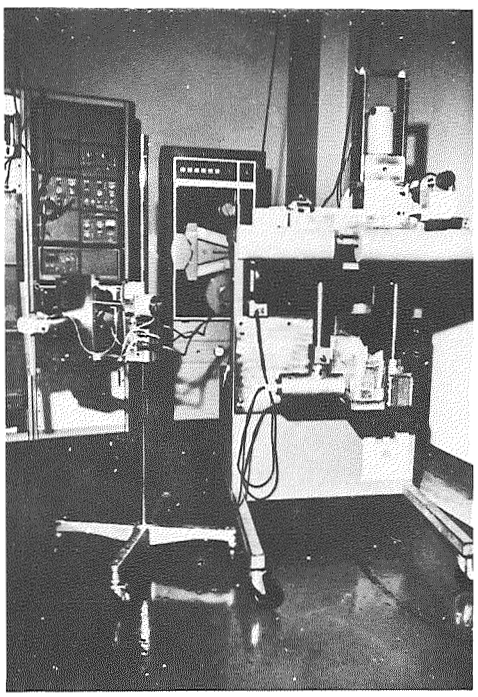

Figure 19.2 Scanning head and other densitometry apparatus

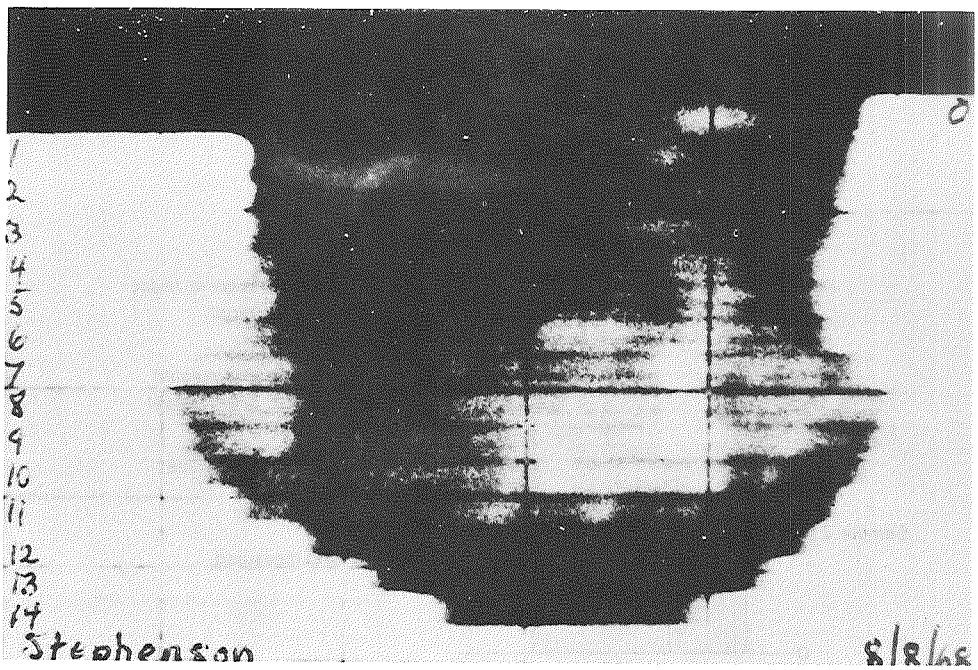

Figure 19.3 Volumetric display of data

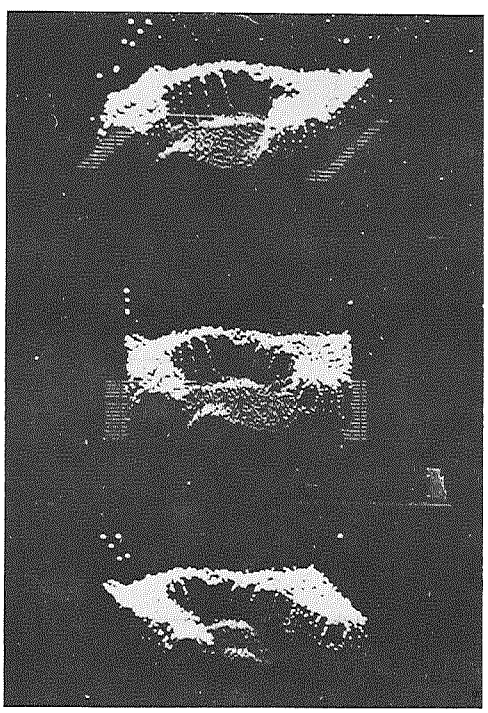

Figure 19.4 Isometric display of data

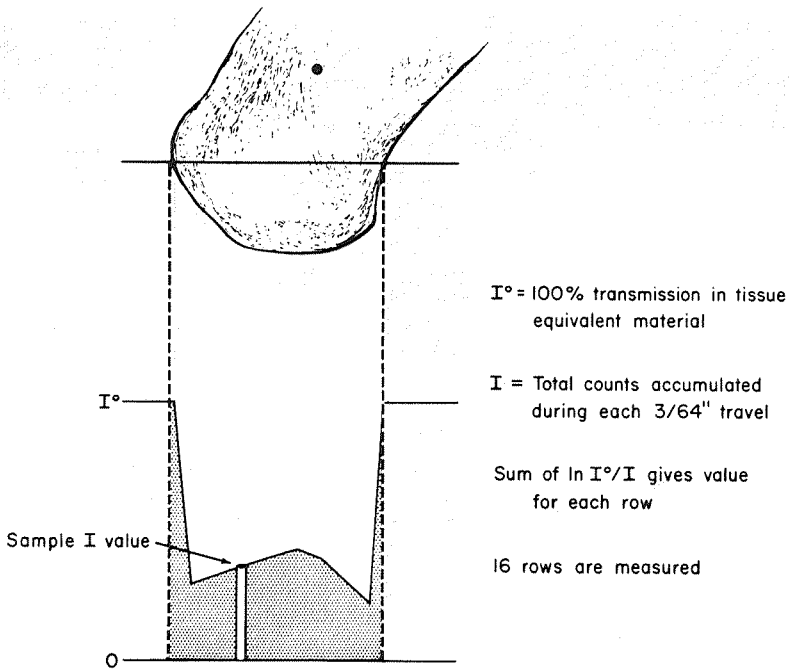

Figure 19.5 Calculation of bone density

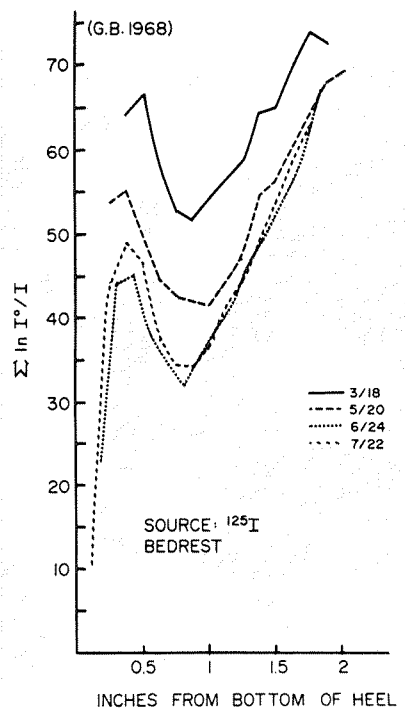

Figure 19.6 Density data obtained during bed rest, subject G.B.

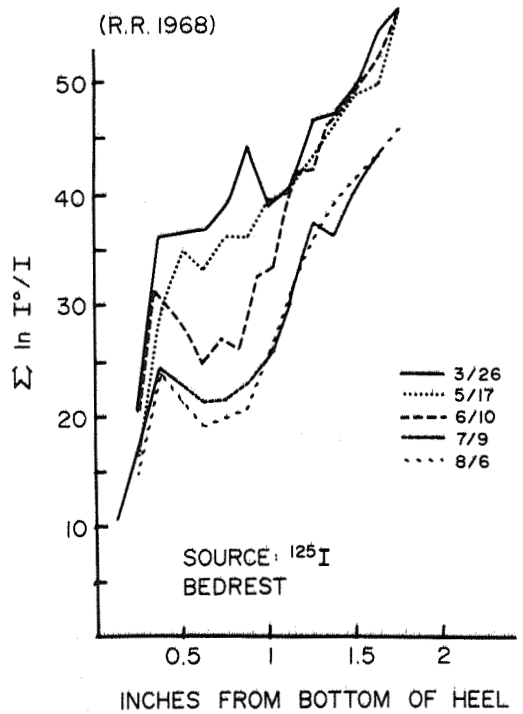

Figure 19.7 Density data obtained during bed rest, subject R.R.

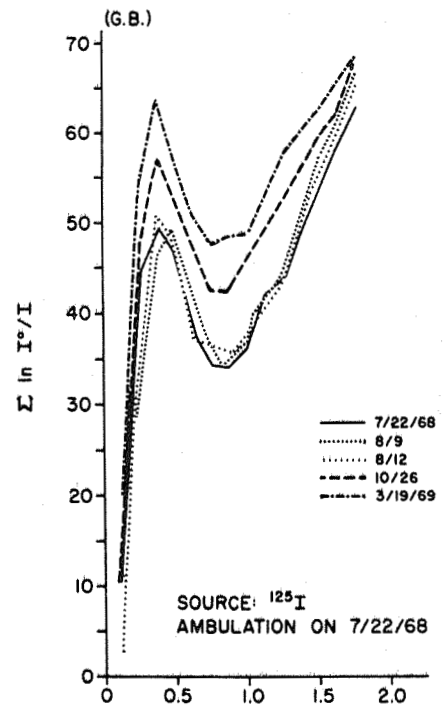

Figure 19.8 Density data obtained on ambulation, subject G.B.

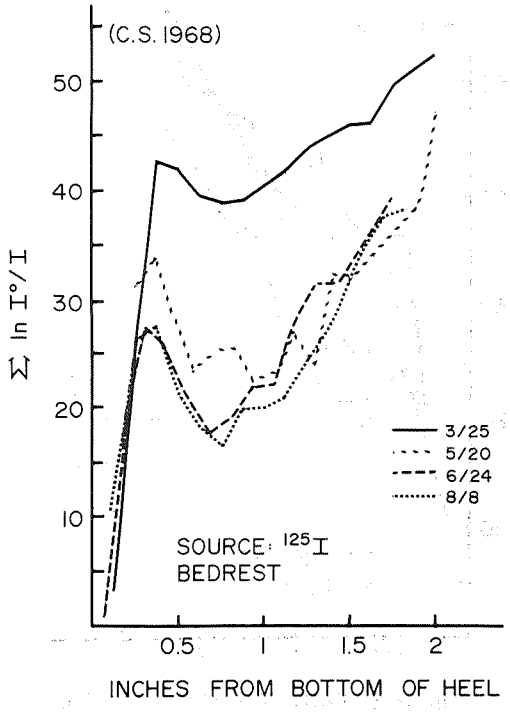

Figure 19.9 Density data obtained during bed rest, subject C. S.

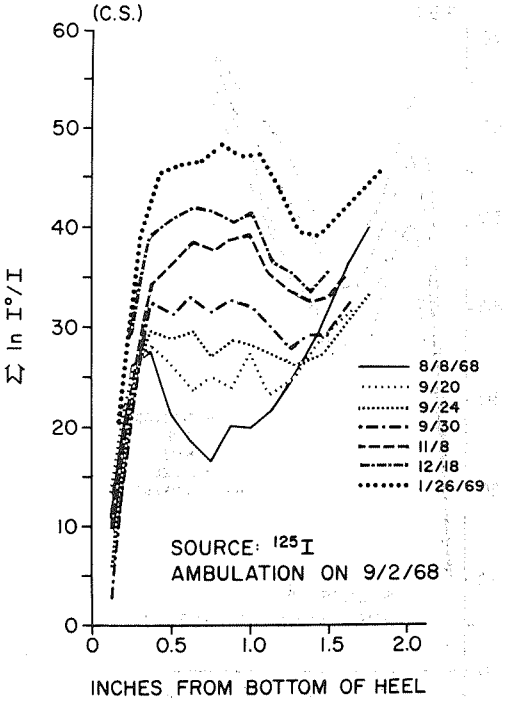

Figure 19.10 Density data obtained on ambulation, subject C. S.

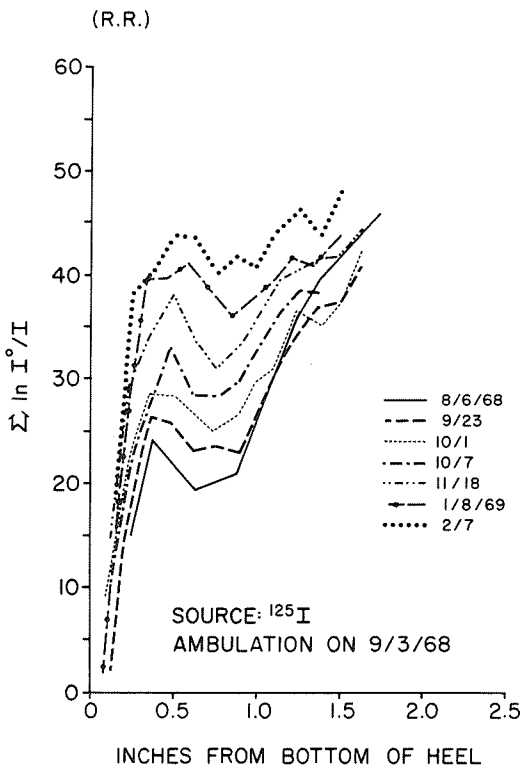

Figure 19.11 Density data obtained on ambulation, subject R.R.

20 THE RELATIONSHIP BETWEEN THE DIURNAL AND MEAL-DRIVEN RHYTHMS OF KIDNEY FUNCTION IN SUBJECTS AT REST*

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INTRODUCTION

This section discusses studies of the relationship between diurnal and meal-driven excretory patterns in the human kidney, and the possible effect on the kidney of the removal of the diurnal "clock." Technical details of the research data are reported in a series of publications (refs. 1–6).

The routine clinical balance studies are based on evaluation of food intake and excretion of metabolites on a daily basis, or on analysis of specimens collected and pooled over periods of several days. The study of the short-term (hour-to-hour) variations in kidney function have been largely neglected, though they may be of significance in assessing the adaptive and regulatory body mechanisms. On earth, the main human activities, such as work and worry, relaxation and sleeping, or food intake, are confined within 24-hr routines. One would therefore expect strong diurnal rhythms (one cycle per day) in most human physiological and metabolic parameters. One also would expect an immediate metabolic transient following a meal or an exercise.

To quantitate the effect of meals on kidney periodic function, for example, one must dissociate the customary food-intake pattern from the 24-hr cycle. Some researchers conducted their studies in deep mountain caves or went far north, above the polar circle, to escape from the day/night cycle. The studies reported here were designed for assessment of the effect of meals on subjects who remained aware of the time of the day.

Another complication in studies of metabolic rhythms is the unavoidable presence of "random biological and environmental variations" in the collected data. Therefore, it was necessary to collect a large volume of data at short intervals over long periods of time, and to devise special mathematical techniques for extraction of periodic functions from the "noisy records." Bed confinement of the studied subjects and rigidly controlled routines of the Metabolic Ward helped to reduce the interfering variations arising from normal physical activities and the environmental disturbances.

METHODS

Subjects

Study subjects included normal controls and patients paralyzed with high spinal cord injury. All were young adults. Rehabilitated quadriplegic patients were investigated intensely at the beginning of this research. Their pathology, resulting in minimal voluntary activity, permanent

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immobilization, and lack of perception of external stimuli from large parts of their bodies, permitted careful assessment of their renal diurnal rhythms and their response to food intake and body position. The use of indwelling urinary catheters allowed continuous collection and hourly fractionation of urine specimens for analysis. The normal controls were allowed periods of activity outside their beds. However, bed rest and activity periods for the controls, and sleep and wakefulness for the quadriplegics were scheduled without any regard for the 24-hr day. The normal controls were able to learn to void at hourly intervals without much disturbance to their routines. All subjects were studied for periods of 14 to 21 days on a given regime.

Synthetic Living Schedules

To disentangle the effect of various factors controlling the rhythmic kidney functions, it was mandatory to design rigid experimental procedures so that only one controlling factor would be operative. Theoretically, four different approaches were possible to assess the effect of food per se.

1. Total avoidance of food. This approach could not be used for longer periods of time without impairing normal body homeostasis.
2. Continuous feeding as by gastric tube or intravenous infusion. This approach again was impractical. However, a compromise "nibbling schedule" was devised that provided small equal meals every 4 hr day and night. This nibbling schedule had, of course, a 24-hr component.
3. Giving food at regular intervals (e.g., every 7 or 19 hr) unrelated to the 24-hr day. A "19-hr schedule" with large meals allowed clear separation of diurnal cycles from the cycles due to the food intake.
4. A "random frequency" meal schedule, which would eliminate meal-driven frequencies and allow the diurnal rhythms to stand out. The timing between meals (each containing 1/6 of the average daily intake) was set at 30 min minimum and 7-½ hr maximum.

Figure 20.1 shows the rhythmic and random schedules of food intake. The meals were chemically preanalyzed and nutritionally adequate. The total average food intake was identical for each subject maintained on various schedules lasting 14 to 21 days. The drinking water was given either with meals or independently at random. Similarly, an effort was made to schedule, when possible, other daily activities of the subjects, including their nursing care.

Numerical Analysis

The large data volume necessitated automated procedures for collection of hourly urine samples and their analysis for volume, creatinine, total nitrogen, potassium, sodium, and chloride. Computer programs helped to (1) compute an average daily output as a function of the time of day; (2) compute a transient response to a selected stimulus, such as a 19-hr meal; and (3) conduct spectral analyses using autocorrelation techniques. The programs were designed to detect frequencies from 1/8 to 12 cycles per day in steps of 1/8 cycle. The strength of each periodicity was measured by its "power" or amplitude.

RESULTS AND DISCUSSION

Figure 20.2 presents schematically the overall picture of urinary excretion patterns in subjects maintained on nibbling, 19-hr, and random schedules.

Diurnal Rhythms in Kidney Function

The urinary excretion of potassium, sodium, and chloride showed strong diurnal periodicity in all subjects maintained on any of the three food-intake schedules. In the power spectra of the subjects maintained on random schedules, this diurnal periodicity of electrolytes was the only one emerging from the "noise" (fig. 20.2(c)).

The diurnal periodicity of the urinary electrolytes is a well-established fact. It was of great interest, however, to demonstrate its presence in the quadriplegics, who were confined to bed over many months and had maintained only minimal voluntary muscle activity. Furthermore, there was no difference in the strength of the diurnal periodicity between the quadriplegics and the normals when compared as groups, although individual variations in the power strength were prominent. It should be stressed that the normal controls maintained little physical activity.

The meal size influenced the amplitude of these electrolyte rhythms. When the meals were small and more frequent, the diurnal cycles of electrolytes were more pronounced.

The daily cycles of potassium, sodium, and chlorides were well synchronized, being lowest between 2 a.m. and 6 a.m. and highest between 10 a.m. and 1 p.m. The 24-hr ensemble average curves were not symmetrical, rising fast from the minimum to the maximum and then declining slowly from the maximum.

The excretion rate of urinary water, total nitrogen, and creatinine apparently was not driven much by "diurnal clocks." The excretion of these metabolites on a random feeding schedule did not show any distinct periodicity, only a considerable level of "noise."

Meal-Generated Periodicities in Kidney Function

The meal frequency of both the nibbling and the 19-hr schedules was always reflected in the excretion rate of all six parameters examined. The rate of excretion of creatinine, total nitrogen, and water was, therefore, dominated by meal size and frequency.

The rate of creatinine excretion is generally considered to be constant throughout the day. It was evident from these experiments that this assumption is valid only when the meals are small and frequent. A large meal significantly depresses creatinine excretion for about 2 hr, in parallel with the water and nitrogen retention.

Urinary electrolytes, namely potassium, sodium, and chloride, show both the meal-driven and diurnal periodicity. This was most dramatically illustrated by the 19-hr schedule, when the meal-driven periodicity, even in the case of potassium, was sometimes higher than that due to the diurnal clock (fig. 20.3).

Diurnal and Meal-Driven Aldosterone Cycles

Figure 20.4 shows the close relationship between the aldosterone excretion and the ratio of potassium to sodium in urine of a quadriplegic subject on a 19-hr meal schedule. The diurnal cycle in the urinary excretion of aldosterone is a well-established fact, but the presence of another meal-driven cycle in the excretion of this hormone is of great interest.

Effect of Constant Light

In experiments conducted within a 19-hr meal schedule and in constant room light, the power of diurnal potassium excretion was reduced by half. This would confirm the generally accepted hypothesis that the light/darkness cycle synchronizes diurnal cycles.

Noise Distribution in Power Spectra

It was observed that the magnitude of the random fluctuations in urinary function, apparent in the individual hourly data, varied from constituent to constituent and from subject to subject.

As expected, the highest noise level was detected on random schedules. It should be noted that the creatinine noise was more marked at higher frequencies, while in the spectra of sodium and chloride, the noise was concentrated at low frequencies (fig. 20.2). For urinary water, the noise tended to be distributed evenly (fig. 20.5). Noise level also increased during transient periods when subjects changed from one living schedule to another.

The magnitude of this noise component was studied as a possible source of information about the human metabolic regulators. It was found that the group of paraplegic patients exhibited a much higher noise level in the kidney periodic function than the group of normal subjects. This might be considered an indication of greater lability of their homeostatic mechanisms resulting from transection of the spinal cord.

SUMMARY

It is not surprising that the kidney, one of the main excretory organs and regulators of the body composition, should be governed tightly in its activities by the intake of food. What is somewhat puzzling is that certain of its homeostatic mechanisms should apparently follow a day/night cycle. The primary triggering mechanism and the purpose of diurnal cycle for electrolyte excretion is still unknown.

In this study, the diurnal kidney cycle was dwarfed with meal cycles by having the subject consume large meals at *regular* frequencies, uncorrelated with the day/night cycle. In addition to dictating the output rate of water, nitrogen, and creatinine, such large meals apparently also trigger aldosterone excretion and thus influence the electrolyte output. One could, therefore, speculate that in prolonged space journeys conducted without the 24-hr day/night sequence, the periodicity of meals alone will determine all periodic kidney functions. In the terrestrial situation, with a more or less routine pattern of life, the meal input is in step with the diurnal metabolic clocks.

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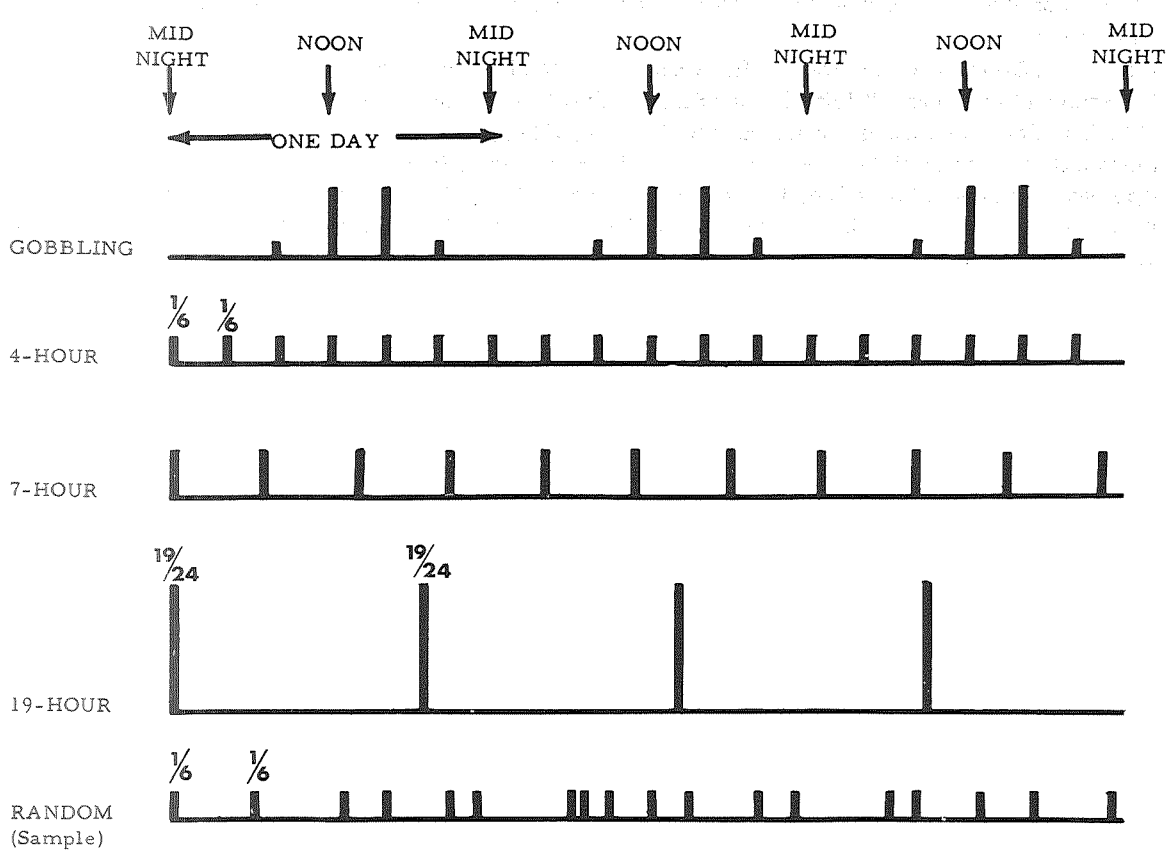

Figure 20.1 Periodic and random meal schedules. The bars indicate meal times and the numbers indicate the meal size as a fraction of average daily food intake. Note that the average daily intake is the same in each schedule. Source: reference 4

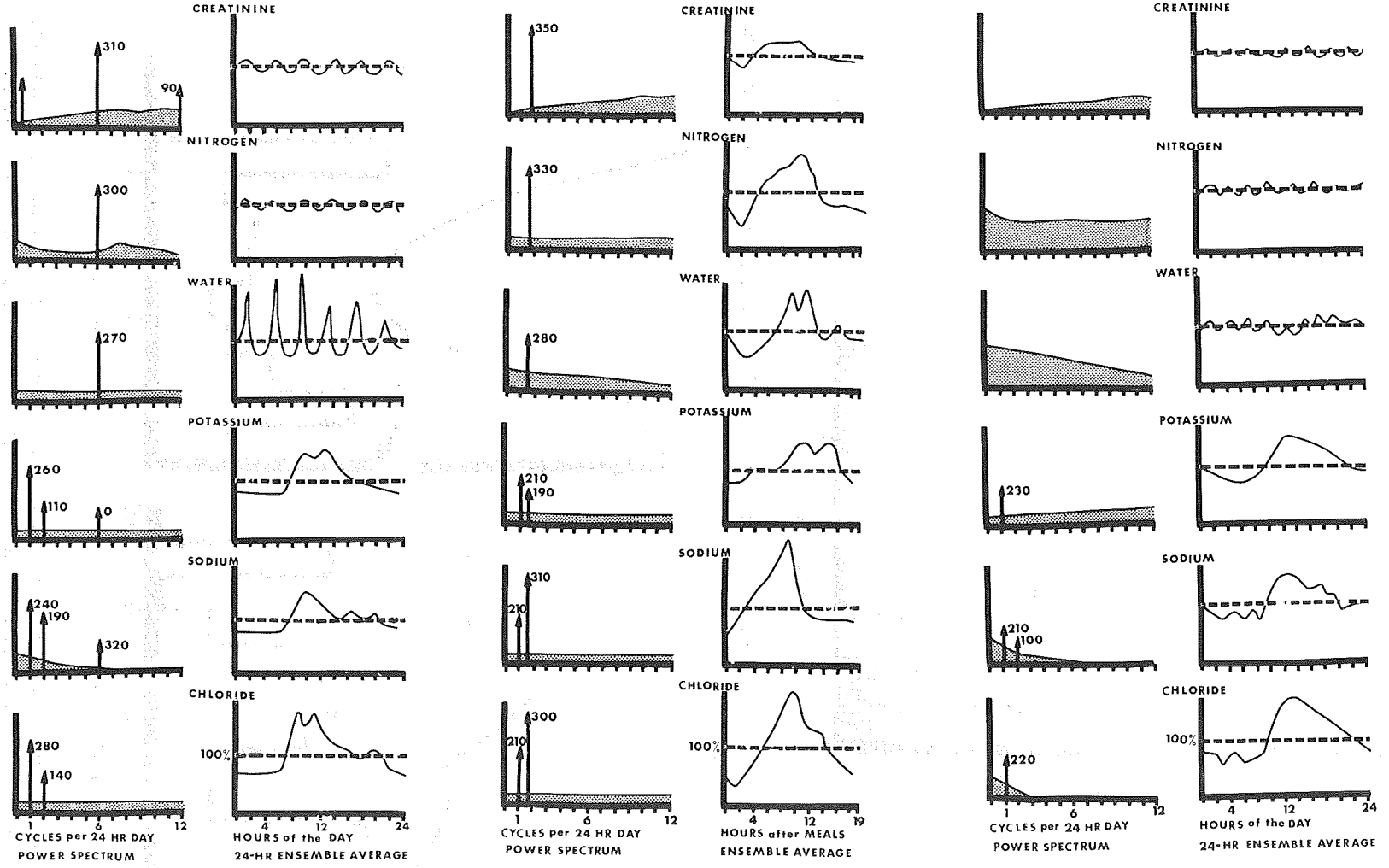

Figure 20.2 Pattern of urinary excretion of metabolites as a function of food intake on (a) a nibbling schedule, (b) a 19-hr schedule, and (c) a random schedule. Power spectra: arrows indicate frequency of periodicities; the arrows' heights show relative strength of periodicities; the numbers above arrows represent time phase shift in degrees from midnight; shaded areas represent "noise" in the data. The 24-hr ensemble average: ordinates are data expressed as percent of the daily mean value

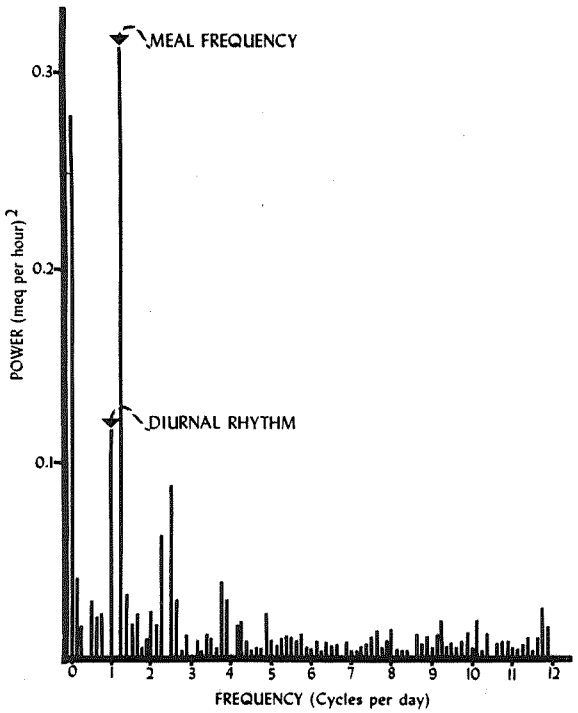

Figure 20.3 Power spectrum of urinary potassium excretion of the quadriplegic on a 19-hr food-intake schedule. Source: reference 6

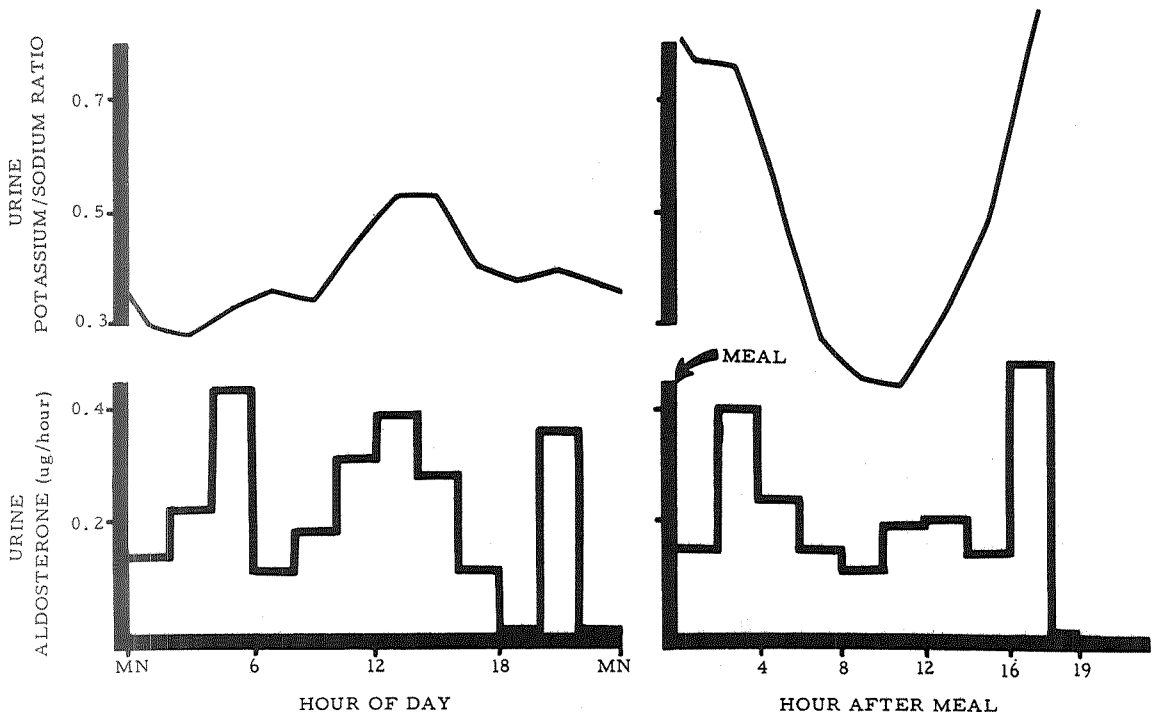

Figure 20.4 Urinary aldosterone excretion by a quadriplegic on a 19-hr meal schedule; average daily pattern and average response following the meal. Note the general similarity between aldosterone level and the potassium-to-sodium ratio content in the urine. Source: reference 6

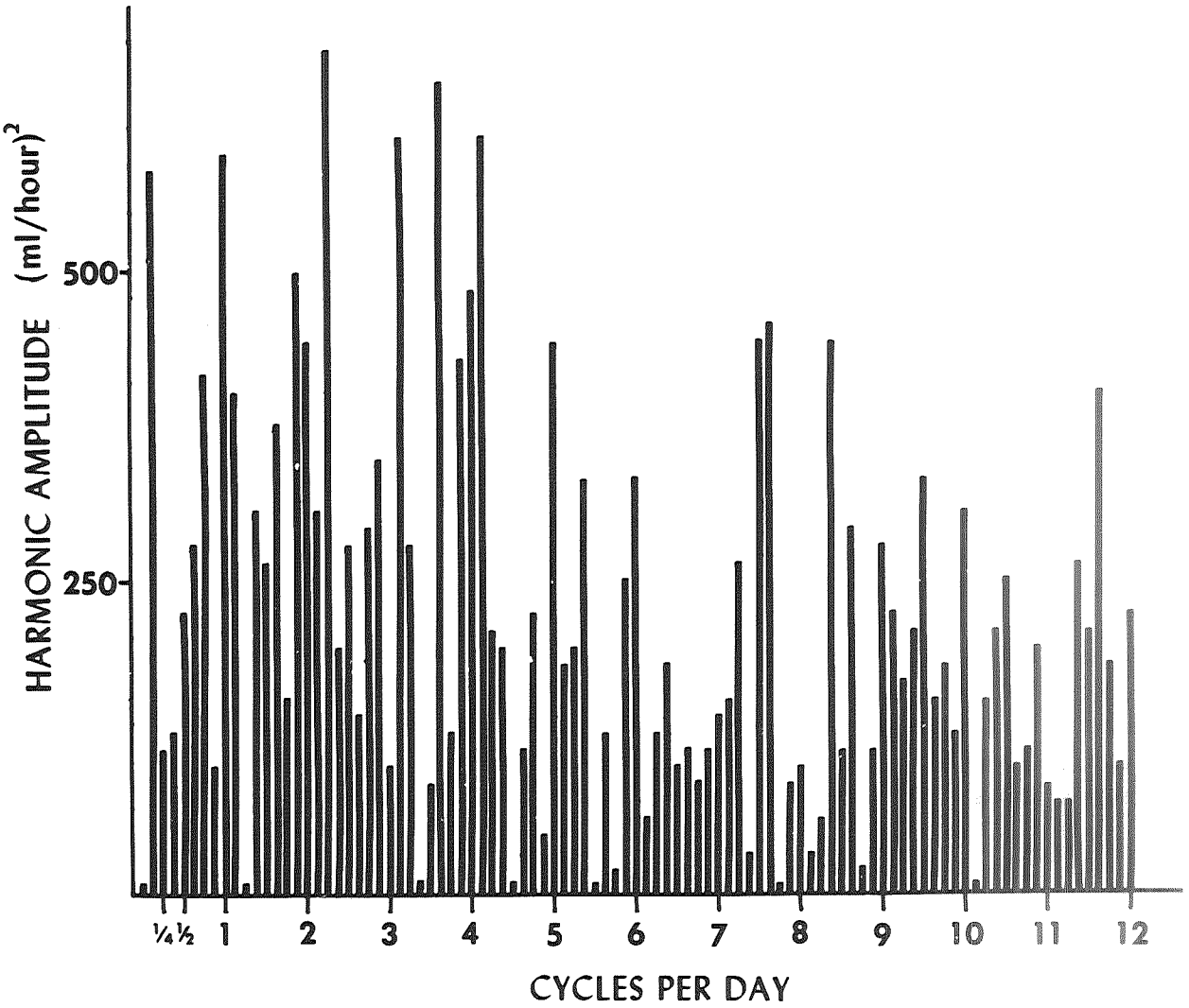

Figure 20.5 Power spectrum of urine water excretion in a subject on a random meal schedule. There is an absence of any predominant periodicity. The wide band of all periodicities represents the "noise" in the urinary output. Source: reference 4

21 EFFECTS OF TWO WEEKS OF BED REST ON CARBOHYDRATE METABOLISM

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INTRODUCTION

The effects of bed rest and physical inactivity on carbohydrate metabolism, as reflected in the glucose tolerance test, has been examined in the past by several investigators. Blotner studied 70 nondiabetic adults and 16 children who had been confined to bed for periods ranging from 1 month to 8 years (ref. 1). The level of blood glucose following a 100-g oral glucose load was found to rise excessively in 63 of the adults and 11 of the children studied. Further, those who had been confined for the longest periods tended to have the most abnormal curves. More recently, in a controlled study by Lutwak and Whedon, 10 normal young adults placed at complete bed rest for 1 to 3 weeks all showed decreased glucose utilization (ref. 2). Within a week of resumption of a program of physical conditioning, the utilization of glucose had returned to baseline levels. Naughton and Wulff (ref. 3) have found that sedentary men respond to a glucose load with significantly higher levels of insulin than do active persons but that the disappearance of glucose is the same in both groups—that is, sedentary individuals seem to require more insulin to handle the same amount of glucose as an active group.

In view of the apparent inefficient handling of glucose during bed rest and inactivity, a carefully controlled study was conducted to investigate the nature of the carbohydrate intolerance observed during bed rest and some of the factors that might produce such intolerance.

METHOD

Seven healthy volunteer students, all in their early twenties, were placed under strict dietary control and supervised activity for a period of four weeks. During this time they were fed and housed on the Clinical Research Unit of the University of Pittsburgh. Total caloric intake and its distribution among foodstuffs were kept constant throughout the experiment. The first and fourth weeks of the study served as control and recovery periods, respectively, during which the subjects were allowed ad lib activity, including a number of hours of rather vigorous exercise. Subjects varied somewhat in the degree to which they engaged in the levels of activity, but this made no apparent difference in the results. The second and third weeks comprised the period of enforced bed rest, during which the subjects conducted all activity with their shoulders no higher than their feet.

On the fifth day of the control and recovery periods and on the tenth day of the bed rest period, the subjects received a standard oral 70-g glucose load. Blood was drawn at appropriate intervals for the measurement of blood glucose and immunoreactive insulin.

RESULTS

All seven subjects showed an elevated peak glucose level and an increased area under the 4-hr glucose curve during the bed rest period as compared with the control period (fig. 21.1). Insulin values measured on the same samples are shown in figure 21.2. Insulin responses to glucose administration were markedly augmented during bed rest in all subjects. The amount of insulin secreted per mg glucose was increased, as indicated by calculated insulin-to-glucose ratios, which more than doubled during bed rest. This would seem to indicate a relative ineffectiveness of insulin in lowering the blood sugar during prolonged recumbency.

As a second well-documented stimulus to insulin release, the infusion of arginine was carried out in each test period on the day following the glucose tolerance test. A total dose of 30 g in 500 ml of water was given intravenously over a 20-min period. Again, glucose and immunoreactive insulin were measured. A small transient rise in blood glucose, followed by a slight decrease, was observed in all subjects (fig. 21.3), but no significant differences were observed among mean values during the control, bed rest, and recovery periods. However, the release of insulin in response to arginine was augmented during bed rest, as it had been in response to glucose administration (fig. 21.4). Once again, there appears to be a relative ineffectiveness of insulin in lowering blood glucose levels during recumbency.

A third stimulus to insulin release, an infusion of tolbutamide, was administered to three of the subjects. Again, a marked increase in insulin values was seen during the bed rest period when contrasted with the control and recovery periods (fig. 21.5). The degree of hypoglycemia induced by tolbutamide was not significantly affected, despite the rise in insulin secretion.

DISCUSSION

The insulin response to each of three different stimuli was augmented during bed rest in all subjects tested. Some inquiry into the mechanism responsible for the apparent decrease in the efficacy of insulin to lower glucose levels would seem to be in order. A number of substances are known to diminish the effectiveness of insulin in lowering blood sugar. Growth hormone is one of these substances.

Floyd et al. (ref. 4) have shown that either glucose administration or the infusion of arginine results in a rise in immunoreactive growth hormone (HGH) independent of their respective actions in provoking insulin release. Thus, one might inquire whether augmented release of HGH during bed rest could be responsible for the observed decline in insulin effectiveness. The normal response of HGH to glucose administration is an early suppression followed by a late rise as glucose levels decline. HGH was measured on plasma samples in all subjects throughout the glucose tolerance test. The usual late rise in HGH levels was not augmented; in fact, it was suppressed. The HGH response to arginine was examined in similar fashion. The responses of HGH to arginine infusion are variable in men, so that interpretation of individual subject test responses is difficult. Nevertheless, if anything, HGH response to arginine was suppressed by bed rest. Thus, it was demonstrated that HGH responsiveness to glucose or arginine stimulation is inhibited during bed rest and therefore cannot be responsible for the observed decrease in insulin effectiveness.

Cortisol is another well-known insulin antagonist. No significant differences were found in measured cortisol levels in the control blood samples taken before glucose and arginine administration in each test period. This finding is consistent with the data of Cardus et al. (ref. 5), whose measurements of diurnal glucocorticoid levels during bed rest are no different from controls. Thus, cortisol does not appear to be responsible for the observed decrease in insulin effectiveness. Provocative stimulation of cortisol release during bed rest has recently been attempted by others.

Elevated levels of catecholamines have been associated with glucose intolerance. Porte et al. (ref. 6) have demonstrated, however, that hyperglycemia seen with catechol excretion is associated with decreased circulating immunoreactive insulin. Furthermore, during a study of venomotor responsiveness as a function of bed rest, reported elsewhere in this symposium, we have been able to demonstrate a decreased store of catechols as a function of bed rest. Thus, catecholamines do not appear to be responsible for the observed intolerance to glucose.

Still another possible insulin antagonist is an increase in the circulating level of free fatty acids. Measurement of free fatty acids in this study has consistently shown a decline during bed rest. Glucagon and prolactin have also been shown to cause an apparent decrease in the ability of insulin to decrease glucose levels but have not yet been investigated in the bed rest setting. Thus, the factor responsible for the observed insulin ineffectiveness remains unknown.

It is interesting to note the close relationship of the present observations to conditions observed in obese persons. Hyperinsulinemia during glucose tolerance testing, arginine administration, and tolbutamide infusion, along with diminished HGH responsiveness, have all been observed in obesity (ref. 7). Bloom and Eidex (ref. 8) recently demonstrated experimentally what has been known empirically for centuries: obese individuals are significantly less active than lean individuals. Thus, inactivity may account for some or all of the observed effects of obesity on insulin-glucose interaction.

Spaceflight, with its attendant absence of a gravity field and enforced inactivity, would be expected to induce relative and transient glucose intolerance. Major Donald Eisele, for example, manifested an abnormal response to oral glucose on return from his Apollo flight. Sequential observation should confirm whether this response was transient as expected, or whether it represents a true prediabetic state. Further observations on astronauts are advisable, both on theoretical grounds and for practical considerations. Dismissal from the program for an abnormal test of glucose tolerance, probably a normal physiologic consequence of spaceflight, would be an unconscionable waste of manpower.

Finally, one clinical point should be made. Tests of glucose tolerance too often are done on hospitalized patients as an afterthought near the conclusion of a prolonged hospitalization. Worse yet, they may be performed following diagnostic and therapeutic procedures requiring caloric deprivation. Conclusions drawn from the revelation of an abnormal test should be guarded, and no such patient can be diagnosed as diabetic without more substantive evidence. Lebovitz et al. (ref. 9) recently reported glucose intolerance in patients during recovery from myocardial infarction. By definition, such patients are kept at enforced bed rest. The responses observed in such patients might well be attributable to bed rest alone.

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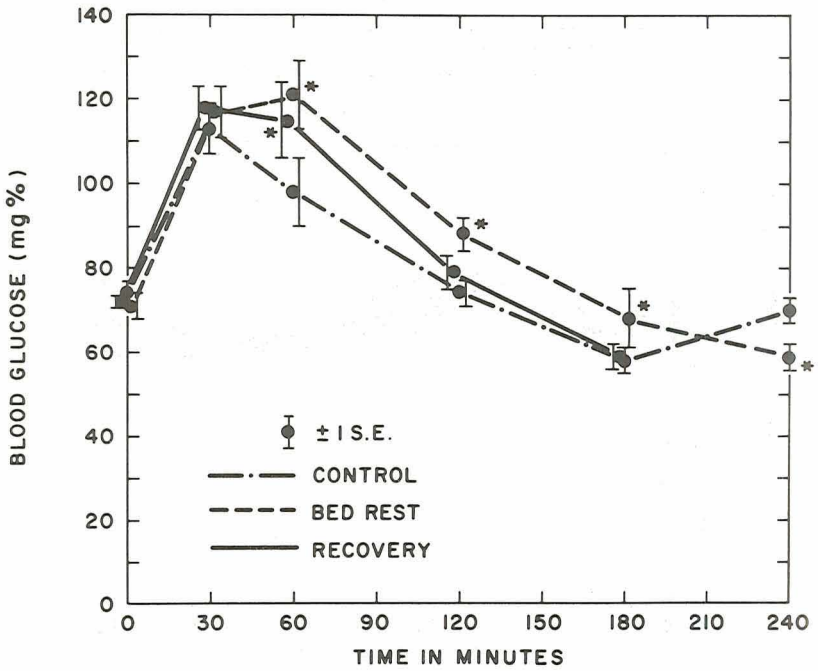

Figure 21.1 Mean glucose response to glucose tolerance test following bed rest. Asterisks indicate values that are significantly different ($p < 0.05$) from control

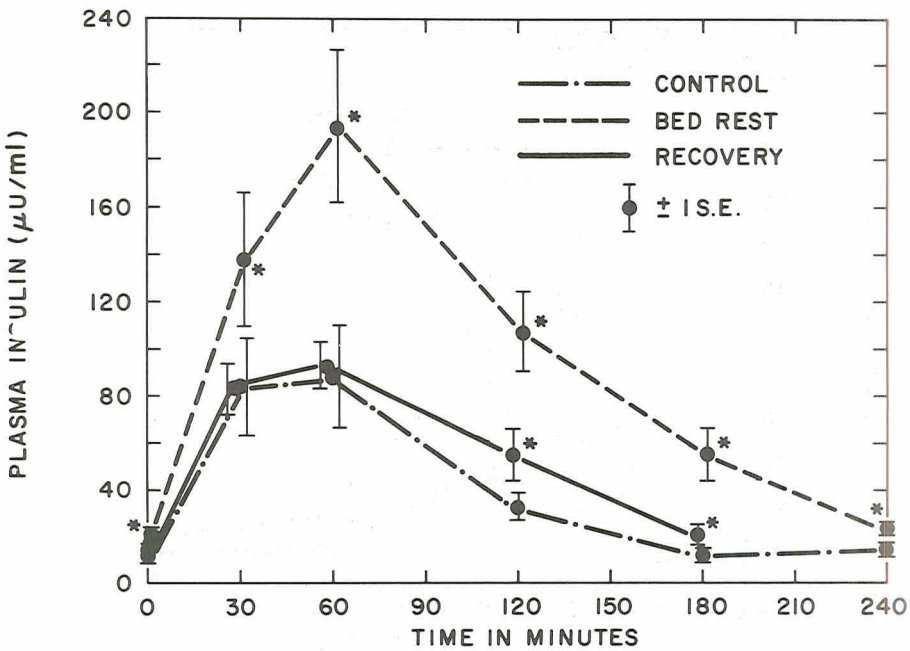

Figure 21.2 Mean insulin response to glucose tolerance test following bed rest. Asterisks indicate values significantly different from control. Levels during bed rest are highly significant ($p < 0.01$ or < 0.001); recovery values are near control but still significantly elevated ($p < 0.05$)

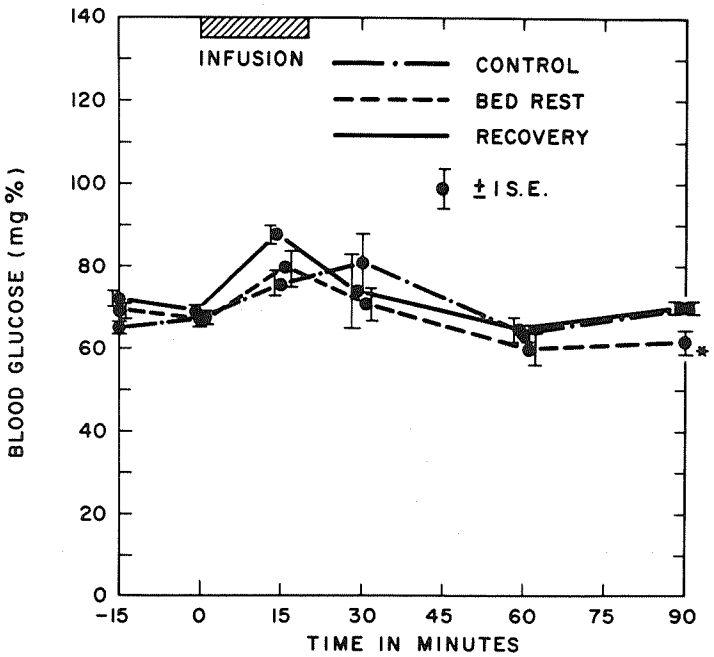

Figure 21.3 Mean glucose response to argininine infusion (30 g) following bed rest. The small transient rise followed by a decline in blood sugar is normal. The 90-min glucose value during bed rest is barely significant ($p < 0.05$)

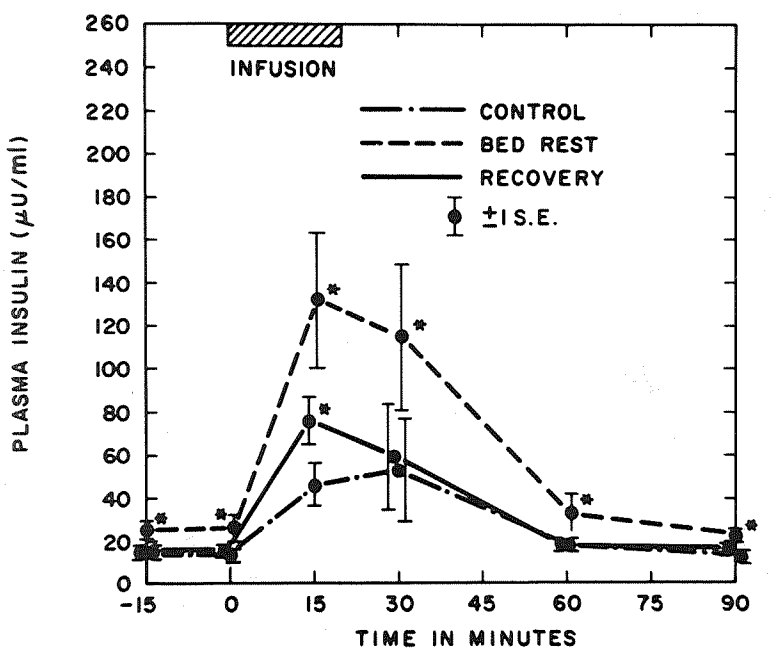

Figure 21.4 Mean insulin response to arginine infusion (30 g) following bed rest. Insulin values are significantly elevated at all times during infusion, and are highly significant at 15 and 30 min ($p < 0.001$). The value at 15 min remains significantly elevated following 6 days of recovery

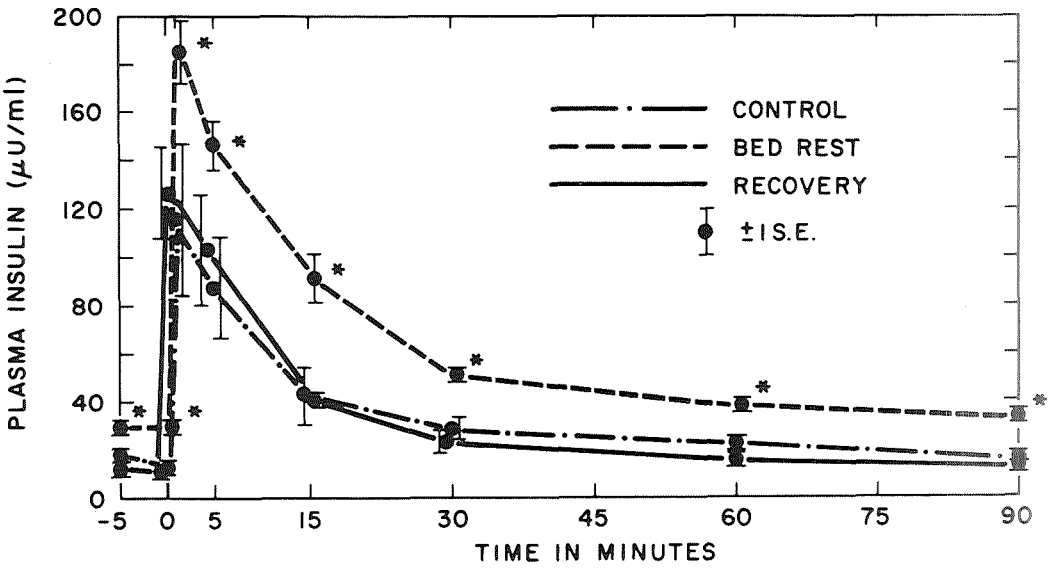

Figure 21.5 Mean insulin response to tolbutamide infusion (1 g/min) following bed rest. Insulin values at 1, 5, 15, and 30 min following infusion are highly significantly different ($p < 0.001$) during bed rest as opposed to control or recovery periods.

22 THE EFFECT OF BED REST ON GLUCOSE REGULATION IN MAN: STUDIES IN PROGRESS

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INTRODUCTION

Humans maintained at bed rest uniformally exhibit impaired oral (refs. 1, 2) and intravenous (L. Lutwak, personal communication) glucose tolerance; the cause for this alteration in glucose balance remains a matter of conjecture. Pawlson et al. (ref. 2) reported an increased insulin response to glucose loading despite abnormal glucose tolerance after 12 days of bed rest, and suggested that an inhibition of peripheral glucose utilization might be responsible for this response. These authors also noted a decreased growth hormone response to arginine infusion in the same subjects, suggesting that bed rest may influence other glucoregulatory hormones. Since the disposition of glucose loads is a complex physiological event invoking many related processes, it is possible that the glucose intolerance at bed rest represents an imbalance of one or more of these processes.

This section presents data from two bed rest studies in which isolated parameters of glucose balance during bed rest are under investigation. In the first study, forearm glucose uptake during glucose loading is measured; in the second study, the effect of an intracellular hypoglycemic agent (2 deoxy-D-glucose) on glucoregulatory hormones is examined. These studies are being performed in collaboration with Capt. Richard Lipman and Dr. Frode Ulvedal, School of Aerospace Medicine, Brooks AFB, Texas; and Lt. Col. Edwin Bradley, Wilford Hall USAF Hospital, Lackland AFB, Texas.

FOREARM GLUCOSE UPTAKE IN SUBJECTS AT BED REST

This study was designed to determine the quantitative significance of peripheral glucose uptake in subjects maintained at bed rest for 14 days. Forearm arteriovenous glucose differences and forearm blood flows were measured during the administration of intravenous glucose loads before, during, and 7 days after the bed rest period.

Methods and Procedures

Five healthy young male volunteers have been studied to date. All were within 10 percent of ideal weight and had normal oral glucose tolerance tests. They were fed a constant 2600-cal diet of which 360 g were carbohydrate. The study lasted 5 weeks comprising a 2-week control, a 2-week bed rest, and a 1-week recovery. Activity was ad lib during control and recovery. Glucose infusions were performed once during the control, on day 14 of bed rest, and on day 7 of recovery.

Glucose infusions were performed 14 hr postabsorptive. Figure 22.1 shows the instrumentation used during the infusions. Arterial blood was obtained through a Cournand needle from the right brachial artery; specimens were withdrawn 15 min before and immediately prior to the infusion and then every 20 min during the 180-min glucose infusion. Venous blood glucose levels were

monitored continuously from an uninterrupted stream of blood (0.18ml/min) introduced into a Technicon Autoanalyzer circuit from an indwelling catheter in a deep left brachial vein. The lag time between aspiration of blood and readout of venous glucose concentrations was 6 min. Glucose was infused as a 20 percent solution through a variable-speed infusion pump into the right brachial vein. Left forearm blood flows were measured at the time of each arterial sampling using Whitney mercury-in-silastic strain-gage venous occlusion plethysmography. Hand flow was occluded during the forearm blood flow measurements.

Arterial and venous whole-blood glucose concentrations were determined by the potassium ferrocyanide-potassium ferricyanide method on a Technicon Autoanalyzer (ref. 3). Peripheral glucose uptake was calculated by multiplying forearm blood flow (ml/100 ml muscle tissue/min) by the arteriovenous glucose difference (mg/100 ml whole blood) and is expressed as mg glucose/ 100 ml muscle/min.

Results

Venous Whole-Blood Glucose Curves In designing this study it was decided that the amount of glucose infused during the bed rest and recovery periods would be adjusted so that the venous whole-blood glucose curves would duplicate the control curve as closely as possible. The infusion rate of the glucose solution during the control period was 15 mg/kg body weight/min. Constant monitoring of venous glucose curves during subsequent infusions and frequent adjustments of the infusion rates permitted duplication of the control glucose curve for each subject during bed rest and recovery studies. The mean venous glucose curves for the five subjects are shown in figure 22.2. There are significant differences between the control values and the bed rest and recovery glucose concentrations at −15 and 0 min preinfusion and during the first several minutes of infusion; however, the mean curves prescribed during the remainder of the infusion are not significantly different.

Table 22.1 lists the mean amount of glucose required for each of the three infusions. It is expressed as net glucose load and was calculated by subtracting the amount of urinary glucose excreted during the 180-min infusion from total glucose infused. The urinary glucose loss was similar during the three periods. As anticipated, much less glucose was required to reproduce the control glucose curve during the bed rest period (80 g vs. 156 g). The mean net glucose load utilized during the recovery infusion was also significantly lower than the control glucose load (104 g).

Peripheral Glucose Uptake There were no significant differences in arteriovenous glucose differences, blood flows, or forearm glucose uptake in the preinfusion data when comparing the control, bed rest, and recovery periods. However, there were marked differences in the data obtained during the glucose infusion. The mean data for the five subjects are listed in table 22.2. Mean arteriovenous glucose differences during bed rest were decreased by more than 50 percent of control. Although mean forearm blood flow was slightly higher during bed rest than during control, peripheral glucose uptake at bed rest was approximately one-half that found during the control period. The mean data found during the recovery period indicate that glucose balance had not recovered during the 7 days of postbed rest activity.

Conclusions

The data from this incomplete study suggest that the glucose imbalance described in humans maintained at bed rest can be attributed, at least in part, to a decrease in peripheral glucose uptake. Although not reported here, serum immunoreactive insulin levels were obtained in these subjects, and no difference in insulin response is apparent when comparing the control to the bed rest and recovery infusion results. Moreover, the change in peripheral glucose uptake is not related to blood flow, since forearm blood flow was actually greater during the bed rest studies than during the control. We postulate, therefore, that the alteration in glucose metabolism that occurs at bed rest reflects changes in cellular glucose kinetics independent of normal control mechanisms.

RESPONSE TO 2 DEOXY-D-GLUCOSE (2DG) IN SUBJECTS AT BED REST

Pawlson et al. (ref. 2) reported that arginine infusion in subjects at bed rest did not provoke the expected rise in immunoreactive growth hormone. Whether this represents an actual bed rest-induced change in pituitary function or a unique change in responsiveness to arginine in subjects at bed rest has not been established. This section gives the results of a study in progress in which, to date, four subjects were infused with 2DG before, during, and after 14 days of bed rest. We chose 2DG infusion as the stress test because it normally induces a wide variety of responses in gluco-regulatory hormones and energy substrates (fig. 22.3).

Methods and Procedure

The four subjects studied met the same criteria and were maintained on a schedule similar to that described above. The first 5 weeks were designated as control, and the subjects were infused weekly with 2DG. This was followed by 14 days of absolute bed rest and then 10 days of recovery during which time ad lib activity was again permitted. The 2DG infusions were carried out on day 14 of bed rest and day 10 of recovery. The 2DG was prepared as a 20 percent solution, passed through a 45 millipore filter, and stored at 4° C. Samples of the solution were cultured on appropriate media regularly to check for bacterial contamination.

Each 2DG infusion was performed at 8 a.m., at which time the subjects were 14 hr postabsorptive. We placed 50 mg/kg body weight of the 2DG solution in a sterile bottle and made up to 100 ml with sterile saline. The mixture was infused at constant rate over a 30-min period through a catheter placed in a brachial vein. Blood specimens were drawn 15 min before and immediately prior to the infusion, then at 60, 105, and 150 min after the start of the 2DG infusion. Blood samples were stored in ice until the end of the study, then immediately separated in a refrigerated centrifuge, and kept frozen at –15° C until analyzed. Plasma glucose was determined on the Autoanalyzer by the potassium ferrocyanide/potassium ferricyanide method (ref. 3), and plasma-free fatty acids were measured by the technique of Dole (ref. 4). Plasma cortisol levels were determined by a modified fluorometric method (ref. 5). Serum immunoreactive insulins were assayed by the Morgan and Lazarow technique (ref. 6) and growth hormone by the method of Lau et al. (ref. 7). Urine was collected for 6 hr starting at the onset of the infusion, and for the same period of time preceding the infusion, for determination of epinephrine and norepinephrine, which were measured according to the method of von Euler and Lishajko (ref. 8). Urines were preserved in hydrochloric acid and refrigerated until the following day, at which time the analysis was performed.

Results

Each subject was infused with 2DG on seven separate occasions (5 controls, 1 bed rest, and 1 recovery), and the only reaction was one transient episode of supraventricular tachycardia and several instances of mild hypoglycemic symptoms.

Response of Glucose and Free Fatty Acid There were no significant differences in the mean glucose or free fatty acid responses to 2DG infusion when comparing control, bed rest, and recovery results (fig. 22.4). In this study and that reported above, fasting glucose values were higher during bed rest than during control, for every subject tested, by day 14 of bed rest.

Response of Plasma Cortisol Also shown graphically in figure 22.4 are the mean results of plasma cortisol response to 2DG infusion. Although control and recovery cortisol responses to 2DG infusion were similar, the response during bed rest was blunted. The data are preliminary, but mean plasma cortisol response to 2DG infusion during bed rest is significantly lower at 105 min than that found during the control infusions.

Response of Serum Growth Hormone Similarly, mean serum growth hormone responses to 2DG infusion were lower during bed rest than during control and recovery (table 22.3). These differences were significant at 60 and 105 min following the onset of 2DG infusion. Mean recovery infusion values were intermediate between control and bed rest and not significantly different.

Response of Insulin, Epinephrine, and Norepinephrine The data are not given since they are preliminary and more observation will be necessary to determine significance. However, it appears that serum insulin response to 2DG infusion is unchanged by bed rest. Unprovoked urinary epinephrine excretion is similar during bed rest and control; however, epinephrine excretion following 2DG appears to be less than the response found during the control period. Conversely, basal norepinephrine excretion is lower during bed rest than during control, but the response to 2DG is similar in the two test periods.

Conclusions

These data confirm and amplify the observations of Pawlson et al. (ref. 2) that the hypodynamic condition imposed by bed rest decreases pituitary growth hormone responsiveness. Since different provocative agents for human growth hormone release were used in these studies (2DG vs arginine), we suggest that nonresponsiveness to the test agent used is unlikely. Moreover, the decreased cortisol response indicates that other hypothalmic or pituitary dysfunction may be present during bed rest. However, the data are not sufficient to indicate whether the decreased cortisol response is primarily adrenal or central (hypothalmic/pituitary) in orgin. Despite the alterations in glucoregulatory hormone response to 2DG, blood glucose rises were appropriate. This may reflect the decreased peripheral glucose utilization rate documented above.

SUMMARY

Significant alterations in both peripheral glucose utilization during glucose loading and glucoregulatory hormone response to interacellular glucopenia are induced by simple absolute bed rest. Whether these changes represent a homeostatic adaption to bed rest or have pathophysiological significance cannot be concluded from these data. However, these findings have significance for both clinical medicine and for the assessment of human response to the effects of prolonged spaceflight.

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Table 22.1 Glucose loads needed to reproduce venous glucose curves

Period	*Net Glucose Loads, g*	*No. of Subjects*
Control	155.5± 7.1*	5
Bed Rest	80.1± 9.1	5
Recovery	103.8±10.5	5

* ±SEM

Table 22.2 Peripheral glucose uptake

	Ateriovenous Glucose Difference, mg glucose/100 ml blood	*Forearm Blood Flow, ml blood/100 ml muscle/min*	*Peripheral Glucose Uptake, mg glucose/100 ml muscle/min*
Control	50.2	2.06	1.03
Bed Rest	19.5	2.70	0.53
Recovery	26.8	1.88	0.50

Table 22.3 Serum immunoreactive growth hormone response to 2DG stimulation serum immunoreactive growth hormone, ng/ml

Time, min	*Control*	*Bedrest*	*Recovery*
0	1.0	0.5	0.5
60	10.2	2.2	9.0
105	14.0	4.2	8.0
150	7.0	3.8	3.8

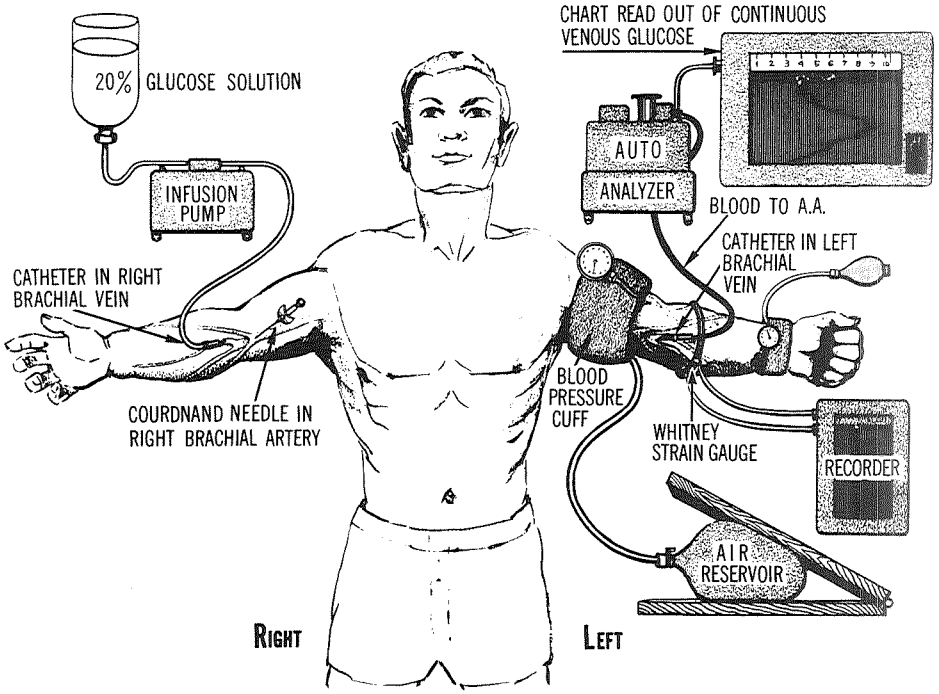

Figure 22.1 Technique for measuring forearm glucose uptake during glucose loading

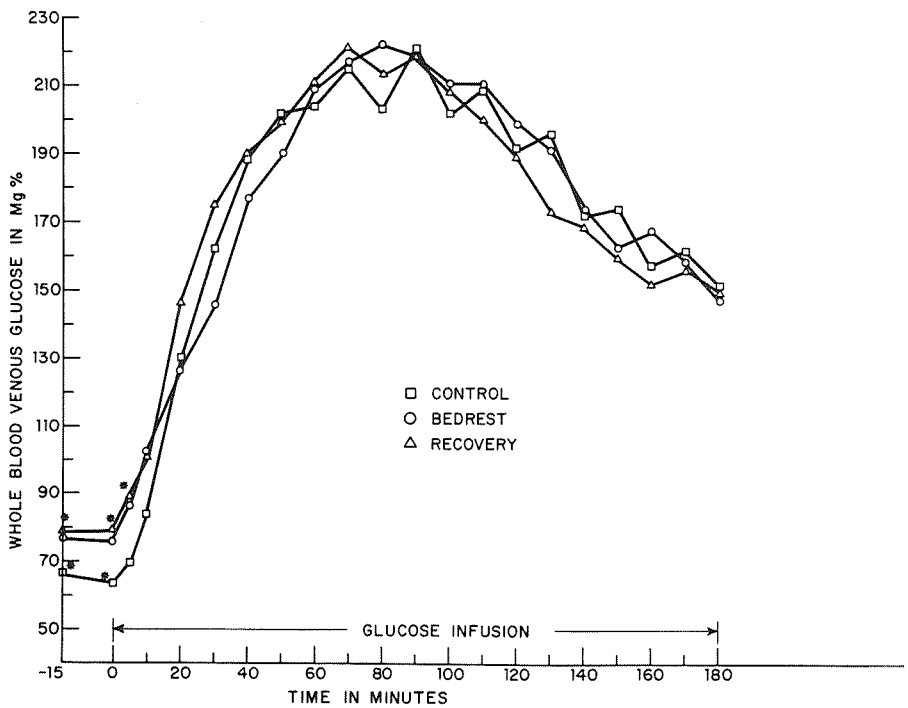

Figure 22.2 Mean glucose curves during glucose infusion

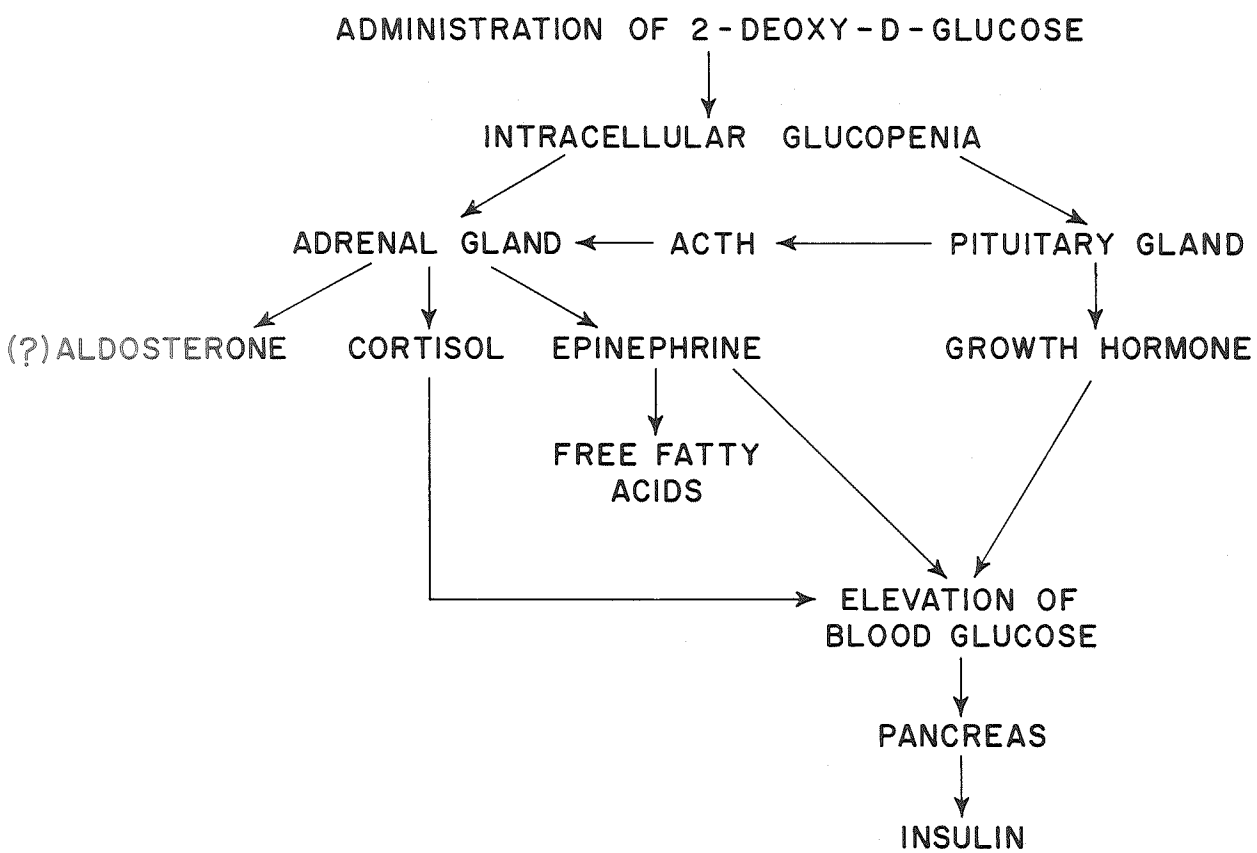

Figure 22.3 Response to 2 deoxy-D-glucose infusion

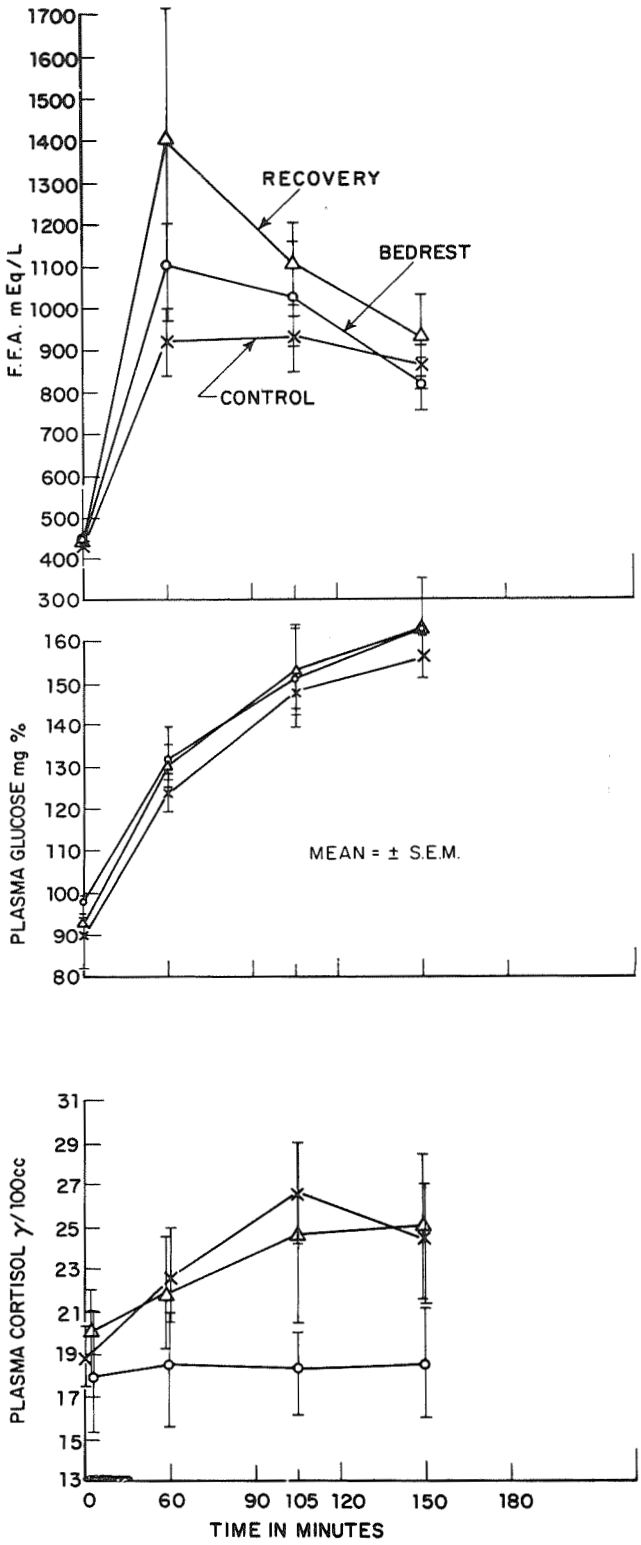

Figure 22.4 Mean plasma-free fatty acid (FFA) glucose and cortisol response to 2 deoxy-D-glucose infusion

23 HEMATOLOGIC ASPECTS OF BED REST

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INTRODUCTION

Data gathered from Gemini missions 4, 5, and 7 (ref. 1) demonstrated some loss of red cell mass (RCM) in all crewmen, in some cases to a rather surprising degree. Red blood cell ^{51}Cr survival studies in some instances also demonstrated shortening, suggesting increased fragility of the red cell. In the first two Apollo flights, only one of the six crewmen showed a significant change in RCM, while two of the three astronauts in the third Apollo flight had some degree of RCM decrease.

Ground-based studies in 100 percent oxygen environment at 5 psi have not produced erythrokinetic alterations in normal subjects (ref. 2). In 1945, Taylor et al. (ref. 3) described a loss of blood and plasma volume and alterations in cardiovascular dynamics after 3 weeks of simple bed rest in normal healthy volunteers. In 1948, Deitrick et al. (ref. 4) confirmed and extended these studies to include a loss of muscle mass, a slight decrease in basal metabolic rate, and negative mineral balances. At the School of Aerospace Medicine in 1964, Miller et al. (ref. 5), in a 4-week bed rest study, observed an approximate 8 percent loss of RCM in addition to the loss of plasma volume. The RCM values were obtained from radioiodinated serum albumin determinations of plasma volume and, therefore, are indirect measurements. Their significance is limited by the uncertainty of the relationship between body and venous hematocrits when the steady state is perturbed. This section reports on studies by Dr. Bernard Morse (ref. 6) in our laboratory, in which the RCM loss that occurs with bed rest was documented by means of direct red cell label.

MATERIALS AND METHODS

Twenty-one healthy male Air Force volunteers participated in the bed rest studies. The experimental period consisted of a 20-day adjustment phase, 35 days of continuous absolute bed rest, and a 20-day recovery phase. During bed rest, the subjects were allowed to lean on one elbow for meals and to sit up for one bowel movement daily. Throughout the adjustment and recovery phases, the subjects were encouraged to participate in occupational therapy, walks, and programed exercise.

In addition, 8 healthy male Air Force volunteers were used to control such variables as age, blood lettings, seasonal changes, and stability of red cell and plasma volumes.

All blood was obtained by venipuncture at 0730 hours in the fasting state. Standard laboratory procedures were used for hemoglobin, microhematocrit, and serum bilirubin. Red cell counts were performed with the Colter Model B counter, and reticulocytes were estimated from peripheral blood smears, supravitally stained with new methylene blue. Serum iron was measured by the automated method of Zak and Epstein (ref. 7), and autohemolysis was performed according to the method of Selwin and Dacie (ref. 8). Saline-washed, ^{51}Cr-labeled, autologous erythrocytes were used for RCM

determinations and red cell half-life estimates. The least-squares method was used to estimate a straightline best fit relating the natural log of radiochromium activity to the time in days. The red cell half-survival ($t^{1/2}$) was calculated from –0.693/slope and corrected for changes in RCM from the change in total circulating erythrocyte-bound ^{51}Cr during the survival study. The RCM determinations were performed in all subjects at the beginning of the adjustment and bed rest phases and after completion of bed rest and recovery. Seventeen of the 21 subjects had an additional RCM determination on day 24 of bed rest. Plasma volumes were derived indirectly from the RCM using the corrected microhematocrit. Ferrokinetic studies were performed on 8 subjects according to the method of Huff (ref. 9) using $^{59}FeCl_3$ incubated with autologous plasma, with serial samples collected over 3 hr and 15 additional samples collected over the next 30 days. Erythropoietin assays were performed on 24- and 72-hr urinary outputs, dialyzed against high molecular weight Carbowax and concentrated 50- to 100-fold. The hypoxic mouse was then utilized as the assay animal. All data have been graphed as a mean ±SE against the time axis. Probability values were derived with the analysis-of-variants technique, each subject serving as his own control.

RESULTS

The RCM decreased during bed rest in 18 of the 21 subjects with an average loss of 140 ml, or 1.6 ml/kg, representing approximately 8 percent of the RCM (fig. 23.1). The loss was gradual and significant (control vs. day 22 of bed rest, $p = 0.025$; control vs. day 35 of bed rest, $p = 0.001$). Three weeks after the completion of bed rest, the RCM remained significantly below the prebed rest determinations.

Plasma volumes determined by indirect estimation of RCM and corrected microhematocrit decreased some 400 ml during bed rest, and plasma volumes determined at the end of recovery by a direct method showed values well above those obtained prior to bed rest (fig. 23.1).

Serial hematocrit values increased from 44 to 47 percent during the first 2 days of bed rest (fig. 23.1). Thereafter, a near steady state was achieved and the hematocrit remained relatively stable, although decreasing slightly throughout the remainder of bed rest. There was a rapid decrease in hematocrit from approximately 46 to near 40 percent when ambulation was resumed. Hematocrit values then slowly increased over the next 3 weeks but never quite returned to the prebed rest values. Reticulocyte counts demonstrated a slight drop in reticulocyte percent during bed rest from a mean control value of 1.15 to 0.9 percent by day 22 of bed rest (fig. 23.1). During the ambulatory phase, a definite reticulocytosis around 1.6 percent developed during the third week of postbed rest.

Stool tests for occult blood were consistently negative. Serum bilirubin and red cell autohemolysis were unaltered during bed rest while, at the same time, the ^{51}Cr red cell $t^{1/2}$ started on days 1 and 24 of bed rest, respectively, were comparable to the prebed rest values. On reactivation, there appeared to be a shortening of the red cell survival curve; however, the corrected $t^{1/2}$ showed no significant difference between the ambulatory period and the earlier period (table 23.1). Body surface counting with ^{51}Cr appeared to exclude splenic red cell sequestration with the spleen-to-heart ratio remaining below 0.5. Additional evidence against sequestration may be inferred from the complete mixing of labeled cells in the circulation within 12 min when RCM determinations were performed.

Ferrokinetic studies using ^{59}Fe disapperance and red cell ^{59}Fe incorporation were measured in 8 subjects in the control period, day 22 of bed rest, and day 21 of postbed rest (figs. 23.2 and 23.3). Plasma iron turnover decreased during bed rest from control values of 35.3 to 27.5 mg/day ($p = 0.01$), and red cell iron turnover decreased from 20.5 to 16.5 mg/day ($p = 0.01$); both parameters increased, but not significantly, during the recovery period. The rate of red cell renewal decreased significantly during bed rest ($p = 0.015$) and paralleled the observed decrease of peripheral blood reticulocytes.

Erythropoietin production (fig. 23.4) was studied in 4 subjects, using the hypoxic mouse assay for 72-hr urinary excretion extractions, and showed a decrease during bed rest and an increase following reactivation. These changes are not statistically significant since the changes fall within the range of error of the procedure; they should be considered a confirmation in trend only.

DISCUSSION

The results reported here agree with the previously described plasma volume changes that occurred during bed rest, where plasma volume was determined by either T-1824 or radioiodinated serum albumin, and RCM was determined indirectly. Taylor et al. (ref. 3) and Dietrick et al. (ref. 4) reported RCM decreases of 54 to 84 ml, respectively, after 3 weeks of bed rest. This range is in agreement with our observed 65-ml decrease in subjects after 24 days of bed rest. However, these values do not differ significantly from those obtained prior to bed rest ($p = 0.025$). Miller and his group (ref. 5) observed a more pronounced decrease in RCM of 180 ml for 12 subjects after 4 weeks of bed rest, but the degree of statistical significance is questionable at the $p < 0.05$ value. Erythrokinetic changes leading to the reduction in RCM were not investigated in these earlier studies, but reference was made to earlier work of Broun (ref. 10) showing that immobilization leads to a decreased rate of erythropoiesis in dogs. Since the total blood volume was derived from the plasma volume in these earlier studies and the reliability of this estimate resides with a fixed relationship between body and venous hematocrit, it is pertinent to note that prolonged bed rest appears to perturb this relationship (fig. 23.5). During the control and recovery phases, there was a high coefficient of correlation between body and venous hematocrits, which is in keeping with the observations of Chaplin et al. (ref. 11). During bed rest, body and venous hematocrits increased; however, significant correlation was no longer evident. Under these conditions, the peripheral venous hematocrit cannot be used with assurance to derive indirect estimates of red cell and plasma volume.

Several mechanisms may be operative to explain the loss of RCM during bed rest. The consistently negative stool tests for occult blood indicate that the blood loss did not occur. In addition, blood loss through testing was not adequate to account for the changes seen, nor did the controls demonstrate comparable changes on the same amount of blood letting. A decrease in reticulocytes and red cell iron turnover, observed in the present study, would not be anticipated if iatrogenic blood loss were responsible for the loss of RCM. Sequestration of red blood cells is a more difficult problem to investigate. Body counts over the spleen, compared to the heart, did not show excessive accumulation of ^{51}Cr during these periods. When ^{51}Cr RCM determinations were performed, no essential difference in counts per minute per ml of blood was noted between the 12- and 25-min samples, suggesting that the injected red cells achieved complete mixing in the circulation during the first 12 min. In contrast, patients with splenomegaly, in whom a significant portion of RCM is sequestered, may demonstrate decreasing red cell radioactivity levels for approximately 30 min

following the injection of labeled cells owing to the exchange with the sequestered compartment. No apparent hemolytic mechanism could be demonstrated during the course of bed rest, as indicated by the normal ^{51}Cr red cell survival studies, the absence of substantial indirect bilirubinemia, and no alterations in autohemolysis.

On the other hand, there is considerable evidence to suggest that erythropoiesis decreases during bed rest. Reticulocyte percentages, as well as total circulating reticulocytes, decreased throughout the period of bed rest. This amounted to a 20 percent decrease in total circulating reticulocytes at days 24 and 35 of bed rest, and it may be inferred that a new steady state of erythropoiesis was achieved. Ferrokinetic studies demonstrated a decrease in both plasma and red cell iron turnover during bed rest, with a significant rise in the postbed rest period.

The regulation of erythropoiesis has been extensively investigated in the past two decades. Jacobson and his associates (ref. 12) have shown that erythropoiesis is influenced by erythropoietin, a humoral agent capable of differentiating the stem cell into an identifiable nucleated erythroid cell as well as of changing the rate of cytoplasmic maturation and hemoglobin synthesis within the nucleated erythroid cell. Reports have indicated that a renal enzyme similar to renin activates a plasma globulin to generate erythropoietin (ref. 13). The analogy of the renin angiotensin system is strengthened even further, since it is believed that the juxtaglomerular apparatus produces both renin and renal erythropoietic enzyme.

The primary stimulus for the secretion and/or activation of erythropoietin appears to depend on the relationship of the oxygen supply and demand of the tissues. Hypertransfusion of red cells, or for that matter hyperoxia, results in an increased oxygen supply to the tissues, which in turn shuts off erythropoietin formation and causes the cessation of erythropoiesis (ref. 14). Erythropoiesis is reinitiated when the hemoglobin concentration or oxygen supply falls to normally maintained levels. During bed rest, the decreased tissue demand for oxygen relative to oxygen supply may be sufficient to alter the secretion or activation of erythropoietin. The studies of urinary erythropoietin excretion during bed rest tend to confirm this hypothesis, at least in trend, even though the actual changes are not of a significant level.

On resuming ambulation after bed rest, there was a rather abrupt decrease in hematocrit and hemoglobin concentrations in the peripheral blood, probably due to augmentation of the plasma volume. The corrected red cell survival curves demonstrated no evidence of hemolysis, and, although increased during the period of reambulation, the 3-week recovery phase was probably insufficient to show complete recovery of the RCM. Such a delay is consistent with the reports by Fowler and Barer (ref. 15) that professional blood donors require an average of 7 weeks to replenish the hemoglobin deficit incurred from the donation of a unit of blood.

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Table 23.1 ^{51}Cr red cell survival studies

Period	*Apparent* $t^{1/2}$	*Corrected* $t^{1/2}$
Control	25.4 ± 1.1†	21.6 ± 1.8
Bed Rest		
First half	26.7 ± 0.9	23.3 ± 1.1
Second half	28.7 ± 0.8	22.9 ± 0.6
Recovery	20.7 ± 0.7	21.6 ± 1.7

*Apparent $t^{1/2}$ derived from plot of common log of red cell ^{51}Cr specific activity against time in days.

**Corrected $t^{1/2}$ calculated from circulating erythrocyte-bound ^{51}Cr remaining at the end of each survival period adjusted for changes in the red cell mass.

†Mean ± SE (five subjects). Underlined value is significantly different from control at the 1 percent level.

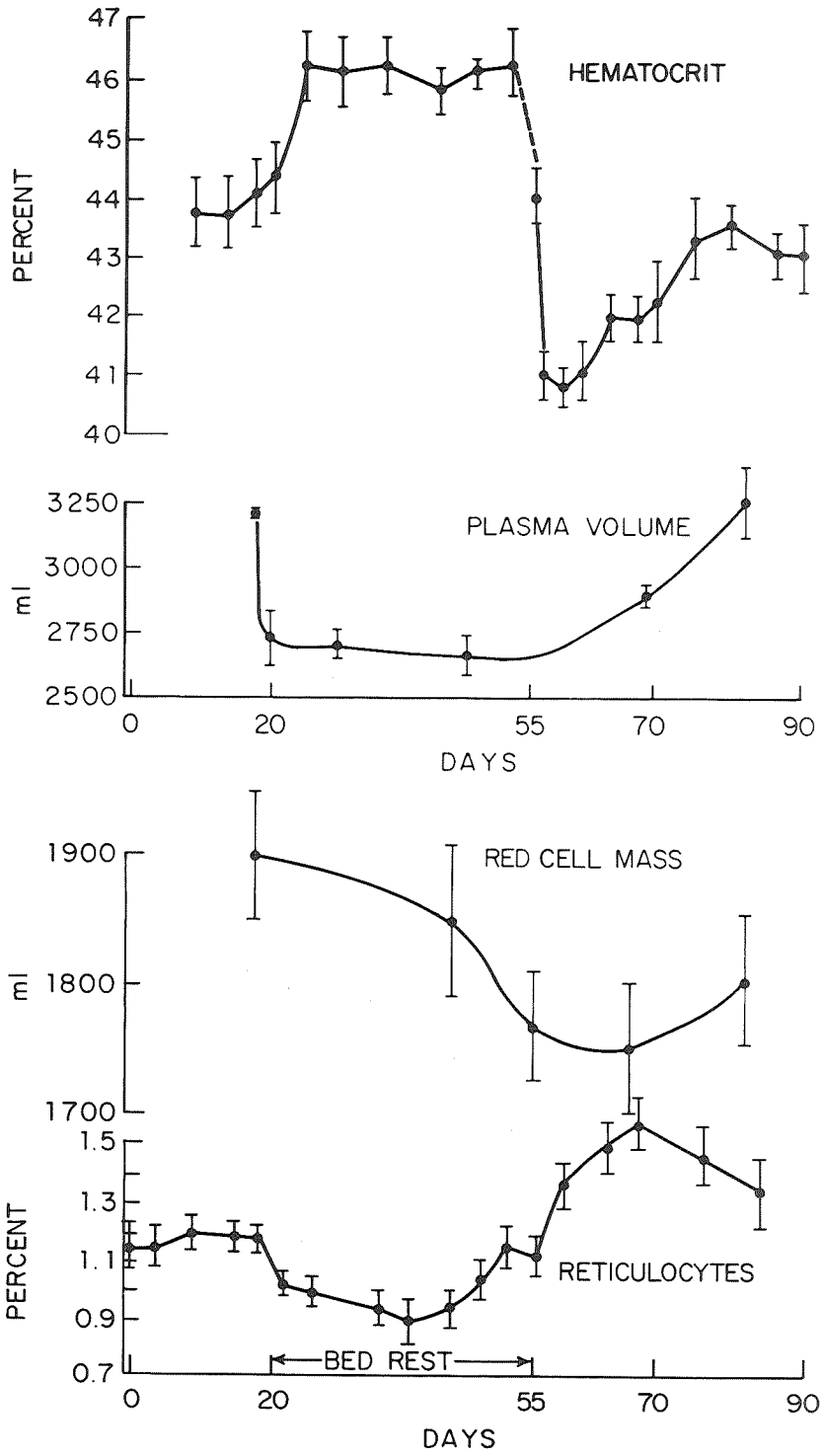

Figure 23.1 (a) Serial determinations of peripheral venous hematocrit (mean ±1 SE) in 21 subjects; (b) plasma volume measurements (mean ±1 SE) in 8 subjects; (c) red blood cell mass estimated by dilution of ^{51}Cr-labeled red cells in 21 subjects at onset and end of bed rest, 9 subjects at day 24 of bed rest, and 20 subjects at end of recovery (mean ±1 SE); (d) serial reticulocyte percentage in peripheral blood

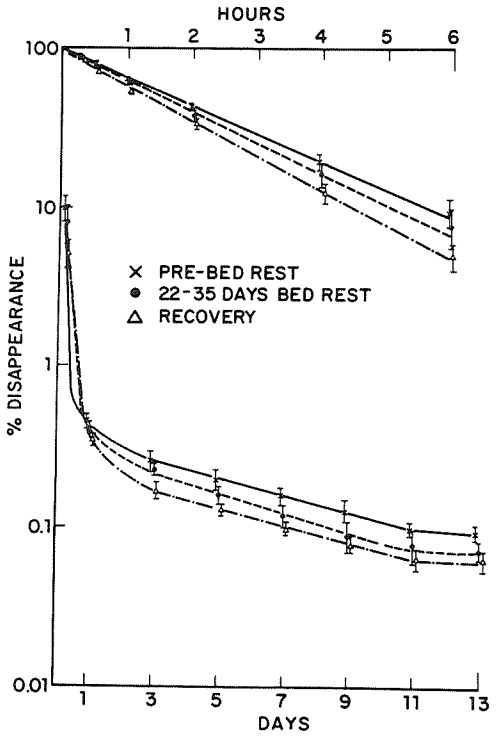

Figure 23.2 Percent of ^{59}Fe in plasma after intravenous injection in 8 subjects. The initial 6-hr segment of each curve is shown in the upper portion of the graph (vertical bars = ±1 SE)

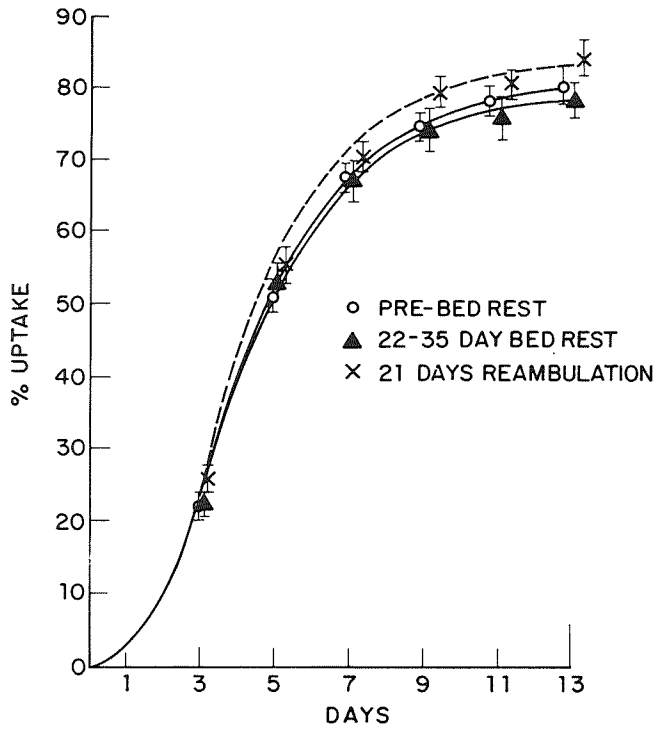

Figure 23.3 Incorporation of ^{59}Fe into red cells in 8 subjects (mean ±1 SE)

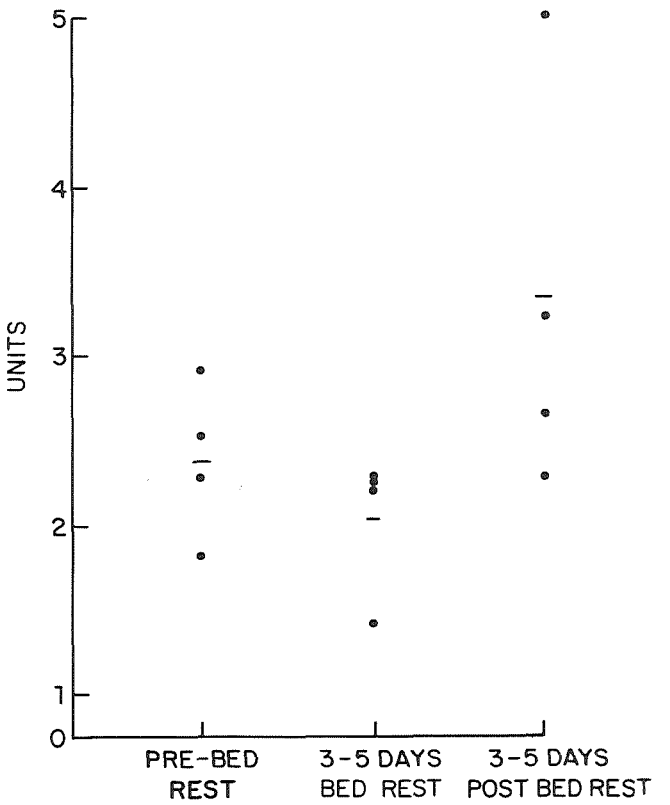

Figure 23.4 72-hr urinary erythropoietin excretion for each period in 4 subjects

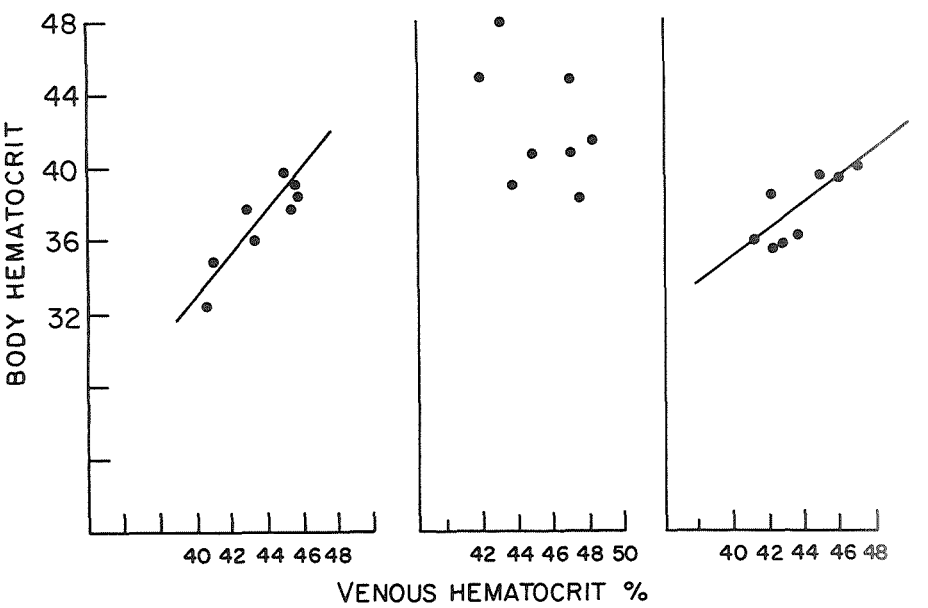

Figure 23.5 Regression of body hematocrit on venous hematocrit in 8 subjects during (a) control phase, (b) bed rest phase, (c) recovery phase. A regression line was not drawn through the bed rest data, since the slope did not differ significantly from zero

24 DISCUSSION

Dr. Lutwak: Your values for basal excretion of calcium in the first and second studies are quite high; many people would be suspicious of hyperparathyroidism. Do you have an explanation for these high values?

Dr. Donaldson: No, I don't have an explanation for them; we have taken them to be within normal limits.

Dr. Lutwak: Were any of these individuals of Scandanavian extraction? It has been suggested that, genetically, such individuals may have relatively high levels of calcium in the urine. This may account for the relatively low percentage of increase in urinary calcium that you saw during bed rest.

Dr. Donaldson: Another possibility is that we were giving them considerably less calcium than they had before. One problem we had in the first study, which wasn't quite so important in the second study, was that the baseline period wasn't really long enough for them to be in equilibrium.

Dr. Whedon: I would also insert the reminder than the degree of immobilization was considerably less in these studies.

Dr. Barzel: You mentioned very little about sodium, potassium, and nitrogen balances; I think that they may be very pertinent and I wonder if you would like to say more about them here. Weren't your subjects in negative sodium balance?

Dr. Donaldson: They did not show very marked changes. The subjects were not in negative sodium balance. There was some decrease in the urinary sodium during bed rest, but this did not reflect a negative balance. The sweat sodium is much higher before, and remains considerably higher after, bed rest, so that this increase in urinary sodium did not really lead to a negative sodium balance. The subjects were also in nitrogen balance.

Dr. Nordin: Dr. Lutwak seems to be under the impression that any urinary calcium over 300 mg/day is hypercalcuria. The relation of urinary to dietary calcium is a function of age and sex. In young men, a urinary calcium of 300 mg for a dietary calcium of 1 g is absolutely normal.

Dr. Whedon: The nitrogen balance is in accord with the experience of others. You get an increase in urinary nitrogen over the first few days, perhaps 10 days to 2 weeks, but then it comes back toward (but usually not all the way to) normal baseline. If you have a very long study, any change you have in the first few days of weeks is obviously going to be washed out in the mean for the entire period.

Dr. Hyatt: How long should we keep a patient on the control diet before beginning balance studies?

Dr. Donaldson: There is no single answer. In certain cases, they seem to reach an equilibrium within a few days. In others, it takes as long as 2 weeks.

Dr. Whedon: It depends mostly on what the prior dietary level was. Obviously, if the prior diet was close to the experimental diet, it will take less time. A practical rule is to put the patient on the control diet for 1 week before beginning the balance study.

Dr. Barzel: Do these patients lose muscle?

Dr. Donaldson: They lose girth of their lower extremities. They do not lose girth in the upper extremities or around the abdomen or the chest.

Dr. Barzel: Shouldn't this be reflected in your nitrogen balance?

Dr. Donaldson: I think this is probably a function of time. The mean balance over an entire 30 or 36 weeks doesn't reflect the kind of change that takes place in the first few days.

Dr. Lind: Could I come back to the problem of sweating? I'm interested in the reduction of sodium concentration in the sweat. Did they keep a fairly level sweat sodium throughout the bed rest?

Dr. Donaldson: We didn't see a variation in the sweat sodium or calcium content.

Dr. Behn: Isn't there some interrelation between sodium and calcium excretion?

Dr. Lutwak: I believe it has been shown that urinary calcium and sodium excretions are parallel, and whatever you do to increase one will increase the other.

Dr. Nordin: You showed losses in absorption of what seemed like 50 percent. Does this represent a 50 percent loss of mineral?

Dr. Hulley: In the area that was described by the slide in question, which was the central area in range of ½ to 1¼ in. from the bottom of the bone, the average loss of mineral, as assessed by this method, appears to be about 35 percent.

Dr. Nordin: Could you see this on the film with the naked eye? If you sorted the films through blindly, could you spot which one it was?

Dr. Hulley: Yes.

Dr. Whedon: You gave the figure of 4 percent mineral loss. Was this for the totalization across the entire bone if you put all of the areas of the X-ray together?

Dr. Hulley: No, this was from the calcium balance data. The average calcium, during the 36 weeks of bed rest, was minus 221 mg/day, and if you add this up over 8 months, it comes to approximately 50 to 60 g, which is about 4 percent of the estimated total skeletal mineral.

Dr. Lind: Is there any evidence to suggest that people who have been at bed rest for a substantial period of time are, in fact, in greater risk of breaking bones?

Dr. Hulley: It is well known that people who are immobilized by disease, such a paraplegia, are prone to fractures. I'm not sure I can answer you more specifically.

Dr. Nordin: A fracture of the calcaneum is a very rare event. This is really only a traumatic fracture. It is one of the interesting pieces of this work at least that the calcaneum is not one of the sites where the elederly fracture their bones. The work that has been done by Weaver and Chalmers in Edinburgh (ref. 1) didn't show that the calcaneum took part in the aging process in bone. One got the impression from their paper that the calcaneum was not involved in metabolic processes at all, so it is a particularly interesting and surprising finding. Mayo (ref. 2) looked at the calcaneum in terms of density in military guardsmen who march up and down outside of Buckingham Palace and stamp their feet every 12 paces. It was felt that the effect of this stamping on the calcaneum would effect the density of the bone. They compared the guardsmen's calcaneal density with nonguardsmen's density but found no difference at all. So the impression has been widespread in the British Isles that the calcaneum is not a very active bone metabolically. But clearly in this country it is.

Dr. Hulley: Dr. Vogel selected the calcaneum to study for three reasons: because it had been studied in previous investigations involving NASA projects, and there did appear to be changes in this bone; it was involved in weight-bearing, which was convenient and easy to study; while calcaneal fractures are rare, they do occur with severe downward trauma. I'm told by Dr. Vogel that this is not a very uncommon fracture among Marine recruits who are forced to stamp their feet hard.

Dr. Nordin: If one extrapolates from your data to Dr. Mack's, doesn't it make Dr. Mack's observations on the 2-week flight much more tenable? I'd like to withdraw what I said last night. I simply hadn't conceived that there could be such a selective loss. The bones, in my experience, behave in a parallel fashion. However, this could well be a very specialized situation where a particular bone is selectively losing mineral that would not significantly affect the overall calcium balance, and perhaps this would reconcile Dr. Mack's data with Dr. Whedon's balance data.

Dr. Vogt: Both of your studies substantiate what Dr. Mack has been finding for 6 or 7 years, and I don't think that there is any reason to be surprised at this correlation.

Dr. McCally: You mentioned that, on reambulation, there was a noticeable difficulty with foot and ankle pain. There had been some suggestion, from animal experiments, of difficulty with joints, such as overgrowing of synovia. Did you see any significant joint pathology?

Dr. Donaldson: I can't be certain exactly what caused the pain, which was intense. This must be a very common finding, for I have heard many comments among people who have done bed rest studies about the discomfort associated with ambulation. Usually, it is not seen in the short-term studies, although, in Dr. Hyatt's 2-week bed rest study, he found typical petechiae and occasionally some edema.

Dr. Hyatt: What was the experience of your subjects in terms of returning to their prebed physical status? What were their complaints?

Dr. Donaldson: Within 4 weeks' time, they looked normal outwardly, but none of them felt normal. When one young man, who had been a cross-country runner a few years before, returned to college, he felt that his stamina was markedly diminished for several months.

Dr. Lecocq: Did you notice any sleep disturbance in your group during bed rest or during recovery?

Dr. Donaldson: We expected that the subjects wouldn't be able to sleep at night with this much inactivity. Actually, it varied considerably, but on the whole, the subjects slept a great deal more than in the control situation. For example, one said that, when the exercise program ended, he slept almost constantly, awakening only for meals.

Dr. Lecocq: Our subjects have required a greatly increased amount of sleep, and this excessive sleeping continues for quite awhile following the bed rest, for as long as 7 to 10 days.

Dr. Hyatt: Do your patients become apathetic during the period of 9 months of bed rest?

Dr. Donaldson: As a matter of fact, they develop a marked lethargy. It's interesting also that on serial testing their IQs went up.

Dr. Oyama: What was the lighting schedule for these various feeding patterns?

Dr. Lindan: We had various light schedules. We generally had daylight and night cycles because the windows were not covered. Some experiments were conducted in a room with constant light and the windows covered. The patients, however, were aware of the day-night cycle because of noisy routine housekeeping activities on the Metabolic Ward. When we had constant light and a random feeding schedule, the power of the urinary diurnal cycle was cut in half. That would add evidence to the postulation that the light-darkness cycle is the triggering mechanism for the diurnal clock.

Dr. Zollinger: Over the last few years, we've been interested in the measurement of extracellular fluid volume using radiobromide. To test the validity of this method, we studied normal volunteers on the Metabolic Ward, with controlled water intake, calories, and activity, for 120 hr after the intravenous administration of radiobromide. Figure 24.1 shows the data of a 27-year-old man, 72 kg in weight. The top curve represents the raw plasma sample fraction of the isotope that had been injected. The large dots represent triplicate fasting values taken at 7 a.m.; other samples are spaced throughout the day, but generally were taken at 12 noon and 5 p.m., just prior to eating. There is a waxing and waning with each day, but some of the morning values were clearly higher than the plasma counts of the night before. Here a diurnal, or more likely a meal-driven, rhythm is definitely present. This amounts to an apparent 1-liter gain in extracellular fluid during the course of a day, while the transient weight gain of these subjects was approximately ½ kg.

We also looked at the radiobromide to chloride ratio in urine and this, too, had a night-day variation, not only in the concentrations one to the other but in the total quantity excreted. It appears as if the kidney prefers to put out chloride more than it does bromide at night. I present this to show you that, in the analysis of serial radiobromide ECF values over a period of days, diurnal and/or meal-driven rhythms need to be taken into account.

Dr. Vogt: Do you think it would be appropriate to talk about a circadian rather than a food-driven rhythm here?

Dr. Zollinger: It is certainly possible, and I can't separate these effects with these data. Radiobromide equilibrates with the succus entericus and gastric juice, and we postulate that it is then reabsorbed as the day goes on, so that you find a rising ^{82}Br activity during the night.

Dr. Young: Were the subjects sleeping between 2 and 6 a.m. when you got this low level of excretion?

Dr. Lindan: Apparently, but we could not control it very well. When we had normal subjects on random schedules, they had to stay in bed during their sleeping time and they were expected to sleep. During the waking hours, the subjects had to be out of bed; but if they decided to sit in a chair and doze, we could not prevent that. In addition, they were allowed to smoke, watch television, and receive visitors. All those variables could have interfered with their biological rhythms and contributed to the "noise" in our data.

Dr. Gatts: Were you able to correlate the sleep periods with the changes in the meal periods? I am wondering if the meals tended to drive the sleep periods.

Dr. Lindan: I can't answer that. We were not able to do electroencephalographic studies and follow the sleep patterns.

Dr. Gatts: If a subject were allowed to sleep whenever he could, his sleep pattern would be different if he were fed once a day rather than every 7 hr.

Dr. Whedon: Would you say that some electrolyte excretions were more "driven" than others?

Dr. Lindan: We didn't analyze this in great detail, but it seems that potassium had the strongest diurnal rhythm of all. But even this circadian rhythm of the urinary potassium was dwarfed when the subjects were on a 19-hr meal schedule.

Dr. Behn: Do you assume that the changes in aldosterone are meal-driven because of the mineral load of the meals?

Dr. Lindan: It may be.

Dr. Oyama: I might add a very interesting observation that we have made in rats. We find that, when we certrifuge rats chronically for periods ranging from 41 days to 1½ years, they show an increased glucose tolerance and a very enhanced insulin sensitivity. This would seem to be the exact inverse of your bed rest study.

Dr. Diamond: Increased secretion of glucogon might also explain these results. There have been recent reports showing that the arginine infusion test has been found to be unsatisfactory for the stimulation of growth hormone release.

Dr. Piemme: Yes, I am aware of this. There are better amino acids to use, but arginine is the cheapest and it is the standard test and quite reproducible. Women respond very consistently but men are more variable.

Dr. Barzel: Would you care to speculate on the effect of a lack of growth hormone and the possible effects on skeletal metabolism?

Dr. Piemme: I think you'll hear, before the evening is over, that there are several hormones "turned off" during bed rest. Growth hormone is just one of them.

Mr. Miller: How does arginine cause insulin release?

Dr. Piemme: I don't know. The insulin release and the growth hormone release are a function of any amino acid infusion. The effect of amino acids on each of these hormones is independent. I don't think anyone knows how the amino acids stimulate release of either insulin or growth hormone.

Mr. Miller: Is it gluconeogenic?

Dr. Piemme: Yes, but that is not the mechanism of action, because the response is immediate.

Dr. Lecocq: Arginine will work on a perfused pancreas to release insulin, so it must have a direct effect at that site.

Dr. Lutwak: When I first saw this program, I was very hesitant to present this material, which we collected over 10 years ago, and only published as an abstract (ref. 3). This study was the outgrowth of a serendipitous observation. In the course of screening normal volunteer subjects, we found an abnormality of glucose metabolism in the form of a very flat oral glucose tolerance test in a trained athlete (fig. 24.2). When we repeated the test after several weeks of inactivity, it was relatively normal. We put her at bed rest for a period of 2 weeks, after which the glucose tolerance curve became abnormally elevated. We then started looking at the effect of bed rest on glucose tolerance in a larger group. Ten normal volunteer subjects, 5 men and 5 women between the ages of 18 and 22, were studied. We ensured adequate carbohydrate intake, about 200 g/day or more, throughout the study. For a period of at least 2 weeks, they were permitted ad lib activity, with at least 2 hr/day in the gymnasium under controlled activity. They were then put at complete bed rest in a metabolic ward, where they were fed and bathed in bed and lifted on and off a commode for toilet function. We did not have a technique for insulin assay available at the time, so we used the Amatuzio calculation for the determination of the half-life of infused glucose in the course of IV glucose tolerance tests. Figure 24.3 shows a set of curves from one individual, with glucose tolerance tests repeated at intervals of several days. The subject was then put at bed rest, and the next three curves were obtained at weekly intervals. Within a matter of days, the slope of the curve became smaller (flatter) and the half-life became prolonged; these effects progressed as bed rest was prolonged. Activity was then started at a gradual pace, increasing with treadmill exercise every day. The final two curves were obtained during the recovery stage. Figure 24.4 shows similar findings in other subjects. There was considerable variation in the half-life of infused glucose among the different subjects; this was related to the degree of activity of these subjects before the study started. All demonstrated a prolonged glucose half-life with bed rest. Each of the points is a mean of at least three measurements taken during bed rest. When activity was reinstituted, the values fell promptly. There was no difference between the sexes. The only apparent relationship was to the previous degree of activity; this was a rather consistent finding. We also tested the response to exogenous insulin with an insulin tolerance test. There were no abnormalities seen in insulin tolerance tests in the five individuals tested. We also carried out glucagon infusion in four individuals and, again, there were no abnormalities when bed rest was compared with the prebed rest period. Recently, another study has been reported by Dr. V. Simko, a physician from Bratislava, now at Cornell University (ref. 4). Figures 24.5 and 24.6 summarize these results.

Two groups of rats were studied. One was considered a control group at normal activity, and the other an exercise group that was trained to swim for several weeks. The mean glucose tolerance curves for the two groups of rats showed a significant elevation in the control group compared with the exercised group. The active animals had much lower glucose tolerance curves after the infusion of the standard dose of glucose. The plasma free fatty acid response to the glucose infusion paralleled the glucose response—but was even more significantly different, with higher free fatty acids in the untrained animals than in the trained animals, showing that less active animals have, again, a different response to glucose load. Dr. Simko has told me that serum cholesterol values were consistently higher in the untrained rats than in the trained rats.

Dr. Lind: Do you have any information on whether your 2-desoxyglucose (2DG) is going selectively to different cells in the body?

Dr. Lecocq: This has not been completely worked out, but it would appear that it is not getting into nervous tissue at the same rate it gets into lean body tissue. It seems that the most insulin-sensitive cells are also those that are most affected by 2DG. In 72 infusions we had two untoward effects: one subject developed (twice) a mild supraventricular tachycardia, and some subjects demonstrated mild sweating and flushing.

Dr. Saltin: I just wondered how much is known about glucose infusion or glucose tolerance tests and the glycogen content of the muscle. We have never been able to increase the muscle glycogen content with a high carbohydrate-enriched diet when the subjects started with a normal muscle glycogen content; but if the muscle glycogen is low, we get an enormous increase and, occasionally, get an overshoot phenomenon with up to double the amount of glycogen in the muscle. What happens in bed rest? Is glycogen depletion a factor in this glucose abnormality?

Dr. Lecocq: I don't know. The subjects were on an adequate carbohydrate diet throughout the study. I doubt whether glycogen depletion is the explanation.

Dr. Oyama: If you starve humans or animals, they show a decrease in glucose tolerance. I wonder if there could be a difference in energy utilization between the bed rest and the activity period that could affect carbohydrate metabolism and, consequently, glucose tolerance? Could at least a partial answer to the glucose intolerance be that the subjects have shifted from carbohydrate metabolism to fat metabolism?

Dr. Lecocq: None of our people are starved in either instance. They are on fixed diets and they cannot change their diets. They are getting adequate carbohydrates, and there is no evidence of ketosis.

Dr. Barzel: In most of our bed rest studies, we attempt to maintain the same body weight. Since we know there is a loss of nitrogen, calcium, and phosphorus, there must be a loss of body tissue in at least the early weeks. If you are maintaining body weight by some artificial means, you may be making and storing more of some other material, perhaps glycogen or something else other than nitrogen, calcium, and phosphorus, which is being lost. So perhaps these patients are overfed. You may have to design a different type of experiment, where the patients would lose as much weight as you would expect from the nitrogen, calcium, and phosphorus loss. You might be dealing with a different metabolic specimen here.

Dr. Whedon: I think it is even more complicated than that, because the subjects certainly are losing some of these elements with which we have been preoccupied. But, at the same time, there is much less physical activity so that calories are being saved and, with calories, certainly fat and perhaps, to a minor extent, some carbohydrate.

Dr. Young: I would suggest you might do a glucose turnover rate and oxidation study. You could resolve this type of problem very easily and better interpret your glucose tolerance test curves.

Dr. Lecocq: I'm just näive enough to think I have it solved.

Dr. Zollinger: If at the end of bed rest these subjects are not pathologically overhydrated, then they probably have normal body compositional ratios. Couldn't one measure total body water at that time and then calculate a value for fat?

Dr. Lancaster: We've demonstrated very little change in the total body water, using both tritium and deuterium dilution techniques; but we have shown a decrease in lean body mass during the bed rest period, with a calculated increase in body fat, which goes pretty well with the expected change anticipated from the nitrogen balance data.

Dr. Murray: Are there further details on the astronauts' postflight carbohydrate intolerance?

Dr. Piemme: As I get the story from NASA MSC, one of the astronauts was found to have an abnormal glucose tolerance test postflight. All the glucose determinations done on the astronauts have run a little bit high, so the flight physicians apparently were never concerned that this astronaut's sugar ran just a bit higher than everyone else's. But they never did a glucose tolerance on this man until they found his postflight blood sugar was near 160 mg percent. His glucose tolerance test, which was done immediately, was grossly abnormal. I am sure that, if it were repeated now, it would be normal. It seems to be that he may well have a predisposition to maturity onset diabetes, but I think they have lost the opportunity to explore this.

Dr. Lutwak: I want to point out that, in our bed rest study, we found return to normal within 2 to 3 days after activity, so this is a rather prompt response.

Dr. Lecocq: I think several of the astronauts are reported to have had a fasting blood sugar of near 90 mg percent before flight and a 15 to 20 mg percent higher value after flight. This often has been explained away as being secondary to the emotional stress involved, that is, a catecholamine response.

Dr. Gatts: Why should one assume that if you put an animal to rest, markedly changing his metabolism, it would be comparable to the metabolic standards obtained when he is up and about? You have a completely different metabolism, thus a different animal. It seems to me that we should be alarmed if it were the same in the two conditions. I think what you're describing is a standard for a bed rest subject as opposed to a standard for an upright subject.

Dr. Lecocq: This has clinical application. It is not uncommon to be called to see a patient who has been at bed rest for a month or more and found to have a high fasting blood sugar and an abnormal glucose tolerance curve. We must think of this bed rest-induced glucose intolerance more often in this situation.

Dr. Murray: I wonder if we know if this phenomenon persists with prolonged bed rest? Has this been studied over a period of weeks, or months, or years?

Dr. Piemme: It persists all right. In the study I quoted, done in 1945, some of the patients had been at bed rest for 8 years. Apparently, the longer they were at bed rest, the more likely they were to have the abnormality, and the more abnormal the curves were.

Dr. Whedon: But in controlled studies in which testing was done before and during bed rest, I think 4 weeks is about the longest time such observations have been made.

Dr. Sandler: Have many paraplegics been found to have the abnormal glucose metabolism?

Dr. Piemme: These patients may have normal fasting sugars and do not develop acidosis, so it would be unlikely that abnormal glucose metabolisms would be recognized often.

Dr. Sandler: There must be glycosuria associated with high blood sugars, such as that 360 level that we were told about. Random urinalysis might find it.

Dr. Lutwak: There are many scattered reports in urology, cardiology, and infectious disease journals on the high incidence of diabetes in patients in bed for prolonged periods, such as patients with paraplegia and tuberculosis. There have been reports in cardiology journals of the onset of diabetes following myocardial infarction (when the patients were at bed rest for a period of weeks) and the disappearance after the patient is sent home and back to normal ambulation.

Dr. Piemme: Lebovitz at Duke (ref. 5) reported doing glucose tolerance tests 2 weeks after myocardial infarction and found glucose intolerance with high insulin secretions.

Dr. Lancaster: I would like to make a clinical observation here. At the School of Aerospace Medicine, we have problems with reproducibility of glucose tolerance curves. We have seen serial glucose tolerance tests in the same individual change from distinctly abnormal findings (by Fajan's and Conn's criteria) to normal in three days or vice versa. This has been a source of considerable consternation to the clinicians in our department. It may well be that variation in levels of physical activity causes a good part of this instability.

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Figure 24.1 Bromine^{-82} kinetics in normal man

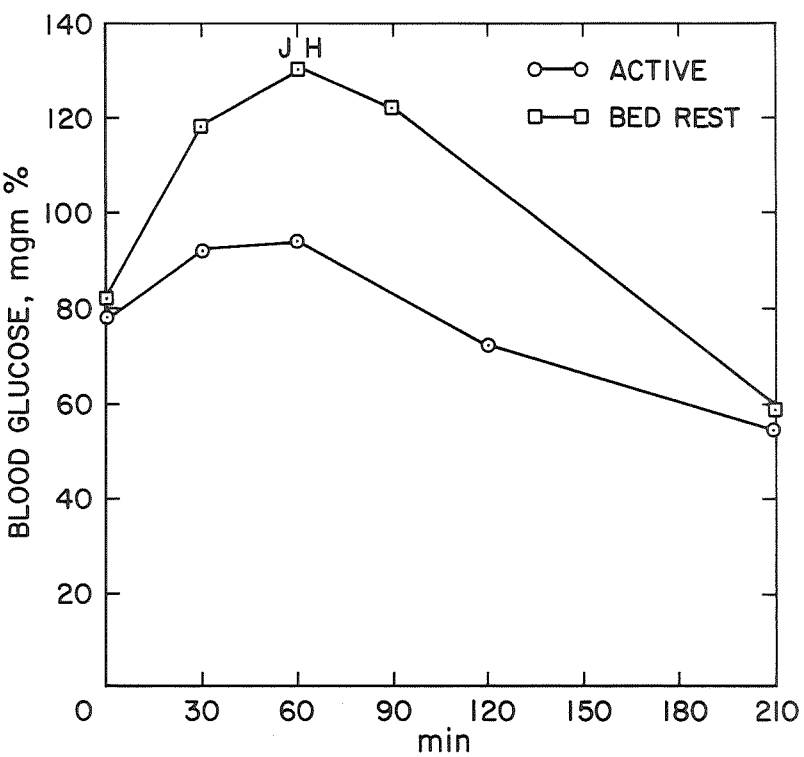

Figure 24.2 Standard oral glucose tolerance

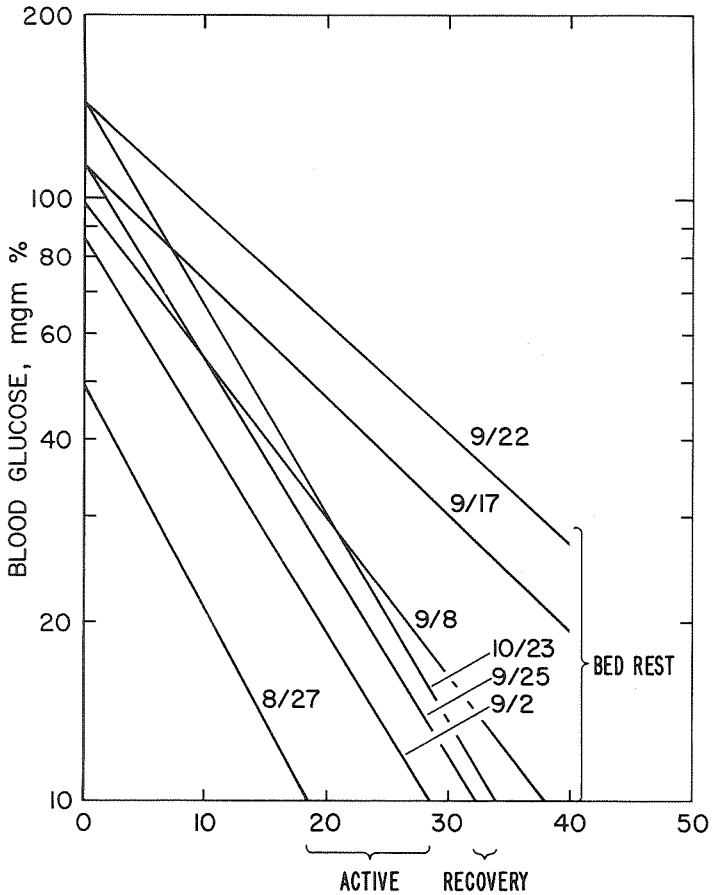

Figure 24.3 IV glucose disappearance

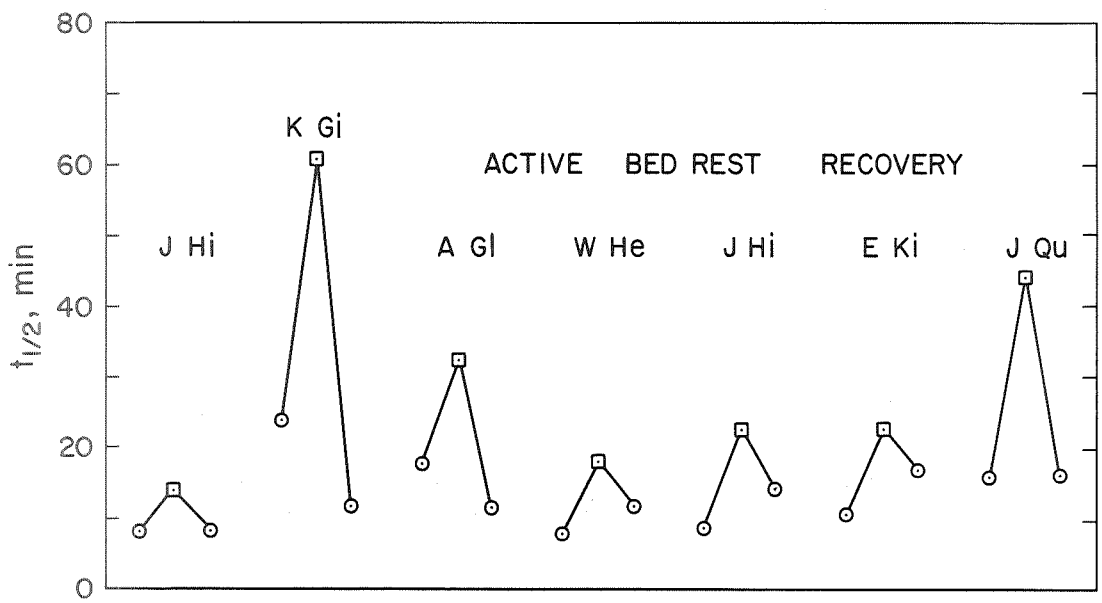

Figure 24.4 Half-life of injected glucose (IV glucose tolerance)

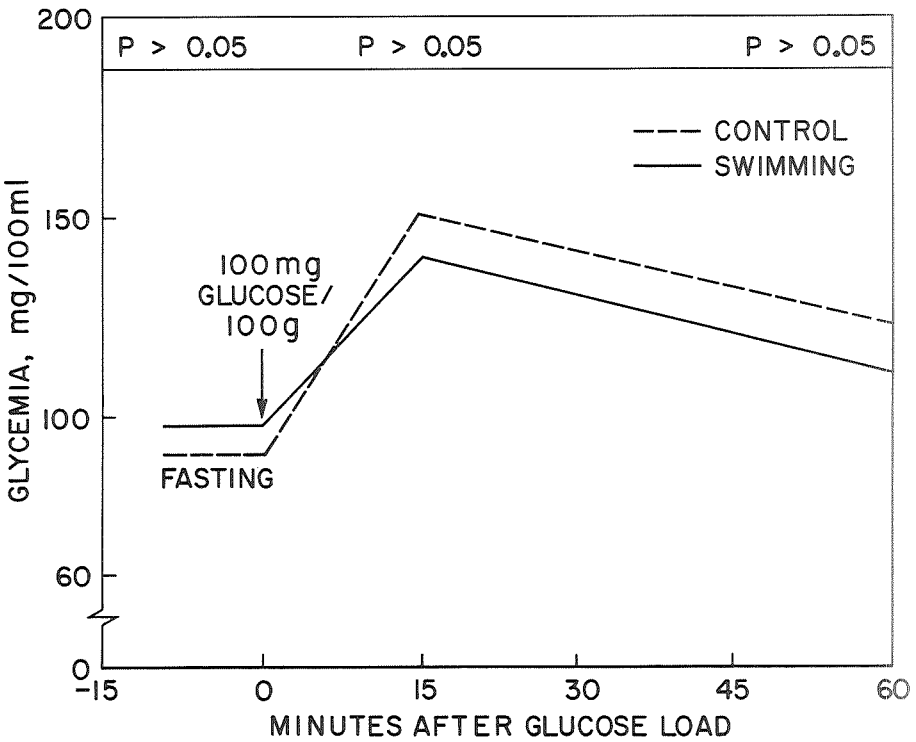

Figure 24.5 Glucose tolerance curves (from ref. 4)

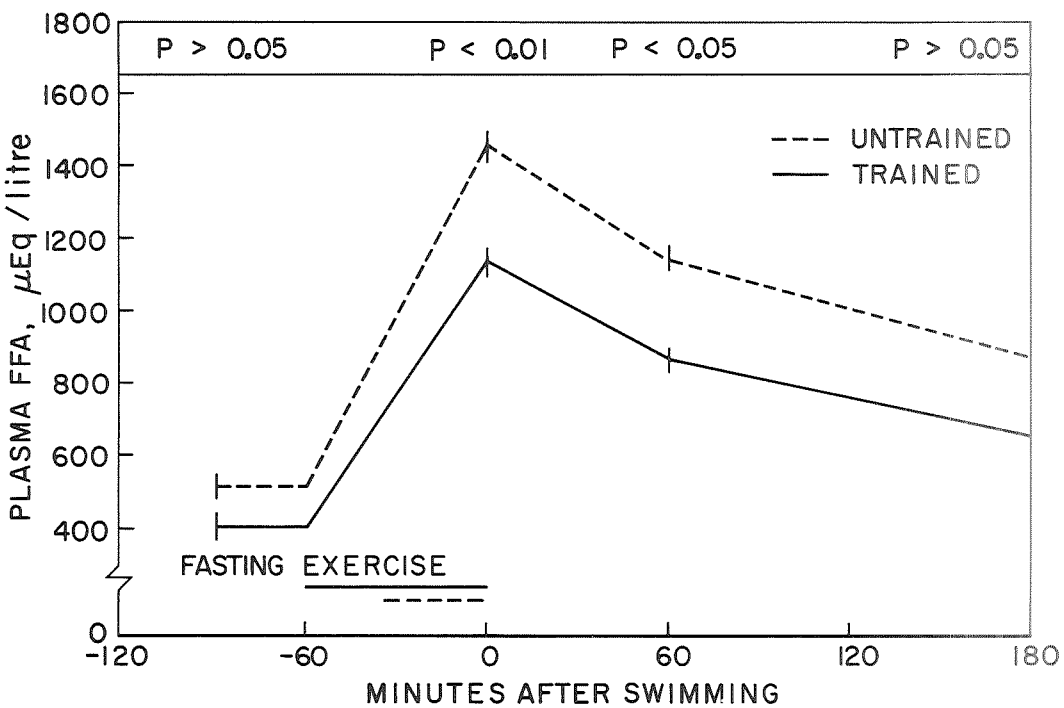

Figure 24.6 Plasma FFA response curves (from ref. 4)

Keynote Paper

25 IMMERSION TECHNIQUES AND THE EVALUATION OF SPACEFLIGHT DECONDITIONING COUNTERMEASURES*

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INTRODUCTION

As a result of man's space exploration efforts, gravity—particularly its reduction or absence—has become an environmental factor of special concern. Gravity is unique because it cannot be eliminated or reduced in any experiment confined to a laboratory on the earth's surface. Gravity as a biological determinant and methods for simulating the weightless state have been reviewed recently and discussed in detail by Wunder and his colleagues (ref. 1). The physical effects of gravity and its major physiological consequences are: reduction in apparent weight, alteration in hydrostatic pressure, and density-dependent phenomena. For any condition in which there is a reduction in weight of an organism, the following effects might be expected: (1) less of the organism's energy expenditure will be used in the processes of support and locomotion, (2) tissues will experience less mechanical stress or deformation, and (3) friction between an object and its support will be reduced.

Bed rest and water immersion are techniques used in simulating certain limited aspects of human exposure to weightlessness. Neither bed rest nor water immersion is a true or in any way a complete analog; rather each attempts to simulate specific anticipated effects of weightlessness, particularly the reduction in apparent weight and the minimization of intravascular hydrostatic pressures.

The use of water immersion as a therapeutic agent dates back to man's earliest days (refs. 2, 3). The writings of all the ancient civilizations—Egyptian, Hebrew, Greek, Persian, Hindu, and Chinese—refer to its healing properties, and Hippocrates wrote in considerable detail on the efficacy of water in the treatment of a variety of maladies. "Hydrotherapy" is an extensive science and has passed through various phases of fashion and popularity. A large source of immersion-effects

*The voluntary informed consent of the subjects used in this research was obtained as required by Air Force Regulation 169-8.

information exists in the 19th and 20th century European medical literature on hydrotherapy, or balneology (from the Latin balneum, or bath) (refs 3–5). A recent advance is the use of a silicone immersion fluid that obviates the problems of skin maceration and allows long periods of immersion. It is now used in burn therapy (ref. 6) and weightlessness simulation (refs. 7, 8).

If a mass m of volume V is immersed in a fluid with density δ, it experiences a buoyant force g equal to the weight of the fluid displaced, $V\delta g$: effective weight of buoyant object = $(m - V\delta)g = m'g$. When the density of the suspending medium is equal to that of the submerged mass m, the gravitational force (weight) acting on the mass is exactly balanced by the buoyant force so that the above equation reduces to zero. Since most structures within an organism possess densities closer to those of body fluids than to that of air, buoyancy should minimize the displacement of one organ with respect to another.

When an experimental subject is immersed in a fluid, the buoyant support provided to the limbs and trunk reduces the work necessary for normal posture and support. Alterations in vascular hydrostatic pressures with a shift in body or limb position will be exactly balanced by a similar change in external hydrostatic counterpressure. As a means of simulating weightlessness, water immersion has certain advantages over bed rest and other means of immobilization. It reduces the force and work requirements for slow movements without the great restriction in motion associated with bed rest. Rapid movements are hindered by the high viscosity of water, but studies on individuals during Keplerian trajectories in airplanes indicate that during weightlessness men tend to execute only slow cautious movements. Furthermore, subjects who have experienced both immersion and Keplerian flight report that the sensations are similar.

The unnatural external environment of immersion produces some important experimental difficulties and is therefore distinct from the true weightless state in several respects:

1. The high specific heat of water results in abnormal heat exchange with the environment. This means that body temperature must be very carefully monitored if temperature artifacts are not to confuse such studies. Additional artifacts may be induced through the restriction of normal cutaneous water losses and ventilation by the use of rubber suits.
2. Immersion in water exposes subjects to ambient pressures of greater than 1 atm.
3. During immersion the ambient pressure over the body is distributed as a gradient, increasing as the depth of immersion increases. This may cause anatomical distortion of the chest and lungs, as the intrapulmonic pressure is uniform.
4. Hydrostatic pressure gradients persist in fluid-filled compliant systems (e.g., the heart, great vessels, and pulmonary vasculature) that are contained in the air-filled, uniformly pressured, thoracic cavity.
5. For comfortable or "eupneic" breathing, breathing air is supplied at a pressure negative to ambient pressure at midchest or right atrial level, producing all the physiological consequences of negative pressure breathing.
6. Viscous resistance to body segment motion is present during immersion.
7. Immersion of human subjects is technically difficult for prolonged periods (days), and although occasionally attempted, immersion is not applicable to most unanesthetized animals (refs. 9–11).

This section reviews the major physiological effects of immersion, specifically the "deconditioning" responses, and some of the above-mentioned artifacts of immersion techniques. The techniques proposed as spaceflight deconditioning countermeasures, including a recent study of the relative effectiveness of certain of these measures, are presented.

A REVIEW OF PHYSIOLOGICAL EFFECTS OF IMMERSION

During his 9-month habitation in the maternal uterus, man is reliving his past history of aquatic existence. Several authors have drawn attention to the analogy of weightless free-floating man in his space vehicle and fetal man in utero (refs. 12, 13). When man first ventured under water is not known, but it probably was at a very early date in prehistory. The physiological effects of immersion were appreciated early but not well studied until the present century. Some excellent texts and reviews of diving physiology are available (refs. 14, 15). The effects of immersion vary with the type and duration of the dive, equipment used, and many other factors.

The simplest and least complicated form of immersion, and the one that can be used as the basic model for comparison with all other immersion modes, is the "bathtub" mode: seated immersion to neck level in neutral temperature (33.5 ± 0.5° C) water or saline without any support equipment. The physiological effects of this type of immersion can be measured, described, and reproduced (refs. 16–22). The addition of breathing equipment, dry suits, and feeding and voiding apparatus complicates the immersion response (fig. 25.1).

Pilot Study

The pilot experiment for the extensive immersion simulations of deconditioning phenomena was performed by Graveline et al. in 1959 at the USAF School of Aerospace Medicine (ref. 20). The coauthors made extensive physiological measurements on the senior author, who was immersed in water to neck level for 7 days. The subject was permitted to emerge from the tank briefly for one period each day for the purpose of body hygiene. The subject reclined on a supporting couch and wore a standard "dry" diving suit. The temperature of the bath was controlled at 35.5° C (92.3° F). Measurements were made of blood morphology, blood volume by the dye-dilution technique, ECG, blood pressure, EEG, water balance, blood and urinary electrolyte levels, and psychomotor proficiency. The subject reported progressively increasing asthenia during the daily period of emersion, which ultimately necessitated termination of the experiment after 7 days. Metabolic rate remained essentially unchanged. The subject's response to tilt-table tests demonstrated progressively severe orthostatic intolerance (fig. 25.2). Water balance studies showed an immediate doubling of the normal urinary output each 24 hr during the first 3 days of the tests, which was associated with similarly increased urinary nitrogen excretion. This striking diuresis during a period of constant intake produced a marked diabetes insipidus-like picture with thirst, hemoconcentration, and weight loss. Biochemical analysis of the blood and serum revealed essentially no difference in the sodium, potassium, calcium, or chloride ion level when comparing the control value to the immersed condition.

Many subsequent studies have demonstrated similar effects of head-out immersion, including the marked diuresis and postimmersion orthostatic intolerance on larger groups of subjects under

varying exposure conditions (refs. 16, 17, 21, 22). Recent studies by Gauer of three subjects during 48-hr head-out immersion confirm the persistent diuresis and diabetes insipidus-like picture (refs. 18, 19, 21).

Cardiovascular Effects

Prolonged water immersion produces circulatory adjustments resulting in orthostatic intolerance (hypotension, tachycardia, vasovagal syncope) on emersion. Factors involved in this response include circulating blood volume; sympathetic nervous system activity; and peripheral vascular reactivity, both arterial and venous. Blood volume is reduced by immersion (ref. 23) and is undoubtedly involved in the response. Using supine subjects and the respiratory regulator at 7 cm posterior to the sternum, Howard et al. found no diuresis, no significant blood volume change, and minimal orthostatic intolerance on emersion (ref. 24); they suggested that the failure to see the deconditioning response was due to the absence of the diuresis. Peripheral vasomotor activity as evidenced by urinary norepinephrine excretion (fig. 25.6) is reduced during immersion (ref. 25). Vascular reactivity during or after immersion has not been studied.

Hood et al. studied changes in hemodynamics in five human volunteer subjects during two separate 8-hr periods of bed rest and total water immersion (ref. 26). During immersion, the subjects showed a decline in pulse rate, arterial blood pressure, peripheral vascular resistance, and elevation of stroke volume compared to the control state. Mean central venous blood pressure fell with immersion, presumably in response to the relatively negative airway pressure supplied to the subjects. These findings suggest that water immersion results in: (1) relative bradycardia with a secondary increase in stroke volume, and (2) peripheral vasodilation with a secondary decline in arterial pressure. These findings are not explained either by loss of plasma volume or by relative negative pressure breathing and, presumably, are a consequence of exposure to the "buoyant state."

Body Fluid Volumes

In recumbency, circulating plasma volume initially increases and is redistributed cephalad with an increased filling of the intrathoracic circulation, followed by a decrease in total plasma volume (ref. 27). Similar responses have been seen during immersion (fig. 25.5). McCally noted a 9 percent plasma volume increase during the first 25 min of immersion, which decreased over the next 4 to 6 hr to approximately 11 percent less than control (ref. 23). A mechanism of body fluid volume regulation during immersion has been proposed and has been supported by considerable direct and indirect evidence (refs. 14, 18, 21, 28). In summary, it is suggested that: (1) immersion produces an increase in both total and intrathoracic blood volume; (2) intrathoracic vascular stretch or "volume" receptors are stimulated; (3) release of the antidiuretic hormone (ADH) is reflexively inhibited; and (4) a predominantly free-water diuresis results, correcting the blood volume increase. Such mechanisms obviously are highly dependent on such factors as temperature and thoracic mechanics.

Renal Effects

Head-out, neutral temperature, water immersion of human subjects produces a profound predominantly free-water diuresis (figs. 25.3 and 25.7). This hyposthernuric diuresis is characterized by natriuresis and by increases in both free-water and solute excretions and glomerular filtration rate (estimated from endogenous creatinine clearance). Although sodium excretion is increased by

immersion, tubular reabsorption (sodium excretion fraction) is not altered (refs. 21, 24, 29, 30). In immersed dogs, however, Myers and Godley observed a diuresis due primarily to an increase in the excretion fraction of sodium, and postulated that the decrease in the tubular reabsorption of sodium was due to either an increase in renal medullary blood flow or the action of a natriuretic hormone (ref. 10).

Lung Volumes

Lung volumes have been measured by spirometry and helium-dilution techniques under many combinations of posture, breathing pressure, and immersion (refs. 31-40). A review of this subject is available (ref. 40). The various authors studied these changes at different intrapulmonary pressures, and comparisons are difficult to make. Jarrett (refs. 37, 38), for example, used a breathing pressure equivalent to the hydrostatic pressure at the level of the centroid* of the chest, and Paton and Sand (ref. 39) used eupneic or subjectively comfortable breathing pressures. In spite of the different intrapulmonary pressures used, most authors agree on the changes resulting from vertical or seated immersion. Tidal volume is unaltered. Vital capacity is reduced during seated or standing immersion. Seated immersion decreases total lung capacity and functional residual capacity, but the supine posture underwater partially restored these decreases. Residual volume is also reduced by immersion in water. Hamilton and Mayo deduced that this was due to an increase in pulmonary blood volume when they observed a 300-ml decrease in vital capacity with vertical immersion to the nipple line; but with cuffs inflated to diastolic pressure on all four limbs, the reduction was only 125 ml (ref. 34). Vertical immersion reduces functional residual capacity, total lung capacity, and expiratory reserve volume but increases inspiratory capacity. These changes follow the same pattern as those produced by recumbency in air.

Intrapulmonary Pressure and Pressure-Volume Relationships

Paton and Sand (ref. 39) first determined that subjects who were requested to choose the pressure that they found most comfortable for breathing while immersed in water invariably chose a pressure—which they termed the "eupneic pressure"—that was more negative than the average pressure over the chest. This pressure was always equal to, or less than, the pressure at the suprasternal notch. Jarrett, however, found that his subjects chose intrapulmonary pressures equivalent to the hydrostatic pressure at the level of the centroid of the chest, and argued that a diver should be supplied with gas at a pressure such that at any depth his functional residual capacity would be unchanged (ref. 37).

A number of procedures have been proposed to minimize or correct for the negative pressure breathing effect of immersion and the resulting diuresis. Carey et al. (ref. 32), Benson et al. (ref. 17), and Agostoni et al. (ref. 31) have attempted to restore normal lung volume compartmentation in the immersed subject and estimate that approximately 20-cm H_2O positive-breathing pressure is necessary. In supine subjects, Torphy applied a positive-breathing pressure equal to the hydrostatic pressure at the midpoint of the anterior-posterior diameter of the chest (refs. 41, 42). Graveline had used balanced or eupneic breathing pressures, maintaining that subjectively selected comfortable breathing pressures would be physiologically optimal (refs. 25, 29, 43). In a detailed discussion of

*The centroid of a given geometrical figure is the point whose coordinates are the mean values of the coordinates of the points of the given figure; it is independent of the choice of axes. The centroid of a geometrical figure corresponds to the center of gravity of a homogeneous material body of similar form.

the problem, Howard et al. suggested that selection of a pressure reference level for the respiratory system during immersion is arbitrary and that no single entirely satisfactory reference can be defined (ref. 24).

Role of the Transpharyngeal Pressure Gradient

Thompson and McCally (ref. 44) have shown that, when breathing through a mouthpiece or a face mask, subjects chose pressures that are negative in relation to the sternal notch (range 0 to −8 cm H_2O). When a helmet alone was used, breathing pressures ranged from −5 to +20 cm H_2O, suggesting that when no transpharyngeal pressure gradient is present, the ability to identify repeatedly a consistently comfortable breathing pressure is reduced. When breathing from a mouthpiece inside a helmet, an increase in breathing pressure resulted in the subject's choosing a helmet pressure that minimized the transpharyngeal gradient (mean range 1 to 7.5 cm H_2O). A wide range of transthoracic pressure gradients (−30 to +40 cm H_2O) is subjectively more comfortable than a slight increase in transpharyngeal gradient, suggesting that during immersion intrapulmonic pressures are selected by the subject to minimize the transpharyngeal pressure gradient.

Temperature Effects

Water has a specific heat a thousand times that of air and a thermal conductivity about 25 times greater than air. Therefore, heat is rapidly lost from the body in cool water, and thermal comfort is maintained only if the temperature is kept at 32° to 33° C (ref. 33). Numerous studies (ref. 45), including basic studies of metabolism and thermal regulation, have used immersion as a means of determining heat loss rates and expected survival times in water at various temperatures and with various clothing combinations. Immersion thus has been a useful tool for the thermal physiologist. The cold diuresis of unimmersed subjects appears to demonstrate well the relationship between intrathoracic blood volume and urine flow. Taking a man from a hot, sweating state to a cold, shivering state produces a decrease in peripheral blood flow, an increase in central venous pressure, and a free-water diuresis apparently mediated by a decrease in ADH concentration in the blood (ref. 46). The effect of temperature on the water-immersion diuresis is not clear cut. Bazett reported that "the diuresis seen in a bath is independent of temperature" in the range 30° to 40° C (ref. 47). DeForest and Beckman, however, have shown that the urine flow rate is considerably greater during head-out immersion at 25° C than at 35° C (ref. 48).

Space Crew Performance

Human performance problems associated with the gravity-free state include the abnormal kinematics of work and locomotion in the absence of normal friction, free body movements in 6 degrees of freedom, and effects on vestibular and other mechanoreceptor systems. The neutral buoyancy, or water immersion, techniques for the study of these problems are valuable because of the length of time allowed for experiments and the size and scale of crew station mockups that may be used. Crew performance data pertinent to locomotion, work, restraint, exercise, orientation, sleep position, crew station design, EVA, etc., can be obtained. Immersed mockups of major manned space systems are in use (refs. 49, 50). Although the immersion technique has the disadvantage of viscous resistance to motion, most of the data from such tests appear valid when compared to similar data from the zero-G aircraft (Keplerian parabola).

RELATIVE EFFECTIVENESS OF DECONDITIONING COUNTERMEASURES

Orthostatic intolerance is seen in normal subjects after exposure to prolonged bed rest (refs. 51–57), immobilization (ref. 58), confinement, (ref. 59), chair rest (refs. 60, 61), and water immersion (refs. 18, 20, 22, 25, 47, 62). Such exposures involve both inactivity and a decrease in the effect of gravity on the longitudinal axis of the body. The physiological mechanisms of the orthostatic intolerance produced in normal subjects by inactivity have been suggested by these studies but are not clearly defined. Available evidence assigns a significant role to the reduction in effective circulating blood volume that occurs in these inactivity states. The roles of peripheral vascular reactivity (in particular, venous tone) and of the sympathetic nervous system activity are less well understood. It has recently become apparent that a major consequence of manned spaceflight is orthostatic intolerance on reexposure to the earth's gravity field. Some degree of orthostatic intolerance is present in the postflight tilt-table tests of the last three Mercury (refs. 63, 64) and all Gemini (ref. 65) and Apollo astronauts (ref. 66). Body weight loss and blood volume contraction are also seen after spaceflight. Inflight urine flow data are not available from either US or USSR manned space programs. The observed blood volume depletion could be sufficient explanation for the postflight orthostatic intolerance. These responses are discussed in detail in recent reviews (refs. 28, 63, 67–69).

It has not yet been established that there is an operational requirement to prevent these physiological events or to protect the astronaut from their effects. However, such changes in body fluid metabolism and subsequent orthostatic tolerance could jeopardize the safety of the astronaut or the successful completion of the mission. A number of protective techniques or countermeasures have been proposed and tested (ref. 70). These include venous occlusive cuffs on the extremities (refs. 22, 43, 62, 71–74), elastic counterpressure garments or leotards (refs. 22, 74), periodic centrifugation (refs. 75–78), positive pressure breathing (refs. 22, 79), lower body negative pressure (LBNP) (refs. 80–84), hypoxia (refs. 84, 85), exercise (refs. 72, 86–92), z-axis trampoline (ref. 52), and the administration of salt- and fluid-retaining hormones (refs. 22, 71, 93). It is clear that a countermeasure should: (1) be relatively antidiuretic, (2) maintain a blood volume and a blood volume distribution approximating that of the erect posture in earth's gravity, and (3) maintain or improve orthostatic tolerance. In recent years, each of a number of countermeasures has demonstrated some degree of efficacy according to such criteria. However, the experimental methods and conditions proposing to evaluate spaceflight deconditioning countermeasures are so varied and undefined that the relative effectiveness of the various countermeasure is impossible to establish.

Accordingly, an experimental study was designed to test the relative effectiveness of six different countermeasures: venous occlusive cuffs (three types of applications), an elastic gradient counterpressure garment or leotard, LBNP, ADH administration, positive-pressure breathing at 15 mm Hg, and mild cold exposure (ref. 22). Six hours of office activity at chair rest were studied as nonimmersion controls (fig. 25.4). With the exception of LBNP, these countermeasures were evaluated in six subjects in a standard 6-hr, head-out, neutral temperature, immersion assay. LBNP was evaluated against a comparable 6-hr bed rest exposure and has been reported elsewhere. Each countermeasure was evaluated for its relative effects on urine flow, blood volume change, norepinephrine excretion, and postimmersion orthostatic tolerance.

Methods

Subjects A total of nine male volunteer subjects between the ages of 20 and 35 were used in these experiments. All subjects were paid volunteers from the US Air Force. All had passed the flying Class III physical examination and were free of intercurrent illness.

Conditions The conditions studied are listed and illustrated in figure 25.4. The experimental period, procedures, laboratory methods, and statistical analysis are described in detail in reference 22.

Results

These studies confirmed that head-out, neutral temperature (34° C), water immersion of human subjects produces diuresis (fig. 25.7), plasma volume contraction (fig. 25.5), diminished urinary excretion of norepinephrine (fig. 25.6), and subsequent orthostatic intolerance to vertical tilt. The elastic leotard, donned after immersion just prior to the tilt, was the most effective measure tested and restored the tilt-table responses to control levels. This garment prevented the orthostatic intolerance seen after immersion, in spite of the fact that norepinephrine excretion and plasma volume were reduced significantly. Four-extremity venous tourniquets inflated to 80 mm Hg (1 min off and 1 min on) were also effective in preventing the postimmersion orthostatic intolerance. This cuff exposure also produced a significant increase in urinary norepinephrine excretion as opposed to the untreated immersion situation, but had no effect on plasma volume contraction. Four-extremity venous tourniquets inflated in a 2-min-on, 4-min-off cycle offered no protection and had no effect on either norepinephrine excretion or a change in plasma volume, suggesting that cycling times are critical for the use of cuffs as a deconditioning countermeasure. ADH administration during immersion produced significant water retention but had no significant effect on the change in plasma volume or on subsequent tilt-table tolerance. Immersion in cold water (30°—31° C) produced a significant increase in urinary norepinephrine excretion, but this was not associated with improved tilt-table tolerance. Estimated plasma volume was reduced by immersion but was not well correlated with the degree of orthostatic intolerance. Urinary norepinephrine excretion was significantly reduced during immersion, suggesting that the sympathetic nervous system is involved in postimmersion orthostatic intolerance.

Positive pressure breathing reduced the diuretic and plasma volume contraction responses to immersion but did not significantly improve postimmersion tilt-table tolerance. Six hours of bed rest produced mild but significant orthostatic tachycardia, which was prevented by 6-hr of intermittent LBNP at -50 mm Hg. Of the various simple countermeasures proposed and evaluated in this program, a simple elastic counterpressure garment is the most effective as well as the simplest to apply.

DISCUSSION

There are two general approaches to deconditioning countermeasures for assuring that an astronaut will not suffer from the adverse effects of cardiovascular adaptations to weightlessness on being reexposed to a gravity environment during exploration of a lunar or planetary surface, or on being subjected to head-to-foot acceleration forces during takeoff, landing, or recovery operations. One approach is to *prevent* the occurrence of cardiovascular adaptations to weightlessness. The other is to *protect* the astronaut from the undesired effects of these adaptations. Methods that

have received significant experimental study are discussed below. Particulars of their application can be obtained from cited references. Although some indication of their effectiveness can be given, it must be remembered that appropriate selection and optimum utilization of a particular countermeasure can be ensured only by more detailed investigation, including specifically the evaluation of a given technique against firm operational requirements or criteria.

Exercise

The greatest attention, from the standpoint of preventing cardiovascular adaptation to weightlessness, has been focused on periodic physical exercise. Since exercise increases blood volume in a normal ambulatory individual on earth, it was thought that an appropriate regimen might minimize the decrease of blood volume associated with weightlessness (refs. 51, 52, 55, 89, 94, 95). It also has been suggested that exercising the lower extremities in particular might reduce the tendency to venous pooling by maintaining muscle tone, strength and mass, and the capacity of arterial and venous vasoconstrictor mechanisms to respond to intravascular hydrostatic forces (ref. 67). However, a number of isotonic and isometric exercise regimens have been studied and found to have no really significant effect on either the blood volume change or the degree of orthostatic intolerance associated with prolonged bed rest (refs. 4, 52, 72, 91, 92). Bungee cord exercises during the 8- and 14-day, two-man Gemini missions did not prevent postflight orthostatic intolerance, even though the cardiovascular response to a calibrated work load postflight was apparently maintained (ref. 65). It appears doubtful that physical exercise is an effective method for the prevention of cardiovascular deconditioning associated with spaceflight. Recent bed rest studies (refs. 4, 86, 87) have demonstrated that exercise may be of some use in the prevention of musculoskeletal deconditioning, but relatively prolonged periods of heavy exertion, including sweating, are required to achieve an effect. Frequent heavy aerobic exercise is probably beyond the life support capability of present manned space vehicles. The problem of inflight exercise raises the unanswered questions of whether the astronaut should be allowed to adapt passively to the spaceflight environment or whether some measure of earth physiological performance should be maintained by active means; and, if the latter, what criteria of cardiovascular, musculoskeletal, or exercise performance should be used.

Venous Occlusive Cuffs

Various combinations of periodically inflated cuffs placed proximally on the extremities have been used in attempts to alter cardiovascular deconditioning effects. It was thought that periodic increases of peripheral venous pressures might maintain not only venomotor capacity but an optimal level of extravascular tissue tension (refs. 43, 67, 96). Another postulated effect was a reduction of the degree of filling of central venous circulation and hence prevention of any reflex decrease of blood volume. Graveline et al. (ref. 43), and later Vogt (ref. 73), found that periodic (1 min on, 1 min off) inflation of cuffs to 80 mm Hg, placed around all four extremities of subjects immersed in water for 6 hr, maintained orthostatic tolerance. When carried out during 2 weeks of bed rest, this technique conferred significant protection from orthostatic intolerance, as tested by a 10° tilt, which presumably simulates the effect of the Moon's gravitational field on the cardiovascular system (ref. 91). On the other hand, none of a variety of cuff configurations applied during a number of water immersion and prolonged bed rest studies has prevented the decrease of plasma volume or orthostatic intolerance (refs. 22, 62, 71, 72, 74, 90). Inflation cycle times appear to play an important role in determining the effectiveness of the occlusive cuff technique. The data presented above confirm Graveline's original observations that four cuffs at 80-mm Hg effective pressure, 1 min on and 1 min off, improve the orthostatic tolerance seen after water immersion. However, it would

appear that frequent cycling is necessary to achieve this effect, as cuffs in a 2-min-on, 4-min-off cycle are ineffective. Periodic inflation of lower extremity cuffs on the pilots of the 8- and 14-day, two-man Gemini missions was also ineffective in lessening postflight orthostatic intolerance, even though there appeared to be some decrease in the degree of postflight pooling of blood in the lower extremities, as judged by the Whitney strain-gage technique (ref. 96). The present study would suggest that four-extremity cuffs and frequent cuff cycling would be necessary for the cuff technique to be effective. In addition, it remains to be established that the postspaceflight and post-immersion orthostatic intolerance are produced by the same physiological mechanisms. Vogt discerned no protective effect from the use of intermittently inflated lower extremity cuffs during a 10-day bed rest study, but felt that the long cuff cycle (5 min on and 10 min off), as well as the use of narrow (3-in.) cuffs, may have been responsible for the observed lack of effect (ref. 72). We agree with Vogt's conclusion that intermittently inflated extremity cuffs may be partially protective in bed rest and water immersion conditions, particularly if large, four-extremity cuffs are worn and cycled frequently and continuously; but this protection is probably not sufficient to warrant their use in spaceflight.

Lower Body Negative Pressure

Exposure to LBNP has been suggested as a method for preventing the adverse effects of cardiovascular adaptations to weightlessness, since its effects on the cardiovascular system are similar to those due to gravitational hydrostatic pressures. Excellent discussions of the physiological effects of LBNP and its mechanisms are available (refs. 82, 97, 98), and a detailed review of the rather voluminous literature is in preparation by the author. A number of studies have shown that LBNP can either prevent or restore the decreases of plasma volume and orthostatic tolerance that result from prolonged bed rest (refs. 80, 83, 84, 87, 97). Of particular importance for space missions of long duration is the fact that orthostatic tolerance was restored by this method when applied over a period of only 2 days (refs. 67, 83). Hence, for space missions of significant duration, orthostatic tolerance could conceivably be reestablished by brief exposures to LBNP just before reentry into a gravity environment. Although exposure to LBNP appears to be a feasible measure for restoring an astronaut's orthostatic tolerance while in space, consideration must be given to the restrictions that this measure might place on an astronaut's activity during a critical phase of a mission. There is also need for studies to determine time and pressure modes that would provide an optimum effect.

Short-Radius Centrifuge

Periodic acceleration on a short-radius centrifuge small enough to be carried in a space vehicle has been assessed for its effectiveness in preventing orthostatic intolerance resulting from prolonged bed rest. White has reported that as few as four 7.5-min rides per day, at +1.7 Gz at the heart on a short-radius centrifuge largely prevents orthostatic intolerance, as judged by the incidence of syncope (refs. 76–78). However, heart rate and blood pressure responses to tilt and decrease of plasma volume during bed rest were essentially unaffected by this measure. Further testing of periodic centrifugation has indicated that its physiological disadvantages, including vestibular stimulation, pulmonary ventilation/perfusion disturbance, and arterial hypoxemia, may outweigh its advantages (ref. 75). The weight, power, and volume penalties imposed by a short-radius centrifuge could be surmounted if the effectiveness of this measure could be well established.

Positive-Pressure Breathing

Various forms of positive-pressure breathing have been examined as deconditioning countermeasures. They have shown, in general, no significant effect on the orthostatic intolerance resulting from head-out water immersion or bed rest (refs. 79, 99). In the present study, the results from continuous positive-pressure breathing at 15 mm Hg for all variables were not significantly different from the simple immersion values. Free-water clearance similarly remained negative and the estimated change in plasma volume did not decrease, suggesting that positive-pressure breathing was effective in preventing the free-water component of the immersion diuresis. In the present study, however, tilt-table tolerance was not improved significantly by positive-pressure breathing. In similar studies with continuous positive-pressure breathing at 20 mm Hg, Hunt found significant improvement in postimmersion tilt-table tolerance, and reduced urine flow rates and free-water clearances. The higher breathing pressure may explain his positive results. Hunt's paper contains an excellent discussion of the physiological mechanism of positive-pressure breathing (ref. 79).

Drugs and Other Techniques

A few other protective measures have been studied for use in space, and all have been essentially ineffective in experiments to date. The oral administration of 9-α-fluorohydrocortisone for a 3-day period at the end of a prolonged bed rest exposure did return blood volume to normal but did not prevent the orthostatic intolerance resulting from the exposure (refs. 71, 93, 100). It should also be noted that this drug produced occasional nausea, an effect that would be highly undesirable in the space situation.

The administration of pitressin (ADH), with and without concomitant water loading, to subjects immersed to the neck in water has prevented the diuresis and associated decrease in plasma volume but not the orthostatic intolerance that results from the immersion (refs. 22, 101).

Based on the fact that many of the physiological responses to hypoxia are the converse of the responses to weightlessness, hypoxia has been suggested as a countermeasure, and individuals have been exposed to 10,000- and 12,000-ft altitudes during bed rest (ref. 85). Although exposure to these mild hypoxic conditions did prevent the decrease in red cell mass that occurred during bed rest exposures at ground level, it did not reduce the orthostatic intolerance produced by bed rest (ref. 83).

Periodic Z-axis bouncing exercise on a railed cart between two trampolines has been carried out on subjects of prolonged bed rest (ref. 52). It was thought that the vascular effects of the exercise, as well as the repetitive "sloshing" of blood, would serve to maintain the capacity of both veins and arteries to compensate adequately for intravascular hydrostatic forces due to gravity. Although this measure was found ineffective, it might warrant further testing.

Vogt et al. have studied a "gravitational acceleration simulation suit" that provided, by means of elastic fittings, a graded force on the musculoskeletal system approximating that experienced at +1 Gz. The suit provided no significant protection from the cardiovascular effects of 3 weeks of bed rest. X-ray density and other measures of musculoskeletal status were made but not reported (ref. 102).

Elastic Leotard

What appears to be the most effective measure assessed to date for the protection of bed rest and water immersion subjects from orthostatic intolerance has been the application of a pressure garment to the lower part of the body during tilt (refs. 22, 55, 67, 74, 91). The external pressure presumably acts to prevent excessive venous pooling and loss of plasma volume through transudation of fluid into the tissues of the lower extremities in the upright position. The garments are lightweight, comfortable, easily ventilated, and may be worn continually for prolonged periods of time without discomfort or impairment of skin hygiene. This form of counterpressure is as effective as that applied by a pilot's antigravity suit with its capstans and bladders, and is considerably more comfortable and simple to operate. Such a garment could be donned by an orbiting astronaut prior to reentry, worn comfortably beneath his spacesuit, and would provide him with considerable protection from exposure to any +Gz acceleration during the reentry and recovery period.

Finally, protection from the adverse effects of cardiovascular adaptations to weightlessness is a major factor in any consideration of whether to provide astronauts artificial gravity in space. It is readily apparent from the above discussion that the operational significance of cardiovascular and musculoskeletal deconditioning remains to be established. Any consideration of physiologic factors in the requirement for artificial gravity is premature until the question of weightlessness adaptation versus prevention is resolved. If artificial gravity is employed, the level required for preventing orthostatic intolerance in the various gravity environments to be encountered during space missions will have to be determined.

CONCLUSIONS

The neutral buoyancy of water immersion has provided a useful laboratory analog of the weightless state. It reasonably simulates specific anticipated effects of weightlessness, including reduction in apparent weight and minimization of intravascular hydrostatic pressures. The post-spaceflight cardiovascular deconditioning phenomena, including loss of orthostatic tolerance, blood volume contraction, and impaired sympathetic nervous system function, were suggested by immersion experiments. Our present space medicine program owes a debt to these studies. However, the problems are now more clearly defined; biomedical data are available from the spaceflight situation; and more appropriate experimental tools, such as bed rest and plaster cast immobilization, have been developed. Water immersion has probably had its day for physiological studies of deconditioning phenomena. Immersion effects on respiratory mechanics, blood volume distribution, and thermal equilibrium make interpretation of experimental results difficult. However, immersion is still a useful technique for the comparative evaluation of countermeasures for deconditioning effects. In addition, the neutral buoyancy of immersion will continue to be important in simulating the problems of motor and kinematic performance of freely moving, suited men within space vehicles and EVA simulations.

Of the various countermeasures for spaceflight cardiovascular deconditioning, LBNP and the elastic gradient leotard would appear to be the most effective, judged by the few criteria presently examined, and the most practical from the engineering point of view. However, it must always be realized that a basic decision necessary to further work on deconditioning countermeasures has not been made. Namely, is it necessary to prevent the astronauts' physiological adaptations to the

hypodynamic and hypogravic conditions of spaceflight? If so, what criteria of performance or integrity of cardiovascular and musculoskeletal structure and function should be maintained? Is any deviation from a physically well-conditioned earthling astronaut undesirable? These questions will obviously be found and answered only with the collection of more biomedical experience from longer duration manned spaceflight. Until such questions are specifically answered and restated as mission requirements with firm criteria, the study of spaceflight deconditioning countermeasures will remain undirected and undisciplined.

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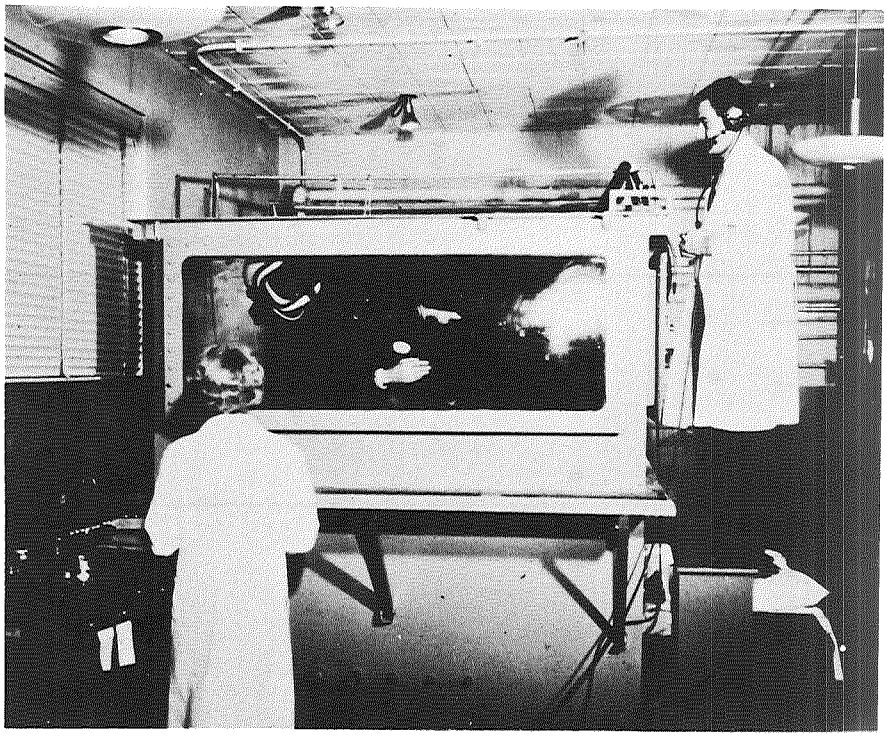

Figure 25.1 Aerospace Medical Research Laboratory immersion facility.

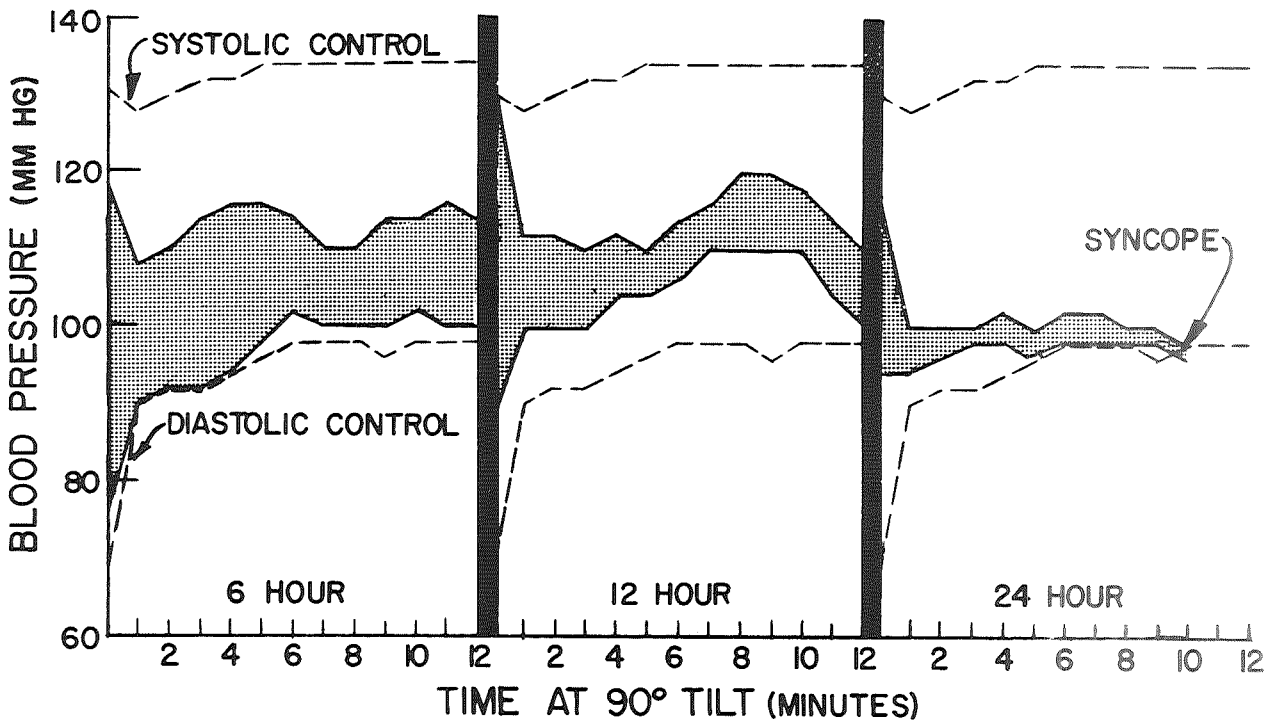

Figure 25.2 Systolic and diastolic blood pressure response of a single subject to a 90° vertical tilt after 6-, 12-, and 24-hr exposures to water immersion

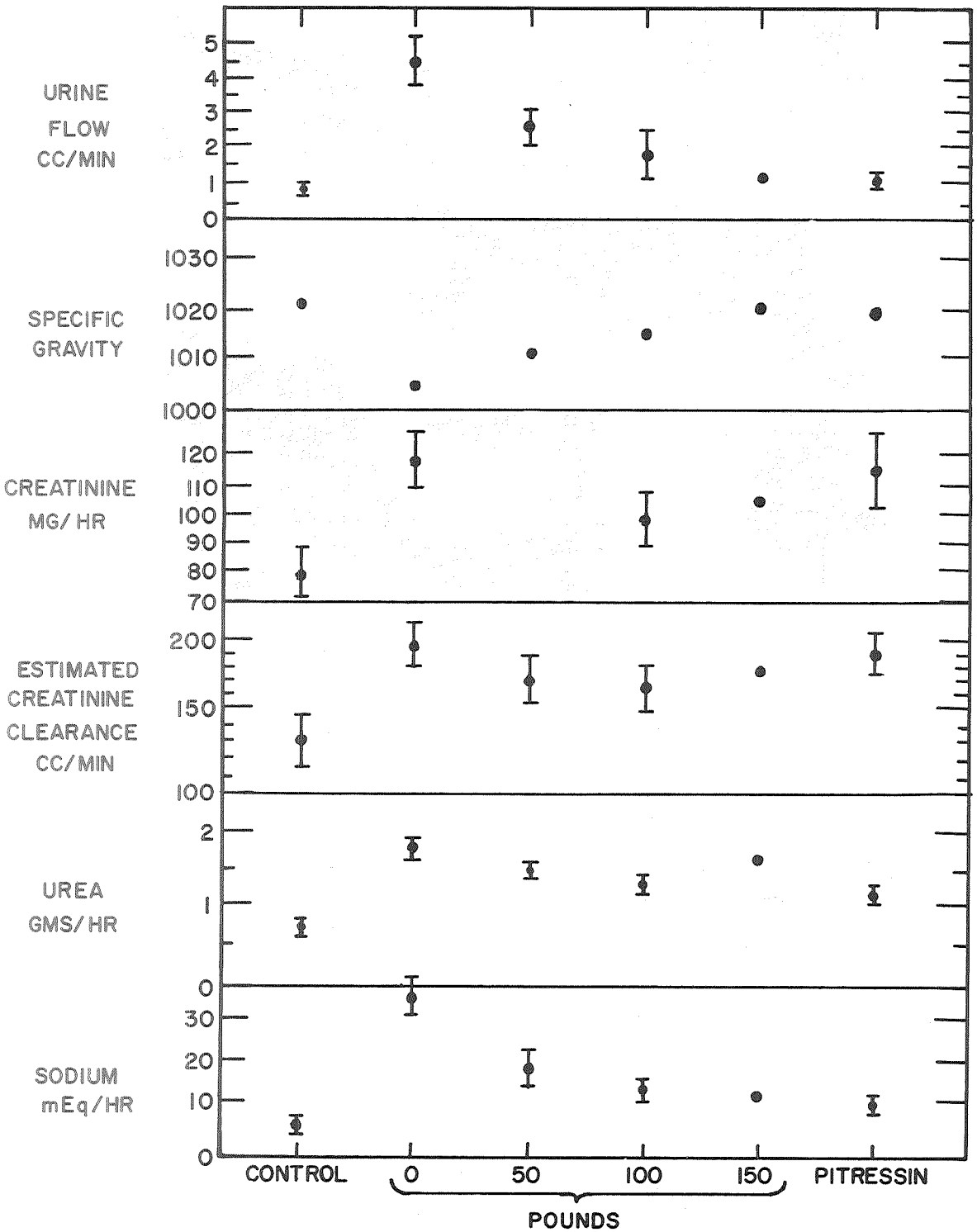

Figure 25.3 Effect of weight-bearing and pitressin (ADH) administration on urine flow, urine solute concentration, and estimated creatinine clearance during 1 hr of water immersion. The mean and range of the values obtained from the ten subjects are shown. Only two subjects were given the 150-lb weighted test. Source: *Aerospace Medicine;* reproduced by permission

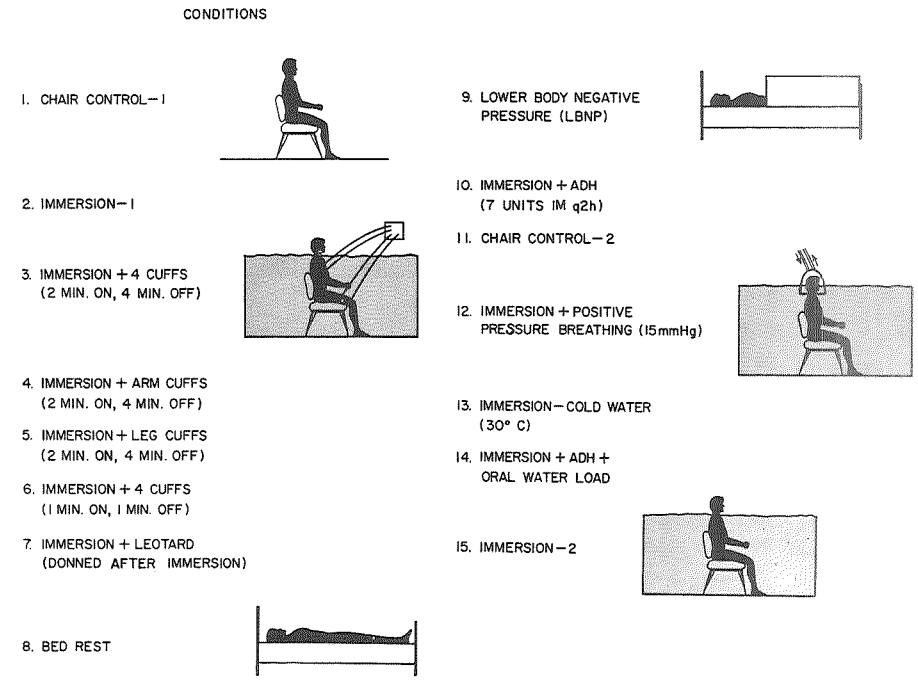

Figure 25.4 The 15 study conditions, in the order of treatment. The sketches present the approximate posture of the subject in each of the major environments including chair rest, immersion, bed rest, and LBNP. Source: *Aerospace Medicine;* reproduced by permission

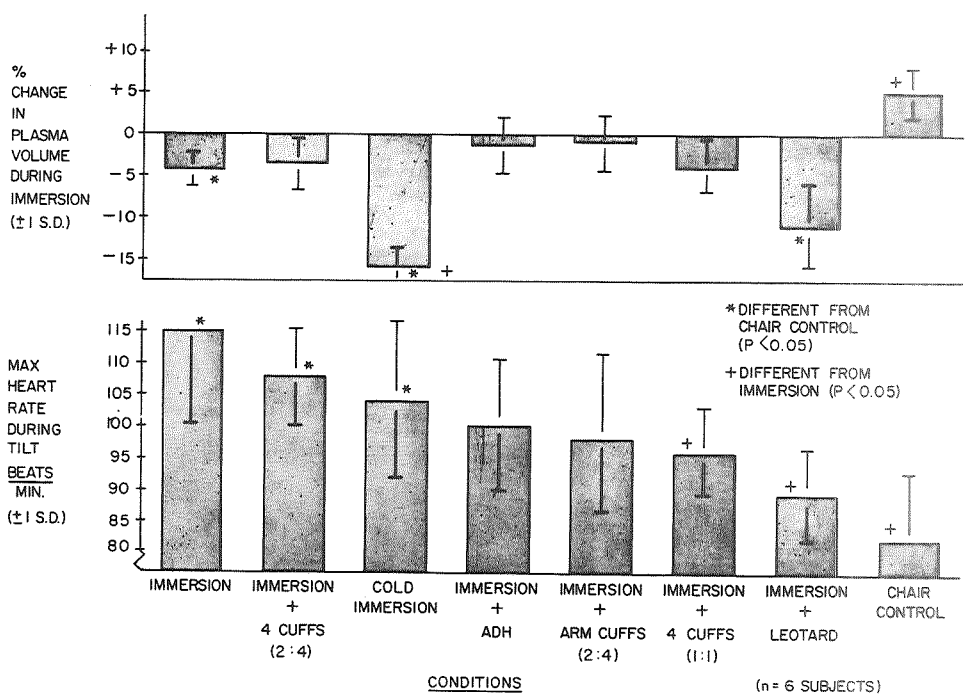

Figure 25.5 Maximum heart rate during the tilt. Note that the vertical axis does not begin at 0 bpm, but shows range of 80 to 115 bpm. The upper panel shows the corresponding percent change in plasma volume presented in a similar fashion. Source: *Aerospace Medicine;* reproduced by permission

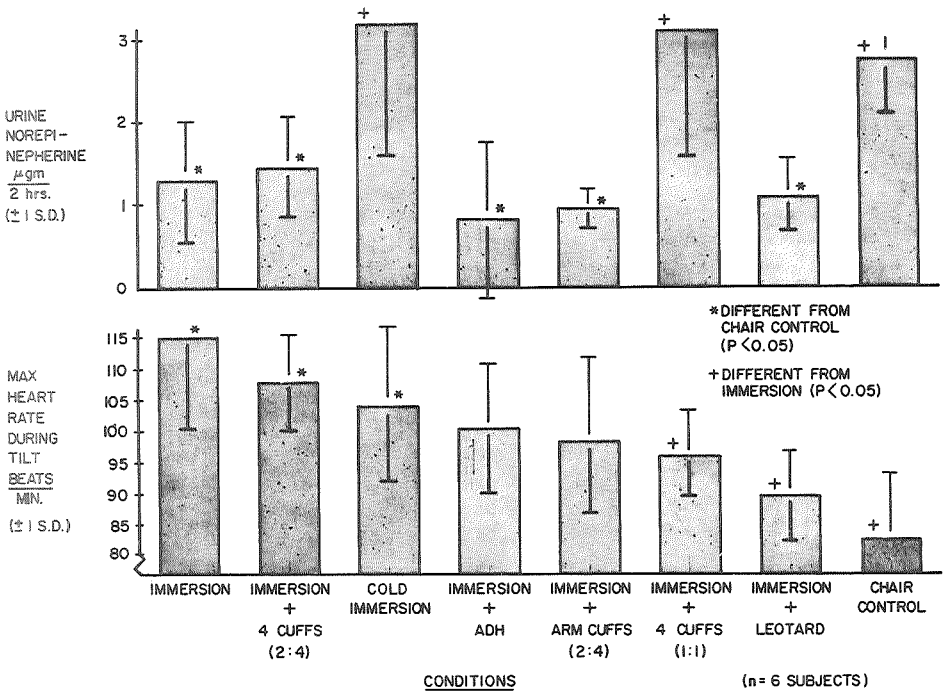

Figure 25.6 Urinary norepinephrine excretion in relation to the ranked tilt-table responses. These values are the excretion rates for the last 2 hr of the 6-hr exposure. Source: *Aerospace Medicine;* reproduced by permission

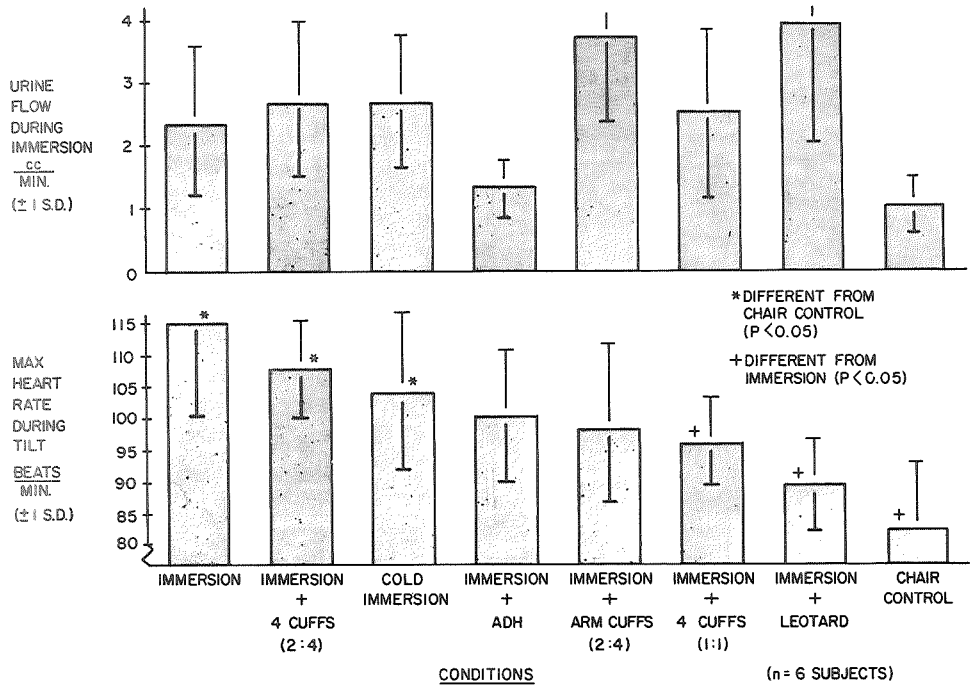

Figure 25.7 Mean urine flow rates during immersion for the total 6-hr experimental collection period. Source: *Aerospace Medicine;* reproduced by permission

26 BODY FLUID REGULATION DURING IMMERSION*

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CIRCULATION AND POSTURE

The human skeleton is always exhibited in the erect posture, demonstrating that every detail of its structure is especially engineered for the upright stance. Human circulation, on the other hand, is usually considered only a slight modification of a rabbit's circulation, with the exception that it has developed a more alert reflex mechanism to actively counteract the so-called "stress of orthostasis" when man stands erect. The doctor usually examines his patients in bed, and the physiologist straps his experimental animals to the table top: neither remembers that man spends much of his day exposed to gravity. However, for a rational analysis of the effects of gravity and weightlessness, it is essential to understand fully that the normal control condition of the human circulation is that of upright posture. This concept, first upheld by the eminent cardiologist F. M. Grödel (founder of the American College of Cardiology), is supported by the not only simple but striking argument that a healthy man spends more than two-thirds of his life in this posture (ref. 1).

Figure 26.1 (refs. 1, 2) depicts the circulatory events accompanying a change in body posture. Let us first assume that the circulation in the supine posture (fig. 26.1(a)) represents the normal control condition. On transition to the upright posture (fig. 26.1(b)), 400 cc of blood pool in the legs. This volume is drained largely from the intrathoracic circulatory compartment, including the heart; as a consequence, stroke volume and cardiac output (despite a cardioacceleration) decrease. Through intervention of the baroreceptor reflexes, heart rate and total peripheral resistance (TPR) rise to prevent arterial pressure at the cerebrum from falling below critical values (ref. 1). Since the plight of the arterial circulation is initiated by the pooling of a considerable volume of blood in the legs, one must wonder why the mechanisms of circulation, when exposed to the "stress of orthostasis," prefer a last-ditch defense of the homeostasis of the arterial pressure by increasing heart rate and TPR instead of meeting the challenge at its root by preventing the initial blood pooling through constriction of the peripheral capacitance vessels.

This paradox is resolved if we assume that circulation in the upright posture is the normal control condition in which the total blood volume, and its distribution, is adjusted to the capacity of the upright vascular bed. Starting from this baseline, the reaction to assuming the horizontal posture would be as follows. As the hydrostatic distending pressure in the peripheral veins falls, approximately 400 cc of blood are poured into the intrathoracic space. The resulting distension of the heart induces an increase in stroke volume and cardiac output. This useless hyperactivity of the

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circulation is promptly attenuated by a reflex reduction in heart rate and a decrease in arterial flow resistance (to meet the "stress of recumbency"). The depressor signals involved in this reflex probably arise from all baroreceptor regions, including those in the heart itself (ref. 3). When the state of circulatory hyperactivity, characterized by a low A-V O_2 difference and rapid depressor signals from the atria, is maintained for some time, a slow mechanism may adjust blood volume to the reduced capacity of the peripheral vascular bed. This consideration suggests that the long-term adaptation of the circulation to changing gravitational fields is achieved basically through control of blood volume.

BLOOD VOLUME CONTROL AND WEIGHTLESSNESS

Reflex Control of Plasma Volume

Figure 26.2 shows the major circulatory mechanisms of plasma volume control, outlined below.

Afferent Pathways Most of the signals indicating the state of filling of the intrathoracic circulatory organs originate in the atrial receptors and are carried in the vagus to the medulla. For hemodynamic reasons, the atrial receptors are very sensitive to small changes in blood volume. Changes in blood volume that are large enough to affect mean or pulsatile blood pressure can be sensed through the receptors in the arterial system (e.g., the carotid sinus).

Effector Pathways — Hormonal Effectors Via poorly explored pathways from the medulla, the hypothalamic-postpituitary system is affected. Increased excitation of the atrial (and arterial) receptors leads to a reduction in ADH secretion and vice versa. In long-term excitation of the baroreceptors in the circulation, the secretion of suprarenal hormones, e.g., aldosterone, is altered, as well as the concentration of pressor substances (renin, angiotensin) in the plasma. As indicated by a dotted arrow in figure 26.2, the efferent pathways are still under debate. A possible third (natriuretic) factor is not shown.

Effector Pathways — Sympathetic Nerves Decreased central blood volume increases the activity in the sympathetic fibers to the kidney and the peripheral vascular beds, resulting in a tendency toward sodium retention and a resetting of the post- to precapillary flow resistance ratio in the tissues in such a way that filtration of fluid from the interstitial space into the vascular bed is enhanced. The reverse occurs with increased central blood volume.

These effects are greatly altered by the state of hydration of the subject. The combined effects of a resetting of fluid balance by the kidney on one side and a redistribution of extracellular fluid volume between the intravascular and interstitial compartments on the other are very efficient means for a reflex control of plasma volume within 6 to 8 hr.

Central Nervous System The alteration of the basic volume control reflex by impulses from higher formations of the CNS, muscular exercise, hypnosis, emotion, etc., is indicated by light arrows arising in the cortex.

Negative-Pressure Breathing vs Whole-Body Immersion

A causal relationship between an increase of intrathoracic blood volume and a concomitant increase in urine flow was established in dog and man, using negative-pressure breathing (NPB) to expand the intrathoracic blood volume (ref. 4). This diuresis is triggered by intrathoracic stretch receptors, as discussed above, particularly those in both cardiac atria. The reflex diuresis following

atrial distension is mediated through vasomotor alterations in the kidney (ref. 5) and changes in the rate of secretion of various hormones, particularly ADH (refs. 4, 6). It was interpreted as evidence for the existence of a sensitive mechanism for the homeostatic control of blood volume.

For the investigation of long-term effects, NPB has serious drawbacks because it causes considerable discomfort to human subjects after 1 hr. The use of whole-body immersion provides better experimental conditions than NPB: it has the same effect on blood volume distribution but it can be continued for hours or even days. The immersion technique was first used extensively by Bazett et al. in 1924 (refs. 7, 8); revival of this technique by Graveline et al. (refs. 9, 10) furnished an indispensable tool when it became evident that certain important aspects of blood volume control, through baroreceptors in the circulation, could only be clarified with stimuli of long duration.

OBSERVATIONS FROM LONG-TERM IMMERSION EXPERIMENTS

In many respects, water immersion clearly is not a perfect substitute for true weightlessness (ref. 11). However, with regard to the gravitational redistribution of blood volume and its initiation of circulatory reflexes, the technique may be ideal since it seems to exaggerate the anticipated effects of zero gravity on the circulation. It is probably correct to assume that the degree of relative engorgement of the heart and thoracic vessels in the weightless state is somewhere between that seen during bed rest, as a lesser stimulus, and that of water immersion, as the more potent stress (ref. 12).

This section discusses the effects of water immersion on various physiological mechanisms. Experimental results similar to those outlined below have been obtained by several independent teams working in this field (refs. 9, 10, 13–16).

Renal Function

Interpretation of the diuretic response to NPB as a blood volume regulatory mechanism has been questioned for two reasons (ref. 17). First, diuresis seemed to subside spontaneously after 45 to 60 min of NPB; second, it is in most cases a pure water diuresis. Preoccupied by the role of salt in edema formation, renal physiologists have been reluctant to accept a change in free-water clearance as an expression of volume control. They insist that salt be considered in the first place. Our immersion experiments indicated that the altered kidney function is maintained throughout the whole immersion period, and that the elimination of fluid — regardless of its composition — has first priority (ref. 18).

The character of the diuresis depends on the state of hydration of the subject. In well-hydrated subjects, a water diuresis is evoked (with a washout of some salt), while the "dry" subject achieves fluid elimination by an increase in osmolar clearance (fig. 26.3) (refs. 19, 20). In order of discovery and solid experimental foundation, the various effector mechanisms of the volume-conditioned diuresis are: (1) ADH; (2) renal hemodynamics; (3) adrenalin; (4) aldosterone; (5) sodium (and water) eliminating factors (third factor?); and, (6) renin, angiotensin.

Evidence for a reduction of ADH activity and an increase of renal filtration with engorgement of the central circulation is abundant (refs. 4, 6, 17). Decrease in noradrenalin with immersion was demonstrated by Goodall et al. (ref. 21). Furthermore, a decrease in sympathetic outflow may

critically affect intrarenal circulation (ref. 5). During 8-hr immersion, a considerable increase of the Na/K quotient was observed, which argues in favor of reduced aldosterone secretion (ref. 20).

Our experiments provided evidence of a diuretic (natriuretic) factor (ref. 22) – a significant decrease in renin-like activity (28 percent) (ref. 23) and in plasma albumin concentration (ref. 24).

Plasma Volume

The claim that the diuretic reflex serves as a control of blood volume requires simply the demonstration that engorgement of the intrathoracic blood compartments for several hours leads to a reduction of blood volume. Several independent investigators furnished proof of a significant decrease of plasma volume after 6 to 8 hr of immersion (table 26.1) (refs. 11, 14, 15, 20, 25, 26). Refer again to figure 26.2

Distribution of Extracellular Fluid Volume

As early as 1924, Bazett found that the degree of hemoconcentration seen during water immersion was not well related to external fluid elimination by the kidney (ref. 7). In two independent sets of experiments in our laboratory, the same discrepancy was observed (ref. 20). Since urine volume is derived from the total extracellular fluid, plasma volume reduction should contribute not more than 20 to 25 percent of the urine volume excreted during immersion. In a group of well-hydrated subjects, this was indeed the case. In slightly dehydrated subjects, however, plasma volume reduction may account for as much as 100 percent of the total fluid loss (fig. 26.4). In some cases the plasma volume reduction was even greater than the fluid loss. This observation can only be explained by a shift of plasma fluid into the interstitial space. Obviously, the same stimulus that induces fluid elimination through the kidney may, at the same time, favor movement of fluid from the intravascular compartment into the interstitium. This observation is in agreement with Öberg's (ref. 27), who found in cats that hemorrhage or stimulation of cardiac receptors leads to a resetting of the post- and precapillary resistance and a subsequent change of fluid filtration pressures.

It appears that "dry" subjects lose fluid more easily into the interstitium than hydrated ones, while their diuretic response is less.

Venous Tone

The susceptibility to orthostatic collapse after immersion of several hours may be attributable in part to the reduction of plasma volume. However, a potential reduction of venous tone must also be considered. To investigate this possibility, a Whitney strain gauge was used to record the change of circumference (Δc) of the lower arm when the venous pressure (p) was elevated by the inflation of a cuff (refs. 28, 29). It was found that $\Delta p/\Delta c$ falls quickly and shows a further slight decline with time. At the end of immersion, return of the distensibility to normal takes several hours. This observation may partly explain orthostatic difficulties that may persist for hours or days after long-term immersion or weightlessness. However, an unsolved question of great interest is why the increased distensibility continues after an exposure of several hours. Furthermore, the increased distensibility by itself is not easily understood. It is generally agreed that the capacitance vessels are normally relaxed and show little vasomotion with moderate stimuli. Therefore, the question arises whether the excess increase in volume of the arm with rise in venous pressure means increased blood content in the muscle or excess filtration into the tissues.

Physical Working Capacity

The observation by Graybiel and Clark (ref. 13) that the working capacity after immersion is considerably reduced has recently been confirmed (ref. 30). This phenomenon is probably related to the symptom of orthostatic weakness.

SPACE PROGRAM OBSERVATIONS

The concept of blood volume control has been very helpful in the interpretation of some clinical conditions characterized by an imbalance in fluid metabolism in the presence of a disturbance of cardiac function or innervation. Similarly, a number of space pilot observations may be interpreted in the light of physiological experience gathered in long-term immersion experiments (refs. 12, 16).

1. Most investigators in the field seem to agree that the disturbance of fluid balance that occasionally results in a considerable weight loss may best be explained by an atrial reflex mechanism (refs. 12, 16, 18).
2. While long-term immersion always leads to a significant reduction in plasma volume and blood volume, variable results have been obtained in prolonged states of weightlessness during the various space missions. Apart from considerable technical difficulties, varying responsiveness of the individual space pilots and factors that strongly influence plasma and cell volume, such as the artificial atmosphere and different water uptake, may be responsible for the inhomogeneity of the results (refs. 12, 16, 31).
3. The orthostatic weakness after landing is most likely due to the long-term attenuation of the regulating centers of blood volume and vascular tone by depressor signals from the heart. Since ADH secretion is reduced during immersion (and probably long-term weightlessness), infusion of this hormone has been tried. By preventing the diuresis of immersion with a slow infusion of vasopressin (refs. 18, 32) in a small group of subjects, orthostatic collapse could also be prevented. Other workers, however, have seen no benefit from this measure (ref. 15).
4. A reduced working capacity is found after long-term immersion (ref. 30) and true weightlessness (ref. 31). Although an explanation is not available, it is probably reasonable to assume that this condition is closely related to the reduction of vascular tone and/or increased tendency toward outward filtration, which seems to be common to both immersion and weightlessness. This tendency toward outward filtration may aggravate plasma volume reduction, which is commonly observed during heavy exercise (ref. 33).
5. Graveline et al. (refs. 9, 10) were the first to observe a most severe disturbance of fluid balance in a subject submitted to an immersion of several days. In our experience this is not the rule, although in one group of five subjects immersed for 48 hr, two showed the same symptoms. One experienced an excess excretion of 15 g Na (40 g NaCl), a weight loss of 5 kg, and an increase in hematocrit. He was always thirsty, although he had free access to water. Possibly, in susceptible individuals, a condition may develop that may be described as the reverse of the salt and fluid retention characteristic of congestive heart failure (cf. ref. 18), induced by the bombardment of the CNS with the continuous signal "too much volume."

   The sleeping behavior of this subject is noteworthy. Most subjects who tolerated immersion well had a tendency to extend their sleeping periods during immersion. This is consistent with the old observation that experimental animals can be put to sleep by stimulating the baroreceptor regions (ref. 34). In contrast, our two poorly adapted subjects appeared alert, took a

very active interest in the experiments, and slept only a few hours. They were obviously of the same "eager beaver" type as the subject of Graveline and one of Graybiel's subjects who, during the long-term immersion, drifted into a very alarming condition. There can be little doubt that the psyche strongly determines the individual response to the effect of immersion (and probably weightlessness). If this is true, the success of the American space program is due, in great measure, to the psychologists who selected the right space pilots.

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Table 26.1 Changes in plasma volume (percent) during whole-body immersion

Author	*Reference*	*6 to 8 hr*	*5 to 10 days*	*n subjects*
Behn et al.	20	-9.7	–	7
Behn et al.	20	-15.3	–	6
Kaiser et al.	26	-14.0	–	17
Korz et al.	23	-14.1	–	8
McCally	14	-11.0*	–	5
McCally	15	-7.9	–	6
White et al.	11	–	-23	10
White et al.	11	–	-10**	10

*9 percent transitory increase after 25-min immersion.
**10-day immersion.

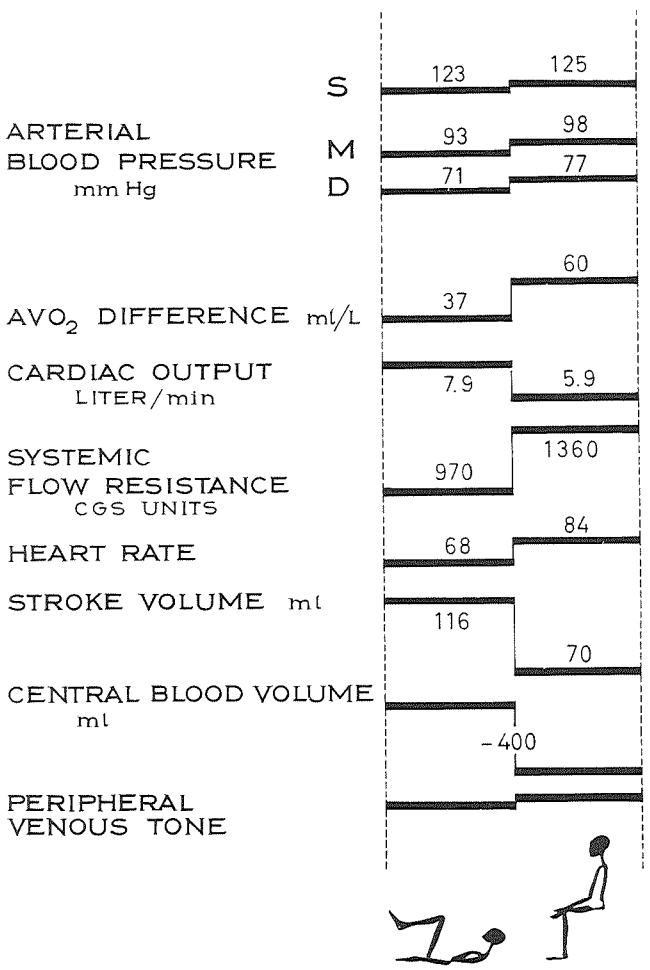

Figure 26.1 Change of hemodynamic parameters with change of position from (a) supine to (b) upright posture. Source: Reference 2

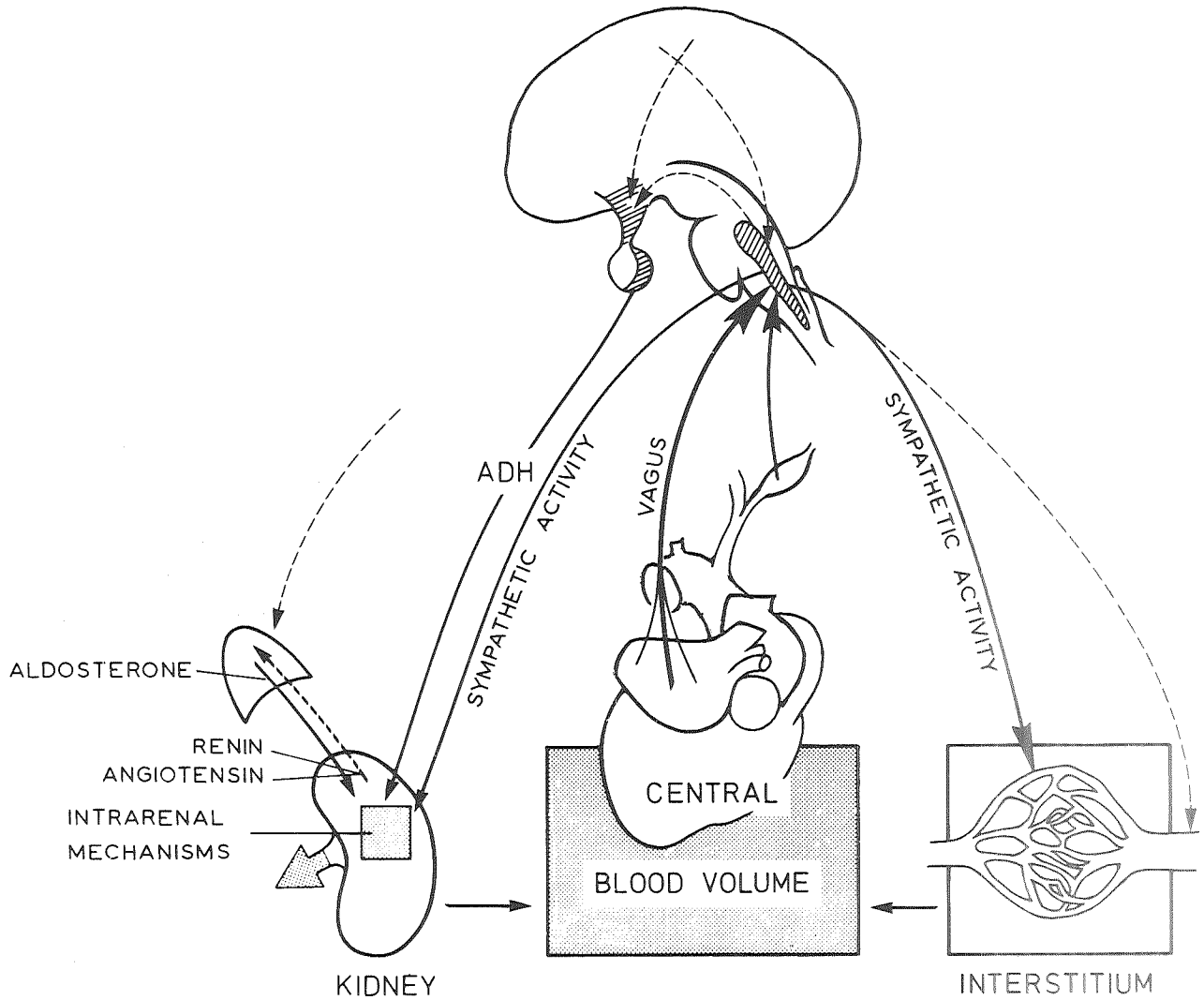

Figure 26.2 Reflex control of plasma volume through stretch receptors

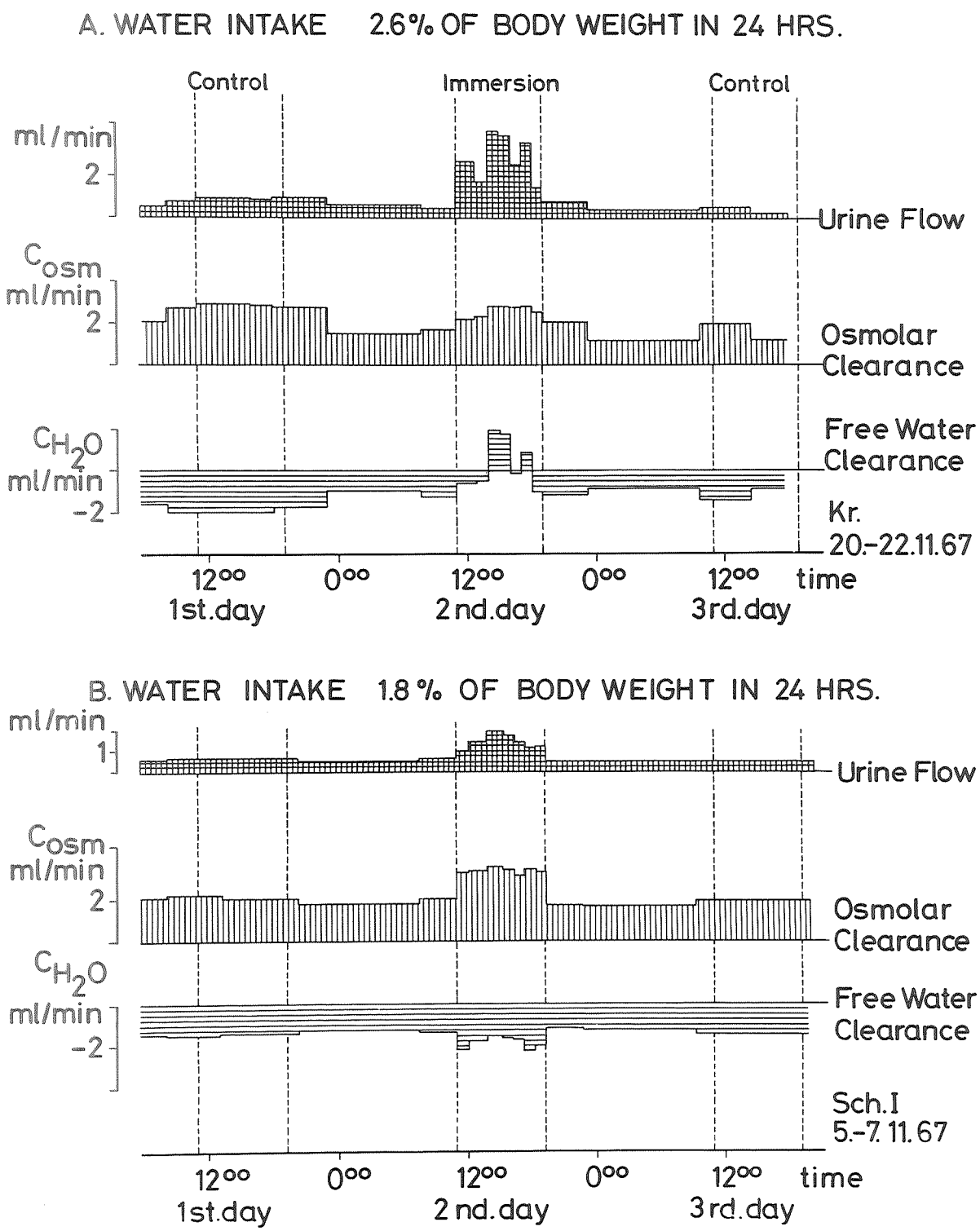

Figure 26.3 Urine flow, solute obligated and solute free water excretion in two subjects (Kr. and Sch. I) submitted to different states of hydration. Immersion is accompanied in both cases by an increase in urine flow, which consists in an increase in free water clearance (C_{H_2O}) in the normally hydrated case (a) and in an increase of osmolar clearance (C_{osm}) in the poorly hydrated one (b). Source: Reference 20

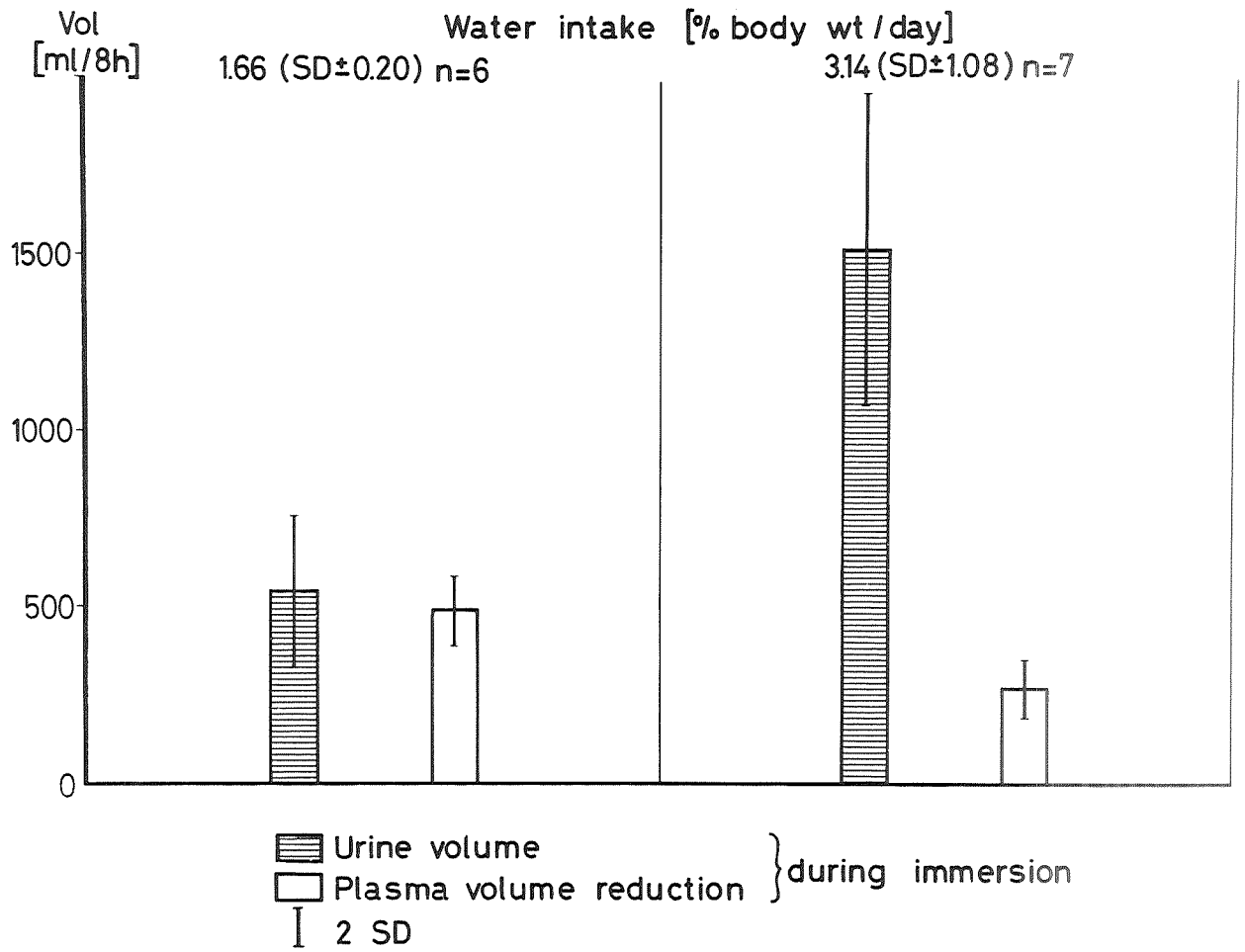

Figure 26.4 The relation between urine volume during immersion and the concurrent plasma volume reduction at different states of hydration. Source: Reference 20

27 DECONDITIONING AND ITS PREVENTION BY SIMULATING THE HYDROSTATIC GRADIENT

Paul Webb
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We know what deconditioning is and how to produce it. After some days of not having to adjust to hydrostatic loading, the veins of the legs and arms relax and no longer react swiftly to filling. Venous tone is decreased. The veins become unusually compliant; that is, they are flabby. If, at the same time, muscles have not been used, circulating blood volume has been reduced; dehydration has occurred; or, if we test for deconditioning when it is hot, the deconditioning is worse. All these factors aggravate the effects of loss of venous tone.

We also know how to detect deconditioning. Tilt-table tests produce changes that lead to fainting—tachycardia, narrowing of the pulse pressure, then sudden hypotension and bradycardia. The heart rate increases significantly from the least exertion; the capacity to work is lessened. Most specifically, tests of venous compliance show that the veins do not resist filling.

As a physiologist whose usual province is biothermal research, I do not hesitate to describe cardiovascular deconditioning in such definite terms. The picture has evolved from several studies I have done, and it is consistent with the studies by circulatory physiologists in the literature. It is not presented here as a new concept, only one that is clear and demonstrable.

Our first study (ref. 1) was of a technique for continuous, prolonged immersion, with the goal being 5 days and nights. Several years ago, when this study was done, a number of water immersion studies had been carried out (refs. 2-8). One recurrent and troublesome problem was that the subject's skin became macerated after only a few hours in water, and when immersions were extended to 24 hr, skin maceration, cracking, and infections were serious limits. Our notion was to substitute for water a silicone liquid that has approximately the same density as water and is biologically inert. With attention to skin hygiene, the expectation was that we could keep the subject immersed for days at a time. Gerow and coworkers (refs. 9,10) had developed a similar technique for treating burned patients.

After several preliminary experiments with 6-hr water immersions to test a breathing system that avoided negative pressure, we then conducted a 16-hr continuous submersion in the silicone liquid without incident. The test was terminated because of discomfort from the breathing system, which had to be revised. In none of these four exposures was there any diuresis, nor was there any sign of orthostatic intolerance to a 70° tilt. Apparently, venous compliance was still normal and circulating blood volume had not changed.

The final experiment was for the full 120 hr, in which the subject was always horizontal and totally submerged for approximately 60 percent of the time, breathing air at a slightly positive

pressure referenced to the midchest position. After the 5 days and nights of being horizontal and neutrally buoyant, it was demonstrated that there was no diuresis associated with the exposure and that only a minor weight loss occurred, which could easily be ascribed to a caloric deficit. The subject's skin was soft and clean. However, he was deconditioned. During a 10-min tilt at 70°, there was a slight narrowing of the pulse pressure; the subject began to sigh; and he felt a bit nauseated between 5 and 9 min. His heart rate was higher than in the preexposure tilt, and his pulse response to exercise was high. Exercise tolerance was low, as shown by the Balke treadmill test. Since the subject had been inactive for 5 days, this was not surprising.

A diagram of the silicone submersion facility is shown in figure 27.1. Figure 27.2 shows the pulse response to the work tolerance test where each minute of work is at a successively higher level. Notice that the subject stopped work after only 4 min following the submersion, whereas he had gone to 17 min prior to the submersion.

Following this successful demonstration of a technique for prolonged neutral buoyancy with total submersion and without diuresis, we returned to our normal studies in biothermal problems. As Dr. McCally has pointed out in his keynote paper, immersion has had its day. There was apparently no great enthusiasm for using the rather difficult technique we had helped to develop. Incidently, another study using silicone submersion had been done almost simultaneously at the Douglas Aircraft Company (ref. 11); but clinical people were more interested in studying the effects of bed rest and immobilization, while space people had begun to accrue real experience with the weightlessness of spaceflight.

My next involvement came when I was gathering material for a report on water exchange in spacesuits and capsules (refs. 12,13). I put together a table of the weight loss experienced by astronauts and cosmonauts in the manned flights through late 1966. All these men had lost weight; the average loss was 2.36 kg with a range of 0.5 to 4.5 kg. The magnitude of the weight loss was entirely independent of the duration of flight.

This loss of from 2 to 5 percent of body weight seemed to be obligatory. As Dr. Gauer has already explained, the prolonged return to the central veins of blood that is normally pooled, about 0.5 liter, causes a reflex suppression of ADH until central blood volume is reduced to normal. The weight loss of men in spaceflight continues through the recent flights. These men have had orthostatic intolerance; but, although the more dehydrated men were often more intolerant of being erect at 1 G, there was not a regular relationship between weight loss and orthostatic intolerance. The resumption of the erect posture in Earth gravity causes the usual pooling, but that pooling is greater if the veins have grown more compliant.

The final chapter in my story began a couple of years ago when we were asked by NASA Langley Research Center, and Mr. Ralph Stone of that center, to make a special antideconditioning garment that would simulate the hydrostatic gradient, with external bladders arranged on the limbs and trunk. We then were to test it on a subject who was kept in a hypodynamic environment for 2

weeks, including 12 hr/day of bed rest and 12 hr/day in a water tank. We proposed two ways of doing this: one with the bladder suit as specified by Langley, and the other a continuous gradient garment of elastic cloth. The first approach was accepted, and the suit was built and tested by Blockley and Friedlander (ref. 14) in our Malibu laboratory. The new cardiovascular conditioning suit (CVCS) was made of 11 toroidal bladders extending from ankle to hip, 7 bladders from wrist to shoulder, and 5 bladders from pelvis to chest. Figure 27.3 is a drawing of the CVCS.

The bladders were filled with water and connected to individual water reservoirs whose height above the immersion tank determined the internal pressures. Figure 27.4 is a diagram of the suit and the water reservoirs located above the immersion tank. The helmet was pressurized to 100 mm Hg, as was the topmost bladder on the trunk. From there down to the ends of the extremities, the bladder pressures decreased until the hands and feet had no pressure on them at all.

The experimental condition extended for 2 weeks with the subject always horizontal. Each day of the 2 weeks, he was in bed for 12 hr. The first 2-week period was a control study to establish the degree of deconditioning that this environment would produce. In the second 2-week period, the subject wore the CVCS only during the 12 hr he was submerged in the water tank. Pressurization was applied during part of this 12-hr period.

The result was a remarkable change between the control exposure and the 2 weeks using the CVCS. The essential differences are outlined in table 27.1. The response to 70° tilt after the control period was an alarming hypotension and bradycardia about the 11th minute, which caused termination of the tilt test. His response to being tilted following the 2 weeks of using the CVCS was normal for him and not at all alarming. The control exposure caused a reduction in his work capacity and he had a high pulse response to exercise; after the CVCS exposure, these tests were normal.

The venous compliance measurement told a most interesting story. We used our own version of the Newberry and Bryan technique (ref. 15) in which the arm is raised to the vertical position and drained thoroughly. Venous return is then blocked by a cuff inflated to 60 mm Hg. When the girth (therefore the volume) change had reached a maximum, it was recorded; the cuff pressure was reduced to 50 mm Hg, where the girth was measured again when a new plateau occurred. This was repeated in 10-mm steps down to zero cuff pressure. Figure 27.5 shows the increase in forearm circumference at these various cuff pressures for two preexposure tests, for the test following the CVCS exposure, and for that following the control exposure. There is an obvious and remarkable increase in venous compliance following the control period of 2 weeks in a hypodynamic environment. The postexposure compliance test with the CVCS is essentially normal.

Even more striking is the history of compliance change during the 2 weeks of hypodynamic exposure with the CVCS. During these two weeks, not entirely by plan, the suit was not on and pressurized much for the first 5 days. Figure 27.6 tells this story. For 5 hypodynamic days, with only about ½ hr of pressurization with the CVCS, the venous compliance rose steadily to a maximum of 3 times the normal level, reached on the seventh day. From day 7 onward, the CVCS was pressurized for an equivalent of 3 hr daily at full pressure (100 mm Hg). Venous compliance dropped dramatically to normal by the tenth day and stayed normal for the rest of the 2 weeks.

In other words, brief exposures to a simulated hydrostatic pressure head (about 3 hr/day) can quickly convert a deconditioned man into a normally reactive one. True, this was only one subject, but our success rate is 100 percent.

To repeat my initial thesis, deconditioning occurs when the veins are not exercised daily by the usual hydrostatic loading, and venous compliance increases. Venous tone can be restored by simulating the hydrostatic gradient with a suit that pressurizes the airway, lungs, and torso, and pressurizes the arms and legs decreasingly out to their terminations. Thus, one causes blood pooling in the extremities just as if one were standing in a gravity field. Loss of venous tone causes orthostatic intolerance, and this effect is worsened by reduced blood volume, which will occur when blood pooling is absent for days. If muscle tone is low from lack of physical exercise, the muscle pump that normally aids venous return would be less active. Also, if the man were hot, his orthostatic intolerance would be worse as he dilated cutaneous vessels to increase heat dissipation. So one should prepare for a return to being upright in Earth gravity by rehydrating, staying cool, exercising, and most of all, using a suit to cause blood pooling.

Is this approach likely to be useful for spaceflight and other conditions where venous tone degenerates? The experimental suit described is bulky and heavy. But consider our other approach, which seems entirely feasible with today's techniques in elastic garment fabrication. (Parenthetically, we are extending elastic garment technology well beyond that required for a new CVCS in another current project in our laboratory.)

Figure 27.7 shows a drawing of the elastic suit concept. It would be a constant wear garment with a special air bladder to produce a simulated hydrostatic gradient. With the bladders unpressurized and the helmet off, the suit simply lies on the skin with mild pressure of approximately 5 mm Hg, which is just enough to keep the suit skintight and wrinkle free. When the helmet is donned, the helmet and bladder are pressurized together to, let's say, 35 mm Hg. The reverse gradient effect is caused by the tapered design of the bladder extensions that run down the arms and legs. Thus, at the shoulders and hips the pressure is 35 mm Hg and decreases to zero at the wrists and ankles. The suit would be of open, porous elastic net, and the bladders would be designed so as to minimize coverage of the skin with impermeable layers when unpressurized. Thus the suit becomes a comfortable thing to wear and need not be donned and doffed for each treatment. In the spaceflight situation, it would be feasible to wear the garment continuously for the last several days of a flight, with a short pressure treatment several times a day to exercise the veins and restore tone.

Please note this is not the usual leotard with pressure gradients from high at the ankle to low at the hip, which McCally et al. found so useful after deconditioning had occurred (ref. 16). The garment I propose here is a reverse gradient garment.

We aim to prevent deconditioning by loading the veins during the several days before reentry to a normal gravity field. Then the astronauts could leap from their capsule and have champagne immediately. Similarly, patients who have been bedridden or immobilized for long periods could wear the garment for the last few days, then leap from bed and remain upright. Prevention is the best treatment.

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Table 27.1 Results of a 2-week hypodynamic exposure, including half days under water

	After Control Exposure	*After CVCS Exposure*
Pulse response to 70° tilt	High, then bradycardia	Normal
BP response to 70° tilt	Hypotension	Normal
Pulse response to exercise	High	Normal
Work capacity	Reduced	Unchanged
Venous compliance	Greatly reduced	Unchanged

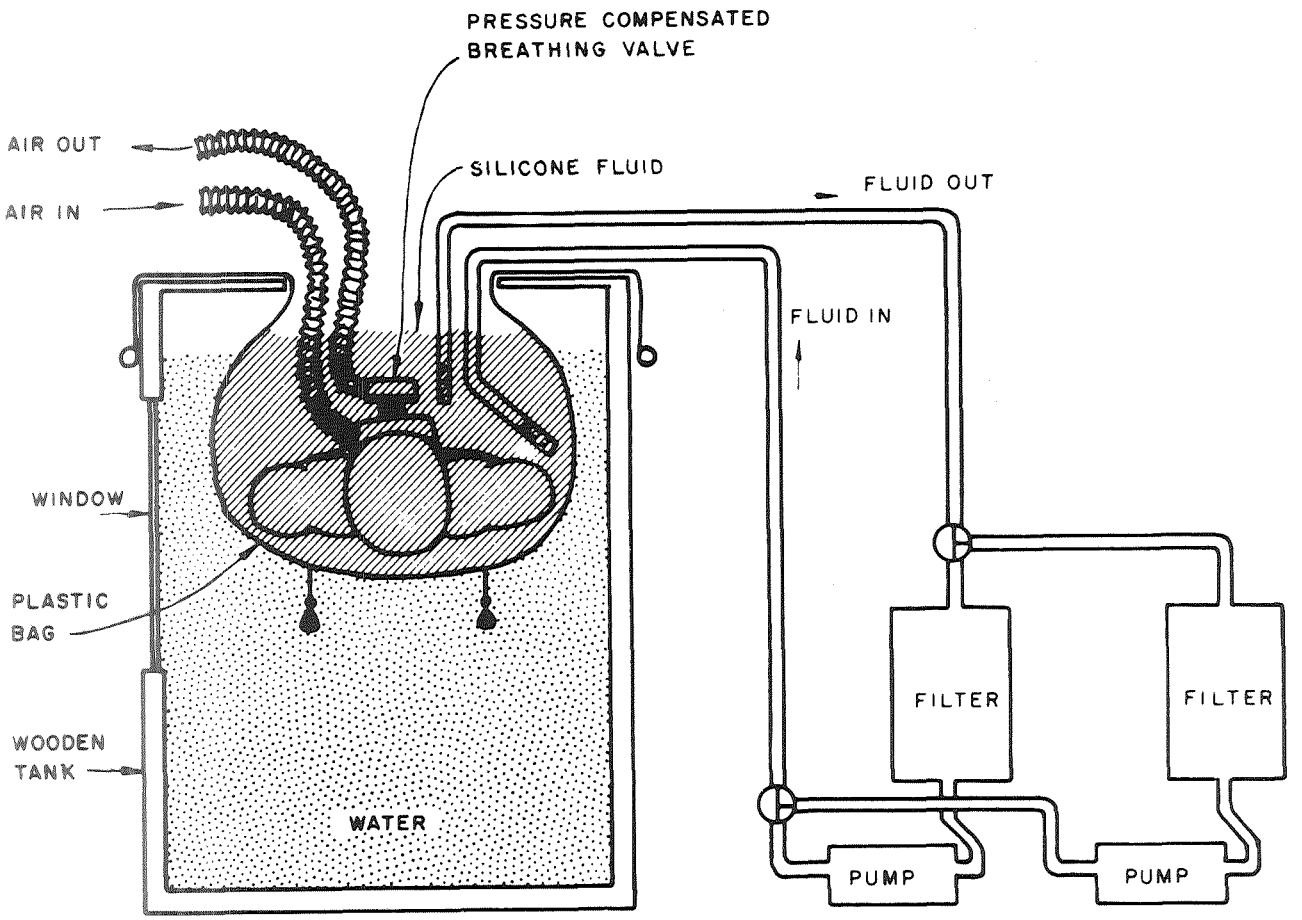

Figure 27.1 Diagram of the apparatus used in the 5-day silicone submersion, showing the subject immersed in the silicone fluid contained in a plastic bag suspended in water. Two large swimming pool filter units containing diatomaceous earth, activated charcoal, and lithium chloride kept the recirculated fluid clean and free of water. The subject breathed from a continuous flow of air through his helmet.

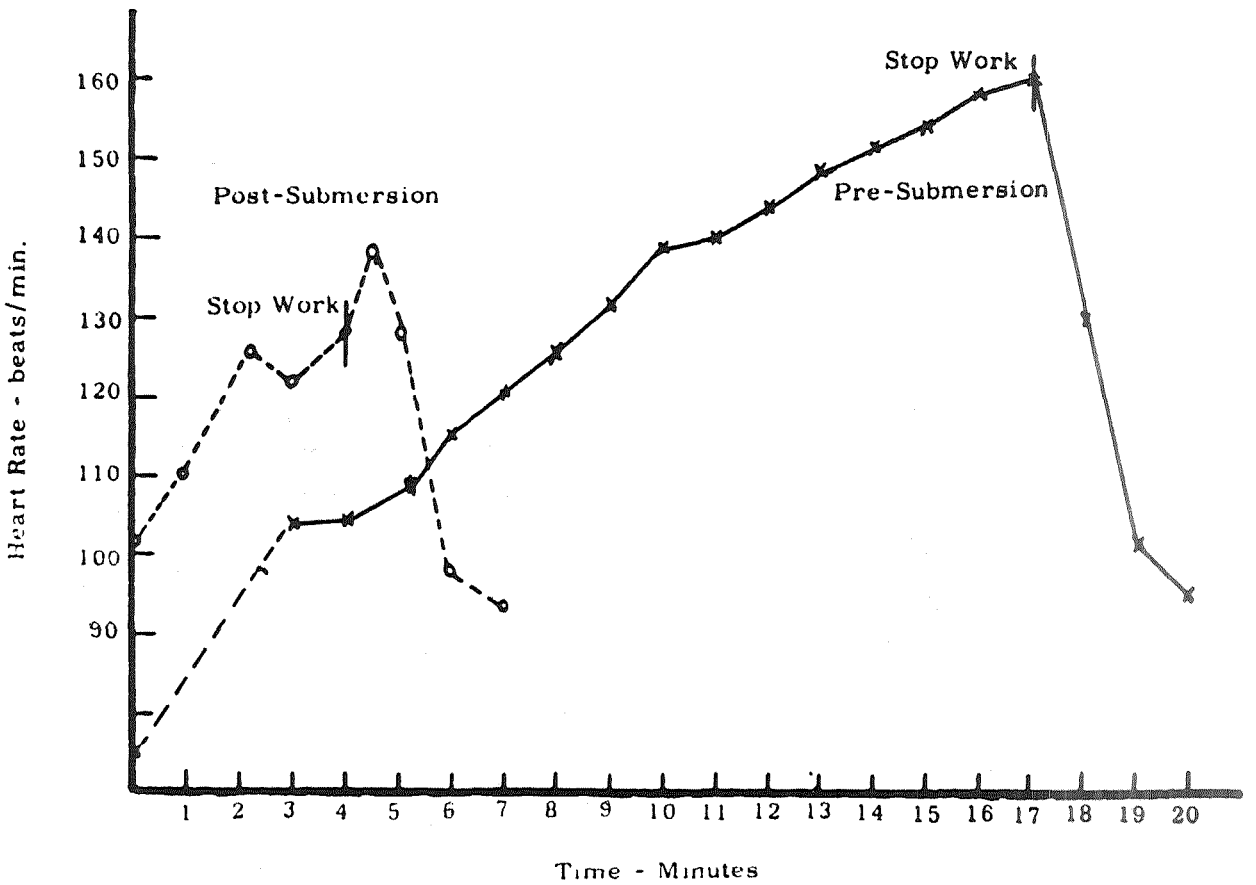

Figure 27.2 Pulse rate response to the Balke exercise test before and after the 5-day silicone submersion. The subject walked at 3.5 mph on a treadmill, starting level the first minute, then uphill at 1 percent grade the second, 2 percent the third, and so on, until he stopped from exhaustion. In the preexposure test he continued for 17 min and reached a heart rate of 160 bpm, which indicates an average level of fitness for a young man and perhaps a low level of motivation, since he could certainly have gone to a higher heart rate. After the submersion, his initial pulse rate was high and went higher than before at low levels of exercise. He chose to stop after only 4 min, feeling weak and ill at ease

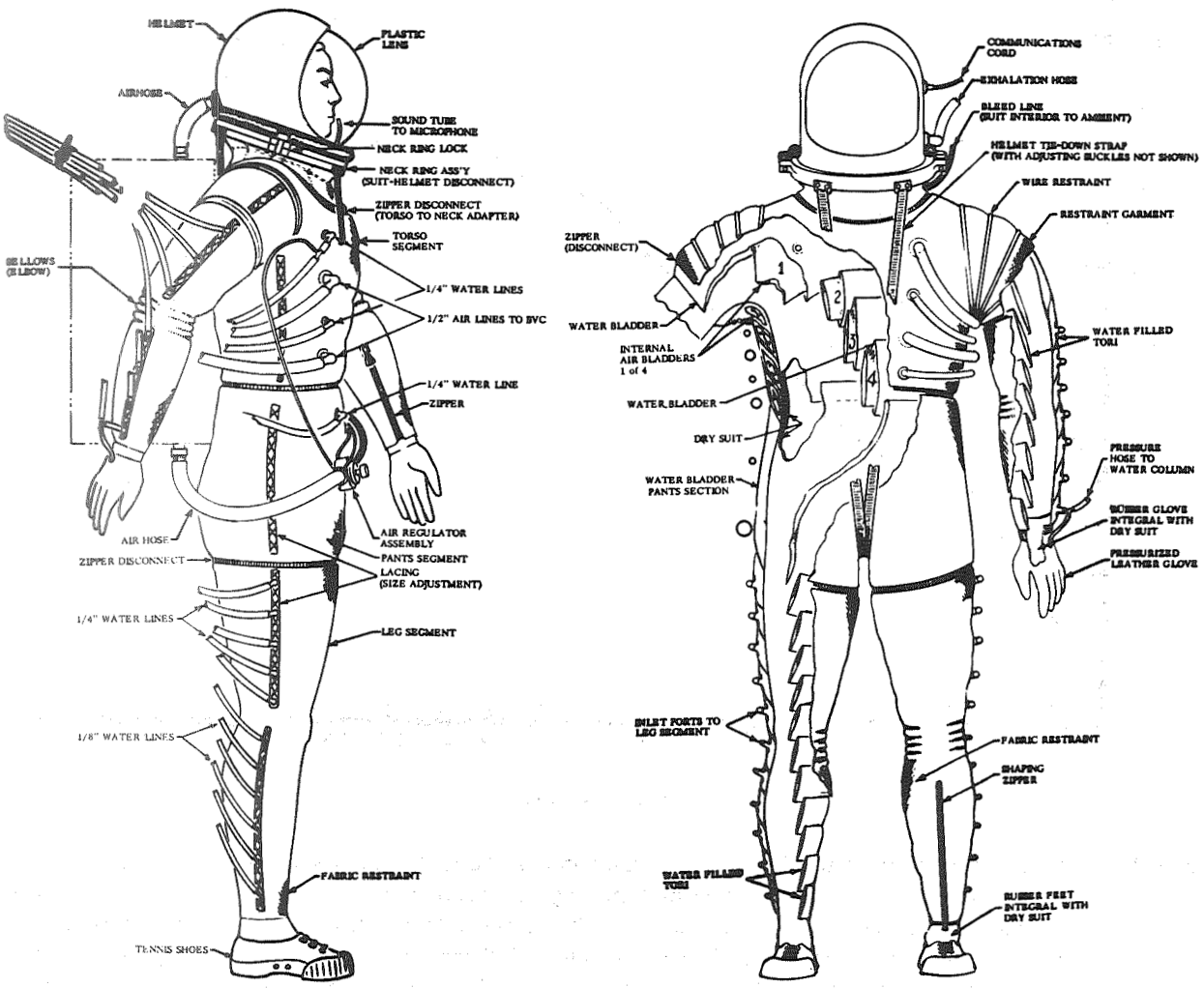

Figure 27.3 The cardiovascular conditioning suit (CVCS) showing the overlapping toroidal bladders covering the arms, legs, and trunk, an underlying skindiver's wetsuit for warmth, and an overlying nonstretch restraint layer to contain the bladders when pressurized. The chest bladders were water filled, as elsewhere in the suit, but also contained internal air bladders connected to a pressure-compensated bellows box to provide volume compensation during respiration. The helmet was weighted with lead to neutralize its buoyancy, and it was kept from rising by tie-down straps passing through the crotch

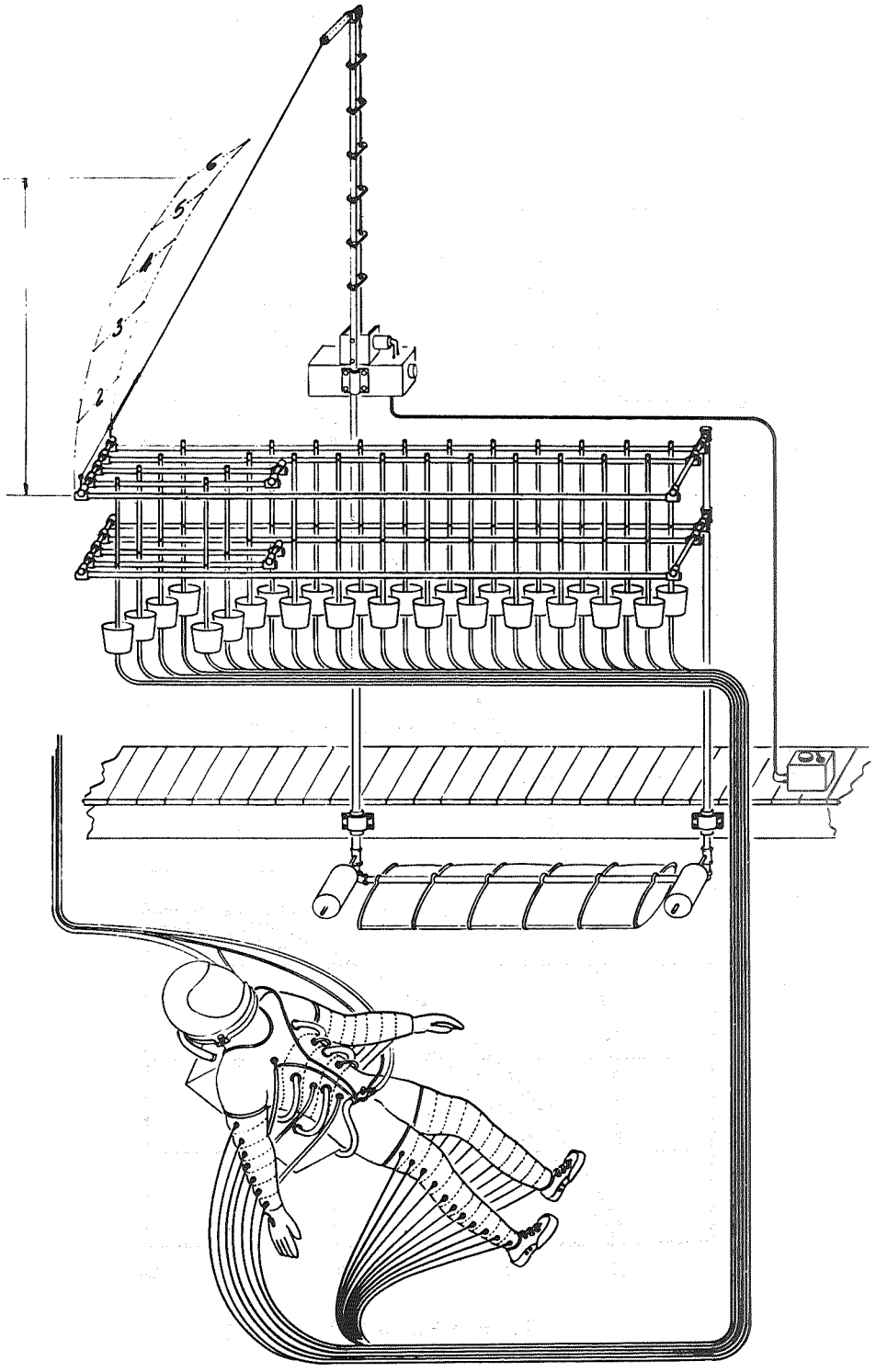

Figure 27.4 Reservoirs used to pressurize the bladders of the CVCS, showing that the left end of the rack could be raised, causing a progressive hydrostatic loading in the bladders connected to the reservoirs from right to left. The subject is shown as if floating in water, the reservoirs being above the water surface, which would be at the level of the boardwalk shown

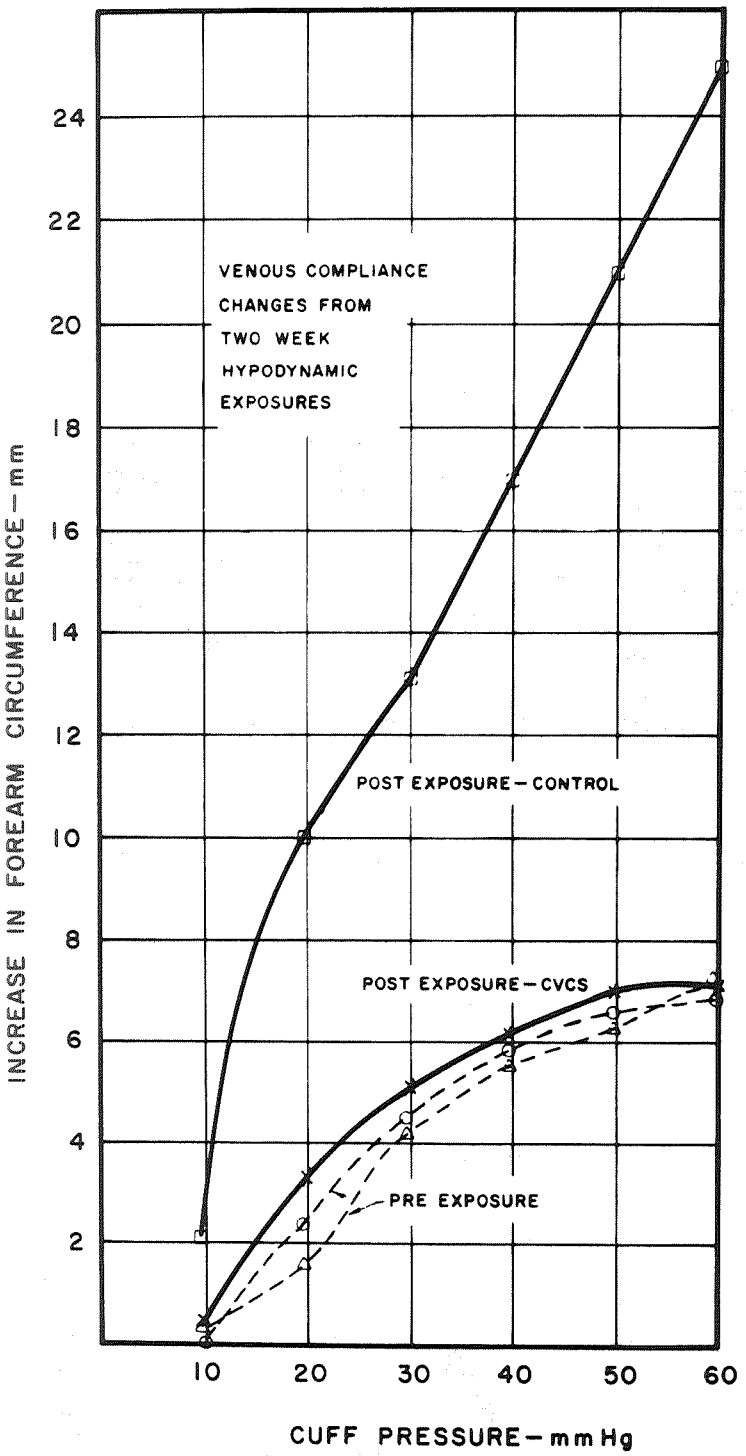

Figure 27.5 Venous compliance measurements using an occlusive cuff, and a mercury-in-rubber strain gage to measure circumferences at the greatest diameter of the forearm. Control values for forearm circumferences are shown as the two lightly drawn preexposure curves below. After the control exposure, the compliance values are markedly increased, as seen in the upper curve; following the exposure using the CVCS, the compliance figures are essentially normal

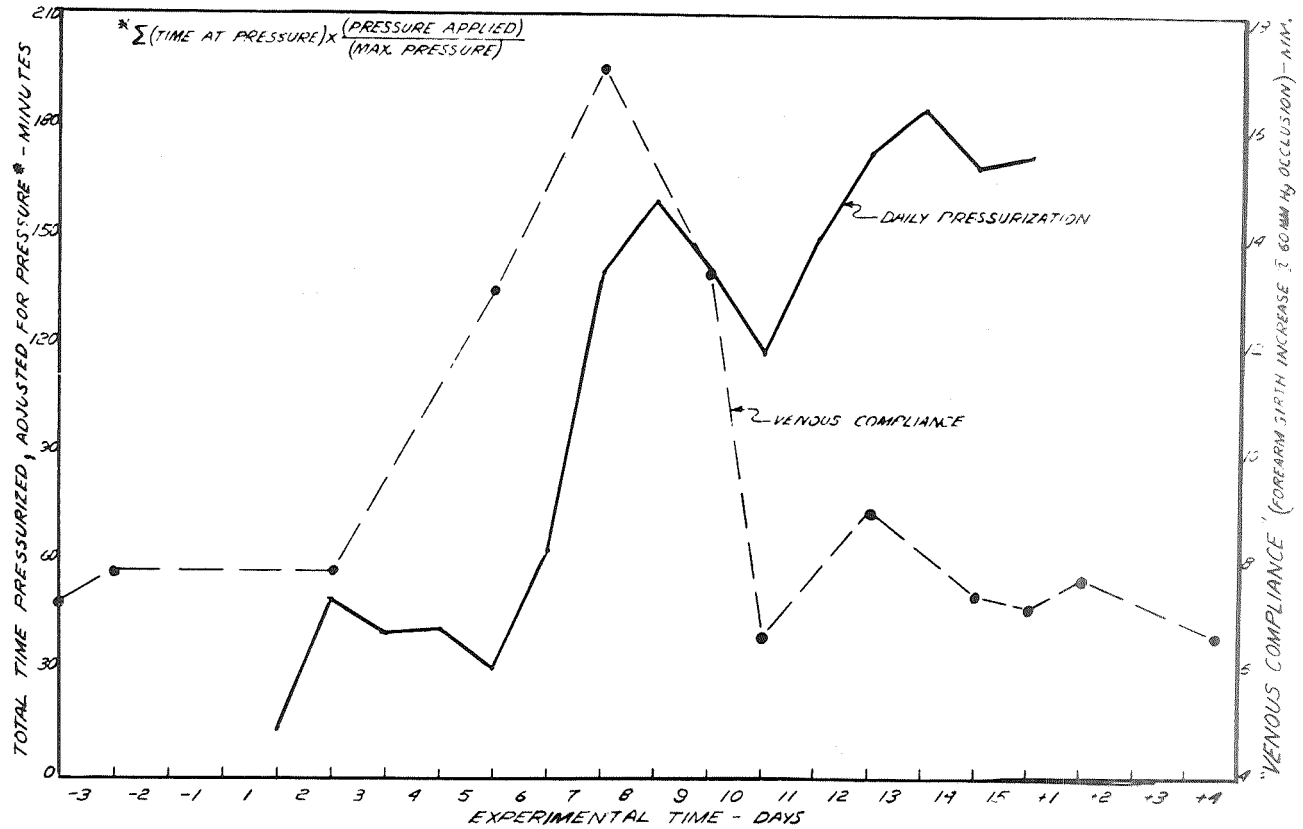

Figure 27.6 The amount of pressurization of the CVCS over the 2-week period is plotted daily in the continuous line curve, and the venous compliance history is plotted with a dashed line. Pressurization is expressed as the time at pressure multiplied by the fraction of the maximum pressure (100 mm Hg) actually used

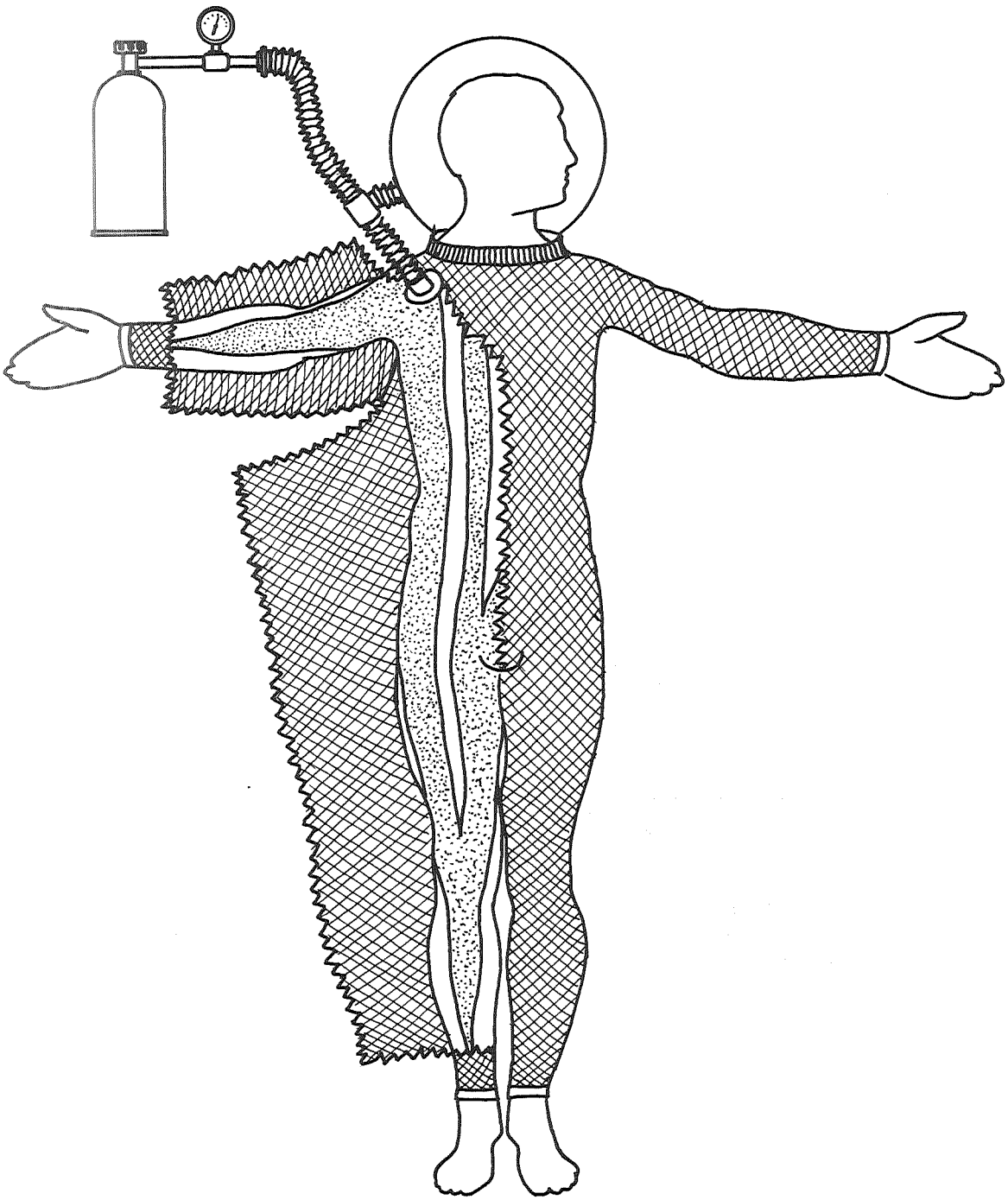

Figure 27.7 A conceptual drawing of a continuous gradient garment that, when pressurized, would cause peripheral blood pooling and would maintain venous tone. The garment would be a snugly fitted elastic net, exerting only mild mechanical pressure (about 5 mm Hg), until the helmet was donned and pressure added. This would produce positive pressure in and over the chest and abdomen; but from the tapered design of the bladders, the pressure gradient would reduce gradually to zero from shoulder to wrist and from hip to ankle. Not shown is a breathing system, either open or closed, which would remove CO_2 and add oxygen to the helmet

28 DISCUSSION

Dr. Piemme: Your sodium and potassium results imply aldosterone is turned off, and the low renin levels fit with this. Did you measure aldosterone? This is particularly important, since Dr. Whedon found a high aldosterone level in the astronauts after spaceflight.

Dr. Gauer: We have no direct determination of aldosterone. The apparent discrepancy between Dr. Whedon's findings and our results can be explained. Dr. Whedon's determinations of aldosterone were made *after* spaceflight, that is, during the period of pending orthostatic collapse. This experience with aldosterone has a close parallel to observations of ADA titers in our immersion experiments. During immersion, the serum of the subjects was free of ADA (antidiuretic activity as tested by rat bioassay). However, when exposed to gravity after termination of the immersion, they immediately showed extremely high ADA concentration in the serum.

Dr. Mitchell: Do you feel that the decreased maximal oxygen uptake is due to an inability to put out an increased stroke volume? How long does the decreased maximal oxygen uptake persist after water immersion?

Dr. Gauer: These experiments were done in Dr. Stegemann's institute (Cologne). As far as I know, cardiac output was not measured, and I forgot to ask how long the reduction of the working capacity lasted after immersion.

Dr. Gatts: We're using the term "deconditioning" here for what seems to me to be two distinct phenomena, one metabolic and the other related to a change in sympathetic tone. Would you lump both under the term "deconditioning"?

Dr. Gauer: I feel that the adaptation to the weightless state should not be called deconditioning. It is a conditioning to the space environment. As long as you stay in space, you will get along very well; and it's your fault if you come back from the angels' realms to earth. When leaving the gravitational field, the blood volume proves to be too large for the capacity of the low pressure system. The resulting congestion (and relative hyperactivity) of the heart is promptly corrected by a reduction in blood and a slight vasodilation.

Dr. Gatts: To return to my previous point, I believe one aspect of deconditioning is a loss of muscle mass and muscle power, and the other is simply a change in sympathetic response due to different inputs to the sympathetic nervous system. I see them as entirely different phenomena, even though they are both called deconditioning.

Dr. Gauer: I quite agree; they are completely different phenomena. I would consider the fast reflex reduction in plasma volume as an expression of a changed input into the autonomic system. The long-term muscular deconditioning, however, is an expression of disuse.

Dr. Lancaster: You mentioned that central venous pressure was reduced after immersion. Have you any central venous pressure measurements immediately on immersion to go along with the change in peripheral venous tones?

Dr. Gauer: We have no measurements of central venous pressure during immersion. We found a great reduction in central venous pressure that may be explained by a loss of approximately 500 cc of blood volume; this venous pressure fall lasts for a period of hours.

Dr. Schmid: What is the temperature of the water that you are using for the immersion studies?

Dr. Gauer: Initially, the temperature is set at 34.5° C. After immersion, the subjects usually request a correction by approximately 0.2° C or so.

Dr. Sandler: What is the effect of distention of the left atrium on the peripheral circulation?

Dr. Gauer: The capacitance and resistance vessels both dilate.

Dr. Vogt: Does your suit impede the return of blood from the periphery?

Dr. Webb: Yes, it impedes it in exactly the same way that hydrostatic gradient forces impede the return of venous blood when standing in a gravity field.

Dr. Vogt: Is there any evidence that the muscular pump is important?

Dr. Webb: We worried initially that the subject might faint in the water if he pooled blood excessively. This never happened, and I can't tell you exactly why. However, apparently venous return was quite adequate, both during activity (when a muscular pump was functioning) and at rest.

Dr. Wunder: Could this suit be engineered so that you could adjust direction of the gradient? It would be very desirable if you could use it first as a conditioning device with the gradient going in one direction (pooling blood peripherally) and then, as the astronaut approaches reentry, reverse the gradient to increase central blood volume.

Dr. Webb: I don't see why we couldn't do that. That does seem to be a good idea.

Dr. Gauer: Have you ever used positive pressure breathing without a gradient suit? Does this give the same kind of protection? You have been saying that it is important to adjust the load on the veins properly, but I believe that it may be more important to keep the central blood volume low.

Dr. Webb: We studied positive pressure breathing by itself and this introduces many problems. The use of positive pressure breathing at 100 mm Hg without limb counterpressure is just not possible. A well-trained subject can maintain positive pressure breathing at a level of approximately 20 mm Hg for an hour or so, which would certainly cause peripheral pooling but not in the same way that we have done it with the suit.

Dr. Gauer: With a more prolonged application, you might be able to get away with smaller positive pressure levels.

29 DISCUSSION

Dr. Whedon: The purpose of this session is to consider what further studies should be done, particularly those studies that would have practical importance for determining whether man is, or will be, qualified for the very long spaceflights in the future. I am strongly in favor of the idea that we ought to have more studies of the astronauts themselves in flight. Such studies are projected for the Apollo Applications Program that will follow the current series of Apollo flights. I think that the data we obtained in Gemini 7 could best be described as provocative, but certainly not definitive. We shall have to confirm a number of these findings: the magnitude of mineral loss, the meaning of the apparent depression in 17-hydroxycortocosteroids, etc. Meanwhile, various studies should be done on this planet. Some of these are already planned. One is to sort out the effects of the many other factors besides weightlessness that make up the spaceflight environment. It might be of interest to study changes in metabolic systems induced by the use of countermeasures, such as the suits that Drs. Webb and Gatts have been working with.

I would like to caution investigators involved in mineral metabolism that a 2-week experiment is too brief to obtain definitive data, especially with regard to calcium metabolism.

We didn't really get into a discussion regarding the mechanism or mechanisms involved in the loss of minerals from the skeletons in bed rest or in a weightless state, or what factors may be related to possible protection against this loss. Without going into a long discussion of the interrelationships between circulatory and neurological systems, I would like to bring to your attention the experience we had with the oscillating bed (ref. 1). You will recall that the same normal subjects who lost a great deal of calcium and had deterioration of the orthostatic reflexes when in a plaster cast in a static bed lost only half as much mineral when immobilized on an oscillating bed for 8 to 20 hr/day. Further, they were protected almost completely from orthostatic deterioration. This slowly tilting bed imparts partial weight-bearing and produces some muscle contraction. When we tried the oscillating bed in paralytic poliomyelitis patients, on the other hand, it did not protect against mineral loss at all. There was, however, partial protection against orthostatic intolerance (ref. 2). It looks to me as though one cannot expect to prevent the loss of mineral by applying pressure directly to the skeleton by static physical loading on the bone per se. You have to have viable muscles. This means that periosteal pull is necessary, or there may be some effect on the circulation to bone that acts indirectly. The relative failure of exercise in bed to provide any protection against mineral loss is simply because this exercise doesn't exert pull of any significant degree on the bones. You've got to have truly resistive, vigorous exercise that stimulates the same force on the limbs that one gets from weight-bearing.

Dr. Cameron: Calcium leaves the bones because the physical forces are not great enough to hold it there. There is some suspicion from previous studies that piezoelectric effects may be related to calcium retention. I would like to propose the use of vibration of the body and of oscillation of the bones. You can vibrate the body rather easily, even in space. This will produce physical forces in the bones that may be very small but perhaps adequate to produce these piezoelectric forces (if that's what is protective) and thereby maintain bone in a normal state. This could be attempted also by using a vibrating bed. An orthopedic surgeon has reported to me that broken bones, with muscle twitching in the same limb, often heal much faster than broken bones in limbs with no such twitching. This would seem to indicate that even small forces may be important to the bone metabolism.

Dr. Whedon: I would predict that the type of vibrating bed one gets in the motels with which I am familiar would not be effective because of the damping effect of the soft mattress, among other things. I think the forces exerted on bone will have to be strong.

Dr. Colbert: There are some ceramic (barium titanate) transducers that can be molded to a limb and excited electrically. Their amplitude of vibration is rather small, but the forces exerted can be extremely high.

Dr. Lind: There is a potential problem with vibration. Recent studies at Wright Patterson Air Force Base have shown that vibration can induce a considerable but transient vasodilation peripherally (ref. 3). I don't know what effect this might have on conditioning. Doesn't vibration produce osteoporosis?

Dr. Cameron: The people who develop osteoporosis from vibration are workers who use pneumatic drills, and this involves force many orders of magnitude greater than the vibration we are considering here.

Dr. von Gierke: You're discussing here, I think, the long-term effect of vasoconstriction associated with severe vibration, and this is not the type of stress one would apply under these conditions. I agree that it would be hard to produce stresses within the skeleton by vibrations which approximate the stresses that might be detrimental. I would propose the use of mechanical stresses on individual bones, which is a quite feasible technique.

Dr. Abendschein: We have been discussing various changes in the skeleton induced by deconditioning, and the question has been raised as to what consequences these changes have on the functional capacity of the bones. The only answer has come from clinical experience with fractures in various osteopenic conditions and general speculation. I was much impressed by the experiments of Drs. Kazarian and von Gierke; and I think this is a very valuable research direction, namely, to relate the functional capacity of the skeleton to the metabolic changes we have seen. I again make a plea for studies, such as our own, involving nondestructive testing and monitoring of the physical properties of bone throughout deconditioning and reconditioning.

Dr. Wunder: I would like to emphasize the fact that we don't know what the mechanism is for this bone loss. It has been suggested that it is a change in piezoelectric forces. I thought at one

time that I got enlarged bones of animals after prolonged centrifugation because of Wolff's law — the concept that mechanical strain would stimulate bone growth, particularly along the axis of greatest strain (ref. 4). Actually, piezoelectric effect might be part of Wolff's law, and I think the best evidence for this is from the unpublished work of Graveline (ref. 5) with chronic centrifugation. Graveline found drastic increases in radio density not only of the weight-bearing bone but throughout the skeleton, indicating that this might be an endocrine effect. Redden (ref. 6) took chick embryos, left them in nature's water immersion (in the eggs), and centrifuged them. Although there was no weight-bearing on any of the bones, he still got quite an acceleration in the growth of a number of the bones in these chick embryos.

Dr. Young: As Dr. Hulley has previously discussed, we have been studying monkeys at bed rest for about 2 years. We apply static forces to the tibia equivalent to half the animal's body weight, and we find that we can reduce the urinary calcium by approximately 50 percent. It is hard for me to see where exercise can create tension in muscles that begins to resemble the strain in bone associated with walking, hopping, skipping, and normal activities.

Dr. von Gierke: If we assume, as Dr. Gauer has said, that "we adapt to the new environment and we readapt on return from space to the earth environment," why are we concerned about bone loss and cardiovascular deconditioning? From a practical point of view, I think we need concern ourselves primarily with the transition from the weightless state to the earth environment, particularly during earth orbital reentry, parachute opening shock, and ground or water impact. We began our studies on bone primarily to determine the relationship that exists between bone strength and impact tolerance, for which there are really very little data. For the normal condition, man in the earth environment, we can predict with a high percentage of accuracy the probability of injury incurred when man is exposed to certain acceleration or impact stresses. We can even predict how the probability of injury increases as a function of increasing age (refs. 7 and 8). However, at the present time, we are not in a position to predict the probability of injury due to mechanical stress following exposure to prolonged weightlessness. Determining changes in one man will not tell us of changes in bone strength, particularly not local changes. Consequently, bone densitometry alone will not provide this final answer for systems design and the operational situation. Animal experiments will be required initially to provide the tolerance data on which to extrapolate the biodynamic response of man in the hypogravic state. Similar practical information is essential on cardiovascular deconditioning due to weightlessness. In Gx impact (where the main mechanical force is applied in the front-to-back direction), the major problem is not bone breakage but cardiovascular shock or "general" shock. We also do not know how deconditioning would affect man's capability to withstand this stress.

Dr. Mitchell: I think that agreement has been reached about what happens to blood volume, red cell mass, and plasma volume after bed rest; however, there is no agreement about what happens to total body water. More studies of the effects of bed rest on total body water should be carried out. There also seems to be good agreement about what happens to the response of the resistance and the capacitance vessels. I think Dr. Schmid's work, which is attempting to get at the mechanisms by which these changes occur, is most interesting, and this approach needs to be continued. There is major disagreement about whether there is a myocardial factor involved in the deconditioning syndrome.

It may be that physical inactivity per se changes the cardiovascular responses and that the lack of gravity stress is really of less importance. The possibility that a myocardial component plays a role is supported by the fact that, after a long period in bed when supine exercise is carried out with the legs well above the head, the stroke volume response is markedly abnormal. In this position, it is unlikely that pooling of blood occurs, and the filling pressure of the heart should be adequate.

It would be very interesting to see whether Dr. Schmid's studies will also show that vein physiology is affected after only a few hours of water immersion. I was impressed by the marked reduction of maximal oxygen uptake that was shown after just 8 hr of immersion. It would be of great interest to know if this would be found in supine as well as in upright exercise. It may be that the venous pooling associated with the upright position may limit the maximal oxygen uptake.

We certainly need to know more about the sympathetic nervous system and its reactions during bed rest, immobilization, water immersion, and spaceflight. Also, we need to know whether ventricular function is depressed during these states. Further studies of the effect of adrenergic stimulation and blockade on cardiovascular functions are needed. I think that the whole idea of countermeasures deserves special attention, for this seems to have been studied very little.

Dr. Saltin: It may also be of interest to look more closely at skeletal muscle function. We know that nitrogen balance is negative during the first few days of bed rest. We also have loss of lean body mass. But are there qualitative changes in these muscles? In training studies, it is well documented in animals that mitochondria and enzyme activities may change within a week after the onset of exercise. I am not aware of any studies of this sort being done in bed rest and immersion.

Dr. Schmid: It is interesting to note that both Dr. Gauer and Dr. Webb have observed what appears to be appreciable relaxation of veins during immersion. This is very difficult to demonstrate under most other conditions. I wonder if there is some special feature of water immersion that makes this evident (such as temperature change), or if they are really measuring something else that is not a reflection of changes in venous compliance. I am impressed with Dr. McCally's observation that cold stimuli don't protect against orthostatic deconditioning in water immersion situation but that the counterpressure garment does. With the counterpressure garment, there is in a sense isometric loading of the venous circulation; whereas with cold and with sympathetic stimulation, there is constriction of the veins, which would seem to be an isotonic condition. In the heart, there seems to be a difference between isometric and isovolumic demands, and the peripheral circulation may well respond differently also.

Dr. Lind: I think, in addition, it would be desirable to look at the muscular metabolism and the consequent vasomotor response. This would probably require the use of a sustained static effort because this is the only way one can measure peripheral blood flows during exercise. Detailed studies of metabolic and cardiovascular responses to exercise in these different situations, or subsequent to them, would be of considerable value.

Dr. Mitchell: I would think that Dr. Schmid's type of studies, done before and after water immersion, would aid our understanding of the mechanisms involved. There is still a question of

whether the effect we see in veins after bed rest is the same as what we see in veins after water immersion.

Dr. Murray: Might the use of vasoactive drugs be helpful to treat the deconditioned subject? Could infusing tyramine or another catacholamine be useful as a possible therapeutic measure? A venotonic drug would seem to be particularly desirable.

Dr. Gauer: Well, we had hoped that Vasopressin would turn out to be a venotonic drug, but this was disappointing. Vasopressin prevented the orthostatic collapse following immersion but had no effect on the distensibility of the veins.

Dr. Gatts: We might be talking about inability to release epinephrine and norepinephrine, due to a lack of proprioceptive stimulation rather than to a lack of manufacture or deficient storage of catecholamine.

Dr. Mitchell: That does not seem to fit Dr. Schmid's work, since tyramine did not release catecholamines. This suggests that the catecholamine stores were depleted.

Dr. Schmid: Recent work would indicate that, with nerve stimulation, it is the newly synthesized norepinephrine that is released, and when you block synthesis with an inhibitor (such as tyrosine hydroxylase, for instance), you block the ability to sustain a response to stimulation. In the same way, the response to tyramine is associated with the release of newly synthesized or freshly labeled norepinephrine, so that the functional change I tried to associate with a change in biosynthesis may be related to an inability to sustain release of norepinephrine with any chronic demand.

Dr. Hyatt: I agree with Dr. Mitchell that we have to look at the possibility that a myocardial factor is playing a role in deconditioning, but the very fact that, in the immersion studies, the same types of changes in oxygen uptake were seen as had been observed following bed rest suggests to me that within 6 hr of immersion one could bring about very great changes in myocardial function. In the bed rest studies, in which we gave our subjects 9-α-fluorohydrocortisone to maintain plasma volume, the responses to exercise, at least in terms of heart rate, were certainly not as great in the 9-α subjects as in the control subjects. Although we are currently beginning studies to evaluate the role of the myocardium in bed rest deconditioning, I will be very surprised if we find that there is any great alteration in myocardial function.

We've heard red cell volume results which indicate that, during the period of bed rest phase, there is a decrease; the bone marrow fails to produce red cells. Those of us who practice clinical medicine often see elderly, debilitated patients who lie in bed for months and are anemic without any good explanation for the anemia. I wonder if this is not bone marrow shutdown due to bed rest?

Dr. Murray: Dr. Schmid, some of your earlier work suggested that certain of the cortisone-like drugs will potentiate the catecholomine effects. I wonder if 9-α-fluorohydrocortisone in

addition to retaining fluids, might have this effect on the peripheral circulation and tend to improve tilt tolerance on this basis.

Dr. Schmid: We observed that the effects of norepinephrine on both resistance and capitance vessels were potentiated by a week of treatment with 9-α-fluorohydrocortisone in a dose of 0.1 mg/day, but this was really not a very pronounced effect.

Dr. Murray: However, in the event of catecholamine depletion, might it potentiate the effect of the remaining catecholamine and become clinically important?

Dr. Schmid: I think this reasonable.

Dr. Nordin: Although weightlessness and bed rest can affect bones, I think one should draw attention to the kidney as well. Everyone is assuming, of course, that the rise in urinary calcium is due to the effect on bone. But there are certain bits of evidence that point to the possibility that the kidney itself is involved to some extent. For instance, there is a very strong relationship between urinary calcium and urinary sodium in all circumstances, and the sodium loss that occurs in weightlessness would itself produce a rise in urinary calcium without necessarily any primary effect at all on bone. Our own data in paraplegia, and Dr. Donaldson's data presented here, show that the rise in urinary calcium is associated with a slightly reduced serum calcium. Everyone assumes that this may be an error in determination; but it is odd that, with a calcium load from bone reabsorption, the serum calcium tends to be slightly below normal, not slightly above it. Again, the suggestion that perhaps tubular absorption is a factor. A third factor that points in this direction is that one can diminish urinary calcium in immobilization by feeding phosphorus. It seems very unlikely that phosphate feeding diminishes bone resorption, but probable that phosphate feeding stimulates the parathyroids and increases tubular absorption of calcium. So there are three points here that suggest that one must not forget the kidney in this situation. Some of the effect being seen in calcium balance might be on the kidney rather than on the bone; bone effect might be secondary to the kidney and not vice versa.

Dr. McCally: Dr. Hyatt, in your studies using 9-α-fluorohydrocortisone, was there a decreased urinary calcium as well as a decreased urinary sodium?

Dr. Hyatt: We have these data, but I haven't looked at it in that way as yet.

Dr. Nordin: I think it unlikely that this relationship would hold here, because 9-α-fluorohydrocortisone works distally, and this relationship between sodium and calcium is always on the proximal tubule. If you would expand the plasma volume and produce the so-called third factor effect, the nature of which is not fully known, you will get a rise not only in urinary sodium but in urinary calcium. It's believed that the common factor between urinary sodium and urinary calcium is proximal rather than distal, so I doubt that 9-α-fluorohydrocortisone would reverse it.

Dr. Hully: I believe the statement about our serum calcium results deserves modification. We really did not show a significant fall in serum calcium during bed rest.

Dr. Nordin: I know you didn't, but *all* the serum calcium values were below the normal mean value rather than above it. I know they are not significantly low, but they were consistently below the mean control value. If you have enough values that are below the control, even though not enough to be really significant, it does look a bit suspicious.

Dr. Donaldson: Dr. Young has carried out some work that shows that bone itself may be the target organ that is important here. The question has been raised as to why supine exercise doesn't seem to be nearly as effective as actual weight-bearing. He has placed monkeys in a compression apparatus that applies pressure to the tibia in the amount of half of the body weight of the animal. Figure 29.1 shows a monkey who was treated in this way for 14 weeks. The urinary calcium excretion in the course of this treatment suggests that compressing the bone alone decreases urine calcium to approximately 50 percent of control value. If there are additional data to support this, then I think it would be certainly worthwhile to look at the bone as the major organ affected by deconditioning.

Dr. Gatts: The pull of the muscle in a nonweight-bearing situation is a mere fraction of what it is in a weight-bearing situation, so it still seems to me that the important component of skeletal deconditioning is the lack of weight-bearing. Immobilization keeps sneaking into the problem; we should call it nonweight-bearing.

Dr. Wunder: I think that bears out the argument that one cannot separate weightlessness from immobilization until one can do control studies with a 1-G centrifuge in a satellite or orbiting vehicle.

Dr. McCally: One subject that recurs often in all of these environments is the possibility of abnormal sleep states and their physiological significance. There are two important considerations here: the behavioral aspect of putting subjects in a foreign environment, and the physiologic effect that the sleep states actually have on both vascular and metabolic systems.

Dr. Webb: I was glad to hear Dr. Lecocq's comment on how much time his subjects spent asleep and certainly in our 120-hr immersion study, this was the major activity for our subject. It was very hard to get him aroused enough to watch his favorite TV program.

Dr. McCally: It would be very interesting to record activity patterns, including time of occurrence, extent, and total amount of activity, and to record sleep states directly. This may prove to be an important variable in some of the phenomena we're seeing. The behavioral aspect of confinement might be extremely important in this regard. Perhaps the bed rest condition should be made a recumbent "office activity" situation. That is, we should provide the subject with all the accoutrements of his normal daily life, except for the continued recumbency.

Dr. Lancaster: We hope to have some of those data for you when we finish the exercise study we are currently carrying on. All of these individuals were prescreened on the basis of psychological testing and about 5 hr of psychiatric interviews by three different psychiatrists. We also have EEG sleep data recorded at night and random tape recordings of activities throughout the control period.

Dr. Bell: We have recently become interested in the biochemistry of Paget's disease, a condition associated with progressive calcium loss from bone. Urinary excretion of proline and hydroxyproline is high from people with this disease. Hydroxyproline, which is incorporated into the collagen calcium matrix of bone, must be synthesized in vivo. The synthesis of this hydroxyproline may be controlled by hormone actions such as those described for growth hormone and insulin earlier in this meeting. It is possible that some of the effects of exercise on calcium metabolism are indirect.

Goldstein et al. showed, in their classic work on the mode of action of insulin (ref. 9), that totally eviscerated animals would show uniform distribution of glucose in the absence of insulin, if the animals performed muscle work. It is quite possible that muscle activity indirectly controls many biological parameters such as hydroxyproline and calcium turnover.

Dr. Gauer: In relation to a discussion of disuse atrophy of bone and muscle, I want to draw your attention to a point that has not been considered so far. An integral part of the CNS is the antigravity reflex system of Sherrington. As you know, this system serves the control of posture and locomotion in a gravitational field. It may also have trophic functions. If this "computer system" deteriorates in the weightless state, preservation of the gross anatomy of peripheral skeleton apparatus (e.g., by isometric training) will be of doubtful use. Man, body and soul, is a creation of gravity. One of the oldest expressions of human culture, architecture, results from an interplay of the creative mind of the artist and the forces of gravity. In the weightless state, the structural elements such as columns, arches, cupolas, and buttresses (e.g. a Gothic hall) (fig. 29.2(a)) lose their purpose and, hence, their meaningful beauty. If we look at the human skeleton (fig. 29.2(b)), the same building elements are found. In other words, in outer space, the elements forming Homo sapiens are as obsolete and out of place as a Gothic cathedral. The creatures that the creator made for weightless floating such as plankton (fig. 29.3(a)) look quite different. There is a quaint similarity to the latest architectural creations of man, the satellites (fig. 29.3(b)). If man succeeds in achieving survival in the weightless state for years and generations, he must embrace the idea that the development of his functional anatomy adapts to the principles of his own technical designs for space. It is fascinating that this tendency becomes apparent, even after a very short time, if only our methods and criteria are sensitive enough.

Dr. Vogt: If we talk about countermeasures and the gadgets that are used with countermeasure devices, we can say that leotards are beneficial. Venous occlusion cuffs have proved their usefulness also, although there is some question as to their mechanism of action. Studies have been performed which seem to indicate that the timing of the inflation and deflation of these cuffs may be very important in determining their effectiveness (refs. 10–14). In terms of other devices and gadgets, such as ergometers, exercisers, and so forth, it seems to me that these may make the subjects feel better (and indeed we want them to feel better), but there is no real evidence that they are effective for preventing or treating deconditioning. If we desire to know what these do to the cardiovascular system or the muscular system, we are going to have to be much more careful in measuring the responses. I should like to say a word about the mercury-in-rubber strain gauge, the so-called Whitney gauge. It seems indisputable that this is an extremely accurate device for measuring changes in length or circumference and that, with very careful experimental technique, it can be quite useful

in measuring a change in the circumference of the calf during tilting. There is, however, a problem that has to be understood; there is a shift in the muscle mass during the initial part of the tilt curve that causes an artifactural change in the calf circumference. After the initial change in volume due to this muscle shift, it seems to me that subsequent circumference changes can be related roughly to changes in fluid volume. The Whitney gauge is particularly useful for the determination of forearm blood flow, and, I think, is very accurate under these conditions.

I don't believe there is enough information for us to state that the use of phonocardiography is of any value in spaceflight. The use of impedance techniques to measure cardiac output might be useful in spaceflight, but these measurements are rather gross and subject to artifacts. We could readily overinterpret some of these data — this has already happened.

We really don't know what happens to the plasma volume when a subject is tilted. We know that plasma volume is diminished after water immersion and bed rest, but we're not sure that, during tilt following deconditioning, there is an excessive pooling of blood or an increased extravasation of plasma as plasma water. It seems that we have the technology now to study this matter in much greater detail.

Dr. Hyatt: Dr. Schmid has shown in studies of the veins that apparently there is a decrease in catacholamines. How does he relate these vein changes to the fact that most of our studies show quite good evidence that following bed rest, all other catacholamine stores in both the myocardium and arterial system are quite intact and function normally? Does he postulate an isolated defect in the veins, and if so, why should this occur?

Dr. Schmid: I showed changes that were similar in resistance and capacitance vessels, so that the defect, whatever it might be, that accounts for a diminished response to tyramine after bed rest is present on both the arterial and the venous sides of the circulation in the forearm. Whether this typifies other vessels in the body or whether this kind of abnormality exists in the heart, I don't know.

Dr. Oyama: There is a good possibility that in the future we may have some artificial gravity-generating devices in space stations or space vehicles. I noticed this was omitted from the discussions of countermeasures. There have been extensive studies employing centrifuges to create artificial gravity, which may be effective in preventing deconditioning. There are, however, various problems of stimulation of vestibular apparatus with this kind of device that must be resolved. Perhaps less emphasis should be placed on studies of the analogs of weightlessness, because the use of "artificial G" generators may overcome any problem with weightlessness.

Dr. Murray: This is an interesting point, and it is unfortunate that we did not have more time to discuss it. There is a great deal of research being done on the short radius centrifuges at the present time, and I know that NASA has studied the possibility of including a short arm centrifuge aboard future spaceflights.

Dr. Wunder: With respect to Dr. Oyama's suggestion of manned, rotating, space stations for studies of weightlessness, I concur that centrifuges in orbit are essential for controlled experiments.

It should be recognized, however, that design criteria for manned, orbiting centrifuges can be expedited at less expense if preceded by space-based centrifuges that are suitable for smaller animals but too small to prevent rotational artifacts with man (ref. 15). It is only when control animals can be maintained in a suitable, 1-G, space-based centrifuge in close proximity to chambers for weightless experimental animals that the effects of weightlessness can be differentiated from other effects of spaceflight.

Earlier in this conference, one of the speakers justified his human studies, without preceding animal studies, by the comment "the *animal* I am interested in is man." I should like to comment that the area of investigation we should concern ourselves with is gravitational biology. We should then select the animals best suited for various problems of gravitational biology. The definitive studies in this investigation can come only by carefully controlled comparisons between animals exposed to zero-G and to 1-G. In this instance, animals that are lower phylogenetically and smaller than man are more suitable for the initial definitive studies. As one who has worked only with animals smaller than man, I acknowledged in 1964 (ref. 16) that man was the most suitable organism for studying the biological effects of weightless simulation by the methods of immersion or bed rest. Hopefully, scientists who worked with those methods will now likewise acknowledge the need for more work with lower animals. This would facilitate the safest and most economical acquisition of biological information basic to man's later flights to our neighboring planets.

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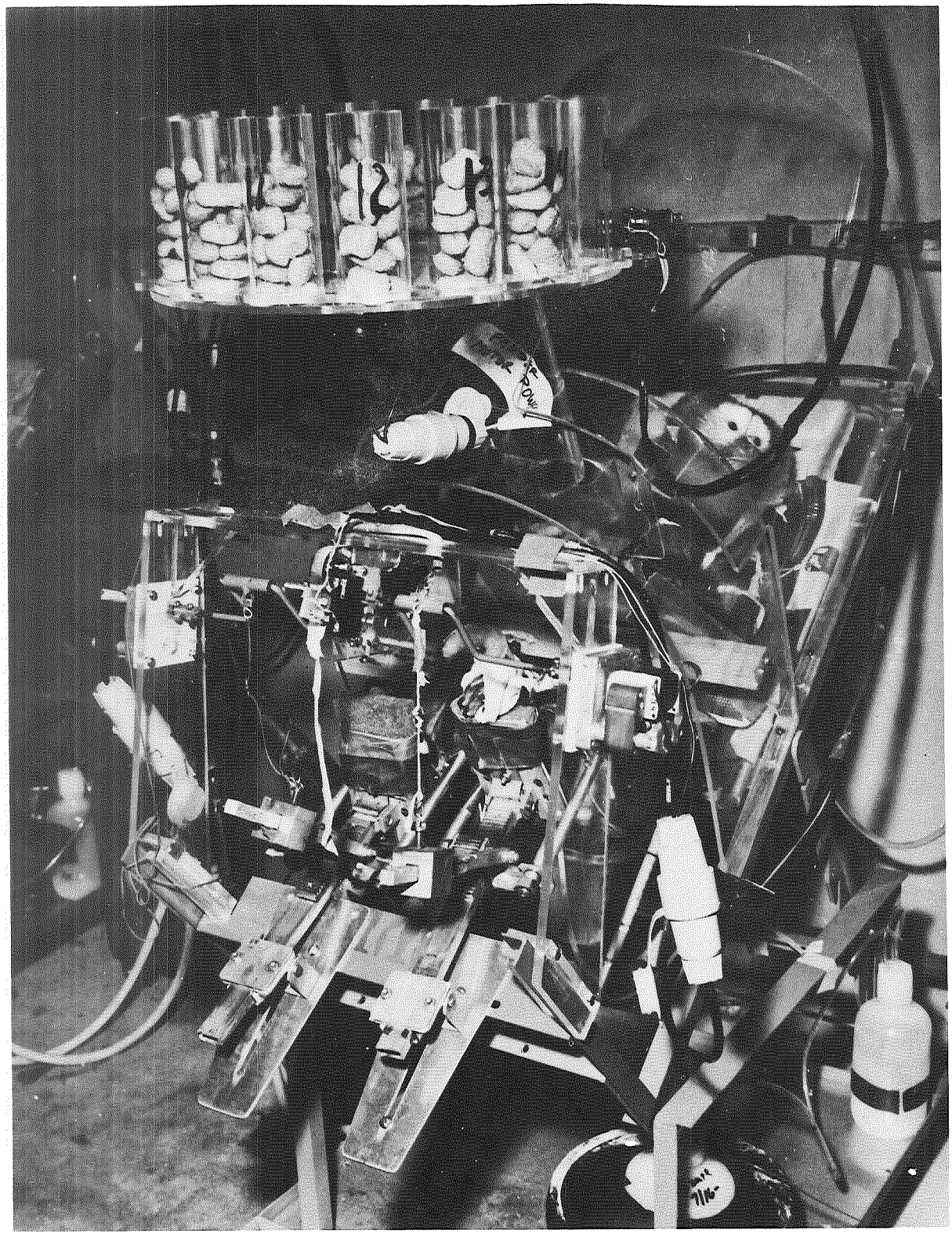

Figure 29.1 Application of axial compressive loads between the knee and heel during recumbency

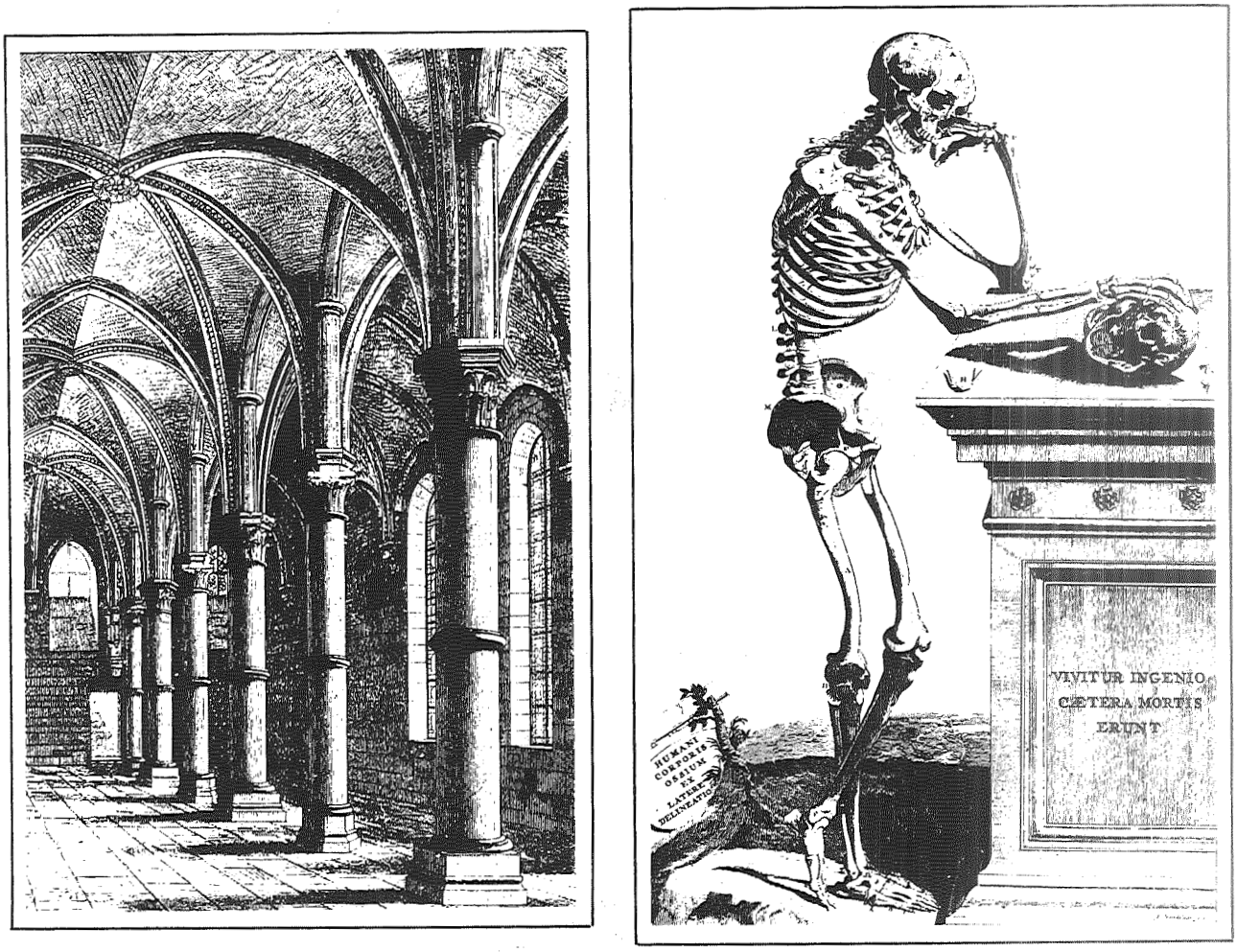

Figure 29.2 A comparison of the architecture of a Gothic hall and the human skeleton

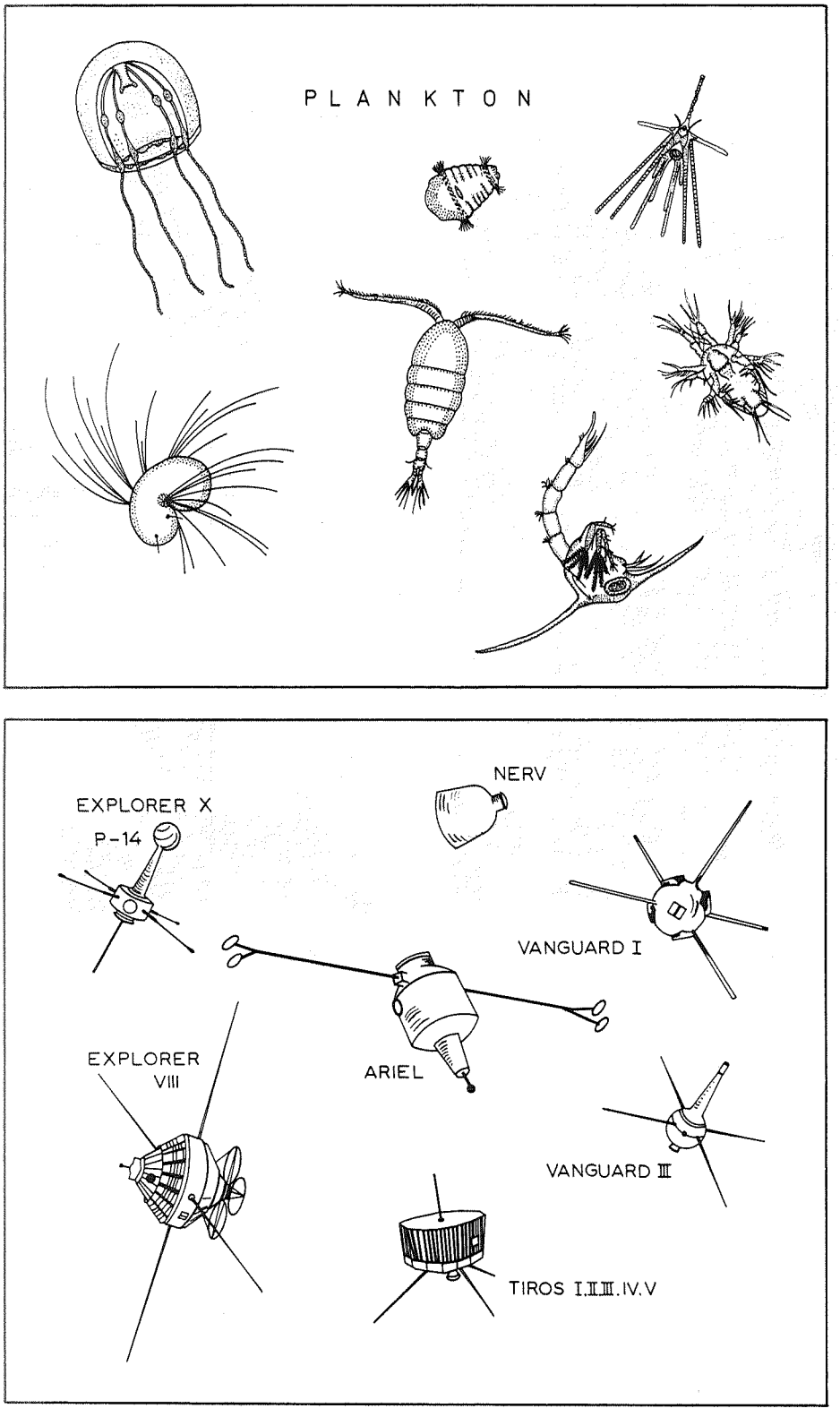

Figure 29.3 Similarity between architecture of plankton and satellites

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