N84-21046

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FINAL REPORT PHASE III

# RESEARCH OPPORTUNITIES IN BONE DEMINERALIZATION

December 1983

Prepared for

The Life Sciences Division
Office of Space Science and Applications
National Aeronautics and Space Administration
Washington, D.C. 20546

under

Contract No. NASW 3728

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# FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB), provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This technical report was developed for the National Aeronautics and Space Administration (NASA) in accordance with the provisions of Contract NASW 3728. It was prepared and edited by Sue Ann Anderson, Ph.D., Staff Scientist, LSRO, with the advice and assistance of Stanton H. Cohn, Senior Scientist, Brookhaven National Laboratory.

The LSRO acknowledges the contributions of the investigators and consultants who assisted with this study. The report reflects the opinions expressed by members of an ad hoc study group that met at the Federation on May 16 and 17, 1983. The study participants reviewed a draft of the report and their various viewpoints were incorporated into the final report. The study participants and LSRO accept responsibility for the accuracy of the report; however, the naming of these individuals in Section VIII does not imply that they specifically endorse each study conclusion.

The report was reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent Society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures, the report was approved and transmitted to NASA by the Executive Director, FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of each individual member of the FASEB constituent Societies.

January 16, 1984
(date)

Kenneth D. Fisher
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Director
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## SUMMARY

Bone demineralization and negative calcium balance have been reported consistently as a physiological response to space flight. Additionally, changes in calcium metabolism have been observed that may be associated with bone loss and a possible increased risk of fracture. The changes include increased fecal loss of calcium and hypercalciuria with a possible change in potential for formation of calcium-containing renal stones. In United States space flights as long as 3 months and Soviet flights as long as 7 months, neither loss of bone mineral nor the resultant hypercalciuria has been associated with impaired functional capacities of astronauts. However, concern for the health, effectiveness, and safety of space crews during and following extended or repeated space flights requires that gaps in knowledge of bone demineralization be identified and priorities for future research efforts be indicated.

The processes underlying bone loss in man during space flight are poorly understood. Histomorphometric studies of bone changes in rats flown aboard Cosmos biosatellites suggest that periosteal bone formation is inhibited and endosteal bone resorption is unchanged in weight-bearing bones in this species. Information concerning bone loss in weightlessness has also been obtained in ground-based studies of suspended rats, immobilized monkeys, and normal human volunteers during bed rest. Comparison of histomorphometric changes in bone of rats during space flight with bone changes in the suspended rat model and with bone changes in a monkey model indicates that some changes are similar for these models despite differences in bone growth and remodeling systems of the two species. Histomorphometric studies of bone changes in man have not been done during space flight or uncomplicated bed rest.

Evidence for loss of bone mineral in man during space flight has been supplied by metabolic balance studies and by noninvasive measures of bone density changes. These studies indicate an overall difference between anabolic and catabolic processes but provide little information concerning the changes occurring in bone during weightlessness. Metabolic balance studies in man during bed rest have shown changes in calcium balance generally similar to those of astronauts during space flight. Increased urinary and fecal losses of calcium have been reported in each situation. It has not been determined whether the fecal losses represent increased endogenous losses or decreased intestinal absorption of calcium. Noninvasive measures of bone density indicate that preferential loss of calcium from weight-bearing bone (os calcis) is common to space flight and bed rest.

Studies of changes in levels of calcitropic hormones (parathyroid hormone, vitamin D, and calcitonin) during space flight and bed rest do not consistently indicate changes of a magnitude that would ordinarily be associated with increased mobilization of bone. It is difficult to draw conclusions from these data, because at the time the measurements were made in space flight and in published reports of bed-rest studies, assays for a number of hormones were not well refined. Plasma levels and urinary excretion of cortisol are increased in man during space flight and adrenal glands are enlarged in rats following flights of the Cosmos biosatellites. However, urinary excretion of cortisol is not increased during bed rest. General systemic effects of endocrine agents cannot readily explain the local and preferential demineralization of weight-bearing bones. However, since physiologic responses during weightlessness differ from those under gravity, it may be possible that responses of bone cells to normal levels of endocrine agents in weightlessness differ from responses on the ground. This may be particularly evident in weight-bearing bones that lack their normal gravity-related stimuli.

Trials of countermeasures to prevent bone demineralization have been conducted in crews during space flight and, more extensively, in ground-based studies of human subjects during bed rest. Exercise during space flight has not completely reversed negative calcium balance or hypercalciuria. However, there is some evidence that use of a treadmill during space flight may have moderated loss of os calcis mineral. In Soviet flights exercise has reportedly been associated with decreased calcium loss. A number of levels of weight-bearing and/or exercise regimens have been tested in ground-based bed-rest studies in the United States in an effort to determine the amount of physical stress necessary to prevent calcium loss. Of all the protocols evaluated, only controlled ambulation on a prescribed course for 4 hours completely alleviated negative calcium balance. Dietary intervention (supplementation of fluoride or supplementation of calcium and phosphorus) did not reverse hypercalciuria or negative calcium balance over a long period of bed rest. A combination of calcium and phosphorus supplements, longitudinal compression and administration of synthetic salmon calcitonin was partially effective for a shorter period; however, administration of synthetic salmon calcitonin or longitudinal compression alone was not effective. Diphosphonates (EHDP or clodronate) administered to normal subjects during bed rest have been the most effective pharmacologic agents tested. However, side effects of these particular compounds contraindicate their further use.

The following report comprises an overview of bone demineralization during space flight, observations of the ad hoc Working Group on the NASA Biomedical Research Program in Bone Demineralization and experiments related to bone loss planned for Spacelab flights, and suggestions for further research. The observations of the ad hoc Working Group focused upon the following topics: (1) pathogenesis

of bone demineralization, (2) potential for occurrence of renal stones consequent to prolonged hypercalciuria, (3) development of appropriate ground-based and inflight models to study bone demineralization, (4) integration of research efforts, and (5) development of effective countermeasures. Priorities for further research are indicated.

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# I. INTRODUCTION

Physiological adjustments to the space environment involve most tissues and organs, including the nervous, cardiovascular, muscular, and skeletal systems. The responses, with the exception of space motion sickness, have not resulted in impaired functioning of crew members during flight. Details of some physiological changes during space flight and readjustment to the Earth's environment are described by Levy and Talbot (1983), Nicogossian and Parker (1982), Talbot (1983), Thornton and Rummel (1977), Smith et al. (1977), and Whedon et al. (1979).

Low bone mass is recognized as a factor in the complex etiology of fracture (Johnston and Epstein, 1981). Thus, bone demineralization during weightlessness is a physiological change that may have the potential to cause both chronic and acute problems, particularly in association with extended or repeated space missions. Other problems that may be associated with the loss of calcium from bone include increased fecal loss of calcium and hypercalciuria, with a possible change in potential for formation of calcium-containing renal stones.

Changes in calcium metabolism and skeletal mineral content associated with space flight have been assessed in man by metabolic balance techniques and $^{125}I$ gamma ray transmission scanning of bone. Total body calcium losses average about 0.3 to 0.4%/mo during space flight (Whedon et al., 1977). Os calcis (a weight-bearing bone) loses about 5% of its mass per month during space flight while radius and ulna (nonweight-bearing bones) show no measurable loss (Smith et al., 1977; Vogel and Whittle, 1976). Similar bone losses (5 to 8%) were reported in os calcis following the 175-day Salyut-Soyuz flight (Oganov, 1981a). Bone loss appears reversible following flights as long as 84 days (Smith et al., 1977) but reversibility of bone loss following more extended or repeated space missions remains to be determined.

Effects of hypercalciuria have received less research attention than the loss of bone calcium. Approximately 75 to 80% of renal stones contain calcium. However, a change in propensity for renal stone formation will depend not only on increased urinary concentration of calcium but also on such factors as urinary pH, concentrations of inorganic constituents (sodium, magnesium, potassium, and phosphorus), concentrations of organic compounds (uric acid, citrate, and oxalate), and crystallization characteristics (Coe and Favus, 1980; Pak, 1981; Pak et al., 1978). Development of renal stones could be a more immediate problem than bone demineralization for susceptible crew members during a space mission; however, in United States space flights as long as 3 months and Soviet flights as long as 7 months, neither loss of bone mineral nor the resultant hypercalciuria has been reported to impair functional capacities of astronauts.

NASA's policy of providing preventive medical care for persons exposed to the space environment requires that all necessary precautions be taken to prevent or counteract any adaptive responses to space flight unless they are shown to be beneficial. Therefore, despite the lack of evidence that bone demineralization during exposure to weightlessness is associated with adverse health effects during or following space flight, NASA must regard bone demineralization and the associated alterations in calcium metabolism as a significant biomedical problem of space flight. Concern for the well-being, effectiveness, and safety of crew members during and following space missions of extended duration dictates that gaps in knowledge of bone demineralization be identified as research opportunities. In order to enhance its research and analysis programming on bone demineralization during space flight, NASA requested that the Life Sciences Research Office (LSRO) of the Federation of American Societies for Experimental Biology (FASEB) review and evaluate its ongoing research effort and provide suggestions for future directions for research in this area. This task was performed with the guidance of the ad hoc Working Group of scientists listed in Section VIII.

## II. OBJECTIVES AND SCOPE OF THE STUDY

The objectives of the LSRO ad hoc review of bone demineralization during space flight are:

(1) to review extant knowledge of the subject;

(2) to examine NASA's current and projected research program;

(3) to identify significant gaps in essential knowledge;

(4) to formulate additional suggestions for future research for consideration by NASA; and

(5) to produce a documented report of the foregoing items that can be used for NASA program planning for future research.

The programs considered by the ad hoc Working Group were the in-house and extramural ground-based research efforts of NASA's Biomedical Research Program and the experiments related to bone loss planned for Spacelab flights 2 and 4 scheduled for 1985 and 1986, respectively. Among the resources examined for information relating to these endeavors were an extensive published scientific and technical literature, unpublished data of the United States and Soviet space programs, the NASA Research and Technology Objectives and Plans (RTOPs) on bone demineralization, Research and Technology Resumes (RTRs) on bone demineralization, and summaries of inflight experiments related to bone loss planned for the Spacelab missions 2 and 4.

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## III. AN OVERVIEW OF BONE DEMINERALIZATION IN SPACE FLIGHT

This section summarizes research findings concerning bone loss associated with space flight and with ground-based models simulating weightlessness. Extensive literature exists concerning bone loss in patients with various forms of osteoporosis or those immobilized for therapeutic purposes. Studies of these conditions have not been included in this overview, because their complex and diverse etiologies may not apply to bone loss observed in healthy individuals during uncomplicated bed rest or space flight. Aspects of calcium and bone loss in osteoporosis and immobilization have been summarized by Johnston and Epstein (1981), Krane and Holick (1980), and Parfitt (1983).

### A. DEFINITION

Bone demineralization is defined as the excessive elimination of mineral or inorganic salts from the skeleton. Accelerated loss of bone mineral has been consistently reported as an effect of space flight (Rambaut et al., 1975a; Smith et al., 1977). Overall, bone and total calcium losses seem to resemble those of healthy subjects during ground-based bed rest: urinary and fecal calcium excretion are increased, calcium balance is negative, and calcium seems to be lost preferentially from weight-bearing bone (Deitrick et al., 1948; Donaldson et al., 1970; Rambaut et al., 1975a,b; Smith et al., 1977; Whedon et al., 1977).

Loss of bone mineral has also been associated with disease states including osteoporosis. Krane and Holick (1980) define osteoporosis as "a group of diseases of diverse etiology which are characterized by a reduction in the mass of bone per unit volume to a level below that required for adequate mechanical support function. The reduction in mass is not accompanied by a significant reduction in the ratio of the mineral to the organic phase, nor by any reproducible abnormality in the structure of either the mineral or the organic matrix." Osteoporosis is generally regarded as the response of bone to many influences, some of which affect bone by altering calcium homeostasis and others by acting on bone directly (Riggs et al., 1981). These influences include dietary inadequacies, progressive impairment of calcium absorption, inactivity, and hormonal imbalances. Osteoporosis is a common feature of many disorders or conditions. The form that seems most similar to bone demineralization during space flight is disuse osteoporosis which develops in healthy persons during immobilization. It is unclear how mechanical forces influence the processes of bone modeling and remodeling, but, according to Lanyon (1981), "it is reasonable to assume that the strains resulting from the commonly encountered loading situations of repetitive coordinated functional activity provide a continuing stimulus."

Bone loss has been evaluated in many studies of patients immobilized as a result of spinal cord injuries, major lower limb fractures, and poliomyelitis or other nontraumatic causes of extensive muscle paralysis. However, the stress, trauma, and denervation associated with these conditions may produce a type of disuse bone loss that is dissimilar in some respects to that observed in healthy individuals during uncomplicated bed rest (Parfitt, 1981).

## B. CALCIUM RESPONSES TO WEIGHTLESSNESS AND SIMULATED WEIGHTLESSNESS

### 1. Calcium losses in man during space flight

Metabolic balance studies conducted during space flights indicate that exposure to weightlessness results in negative balances of calcium and phosphorus as well as magnesium and nitrogen (Lutwak et al., 1969; Rambaut et al., 1975b; Whedon et al., 1977). The earliest observation of changes in calcium and phosphorus balances occurred during the 14-day Gemini VII orbital space flight (Lutwak et al., 1969). Although interpretation of the data was qualified because of technical difficulties, calcium balances in the two astronauts became less positive than pre- and postflight values by the end of the first week of flight and phosphorus balances were negative throughout the flight (Lutwak et al., 1969).

More extensive metabolic data were collected for 5 days during the 12 day Apollo 17 flight and compared with data gathered on the Apollo crewmen for 5 days approximately 2 months prior to the flight (Rambaut et al., 1975b). Each of the three crewmen was in positive calcium balance (318, 360, and 235 mg/d) during the baseline period. Inflight mean daily calcium intake was 674 mg. Urinary excretion of calcium differed little from baseline values but fecal losses were two to three times greater during flight than before flight. Calcium balances during flight were +18, -199, and -37 mg/d, apparently caused by increased fecal losses. In a partial metabolic study conducted during the 11-day Apollo 16 flight (Rambaut et al., 1975b), fecal calcium losses greatly in excess of intake were also recorded for each of the three crewmen. Similar results regarding fecal calcium excretion have been reported in association with intense stress (Malm, 1958; Ohlson and Stearns, 1959; Roberts et al., 1948), and in patients with osteoporosis (Gallagher et al., 1973).

Phosphorus intake was approximately two times the calcium intake and fell somewhat during the Apollo 17 flight from preflight intakes. Urinary and fecal excretion of phosphorus increased during flight, bringing each of the crewmen into negative balance (-117, -507, and -325 mg/d) (Rambaut et al., 1975b).

Calcium and phosphorus balances were also measured in the nine astronauts participating in Skylab missions 2, 3, and 4 of 28-, 59-, and 84-days duration, respectively. Mean calcium and phosphorus balances for the six astronauts in Skylab missions 2 and 3 fell from positive balances preflight to negative values inflight and returned to positive values postflight (Whedon, 1979; Whedon et al., 1977). Calcium intakes were reported as nearly constant before, during, and after the flights. The mean daily dietary calcium intake of the Commander of Skylab 3, considered representative for the members of that mission, was 725 mg preflight, 729 mg inflight, and 742 mg postflight (Whedon et al., 1977).

The mean shift in calcium balance for the six astronauts of Skylab flights 2 and 3 from the preflight control periods (18 and 28 days) to the last 16 to 18 days during flight was -184 mg/d. The mean calcium balance during the second month in flight for the three crewmen of the 59-day Skylab 3 flight was -140 mg/d. Phosphorus balance was also negative during Skylab flights 2 and 3: in Skylab 2 the mean shift from preflight values was about -400 mg/d while in Skylab 3 it was -222 mg/d (Whedon et al., 1977).

Postflight, phosphorus balances of all and calcium balances of two of the three astronauts from Skylab 2 returned to preflight values within 17 days. For the three Skylab 4 astronauts, calcium balances became less negative, but had not returned to preflight positive balances by the end of the 18-day postflight evaluation period (Whedon et al., 1979).

Urinary excretion of calcium during the three Skylab flights increased steadily during the first 2 to 4 weeks of flight until losses were approximately 80 to 100% greater than preflight levels. Urinary calcium excretion remained at these elevated levels throughout flight (Whedon et al., 1977). Considerable inter-individual variation in the degree of hypercalciuria was present, but the overall trends followed the same patterns for all of the astronauts. Urinary phosphorus excretion increased markedly during flight. Urinary excretion of calcium and phosphorus followed the same pattern of increase and was of the same magnitude as that of immobilized healthy male subjects (Deitrick et al., 1948; Donaldson et al., 1970; Whedon et al., 1977). Urinary excretion of hydroxyproline and total and nonglycosylated hydroxylysine was elevated during flight for the nine crew members of the Skylab flights (Leach and Rambaut, 1975, 1977; Whedon et al., 1977). The mean increase in hydroxyproline excretion was 30%, although marked individual differences were noted (Whedon et al., 1977; Whedon, 1982). Investigators in the Soviet Union have also reported increased excretion of calcium during space flight (Biryukov and Krasnykh, 1970; Gazenko et al., 1980). During the postflight recovery periods urinary calcium excretion returned toward preflight baseline levels.

Urinary excretion of uric acid was decreased during flight for most of the crew members of the Skylab missions (Leach and Rambaut, 1977). Plasma uric acid levels were not measured during flight but were significantly decreased postflight, confirming findings reported following the Apollo flights (Leach and Rambaut, 1975; Rambaut et al., 1975b). Factors responsible for the low levels of uric acid in plasma were not readily apparent.

In contrast to the very large increases in fecal calcium and phosphorus excretion reported for the Apollo 17 astronauts (Rambaut et al., 1975b), smaller overall increases in fecal calcium and phosphorus losses were observed in the crewmen of the Skylab flights (Rambaut et al., 1979; Whedon et al., 1977). When fecal calcium losses were expressed as a function of flight duration, however, a different excretion pattern became apparent. During the early phases of flight fecal calcium excretion decreased to levels below preflight values, then rose steadily for the remainder of the flight and showed no tendency to plateau (Rambaut and Johnston, 1979). The investigators suggested that the continuous increase in fecal calcium excretion during space flight represented the major route of calcium loss and might represent either progressive malabsorption of calcium or increased losses of endogenous calcium. Another analysis of these data indicated the changes in fecal calcium excretion, net calcium absorption, and relative calcium absorption (net absorption expressed as a percentage of dietary intake) were not statistically significant, thereby suggesting that the negative calcium balance of space flight resulted from an increase in urinary calcium excretion (Parfitt, 1981).

The amount of calcium lost during each month of space flight was calculated to represent about 0.3 to 0.4% of total body calcium. An extrapolation to a 6-month space flight indicated that 2 to 3% of total body calcium would be lost by astronauts during that length of time (Bricker, 1979; Whedon et al., 1977). However, an extrapolation based on the analysis of Rambaut and Johnston (1979) predicted that the average quantity of calcium lost in one year of space flight would be over 300 g or 25% of the overall body pool of calcium (1250 g). Parfitt (1981) calculated the relative loss of cortical and trabecular bone to be 0.5% and 5.0%, respectively, based on his analysis of the calcium loss data and on the assumption that bone loss is partitioned between cortical and trabecular bone in proportion to their rates of turnover.

Measurements of bone density corroborate the evidence of bone calcium loss implied by balance studies during space flight. Data collected by radiographic densitometry before and after the early Gemini and Soyuz missions suggested large losses of bone density in os calcis, radius, and ulna in short-term flights of 4 to 14 days (Mack and Lachance, 1967). However, reevaluation of some of these data indicated significant overestimates of the losses (Vose, 1974). In these very short flights, the losses of bone mineral were likely very slight.

Mineral content of the radius, ulna, and os calcis of 18 astronauts was determined by photon absorptiometry immediately before and after Apollo 14, 15, and 16 missions and the Skylab 2, 3, and 4 flights. This method was considered a more reliable estimate of bone mineral loss (Rambaut et al., 1972). Two crewmen from the Apollo 15 mission (14 days) showed loss of os calcis mineral. No significant mineral losses were reported in the radius or ulna of the crewmen on the Apollo missions (Rambaut et al., 1975a). No bone mineral losses were detected during the Skylab 2 mission (28 days); however, significant losses in os calcis mineral (-7.4%, -4.5%, and -7.9%) occurred in three of the six crewmen of the Skylab 3 and 4 missions. These crewmen also had the largest increases in urinary calcium losses and the greatest negative shifts in calcium balances (Smith et al., 1977). No losses were detected in radius or ulna. Similar mineral losses (5 to 8%) from os calcis were reported for cosmonauts following the 175-day Salyut-Soyuz flight (Oganov, 1981a).

It is understood that a joint program between the United States and the Soviet Union is underway to evaluate by computed tomography (CT) techniques mineral content of vertebral columns of the Soviet cosmonauts on the 211-day flight (Cann et al., 1983). In a previous study of vertebral density of Soviet cosmonauts and bed-rested subjects, Cann (1981) reported that spinal bone loss appeared to correlate well with calcium balance data.

Little is known concerning degradation of organic bone matrix during space flight. Urinary excretion of total hydroxyproline, peptide-bound hydroxyproline or hydroxylysine glycosides was increased in astronauts during space flight, but to a lesser extent than in quadriplegic patients (Claus-Walker et al., 1977; Rambaut and Johnston, 1979; Rambaut et al., 1979). Analysis of autopsy samples of os calcis of three cosmonauts of Salyut-1 indicated no abnormalities in amino acids, glycoprotein, or organic matrix content after 28 days of space flight (Prokhonchukov and Leont'yev, 1980; Prokhonchukov et al., 1980).

The question of reversibility of the loss of bone mineral during space flight has not been completely resolved. Os calcis mineral content of the Skylab 3 crewman (59-day flight) was regained by 87 days postflight. However, os calcis mineral of the two Skylab 4 crewmen had not returned to preflight values by 95 days postflight (Smith et al., 1977). Os calcis mineral content measured 5 years following the Skylab program was significantly lower in the nine crew members participating in space flights than in eight back-up counterparts (Tilton et al., 1980). The investigators were unsure of the clinical significance of this finding and advised cautious interpretation of the data. Although low bone mass has been recognized as a factor contributing to fracture, many individuals with low bone mass do not experience bone breakage, whereas some persons with higher than normal bone mass for age do develop fractures (Johnston and Epstein, 1981). It has been suggested that even large initial deficits resulting from increased bone turnover may be completely reversible with no intervention other than removal of the stimulus responsible for the increased activities and turnover (Parfitt, 1981).

### 2. Calcium losses in man during simulated weightlessness

Bed-rest studies of healthy men have been used as the ground-based simulation of weightlessness for evaluation of mineral metabolism during space flight. Protocols for bed-rest studies in the United States have included horizontal recumbency with or without partial raising of the upper body for meals or reading, with or without bathroom privileges, and with use of partial body casts for complete immobilization. Anti-orthostatic (6° head-down tilt) bed rest is the position described by cosmonauts as most closely approximating their sensation of head fullness experienced during space flight. This protocol may be employed during future ground-based studies of bone demineralization in the United States.

During the early phases of bed rest, plasma ionized calcium showed very small fluctuations and parathyroid hormone (PTH) levels were normal or below baseline values (Heath et al., 1972). However, it was reported in studies from the U.S.S.R. that plasma concentrations of calcium and PTH were increased in bed-rest studies lasting 3 to 6 months (Grigor'yev, 1981). Urinary calcium excretion of healthy persons begins to rise immediately with onset of bed rest (Deitrick et al., 1948; Donaldson et al., 1970; Hulley et al., 1971). In these studies, urinary calcium concentrations peaked at about 6 to 7 weeks after initiation of bed rest and then began to subside, but hypercalciuria persisted throughout bed-rest periods as long as 36 weeks.

Hypercalciuria and development of renal stones have been associated with immobilization in patients (Albright et al., 1941; Leadbetter et al., 1945; Tori and Kewalramani, 1978). Parfitt (1983) listed several factors associated with immobilization including impaired drainage of the kidneys, increased phosphate excretion, and increased urinary pH which might increase the risk of development of renal stones during uncomplicated bed rest and possibly space flight.

Fecal calcium excretion was elevated throughout the studies of Donaldson et al. (1970) and Hulley et al. (1971), suggesting either decreased absorption or increased endogenous loss of calcium into the gastrointestinal tract. The sources of the increased fecal calcium excretion have not been ascertained. Two experimental approaches suggest different origins of the calcium. Studies of calcium kinetics suggested increased secretion of endogenous calcium into the gut; however, measurement of intestinal calcium absorption after 5 weeks of bed rest indicated decreased, increased, or unchanged absorption among the subjects (Lockwood et al., 1975). Study of calcium kinetics in patients immobilized by poliomyelitis suggested that absolute absorption and efficiency of absorption of calcium were greatly depressed during the acute development stage but not the chronic, stable stage of disuse osteoporosis (Heaney, 1962).

Calcium balance during 36 weeks of bed rest was about -200 mg/d, a loss of total body calcium of about 0.5% each month or 4.2% for the entire period. Mean phosphorus balance was -34 mg/d. Urinary excretion of hydroxyproline peaked during the second month of bed rest with a mean excretion level for the entire period approximately 8% above baseline levels (Donaldson et al., 1970). Schneider et al., (1981a) reported that calcium balance in older male subjects (35 to 45 years of age) was similar to that of younger subjects (ages 19 to 35 years).

Measurements of bone mineral content during bed rest by $^{125}I$ gamma ray transmission scanning showed no losses of bone mineral in the radius, but substantial losses of mineral content (25 to 40%) in the weight-bearing os calcis during periods of 24 to 36 weeks (Donaldson et al., 1970; Hulley et al., 1971). This rate of loss from the os calcis (approximately 4% each month) was disproportionately greater than the overall rate of loss of body calcium (0.5%/mo) but rates of mineral loss of other weight-bearing bones were not measured. Calculations by Rambaut and Johnston (1979) indicated that loss of calcium from the calcaneus accounted for only a small proportion of the total amount of calcium lost. Data of Hesp et al. (1982) indicated differences in regional bone density measurements compared with total body calcium in osteoporosis. Dual-photon ($^{153}Gd$) absorptiometry measures of bone mineral of the lumbar vertebrae indicated a loss of 0.9%/wk during bed rest periods of 11 to 61 days (mean 27 days), suggesting a rate of loss of bone mineral from the lumbar vertebrae similar to that of the os calcis during bed rest (Krølner and Taft, 1983). However, no significant changes in urinary excretion of calcium or phosphorus were reported in this study.

When zero balance was used as the baseline for calculating change in calcium balance during space flight, values reported for urinary loss of calcium, negative calcium balance, and loss of os calcis mineral by astronauts during exposure to weightlessness were within two standard deviations of the means for these parameters measured in healthy subjects during bed rest (Parfitt, 1981). An alternate method for calculation of the calcium loss in the Skylab astronauts utilized the positive balances observed preflight as the baseline for computing the changes in calcium metabolism occurring during space flight, thereby maximizing the changes observed (Rambaut and Johnston, 1979). Based on these calculations, Rambaut and Johnston (1979) concluded that bone demineralization during space flight was more severe than predicted on the basis of observations of immobilized, bed-rested, or paralyzed subjects and suggested that the process might not be totally reversible.

The changes in calcium balance and in mineral content of os calcis and lumbar vertebrae of subjects during voluntary or therapeutic bed rest appear reversible upon reambulation (Donaldson et al., 1970; Hulley et al., 1971; Krølner and Taft, 1983). Donaldson et al. (1970) reported that remineralization of os calcis began immediately upon reambulation and that remineralization occurred at a rate similar to the loss. Initial values for bone density were regained by 36 weeks of reambulation. Following a bed-rest study of 24- to 30-weeks duration, calcium balances became positive during the first month of reambulation and remineralization of the os calcis occurred within 10 to 20 weeks (Hulley et al., 1971). Vertebral losses were nearly restored 4 months after therapeutic bed rest of 11 to 61 days (Krølner and Taft, 1983). However, Rambaut and Johnston (1979) referred to unpublished studies in which calcium balance returned to zero balance in middle-aged men before all bone mineral could have been restored.

Studies of calcium kinetics in healthy subjects during bed rest suggested that the rates of bone accretion and resorption were both increased, with a greater increase in bone resorption (Lockwood et al., 1975). Similar findings were reported in poliomyelitis patients with acutely developing disuse osteoporosis; however, bone formation and resorption rates were normal or slightly low in patients with chronic, stable disuse osteoporosis (Heaney, 1962). Changes in bone histomorphometry have not been measured in studies of healthy subjects during uncomplicated bed rest. The applicability of measurements of skeletal histomorphometry to the study of bone changes was reviewed by Meunier (1983). Quantitative histological data are available from iliac crest biopsies of 28 patients immobilized by spinal cord trauma, fractures, Schneider's syndrome, or cervical myelopathy (Minaire et al., 1974). Bone samples used as control comparisons were obtained at autopsy from 236 accident victims. Trabecular bone volume of the immobilized patients was less than that of the control samples. In the immobilized patients, trabecular bone volume decreased by an average of 33% prior to the 25th week of immobilization, after which time it appeared to become constant at a new, lower steady state value. The trabecular osteoclastic resorption surface varied during the period of immobilization. During the first 16 weeks following trauma it increased from normal (3%) to a high (5.3%) value. From 16 to 40 weeks the resorption surface decreased to normal values and stabilized at this level. The periosteocytic lacunae also enlarged temporarily after 12 weeks of immobilization.

Osteoblastic bone formation was greatly decreased and the internal and external cortices thinned about 50% during the 40 weeks following onset of immobilization (Minaire et al., 1974). Relative osteoid volume of cancellous bone decreased during the early part of immobilization, then rose to normal values, and eventually stabilized at values slightly below normal. The calcification rate as measured by tetracycline labeling was also very slow. Urinary excretion of calcium and hydroxyproline was elevated. Plasma calcium concentration remained within the normal range. The investigators suggested several possible interpretations of these findings in terms of changes in bone cell metabolism (Minaire et al., 1974).

## C. ENDOCRINE RESPONSES TO WEIGHTLESSNESS AND SIMULATED WEIGHTLESSNESS

Bone metabolism is regulated in part by endocrine factors. Several recent comprehensive reviews should be consulted for a detailed discussion of specific roles of hormones and their mechanisms of control of bone metabolism (Raisz, 1980; Raisz and Kream, 1983a,b; Raisz and Lorenzo, 1980).

### 1. Endocrine responses in man during space flight

Endocrine responses were measured in astronauts before and after the Apollo flights and during Skylab missions 2, 3, and 4 and for at least 21 days before flight and 17 days after the Skylab missions (Leach and Rambaut, 1977; Leach et al., 1975; Whedon et al., 1979). Calcitropic hormones showed no changes that would explain satisfactorily the mobilization of bone mineral during space flight. PTH levels fluctuated above and below preflight values during the Skylab flights. There was a tendency towards a slight increase in PTH level during the third month of the Skylab 4 flight; however, plasma samples from only three of the nine Skylab astronauts were available for these analyses and the increases were not statistically significant (Leach and Rambaut, 1975). Similarly, no change was noted in serum PTH concentrations prior to or following the Apollo missions (Leach et al., 1975). Plasma levels of 25-hydroxycholecalciferol were not different before and after the Skylab flights of 28 and 59 days; however, plasma 25-hydroxycholecalciferol levels were decreased by about 9% immediately postflight in the Skylab 4 astronauts exposed to weightlessness for 84 days. Calcitonin levels in plasma were too low to be measured by the assay used and therefore were considered not elevated to any clinically significant extent (Leach and Rambaut, 1975, 1977; Whedon et al., 1979). Total concentrations of calcium and phosphate in plasma rose slightly but significantly during the Skylab flights, returning to preflight values during the postflight periods (Leach and Rambaut, 1975). During space flight plasma calcium and phosphate levels were within ranges considered normal (Guyton, 1981).

Changes in urinary excretion of adrenal hormones were observed following the Mercury, Gemini, and Apollo flights (Leach, 1971). Plasma and urinary levels of adrenal hormones were measured during the Skylab flights. Glucocorticoids are of particular interest because of their direct and indirect effects on calcium metabolsim (Raisz, 1980). Evidence indicates interactions of glucocorticoids with PTH (Chen and Feldman, 1979; Wong, 1979) and vitamin D (Chesney et al., 1978; Hahn et al., 1979; Slovik et al., 1980).

Plasma adrenocorticotrophic hormone (ACTH) concentrations were generally decreased during and after flight, but adrenocortical hormones followed no consistent patterns during space flight. Plasma and urinary cortisol concentrations were elevated above baseline levels throughout the three Skylab flights, returning towards baseline levels postflight (Leach and Rambaut, 1975, 1977; Leach et al., 1975, 1976). Urinary but not plasma cortisol levels were elevated following the Apollo missions (Leach et al., 1975). Urinary excretion of 17-hydroxycortisone was elevated in the three crew members of the Apollo 17 flight. Total urinary 17-hydroxycorticosteroids did not change or decreased only slightly during the flight; however, the proportions of individual components varied, with decreases in pregnanetriol and tetrahydrocortisone and a slight increase in tetrahydrocortisol during the Skylab flights (Leach and Rambaut, 1975; 1977). Postflight, a significant decrease of total urinary 17-hydroxycorticosteroids was observed for astronauts from both the Skylab and Apollo series (Leach and Rambaut, 1977; Leach et al., 1975, 1976). Urinary excretion of total 17-ketosteroids rose with increases in androsterone and etiocholanolone excretion specifically (Leach and Rambaut, 1977). Following the Apollo missions, however, urinary excretion of androsterone and etiocholanolone fell by 49 and 35%, respectively (Leach et al., 1975). A slight downward trend was reported in urinary excretion of epinephrine and norepinephrine inflight; however, both showed little change (Apollo) or tended to increase immediately postflight (Skylab) (Leach and Rambaut, 1977; Leach et al., 1975, 1976; Whedon et al., 1979).

Other hormones indirectly implicated in calcium metabolism were measured as a part of the Apollo and Skylab studies. Thyroid hormone concentrations showed changes postflight when compared to preflight values. Thyroid stimulating hormone (TSH) and thyroxine ($T_4$) concentrations in plasma were increased; however, no change occurred in tri-iodothyronine ($T_3$) uptake postflight (Leach and Rambaut, 1977; Leach et al., 1975). Plasma human growth hormone increased significantly on the first day of flight and showed an even greater increase following flight (Leach et al., 1975; Whedon et al., 1979). For the Skylab astronauts fasting plasma insulin concentrations varied during the first month of flight, then decreased by 20 to 60% for the remainder of the longer flights. However, fasting plasma glucose levels did not change during the first month of flight and fell only about 5% during the remainder of the longer flights. After the Apollo and Skylab flights, fasting plasma insulin levels were somewhat elevated, but plasma glucose increased only slightly (Leach and Rambaut, 1977; Leach et al., 1975; Whedon et al., 1979).

Some measures of endocrine changes during space flight are also available from the two cosmonauts of a U.S.S.R. 63-day flight, Salyut-4. Increased concentrations of total 17-hydroxy-corticosteroids and norepinephrine in urine and TSH in blood were reported (Tigranyan and Ushakov, 1978).

In summary, plasma levels of the calcitropic hormones (PTH, 25-hydroxycholecalciferol, and calcitonin) measured to date in crew members during space flights have not shown changes that would account for the mobilization of bone calcium. Plasma levels and urinary excretion of other hormones related to calcium metabolism (cortisol, insulin, and human growth hormone) increased during space flight. The role of these hormones in calcium metabolism of man during space flight has not been established.

Experiments related to study of bone loss are planned for upcoming Spacelab flights (See Appendix B). Plasma levels of 1,25-dihydroxycholecalciferol and other vitamin D metabolites will be measured in the astronauts of the Spacelab 2 mission scheduled for 1985. A more comprehensive study of factors affecting calcium metabolism is planned for Spacelab 4. The crew of that flight will serve as experimental subjects. This study will combine investigation of changes in plasma levels of the calcitropic hormones, PTH (intact, N-terminal, and C-terminal assays), calcitonin, and the vitamin D metabolites (25-hydroxycholecalciferol, 1,25-dihydroxy-cholecalciferol, and 24,25-dihydroxycholecalciferol) with an examination of changes in intestinal calcium absorption done by a dual-isotope technique, using stable calcium isotopes. These studies should provide information concerning primary defects in renal or intestinal handling of calcium, on rates of total body calcium turnover, and, by inference, information concerning early changes in bone turnover.

### 2. Endocrine responses in man during simulated weightlessness

In a short-term (10-day) study of male and female subjects in the age ranges 36-45, 46-55, and 56-65 years, bed rest was associated with different effects on urinary excretion of hormones among the age and sex groupings. Calcitropic hormones were not measured in the study; however, urinary excretion of cortisol was found to be essentially unchanged after bed rest (LaRochelle et al., 1982).

In a long-term experiment involving six subjects, Hantman et al. (1973) noted that PTH levels did not change in a consistent manner during 19 weeks of bed rest. Schneider (1981) also reported that studies of PTH concentrations during several bed-rest studies were inconclusive and attributed this in part to the type of PTH assay performed. Calcitonin and 1,25-dihydroxycholecalciferol concentrations measured in normal volunteers during bed rest are not yet available. However, in 14 patients immobilized by traumatic spinal cord injury and fed a diet containing 400 mg calcium and 800 mg phosphorus, serum levels of 1,25-dihydroxycholecalciferol, immunoreactive PTH, and nephrogenous cyclic AMP were markedly reduced. Serum levels of 25-hydroxycholecalciferol and calcium were normal and urinary excretion of calcium was elevated. None of these patients had a prior history of nephrolithiasis, hypercalciuria, or metabolic bone disease (Stewart et al., 1982).

Some indicators of adrenal function have been measured during bed rest. Leach et al. (1973) reported that urinary excretion of epinephrine and norepinephrine fell slightly and that exercise prevented the decrease in norepinephrine excretion. Later, Sandler et al. (1983) reported that excretion of norepinephrine, but not epinephrine, was significantly increased during bed rest. Excretion of 17-hydroxysteroids (Sandler et al., 1983) and hydrocortisone (Leach et al., 1973) showed a slight but nonsignificant tendency to be elevated. Urinary excretion of cortisol was not significantly increased (LaRochelle et al., 1982) in contrast to higher levels reported in plasma and urine of astronauts during space flight (Leach and Rambaut, 1976; Leach et al., 1977).

Insulin-glucose relationships were abnormal during bed rest. Baseline glucose and insulin levels remained normal but, by the first week of bed rest, administration of a glucose challenge provoked an exaggerated insulin response which persisted as long as 2 weeks of recovery following bed rest (Dolkas and Greenberg, 1977; Lipman et al., 1970a; Sandler et al., 1983). A linear association between urinary calcium excretion and insulin levels has been reported (Allen et al., 1981). Production of the insulin antagonists, glucagon and growth hormone, changed little during bed rest (Lipman et al., 1970b; Pawlson et al., 1968).

## D. MECHANISMS OF BONE LOSS

### 1. Studies during space flight

Information concerning mechanisms of bone loss and histologic changes in bone during weightlessness has been derived from studies of rats flown aboard spacecraft, ground-based immobilized animals (suspended rats or restrained monkeys), and patients exhibiting bone loss following spinal cord injury. See Section III-B (p. 6) for a description of histomorphometric findings in trauma patients. Histologic changes in bone during exposure to weightlessness and the underlying causes thereof have not been studied in man or in an animal model (e.g., monkeys, dogs, miniature pigs) having a bone remodeling system similar to that of man. However, some histomorphometric changes in bones of rats flown aboard spacecraft are similar to those reported for restrained monkeys in ground-based studies (See Section III-D, p. 19).

Collaborative studies between the U.S. and U.S.S.R. of skeletally immature male rats flown onboard Cosmos biosatellites 782, 936, and 1129 have been useful in the investigation of some of the changes in bone occurring during space flight. In comparison to ground-based control rats, parameters associated with periosteal bone formation were decreased significantly in tibiae of rats flown for 19 days onboard the Cosmos 782 and 936 biosatellites. Parameters associated with bone resorption, longitudinal growth, and bone mineral density showed no significant changes. Arrest lines at both the periosteal and endosteal surfaces implied that bone growth stopped completely in the flight animals. A rebound effect was observed in bone formation parameters in the flight rats during a 26-day postflight period (Morey and Baylink, 1978; Turner et al., 1979; Wronski et al., 1980).

Direct measurement of resorption of bone from the rib cage of space-flown rats suggested that mobilization of bone mineral continued at the same rate as in ground-based synchronous control rats for the first 10 to 12 days of flight. Study of kinetics of bone resorption measured as calcium excretion suggested that breakdown subsequently decreased, falling to a level 20 to 25% below that of the controls at the end of flight. However, normalization of resorption rates by calcium turnover indicated that the decrease was probably secondary to an overall slowdown in total body calcium turnover (Cann and Adachi, 1983). Adrenal glands were enlarged in rats flown aboard Cosmos 782 (Morey and Baylink, 1978; Morey-Holton et al., 1982) and it was suggested that increased glucocorticoid levels may have contributed to the bone changes observed during space flight. An extensive literature exists concerning the effects of corticosteroids on calcium metabolism and bone changes in man and in animal models. (For examples, see Bressot et al., 1979; Clark and Roth, 1961; Clark et al., 1959; Hahn et al., 1979; Jee et al., 1970; Klein et al., 1977; Lukert and Adams, 1976; Simmons et al., 1979; and Yasumura et al., 1976).

Breaking strength determined by torsion loading was reduced in femurs of rats flown aboard Cosmos 936; however, the defect was corrected by the end of the 25-day postflight observation period (Spengler et al., 1979). Femurs of rats maintained in a 1-g centrifuge during the Cosmos 936 flight did not display the adverse effects noted in bones of rats subjected to weightlessness during flight. Results of tests of bone strength in vertebrae of rats flown aboard Cosmos 1129 also suggested loss of bone strength (Kazarian et al., 1980).

Studies of bone changes in rats during the 18.5 day Cosmos 1129 flight confirmed and extended the findings of the previous studies. Periosteal bone formation was decreased in the tibial and humeral diaphyses in proportion to their weight-bearing function, while endosteal bone resorption was not measurably changed (Wronski and Morey, 1983). In trabecular bone (proximal tibial and humeral metaphyses) the mass of mineralized tissue was decreased and the fat content of the bone marrow increased. The osteoblast population was reduced near the growth cartilage-metaphyseal junction, suggesting inhibition of bone formation during space flight. Osteoclast numbers were not changed in the flight animals (Jee et al., 1983). These histologic changes are similar to bone responses in rats or rabbits following administration of corticosteroids (Hulth and Olerud, 1963; Jee et al., 1966; Wang et al., 1977) and those resulting from disuse (Wronski and Morey, 1983). Except for the trabecular bone mass of the proximal tibia, the metaphyseal tissue returned to normal within 29 days postflight (Jee et al., 1983).

Lumbar vertebrae of the rats flown aboard Cosmos 1129 showed a decrease in calcium content of trabecular and cortical bone at 6 days postflight. This change was not present immediately postflight or at 29 days postflight and, in combination with postflight changes in keratosulfate in trabecular bone and chondroitin sulfate in vertebral bone, indicated a possible slowing of bone turnover during space flight, resulting in accumulation of older bone (Eurell and Kazarian, 1983). They suggested that the older bone was degraded by osteolysis immediately postflight, an interpretation supported by an earlier observation of perilacunar osteolysis in the metaphyses of long bones of rats 2 days postflight (Yagodovsky et al., 1976). In both instances, replacement of bone mineral content occurred by 27 to 29 days postflight.

In contrast, sections of rat mandible covered by masticatory muscle did not incur the decreased periosteal bone formation noted in weight-bearing bones. Additionally, growth and maturation of the mandibular incisor seemed to be maintained during space flight (Simmons et al., 1980). However, sections of the jaw that had no contiguous muscle exhibited evidence of impaired bone formation-calcification rates. A similar finding was reported for tibial diaphysis of rats flown aboard the Cosmos biosatellites; Spector et al. (1983) concluded that cessation of bone formation did not occur where intrinsic muscle forces continued to act on the tibia. Gravity density fractionation studies and ultrastructural studies of rat alveolar bone suggested delayed maturation of bone mineral and matrix and differences in metabolic activities of osteoblasts and osteoclasts. Recovery was complete at 19 days postflight (Simmons, 1981; Simmons et al., 1983). Suppression of osteoblast differentiation was suggested by a change in relative distribution of large to small nuclei in periodontal ligament adjacent to alveolar bone (Roberts et al., 1981).

Based on the results observed in rats flown aboard the Cosmos flights, further studies of changes in calcium homeostasis are scheduled for an upcoming Spacelab flight (See Appendix B). Quantitative histomorphometric analyses are planned to define more specifically the time course of inhibition of bone formation and differences in regional bone turnover at several skeletal sites in juvenile and adult rats. In addition, metabolic studies will examine changes in intestinal calcium absorption, routes of excretion, and total skeletal turnover.

### 2. Ground-based studies

In ground-based studies weightlessness has been simulated in the rat by suspending the animal in a harness made of orthopedic casting material, thereby removing weight from the hindlimbs and the lower vertebrae while allowing the forelimbs to be normally weighted (Morey, 1979). Comparison of the effects of this head-down suspension and space flight on the skeletal system showed similar reductions in periosteal bone formation at the tibiofibular junction, in trabecular bone volume, and in osteoblast populations. The osteoclast population was increased in the head-down suspension model but not changed in the space flight animals (Morey et al., 1979; Morey-Holton and Wronski, 1981). Rats suspended for 15 days absorbed calcium normally but showed progressive loss of bone mass and mineral in unweighted bones only (Bikle et al., 1982). Such differential bone loss may indicate that local rather than systemic factors mediated the bone loss, a finding supported by lack of inhibition of periosteal bone formation by corticosterone administration to nonrestrained cold-stressed rats (Morey-Holton et al., 1982).

In the suspended rat model $^{45}Ca$ uptake by vertebrae and tibia was depressed initially, but returned to control levels at 10 days, and then rose to levels significantly greater than those of control animals after 15 days of suspension (Doty and Morey-Holton, 1982). The investigators concluded from these data that bone resorption must be increased. However, electron photomicrographs of osteoblasts from femurs of rats suspended for 14 days showed a reduced number of gap junctions between cells in the periosteum and endosteum, suggesting reduced activity of bone-forming cells (Doty and Morey-Holton, 1982).

Histomorphometric bone changes have also been studied during chair-restraint of *Macaca nemestrina* or full-body casting of *M. mulatta*. In *M. nemestrina* restrained for as long as

6 months, loss of cancellous bone from the axial skeleton (vertebrae) was significantly greater than loss of cortical bone from three appendicular sites (tibia, radius, and ulna) (Cann et al., 1980). No losses were reported in radius and ulna, while loss in tibia varied regionally. In the proximal tibia, trabecular and cortical thinning was evident, while cortical striations were apparent in the tibial midshaft. Serial measurements (computed tomography or photon absorptiometry) indicated that vertebral mineral loss did not level off as soon as mineral loss in peripheral bone. In addition, the decrease in vertebral mineral content was more closely related to negative calcium balance than was mineral loss from peripheral sites, suggesting that the axial skeleton may be more adversely affected than the appendicular skeleton by extended immobilization (Cann et al., 1980). Electron probe analysis showed decreases of approximately 25% in calcium and phosphorus content of cortical bone (Bunch et al., 1982).

Further study of loss of cortical bone mass in the proximal tibia during 6 months of restraint indicated that resorption cavities were present within 1 month and large subperiosteal, endosteal, and intracortical resorption cavities were apparent after 10 weeks of restraint; that losses were localized predominantly in the proximal tibia with cortical thinning and endosteal widening; and that bone mineral content in a cross section of tibia decreased by 17 to 21% (Young and Schneider, 1981; Young et al., 1983). In addition, bending stiffness of the tibia was decreased by as much as 40% during restraint. Normal bending properties were restored after about 8.5 months of recovery. However, mineral content of the proximal tibia was not restored in all cases after 15 months of recovery, at which time the cortex was comprised mainly of first generation haversian systems. After 40 months of recovery, normal properties of this compact bone appeared to be restored (Young et al., 1983). Information presented at the ad hoc Working Group meeting indicated that use of electromagnetic coils for muscle stimulation, a technique reported successful for prevention of bone rarefaction in rodents (Geiser and Trueta, 1958) had been ineffective as a countermeasure in the restrained monkey and that compressive loading on the tibia to simulate walking showed a positive effect about 50% of the time.

Striking changes were observed in an integrated series of studies of metabolic, endocrine, and histomorphometric alterations of calcium metabolism and bone in rhesus monkeys immobilized in full-body casts in an upright position for 14 days (Cann and Arnaud, 1981; Kazarian and Collins, 1981; Mathews et al., 1981; Russell and Simmons, 1981; Simmons and Walker, 1981). Bone strength, energy to ultimate load (the capacity of a specimen to absorb or store energy), and bone stiffness of vertebrae were decreased at the end of the casting period, but all showed a further decrease following a recovery period of 14 days (Kazarian and Collins, 1981; Kazarian and von Gierke, 1981).

Study of the histomorphometry of trabecular bone of vertebrae indicated that bone formation surface decreased from 24% of total surface in control animals to 3% in immobilized monkeys (Mathews et al., 1981). Osteoblasts were absent and osteoid surface was greatly reduced; however, mineralization continued. During the recovery period, the bone formation surface increased to 13%. Bone resorption surface was increased dramatically, from about 28% for control animals to 46% for the immobilized monkeys. At the end of the recovery period the resorption surface remained elevated at 36%. The area of bone surface covered by osteoclasts showed a 200 to 300% increase during casting and returned to normal levels by the end of the 14-day recovery period. Cortical bone formation in the ulna virtually stopped during the casting period and resumed during recovery, while resorption spaces did not differ between the control and immobilized monkeys (Mathews et al., 1981). The changes observed in vertebrae and ulna of these monkeys were similar to those in rats flown aboard Cosmos biosatellites. Small changes in bone growth and maturation of mandibular bone were reported (Russell and Simmons, 1981; Simmons and Walker, 1981); however, these changes were different from those in mandibles of rats flown in space.

In a preliminary report of analysis of serum biochemical parameters in the immobilized rhesus monkeys, Cann and Arnaud (1981) reported that total serum calcium decreased progressively throughout immobilization. During the recovery period a large transient increase in serum calcium occurred. Measures of 25-hydroxycholecalciferol and PTH in some of the animals provided tentative evidence for changes in concentrations of these hormones related to changes in serum calcium concentration (Cann and Arnaud, 1981).

## E. COUNTERMEASURES

Approaches to prevent the calcium losses observed during space flight have focused on dietary modifications, physical techniques, and pharmacological intervention. See Whedon (1981) for a discussion of the influence of physical activity and nutrition on maintenance of bone mass.

Dietary modifications first included supplemental oral phosphate (1.3 g/d as the potassium salt). In human subjects the supplemental phosphorus ingestion prevented the hypercalciuria of bed rest but failed to prevent negative calcium balance or loss of os calcis mineral (Hulley et al., 1971). Administration of calcium <u>and</u> phosphate supplements (1.8 g calcium as calcium lactate and 3.0 g phosphorus) tended to reduce the hypercalciuria and urinary excretion of hydroxyproline. During the first 12 weeks of bed rest calcium balance was significantly less negative than that of untreated control subjects, but after 12 weeks, mean calcium balance fell to -100 mg/d (Hantman et al., 1973).

Responses to other dietary modifications have also been examined. Fluoride supplements (10 mg F/d) improved calcium balance in healthy ambulatory subjects; however, the supplement of 10 mg F/d did not prevent calcium losses during bed rest (Maheshwari et al., 1981; 1982). Urinary calcium excretion and calcium balance also varied with protein intakes of 69, 102, and 156 g/d in five ambulatory males. Mean daily protein intakes for astronauts in the Apollo flights ranged from 51 g to 112 g (Rambaut et al., 1975b), while the mean protein intake of the Commander of Skylab 3 was 85 g/d (Whedon et al., 1977). Increasing the protein intake of the five ambulatory subjects to 156 g/d increased urinary calcium losses, while decreasing the protein intake to 69 g/d lowered urinary calcium excretion, resulting in slightly more positive calcium balance. Fractional renal tubular reabsorption of calcium was increased in subjects consuming the diet containing 69 g protein and decreased in subjects consuming the diet containing 156 g protein (Schneider et al., 1981b).

Exercise regimens have been associated with slower bone loss in patients with osteoporosis (Aloia et al., 1978; Krølner et al., 1983) and with increased bone density in athletes (Nilsson and Westlin, 1971). Exercise plans employing a bicycle ergometer, a Cybex Isokinetic Dynamometer, or a treadmill, were included in the Skylab flights (Thornton and Rummel, 1977). While exercise was not generally associated with decreased urinary calcium loss, Whittle (1979) suggested that performance of "toe springs" on the treadmill may have prevented loss of bone mineral from the os calcis in one of the Skylab 4 crewmen. In U.S.S.R. space flights, 1.3 to 2.5 hours of exercise (warm-up exercises, rowing and bungee cord exercises, and treadmill running and bicycling) are provided for the cosmonauts. In some flights these protocols have been associated with decreased calcium losses and it has been stated that changes in bone can be associated with changes in contractile properties of corresponding muscles (Gazenko et al., 1981; Oganov, 1981b).

Various levels of weight-bearing and/or exercise have been tested in order to determine the amount of physical stress on the skeleton required to prevent calcium loss during bed rest. In subjects at bed rest, mineral loss from the os calcis and hypercalciuria persisted during vigorous exercise regimens in the supine position (Hulley et al., 1971; Issekutz et al., 1966). Lower body negative pressure and static and intermittent longitudinal compression in the supine position were also ineffective in improving calcium balance (Hantman et al., 1971; Hulley et al., 1970). Use of a reverse gradient garment to induce venous pooling during bed rest was associated with a greater increase in urinary calcium excretion than bed rest alone (Sandler et al., 1983).

Quiet standing produced less clear results. Issekutz et al. (1966) reported that urinary calcium levels fell slowly to the basal level with 3 h/d of quiet standing with the remainder of the day in bed, but a report of Schneider et al. (1981c) indicated that neither calcaneal mineral density nor calcium balance was protected by this regimen. No further improvement was noted with 20 or 40 minutes of upright exercise on a bicycle ergometer (Issekutz et al., 1966; Schneider et al., 1981c). Impact loading (loads of 25 or 40 pounds to each heel and compression of 80% of body weight to the axial skeleton for 6 or 8 h/d) to create a piezo-electric stimulus did not affect overall calcium loss but did maintain or increase calcaneal mineral density (Schneider et al., 1981d,e). A second type of impact loading procedure (repetitive brisk dropping of the erect body weight from a "tiptoe" position to the heel pads once per second for 15 minutes four times daily) was associated with improved calcium balance (Schneider et al., 1981b).

The increased urinary excretion of calcium was reduced by about 50% by use of a bed oscillating through an arc of 25° once every 2 minutes for 8 hours, a technique that partly reproduces the effect of weight bearing (Whedon et al., 1949). Three hours of random ambulation daily resulted in less negative calcium balance for subjects otherwise at bed rest; however, 4 hours of controlled ambulation daily on a measured course during 6 weeks of bed rest (20 h/d) was considered necessary to completely alleviate negative calcium balance (Schneider et al., 1981c). These results indicate that both exercise and weight bearing are necessary to counteract the bone mineral loss that occurs during prolonged bed rest.

Administration of pharmacological doses of synthetic salmon calcitonin (100 Medical Research Council Units/d intramuscularly) did not prevent negative calcium and phosphorus balances during bed rest (Hantman et al., 1973). However, a combination of synthetic salmon calcitonin, longitudinal compression, and supplementary calcium and phosphate administered to two subjects during 8 weeks of bed rest resulted in maintenance of calcium balance and a smaller increase in hydroxyproline excretion in both subjects, and prevention of calcaneal mineral loss in one of the two subjects (Hantman et al., 1973).

Effects of two disphosphonate compounds, EHDP (disodium ethane-1-hydroxy-1,1-diphosphonate) and clodronate (disodium dichloromethylene diphosphonate), on calcium metabolism have been investigated during bed rest. Diphosphonates are synthetic compounds structurally related to pyrophosphate and have been shown to inhibit both soft tissue calcification and bone resorption in several in vitro and in vivo systems (Fleisch et al., 1969; Francis et al., 1969; Martodam et al., 1983). EHDP is thought to act on two aspects of bone metabolism: to reduce the rate of bone resorption and inhibit bone mineralization. At a dose of 20 mg EHDP/kg/d, calcium and phosphorus balances improved only during the last 8 weeks of a 20-week study involving four healthy men at bed rest. Urinary hydroxyproline excretion and calcaneal mineral losses decreased during the final 3 weeks of the study. However, this dose level was subsequently associated with development of osteomalacia (Bone et al., 1979). A lower dose of EHDP (5 mg/kg/d) had no effect (Lockwood et al., 1975).

Clodronate treatment was a more effective means of decreasing the hypercalciuria observed during bed rest (Schneider, 1981; Schneider and McDonald, 1981). During a 6-week ambulatory preloading period, nine subjects received 1.6 g/d of clodronate while five control subjects received a placebo. During the ambulatory preloading period both urinary calcium and urinary hydroxyproline dropped significantly in the clodronate-treated group but not in the control group. Filtered urinary calcium, renal calcium reabsorption, and serum concentrations of total and ionized calcium did not change in the clodronate-treated group. During the 17-week period of bed rest, hypercalciuria was absent in the clodronate-treated subjects and calcium and phosphorus balances were significantly less negative than in the control group. Seven of the nine subjects receiving clodronate exhibited no loss of calcaneal density while only two of the five placebo-treated subjects maintained calcaneal mineral content during bed rest (Schneider, 1981; Schneider and McDonald, 1981). However, subsequent identification of serious side effects in patients receiving clodronate have contraindicated further use of this drug (Bounameaux et al., 1983).

Material presented at the ad hoc Working Group meeting reported that administration of acetazolamide, a carbonic anhydrase inhibitor, did not inhibit bone loss in rats. Use of carbonic anhydrase inhibitors has been associated with increased formation of renal stones (Parfitt, 1969).

# IV. THE NASA BIOMEDICAL RESEARCH PROGRAM IN BONE DEMINERALIZATION

The NASA Biomedical Research Program in bone demineralization is conducted through in-house research efforts at Ames Research Center, Johnson Space Center, and the Jet Propulsion Laboratory, and through grants to university scientists. The bone demineralization research presently conducted at the three space centers and the universities is ground-based. Work at the Ames Research Center has centered on development of restrained monkeys (*M. nemestrina*) and suspended rats as ground-based models for analysis of bone alterations during weightlessness. Work at the Johnson Space Center has focused on biochemical and endocrine factors associated with bone loss during weightlessness and development of effective countermeasures. The human bed-rest research program on bone demineralization now located at the University of Texas is associated with the research program at Johnson Space Center. Projects housed at the Jet Propulsion Laboratory are related to development of noninvasive measures of bone loss. In addition to these ground-based efforts, several experiments pertaining to bone demineralization are scheduled for upcoming Spacelab flights. See Appendix A for summaries of the 16 in-house and extramural projects relating to bone demineralization supported by the NASA Biomedical Research Program in Fiscal Year 1983, and Appendix B for summaries of inflight experiments planned for Spacelab missions.

The LSRO ad hoc Working Group concluded that, in general, the research projects in bone demineralization sponsored by the NASA Biomedical Research Program fall within the guidelines for areas of research recommended in the reports of the Space Science Board Committee on Space Biology and Medicine (Bricker, 1979) and the NASA Life Sciences Advisory Committee Report of 1978 (Whedon, 1978). The total program is periodically reexamined and reviewed in response to revisions in scientific guidance and research priorities, addition of new research tasks, and deletion of completed projects or efforts of decreased importance. In contrast to the recommendations of one of the earlier Committees which emphasized the endocrine approach for the study of bone loss during space flight (Bricker, 1979), the LSRO ad hoc Working Group considered the study of the pathogenesis of bone changes in man and validated animal models of primary importance to NASA's research program in bone demineralization. The propensity for formation of renal stones during space flight should also be examined and these studies coordinated with studies of bone loss.

The LSRO ad hoc Working Group concluded that the NASA Biomedical Research Program includes areas of research important for study of bone demineralization during space flight. However, they agreed that the program needs improved definition of short- and long-term objectives. While cognizant that their opinions were based in part on abstracted information that might not completely represent all aspects of each project to NASA's mission-oriented research needs, the Working Group recommended that projects be more clearly focused to meet NASA's research objectives. A strong in-house and extramural ground-based program closely coordinated with inflight experiments was considered critical for documentation of bone change and prediction of its consequences and for development of countermeasures to prevent or minimize these losses.

# V. OBSERVATIONS OF THE AD HOC WORKING GROUP ON BONE DEMINERALIZATION

The observations of the ad hoc Working Group centered upon the following topics: (1) pathogenesis of bone demineralization, (2) potential for occurrence of renal stones consequent to prolonged hypercalciuria, (3) development of appropriate ground-based and inflight models to study bone demineralization, (4) integration of research efforts, and (5) development of effective countermeasures. These discussions and suggestions for further research in bone demineralization will aid NASA in planning research programs and stimulate investigators to initiate research directed toward resolution of the problems of bone demineralization during space flight.

## A. PATHOGENESIS

### 1. Assessment

Bone loss during space flight is poorly understood in terms of the processes underlying bone alteration. It was the opinion of the Working Group that the mechanisms are undoubtedly complex and their elucidation will require much more effort. Loss of bone mineral during space flight is indicated by metabolic studies showing negative calcium and phosphorus balances and by noninvasive measures of bone density changes. Such findings indicate a net difference between anabolic and catabolic processes, but provide little information concerning the mechanisms involved under conditions of weightlessness.

Knowledge of the mechanisms of changes in rat bone has been obtained during space flight. As detailed in Section III-D (p. 16), data from rats flown aboard the Cosmos biosatellites indicate that bone formation rates are decreased, but that bone resorption rate is not affected by space flight, suggesting a slowed bone turnover during space flight (Cann and Adachi, 1983; Eurell and Kazarian, 1983; Jee et al., 1983; Wronski and Morey, 1983). Although the bones of rats exhibit continued growth in contrast to the remodeling system of many other species, including man, examination of histomorphometric changes in bones of rats flown aboard the Cosmos biosatellites showed alterations quite similar to those described for bones of monkeys (*M. mulatta*) immobilized for 14 days (See Section III-D, p. 16).

Studies of histomorphometric changes in bone done in two species of monkeys (*M. nemestrina* and *M. mulatta*) were considered by the Working Group as not directly comparable to each other because of differences in techniques of immobilization, differences in lengths of restraint, and differences in behavioral response to immobilization. It remains to be established whether changes during space flight will be similar to changes observed in ground-based experiments in either of these models. Their relevance for study of bone changes in humans also remains to be determined.

Knowledge of primary changes in bone of normal human subjects during bed rest is lacking and the ad hoc Working Group was aware of only one study of quantitative histomorphometry of paralyzed patients (Minaire et al., 1974). The Working Group emphasized that study of bone changes in human subjects during simulated as well as actual weightlessness is essential to gain an understanding of the pathogenesis of bone loss during space flight. Understanding of the mechanisms of bone loss during space flight in human subjects and in an animal model having a bone-remodeling system similar to that of man was considered a most important research goal for the NASA Biomedical Research Program. Knowledge of the types of changes occurring in bone in man is essential for validating any model of immobilization bone loss, for predicting reversibility and consequences of immobilization bone loss, and for developing effective countermeasures.

### 2. Research suggestions

The effects of real and simulated weightlessness on alterations in bone histomorphometry should be investigated in human subjects and relevant animal models. Quantitative histomorphometric analyses to indicate rates of bone formation and bone surfaces involved in resorption should be conducted in normal human volunteers during bed rest and, if possible, during exposure to weightlessness of space flights. If indicated by results of quantitative histomorphometric analyses, studies of bone cultures exposed to weightlessness might subsequently provide a means of identifying specific aberrations in bone cell metabolism.

Types of bone preferentially mobilized should be determined. The site(s) releasing calcium from bone should be identified, i.e., trabecular or cortical bone and specific sites within these types of bone. In addition, the organic matrix should be examined for changes to determine whether existing collagen is undergoing physical change or if osteoblast function is altered, resulting in synthesis of faulty collagen. It was suggested that measurement of urinary excretion of peptides containing hydroxyproline (Krane et al., 1977) or of serum bone GLA protein, (Delmas et al., 1983; Patterson-Allen et al., 1982; Price et al., 1982) might provide a better index of bone remodeling than the total urinary hydroxyproline excretion measurements. Monitoring of the progression of such changes is important in terms of detecting early, subtle, subclinical changes and determining the reversibility of the bone loss.

Exposure to weightlessness may also affect local events in the bone microenvironment of primary importance to skeletal maintenance (Urist et al., 1983). Removal of physical-mechanical stress and changes in muscle action may result in alterations of piezo-electric forces and local factors such as osteoclast activating factor, prostaglandins, and growth factors influencing bone cell function. Circulatory changes and fluid shifts occurring during exposure to weightlessness (Nicogossian and Parker, 1982; Thornton and Hoffler, 1977; Thornton et al., 1977) may also affect bone cell metabolism in a localized fashion (Geiser and Trueta, 1958; Heaney, 1962). It was the opinion of the ad hoc Working Group that the role of such factors must be considered more extensively in future research efforts.

## B. ENDOCRINE EFFECTS

### 1. Assessment

Direct roles of hormones (PTH, 1,25-dihydroxycholecalciferol, and calcitonin) in bone metabolism have long been recognized and mechanisms of these hormones in bone formation and resorption have been the subject of extensive research efforts. The studies of plasma levels of PTH, 25-hydroxycholecalciferol, and calcitonin in Skylab astronauts during space flight did not indicate consistent changes of a magnitude that would ordinarily be associated with increased mobilization of bone (Leach and Rambaut, 1977) and general systemic effects cannot explain the local and preferential demineralization of weight-bearing bones. However, the ad hoc Working Group did not dismiss possible effects of PTH, 1,25-dihydroxycholecalciferol, or calcitonin on bone mobilization during space flight. At the time the hormone levels were measured during previous space flights, assays for a number of hormones were not well refined and it is difficult to draw conclusions from those data.

In light of the findings of decreased plasma levels of 1,25-dihydroxycholecalciferol in immobilized patients (Stewart et al., 1982), analyses of vitamin D metabolites planned for the Spacelab 2 astronauts may provide information concerning the changes in calcium metabolism during space flight. However, the opinion of the Working Group was divided over the value of information obtained by analysis of vitamin D alone.

Development of improved techniques for quantitation of PTH (intact, N-terminal, and C-terminal) (Goltzman et al., 1980; Kao et al., 1982; Parthemore et al., 1978; Roos and Deftos, 1979; Roos et al. 1981; Slovik et al., 1980) may provide a more accurate indication of changes of levels of this hormone during space flights. Since physiologic responses during weightlessness differ from those under gravity, it may be possible that responses of bone cells to normal levels of hormonal agents in weightlessness differ from responses on the ground. This may be particularly evident in bones that lack their normal stimuli.

Observations of unpublished radiographic data on changes in bones of restrained monkeys presented at the ad hoc meeting suggested to some members of the Working Group that the changes were similar to those associated with hyperparathyroidism, senile osteoporosis, and renal osteodystrophy. They concluded that determination of changes in bone was necessary to indicate future directions for study of hormonal changes and that further research at the present time to find a role for calcitropic hormones in bone demineralization during space flight should be coordinated with direct study of bone histomorphometry.

The Working Group considered possible effects of other hormones influencing bone metabolism. Urinary excretion of cortisol in astronauts was elevated during space flight (Leach and Rambaut, 1977) and adrenal glands of rats flown aboard the Cosmos 782 biosatellite were enlarged (Morey and Baylink, 1978). It was the opinion of the Working Group that an elevation in cortisol secretion may be an accessory factor in bone demineralization during exposure to weightlessness. However, it did not seem likely to most members of the Working Group that cortisol is the sole stimulus for the increased bone mobilization observed during space flight. This opinion was based on observations that urinary cortisol excretion was not increased in subjects during bed-rest studies (LaRochelle et al., 1982), yet urinary calcium excretion is still elevated to a similar extent during bed rest as during space flight (Donaldson et al., 1970; Hulley et al., 1971). Roles of other endocrine agents such as thyroid hormone, aldosterone, and prolactin were considered peripheral to the problem of bone demineralization during space flight.

### 2. Research suggestions

Although the ad hoc Working Group recognized the extremely important influence of the endocrine system on bone, they considered that changes in circulating levels of hormones which regulate calcium metabolism probably were not a primary stimulus for changes in bone metabolism during space flight. They suggested further study of hormonal influences in conjunction with study of hypercalciuria and histomorphometric changes in bone of astronauts and subjects at bed rest and in appropriate animal models (See Sections III-D, p. 16; V-C, p. 31; and V-D, p. 33). PTH and cortisol were the endocrine factors noted most likely to influence skeletal calcium mobilization under conditions of real and simulated weightlessness. Quantitative determinations of the circulating levels of PTH and of 24-hour urinary excretion of free cortisol were considered the most sensitive estimates of changes in levels of these hormones.

## C. MODELS

### 1. Assessment

Several models have been used for evaluation of bone demineralization during real or simulated exposure to weightlessness. None has been completely validated and it is possible that the models do not correlate very well with each other or with the changes occurring in humans in space. During actual space flight and its ground-based analog of bed rest, changes in calcium balance, and bone density have been measured in man. No histological information has been published on human bone during space flight or on an animal model having a bone remodeling system similar to that of man.

Changes in bone of rats flown in space have been compared to some extent to a ground-based immobilized rat model and found similar (Morey, 1979). Some bone changes in the rats flown in space are also similar to bone changes in ground-based immobilized monkeys (Mathews et al., 1981). The Working Group recognized that the separation of bone formation and bone resorption sites in rat bone afforded an opportunity to study each process independently. However, differences in the steady-state relationship of $^{45}Ca$ between bone and blood in growing rats and dogs suggest a significant difference in skeletal dynamics for the two species (Klein, 1981). A majority of members questioned the appropriateness of the continually growing rat bone as a model for the bone remodeling system of human adults, but the opinion was also expressed that study of selected aspects of bone metabolism in rats would provide valuable information concerning bone loss during space flight. For example, trabecular bone in the tail vertebrae of rats is remodeled in a manner somewhat similar to that of adult human iliac crest (Vignery and Baron, 1980). It may be appropriate to examine changes in the histomorphometry of the trabecular spongiosa of this bone source.

Nonhuman primates, _M. nemestrina_ and _M. mulatta_, have also been used as models in ground-based studies of bone loss during immobilization. The ad hoc Working Group concluded that there are insufficient comparative data to indicate whether the changes occurring during immobilization are similar for the two species or whether the changes observed in ground-based studies are the same as the changes that may occur during space flight in either species. Because the bone remodeling system in nonhuman primates may be similar to that of the human, the Working Group suggested their continued use as models.

Based on consideration of the strengths and weaknesses of the above mentioned animal models, the ad hoc Working Group concluded that it has not been determined that any of the models used thus far is completely adequate for the investigation of human bone loss during weightlessness. The Working Group considered it essential to know that the bone changes in a particular model approximate changes in human bone during space flight. They recommended that an animal model be systematically developed in a ground-based situation with assurances that the changes occurring in space flight are the same as those occurring in the ground-based counterpart and in man. Mature monkeys, dogs, and miniature pigs were named as the species that have bone-remodeling systems similar to that of man.

After reviewing the ground-based studies of calcium loss in the human bed-rest experiments and the various animal models, the ad hoc Working Group considered that human volunteers at bed rest probably provide the best ground-based model for study of effects of weightlessness on bone changes of astronauts. Calcium balance and bone mineral loss from os calcis are similar in ground-based subjects during bed rest and in astronauts. However, histomorphometric evidence that changes in bone are similar is lacking. Comparisons of hormonal profiles during bed rest and space flight are incomplete but do indicate that levels of cortisol in subjects at bed rest do not increase (LaRochelle et al., 1982), whereas urinary excretion of cortisol was markedly increased in astronauts (Leach and Rambaut, 1977). The Working Group emphasized that while the study of astronauts inflight and human subjects at bed rest is essential, there is little chance of finding an answer to the problem of bone demineralization using studies in man alone because of the inherent nature of experimentation with human subjects.

Mathematical models may have heuristic value for contributing to the understanding of bone demineralization during space flight. Several models have been proposed (Brand, 1983; Leonard et al., 1979; White et al., 1982). The Working Group cautioned against premature use of mathematical models and stressed the importance of generating valid physiological data with bone as the central component for mathematical models.

### 2. Research suggestions

The ad hoc Working Group recommended development and validation of a ground-based model of bone demineralization that shows biochemical, hormonal, and histomorphometric changes that correspond to the changes occurring in that species and in man during space flight. Observation of histomorphometric change of bone in astronauts is essential to this effort to provide a benchmark for comparison and validation of any ground-based or space-flown model. Mature monkeys, dogs, and miniature swine were suggested as animal models having bone remodeling systems similar to that of man. However, the Working Group urged continued caution in extrapolation of data from animal models to man.

Expansion of the studies of human volunteers during bed rest was recommended. These studies should include women and should examine the problem of calcium metabolism, bone loss, and hypercalciuria comprehensively, from histomorphometric, biochemical, and endocrinologic aspects in order to maximize the information obtained. Histomorphometric study of bone changes should be given high priority and related to changes in bone as evaluated by noninvasive methods and to changes in hormonal profiles. Rapid advances in the development of programmed and fully automated instruments will expedite the acquisition of quantitative histomorphometric data. Studies of parameters associated with renal stone development should be conducted in bed-rested subjects and in astronauts.

## D. INTEGRATED RESEARCH APPROACHES

### 1. Assessment

The ad hoc Working Group observed that much of the research on bone demineralization was performed and published in a fragmented manner. Some information has been obtained using various animal models (See Section III-D, p. 16); however, because the models vary considerably from each other (for example, the behavioral responses of the two species of immobilized monkeys and thus probably their endocrine profiles), the Working Group questioned whether information obtained using one model could be extrapolated to other models or to man.

In addition to bone mineral loss from os calcis, muscle deconditioning and loss of muscle mass has been observed, particularly in the lower extremities (Thornton and Rummel, 1977; Whittle, 1979). Some effects of exercise on muscle mass and deconditioning have been described (Leonard et al., 1983; Thornton and Rummel, 1977; Whittle, 1979). However the association between bone loss, muscle loss and deconditioning, and exercise remains to be thoroughly examined. A parallel study by LSRO ("Research Opportunities in Muscle Atrophy," edited by G.J. Herbison and J.M. Talbot) addresses the occurrence of muscle changes during space flight.

Renal stone formation is associated with hypercalciuria, hyperuricosuria, and hypocitraturia (Coe and Favus, 1980; Nicar et al., 1983; Pak, 1981) and with bed rest in convalescent patients (Albright et al., 1941; Leadbetter and Engster, 1945; Tori and Kewalramani, 1978). While there is evidence of hypercalciuria in space flight, there are no reports of urolithiasis in those astronauts participating in metabolic balance studies inflight. Thus, the potential for renal stone formation remains a possibility. Despite the absence of reports, evidence from inflight studies suggests the occurrence of changes in urine composition that may alter the propensity for renal stone formation. Decreases in plasma levels and excretion of uric acid suggest a possible alteration in metabolism of that compound during space flight (Leach and Rambaut, 1975). Because of potassium loss during space flight (Leach and Rambaut, 1975), renal citrate excretion may be impaired (Fourman and Robinson, 1953). Defective citrate excretion was also suggested from studies of normal volunteers during bed rest (Deitrick et al., 1948). Taken together, these observations suggested to members of the Working Group that the tendency for renal stone formation might be affected during space flight.

Useful information concerning the amount of bone mineral lost during space flight and immobilization has been obtained with photon absorptiometry and computed tomography techniques. Each of the methods has specific advantages and limitations (Cohn, 1981; Elsasser and Reeve, 1980; Genant et al., 1983; Mazess, 1983). Immobilization and corticosteroid use have been associated with preferential loss of trabecular bone (Hahn et al., 1974; Minaire et al., 1974). A measurement that provides an indication of the density of trabecular bone itself might prove useful in evaluating bone loss induced by weightlessness; however, according to a recent review (Mazess, 1983), existing computed tomography and photon absorptiometry techniques are not presently sensitive enough for such measurements. The ad hoc Working Group considered it unlikely that continued development and refinement of computed tomography or photon absorptiometry would show either method preferable for all uses. It was the opinion of the Working Group that continued use should be made of these techniques as well as use of other non-invasive methods of calcium measurement such as neutron activation.

2. Research suggestions

For those investigators whose research would lend itself to a broader approach, NASA should encourage projects integrating several areas possibly related to bone demineralization. For example, some studies of bone loss could be expanded to include evaluation of the tendency for renal stone formation and of other changes in body composition, i.e., loss of muscle mass and electrolytes. Neutron activation analysis may be a useful technique for studies of changes in body composition. It was also suggested that coordinated research projects include quantitation of muscle fiber changes from biopsy samples and measurement of glycogen content and activity of enzymes involved in aerobic and anaerobic metabolism in muscle fibers in conjunction with comprehensive studies of bone loss.

Biomedical studies in space flight should include as many analyses as possible. Much more valuable information could be obtained if plasma samples of crew members of Spacelab 2 were analyzed for PTH, corticosteroids, and total and ionized calcium in addition to the vitamin D metabolites. Analyses of plasma and urinary levels of corticosteroids in the crew members of Spacelab 4 would be a worthwhile addition to the more comprehensive metabolic experiments planned for that flight. If compatible with other biomedical experiments utilizing nonhuman primates scheduled for Spacelab 4, direct observation of quantitative histomorphometric changes in bone of these animals could supply detailed information concerning mechanisms of bone loss.

Development of newly emerging techniques for noninvasive measures of skeletal status should be continued and application of existing techniques should be expanded. While it is unlikely that development of these techniques will provide new clues to the etiology or pathogenesis of bone loss, they may be very useful for testing various pharmacologic agents or other countermeasures used to inhibit bone loss.

## E. COUNTERMEASURES

### 1. Assessment

Attempts to prevent bone demineralization during exposure to weightlessness have followed three approaches: manipulation of the dietary intake of nutrients, application of physical stress by various forms of exercise, and pharmacological intervention. Some trials of countermeasures have been conducted during space flight in astronauts and more extensively in ground-based studies of human volunteers during bed rest and in an immobilized monkey model (*M. nemestrina*).

Although fecal calcium excretion is increased during bed rest and space flight, it has not been ascertained whether intestinal calcium absorption is impaired. Calcium balance could not be maintained for more than 3 months by administration of supplemental calcium and phosphorus to human volunteers during bed rest (Hantmann et al., 1973; Schneider, 1981). Based on this work, the ad hoc Working Group agreed that dietary supplementation of calcium and phosphorus was not an effective approach for preventing negative calcium balance during space flight. The effect of calcium supplementation alone with a dietary Ca:P ratio greater than 1:1 has not been assessed. The ad hoc Working Group expressed concern that the hypercalciuria was slightly aggravated and not alleviated by the simultaneous addition of calcium and phosphorus, thereby possibly enhancing the potential for formation of renal stones.

Because renal excretion of calcium varies directly with the level of dietary protein intake (Altchuler, 1982; Schneider, 1981) the role of dietary protein in hypercalciuria requires further study. The ad hoc Working Group suggested that reducing of the dietary protein intake (and thereby probably decreasing the acceptability of the diet) during space flight would not be a practical means of decreasing renal excretion of calcium. They did state, however, that increase of dietary protein was not indicated on the basis of nutritional needs and might be contraindicated by a possible propensity for stone formation. A high protein intake may contribute to renal stone formation by increasing urinary excretion of uric acid and decreasing urinary excretion of citrate (Robertson et al., 1979). In regard to the possibility of increased renal stone formation during space flight, the ad hoc Working Group recommended that high fluid intake of at least 3 l/d be maintained throughout space flight as a possible means of reducing this potential.

Although addition of optional exercise regimens to space flight schedules did not completely prevent negative calcium balance or modify the hypercalciuria in astronauts, it has been suggested that use of the treadmill may have moderated loss of os calcis mineral in one instance (Whittle, 1979). See Section III-E (p. 21) for information concerning exercise routines employed during space flights and bed-rest studies. Walking a prescribed course for 4 hours each day fully prevented the negative balance in ground-based bed-rest studies (Schneider, 1981); and, as a protective measure in weightlessness, some forms of stress on the skeleton equivalent to 4 hours of walking on Earth would have to be devised.

Pharmacologic preparations to prevent loss of bone calcium have been investigated in human volunteers at bed rest and in rats (See Section III-E, p. 21). Based upon their review of the pharmacologic preparations tested, it was the opinion of the ad hoc Working Group that diphosphonate compounds (EHDP and clodronate) have shown the most promise as potential countermeasures. However, as recognized by NASA investigators, serious side effects of clodronate and higher doses of EHDP recognized subsequent to the bed-rest studies of these compounds, indicate that these particular diphosphonates are unacceptable for further use. Again, the Working Group stressed the importance of knowledge of the factors primarily responsible for the bone loss during exposure to weightlessness in identification of a safe and effective countermeasure.

### 2. Research suggestions

Although plasma levels of 1,25-dihydroxycholecalciferol are decreased in immobilized patients (Stewart et al., 1982), it has not been established that intestinal absorption of calcium is impaired in healthy subjects during bed rest (Lockwood et al., 1975). Administration of supplemental calcium and phosphorus, though temporarily maintaining calcium balance at close to zero, did not prevent hypercalciuria during bed rest (Hantman et al., 1973). Because of these findings and because of their concern over the effects of prolonged hypercalciuria, the ad hoc Working Group was reluctant to recommend further extensive investigation of dietary intervention as a countermeasure. However, one consultant suggested that dietary supplementation of calcium alone be considered in any further studies of dietary intervention.

The Working Group considered that study of the changes in bone taking place during an effective exercise regimen (walking for 4 h/d) might provide insight into the mechanisms responsible for changes occurring in bone metabolism during immobilization and reambulation. Such studies may help to identify roles of such factors as muscle pull, mechanical stress, piezo-electric forces, and altered circulation on bone demineralization during actual or simulated weightlessness. In view of effects of previous exercise protocols on calcium loss in the United States and U.S.S.R. space programs, the Working Group considered that exercises placing stress on the weight-bearing bones equivalent in forces to 4 hours of walking on Earth might prove efficacious. Other suggestions of the members of the ad hoc Working Group included more vigorous use of a bicycle ergometer, strenuous jogging or other exercise on a treadmill, or lifting the lower limbs against varying fixed resistance or loads such as those found on a Cybex machine. Use of a Cybex machine, in particular, would allow exercise of individual muscle groups such as the quadriceps and hamstring muscles, an approach that might help to counteract calcium loss during space flight.

Further research on pharmacologic preparations was suggested as a feasible means of identifying an efficacious countermeasure for bone demineralization. Human calcitonin, administered at intermittent intervals to avoid the "escape phenomenon" was considered as a possible approach. Because administration of anabolic steroids was shown to promote nitrogen balance and to have a possible slowing effect on progression of postmenopausal osteoporosis (Chestnut et al., 1977) and disuse osteoporosis (Heaney, 1962), one member of the ad hoc Working Group suggested consideration of use of anabolic steroids as a countermeasure. Investigation of doses of fluoride greater than the 10 mg F/d previously tested may be indicated if studies of bone dynamics show that bone formation is inhibited during exposure to weightlessness. Because two diphosphonate compounds (clodronate and EHDP) were partially effective in reversing calcium loss during bed rest, a third diphosphonate compound, amino propylidene diphosphonate (APD), was considered the most appropriate pharmacologic agent for testing as a means of alleviating bone loss during space flight. Depending on results of safety testing of this compound, the Working Group recommended that studies of countermeasures next address the efficacy of APD during bed rest.

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## VI. PRIORITIES FOR SUGGESTED RESEARCH

The discussions and deliberations of the ad hoc Working Group led to the proposed priorities for suggested research listed in Table 1. The categories of research are interrelated and all were regarded as important for future research consideration. Within the separate categories the suggestions for research are listed in decreasing order of priority.

Table 1. Suggested Priority for NASA Research in Bone Demineralization*

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A. PATHOGENESIS

- Distinguish processes causing primary changes in bone during weightlessness from associated influences such as changes in hormone levels that may result from, rather than cause, changes in bone.

- Analyze and compare quantitative histomorphometry of bones of human subjects exposed to real and simulated weightlessness to determine the gravity-dependent responses of bone itself.

- Study effects of real and simulated weightlessness on histomorphometry of trabecular and cortical bone from different sites (axial and appendicular) in an appropriate animal model in inflight and ground-based studies to determine the type of bone preferentially mobilized.

- Examine collagen at bone sites most severely affected by weightlessness to determine whether existing collagen is modified or if newly-synthesized collagen is defective.

- Continue to study changes in bone density resulting from exposure to zero-gravity. Examine changes at sites in addition to those previously observed to assess change in a more comprehensive manner.

- Extend the length of follow-up studies of bone loss in astronauts and subjects at bed rest to determine if effects of real and simulated weightlessness on bone demineralization are reversible.

- Determine the results of weightlessness on local bone mechanisms: e.g., effects of removal of physical-mechanical stress and piezo-electric stimuli on bone, changes in influences of muscle action on bone, and consequences of altered circulation induced by weightlessness on bone cell metabolism.

- Examine bone cell metabolism by tissue culture techniques to extend the definition of changes occurring during space flight.

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* Listed in suggested decreasing order of priority within each of four separate categories. See Section V, A-D for detail.

Table 1. (cont.)

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B. ENDOCRINE EFFECTS

- Determine endocrine changes simultaneously with bone changes in human subjects and appropriate animal models.
- Determine and compare effects of weightlessness on plasma concentrations and urinary excretion of hormones in astronauts and subjects during bed rest.
- Analyze plasma and urine samples from subjects of ground-based experiments to determine if there is a substance that correlates to loss of bone mineral and could serve as a marker of bone demineralization.

C. MODELS

- Study bone histomorphometry and associated biochemical and endocrine changes of astronauts during space flight to establish a basis for development and validation of a ground-based and space-flown animal model to evaluate bone demineralization, potential for formation of renal stones, and efficacy of countermeasures.
- Expand the studies of subjects during bed rest. Compare the bone changes and biochemical and hormonal alterations in astronauts with those of healthy male and female subjects during bed rest.
- Develop and validate a ground-based animal model exhibiting histomorphometric, biochemical, and hormonal changes that correspond to the changes observed in that species and in man during space flight.
- Develop mathematical models for bone loss based upon observations of changes in bone during space flight in man and in validated animal models.

Table 1. (cont.)

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D. INTEGRATED RESEARCH APPROACHES

- Examine the problem of bone demineralization from histomorphometric, endocrine, and biochemical aspects simultaneously. Integrate study of the potential for renal stone formation into these studies. Correlate bone loss during space flight and bed rest with loss of muscle mass and electrolyes.

- Maximize the amount of information obtained from in-flight experiments. Two suggestions for accomplishing this were increasing the number of analyses of blood and urine samples collected inflight as well as pre- and postflight and, whenever possible, combining studies of bone loss with studies of other physiological changes during space flight.

- Continue to develop and refine newly emerging techniques for noninvasive measures of skeletal status. Expand application of these techniques for evaluation of bone loss.

E. COUNTERMEASURES

- Evaluate the effect of amino propylidene diphosphonate (APD) on bone loss and hypercalciuria during bed rest if safety tests of the drug prove satisfactory. Other drugs that might be considered for testing are fluoride (in larger doses than previously tested), human calcitonin administered on an intermittent schedule, and anabolic steroids.

- Assess in a ground-based program the changes in bone and muscle occurring during an exercise program effective in counteracting calcium loss to help identify the influence of such factors as muscle pull, mechanical stress, piezo-electric stimulation, and altered circulation on bone demineralization.

- Investigate in space flight, protocols for exercise that would place stress on weight-bearing bones equivalent in forces to 4 hours of walking on Earth.

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APPENDIX A

## RESEARCH PROJECTS IN BONE DEMINERALIZATION FUNDED IN FISCAL YEAR 1983 BY THE NASA BIOMEDICAL RESEARCH PROGRAM

### Investigations in Hormonal Control of Calcium and Bone Metabolism (199-20-31-03)

Robert M. Neer (Massachusetts General Hospital)

This investigation seeks to understand the role played by the principal calcium regulatory hormones in the shifts in calcium metabolism observed during space flight. Using more sensitive new techniques, stimulation and suppression tests are being performed to characterize parathyroid hormone and vitamin D responses that occur in normal subjects as a result of immobilization and glucocorticoid administration.

### Determination of Bone Density by Computer Tomography (199-20-31-10)

Harry K. Genant (University of California)

This task aims to develop sensitive computerized tomographic techniques for measuring changes in astronauts' bone density occasioned by space flight. Computerized tomographic techniques for measuring changes in each bone envelope, particularly in the vertebrae, are being developed and will be used to collect baseline data on human subjects. Using specialized software in conjunction with a dual-energy X-ray tomography, cross-sectional data have been collected on numerous subjects of different ages and of both sexes to determine the normal variance in vertebral density, and to determine the changes in density with age. As part of a U.S. - Soviet collaborative effort, analyses have been performed of tomographic data on Soviet cosmonauts.

### Vitamin D- and Vitamin K-Dependent Regulatory Mechanism in Calcium Homeostasis (199-20-31-12)

Nitza M. Cintron-Trevino (NASA Johnson Space Center)

The objective of this task is to understand the mechanisms of action of the vitamin D-derived metabolites and the vitamin K-dependent calcium-binding proteins (CaBPs) of bone and kidney in effecting calcium homeostasis. Major emphasis is being placed on studies of human subjects to determine the relationship between vitamin D status and that of the vitamin K-dependent CaBPs. The status of each vitamin will be controlled by diet. Plasma and urine samples will be analyzed for vitamin D metabolites and vitamin K-dependent CaBPs, among other compounds. Amino acid analyzer methods will be used to determine the levels of urinary CaBP secretion under varying physiological conditions such as bed rest and calcium-associated disorders such as osteoporosis. Baseline levels for 48 normal human subjects have been obtained and are found to agree with those in the literature determined by conventional means.

Clinical Bedrest Research Study (199-20-31-14)

Victor S. Schneider (University of Texas Health Science Center)

Bed-rest simulation of weightlessness is used to study the effect of a hypogravic situation on the skeletal system. One specific objective is a study of the effect on bone loss of "disuse" such as would occur in multiple Space Shuttle trips, leading to a determination of whether 1-g reexposure allows recovery. A second aim is examination of countermeasures including mineral supplements, diet modification, pharmacologic agents, and physical modalities. Changes in calcium absorption and secretion in the gastrointestinal tract during the first two weeks of bed rest and sources of urinary calcium (skeletal or gastrointestinal) are being studied. Human subjects are being subjected to anti-orthostatic (head-down) bed rest to compare changes in calcium metabolism with those seen in horizontal bed rest. Study is being made of the minimim bed-rest period producing skeletal changes, and the recovery time needed between short-term bed-rest periods simulating Shuttle missions. Skeletal system changes will be monitored with an improved computer isotope densitometer which is already in use and a dual beam photo densitometer which is now undergoing testing.

Bone Metabolism and Biomechanics (199-20-32-01)

Donald R. Young (NASA Ames Research Center)

Chronically restrained, nonhuman primates were used to investigate the mechanisms of disuse osteoporosis (increased porosity and fragility of bones). Histologic and radiographic evidence indicated that bone loss occurred in the intracortical and subperiosteal areas of the tibia following prolonged restraint. The reduction of bone mass resulted in a loss of bending rigidity in the tibia. Future research will focus on the role of parathyroid hormone and aldosterone in acid-base status and bone loss; study will also be made of the cellular mechanisms and the time course of recovery associated with disuse osteoporosis.

## Bone Elasticity (199-20-32-02)

Charles R. Steele (Stanford University)

The Steele Oxbridge Bone Stiffness Analyzer developed by NASA was used for rapid, reproducible, and noninvasive mechanical evaluation of bone stiffness. Decrease in bending rigidity of the tibia in the nonhuman primate correlated well with histologic changes. Animal studies are being conducted to establish the relationships between new bone formation and bone stiffness. Human subjects will be used in making noninvasive tests of bone bending stiffness to determine the suitability of such tests to monitor changes associated with fracture healing, senile and disuse osteoporosis, osteoporosis associated with metabolic disorders, and during therapeutic use of dietary fluoride and phosphate.

## Calcium Homeostasis in Altered Gravity (199-20-32-06)

Emily M. Holton (NASA Ames Research Center)

Evidence from prolonged space flight suggested that excessive steroid secretion from the adrenal cortex or increased sensitivity to steroids by target cells such as osteoblasts (OB), may contribute to reduced bone formation. Diverse rodent species exhibited different responses to dexamethasone, a synthetic glucocorticoid, but similar responses to 1,25-dihydroxyvitamin D. Receptor binding of vitamin D accelerated with an increase in growth of rodent OB cultures. Dexamethasone slowed cell division at all growth phases of mouse OB, while the effect on rat OB was dependent on the period of growth. Dexamethasone inhibited OB growth in rats when cells were sparse, had no effect on intermediate densities, and stimulated growth in confluent cultures.

## Pharmacological Prevention of Bone Loss (199-20-32-14)

Alexander D. Kenny (Texas Tech University)

In testing drugs to block bone loss induced by denervation, rats were given carbonic anhydrase inhibitors. Results included a decrease in urinary calcium and no significant effect in blood acid-base balance in denervated rats. Estradiol enhanced acetazolamide's action in the liver of denervated rats. Study will continue to characterize further the interrelationships of acetazolamide, estradiol, sex, and age on carbonic anhydrase activity and bone loss. The ultimate objective is to discover a therapeutic combination of drugs which may be administered at doses which minimize side effects.

Mechanisms of Action of Glucocorticosteroids and Fluoride Ion on the Growing Skeleton (199-20-32-18)

Webster S. Jee (University of Utah School of Medicine)

Effects of glucocorticoids and sodium fluoride (NaF) on skeletal tissue were studied. Preliminary data from doses of NaF given orally to growing rabbits for 15 to 30 days indicated a decrease in bone formation and longitudinal growth; the higher the dose, the greater the loss in body weight compared to controls. Cortisol suppressed, and then stopped completely, the rate of longitudinal growth and formation of the periosteal bone in rats. Studies will be completed of the effects of orally administered NaF on bone formation and resorption in growing rabbits. The cortisol dose-effect study will be completed on the growing rat model and experiments will be performed on the most effective mode of administering NaF to rabbits.

Electron Probe Analysis of Trabecular and Cortical Bone (199-20-32-19)

Theodore L. Bunch (NASA Ames Research Center)

The purpose of this task is to evaluate the microcomposition and crystalline structure of bone so as to define bone resorption relative to mineral distribution and crystallinity. Electron probe analysis has been used to determine in tibial cortical bone of monkeys the distribution of Ca, P, Mg, Na, Cl, F, S, C, N, O, and K in normal bone, during the development of disuse osteoporosis, and again during subsequent recovery. Future efforts will focus on mineral changes occurring in bone. In addition to electron probe analysis, X-ray and electron diffraction studies will be employed to characterize the crystallites of bone in normal subjects and in patients with osteoporosis. Emphasis will be placed on determining whether bone collagen loss is preceded by an initial demineralization of bone.

Biochemical Changes in Bone in a Model of Weightlessness (199-20-32-20)

Gerald Mechanic (University of North Carolina)

The purpose of this task is to investigate the changes in collagen chemistry associated with bone mass loss and loss of crystallites. Using animal models of disuse osteoporosis, bones will be analyzed for collagen crosslinks, collagen turnover, and tissue levels of cAMP. Mineralized versus nonmineralized collagen content will be determined. Skin samples from each animal will be subjected to identical analyses to insure that any effects seen are bone-specific. Both bone resorption and recovery bone formation will be evaluated. Results will be correlated with those from other studies related to alteration of crystallites and of mineral distribution within bone as a result of osteoporosis. The sequence of events will be defined beginning with demineralization and extending through subsequent collagen loss.

Identification of an Unknown Humoral Agent Responsible for Bone Mobilization (199-20-32-21)

Hector F. DeLuca (University of Wisconsin)

The purpose of this investigation is to determine the nature and the identity of as yet unidentified factors involved in bone mobilization. One of the major initial objectives is to determine whether prolactin or some other pituitary hormone is responsible for bone mobilization during pregnancy and lactation and which might be involved in bone mobilization during space flight. Specifically, it is planned to isolate and identify the factors responsible for bone mobilization during lactation in vitamin D-deficient rats, and to determine the quantity of bone loss induced by lactation.

Bone Loss (Tomographic Imaging) (199-20-34-01)

Stanley L. Manatt (Jet Propulsion Laboratory)

This project seeks to understand the various methods for in vivo bone mineral measurements and to establish which of several competing approaches best satisfies the needs of NASA. In addition, ways will be sought to use Jet Propulsion Laboratory (JPL) expertise in image analysis, photoemission and detection, and biochemistry in solving the problems of bone mineral loss and measurement. Two approaches will study computer tomography (CT). One involves development and testing of a dual photon, gamma-ray CT system for bone mineral measurements. The second involves X-ray CT and will assess accuracy and precision of measurements of bone mineral changes associated with various metabolic bone disorders, immobilization, and weightlessness. A third effort is directed toward further improvement of both single and dual photon gamma-ray absorptiometry through in vivo and phantom studies; computer algorithms for improved data analysis are also being developed. Jet Propulsion Laboratory personnel will monitor the three efforts described above and will recommend to NASA the most suitable approach to receive major effort. To aid in evaluating the three approaches, JPL has assembled a small dual photon, gadolinium-153 gamma-ray scanner, capable of operating in both CT and absorptiometry modes. Results from the scanner will be used to prepare standards for analysis by the three systems being compared, to contribute to definition of error models, and to provide data from which images can be generated.

Feasibility of Improved CT System (199-20-34-02)

Douglas Boyd (Jet Propulsion Laboratory)

This task deals with the development of an improved dual photon gamma-ray prototype with gadolinium-153 as the radioactive source. The objective is to demonstrate the accurate measurement of bone mineral density with a prototype device and verify through analysis and independent testing the potential for full-scale engineering development of an operational unit. A breadboard model has been assembled incorporating a 3-Curie, single fan gadolinium-153 source and a cryogenically-cooled, 15-element germanium detector. The breadboard includes data acquisition, electronics for pulse counting and a computer system for data acquisition, reconstruction, and display. Preliminary tests of contrast and resolving power have been conducted on phantoms. Continuing tests with phantoms will be conducted to define prototype system characteristics and determine final specifications for the complete system.

CT Determination of Spinal Bone Mineral Content (199-20-34-03)

Harry K. Genant (University of California School of Medicine)

The objective of this task is to develop X-ray Computer Tomography (CT) methods for accurate and reproducible measurement of vertebral trabecular mineral content. These methods will be used to measure mineral content and mineral changes in various metabolic bone disorders including immobilization and other osteoporoses. In addition, normative data and data from fracture populations will be obtained for use in predicting risk of vertebral fracture. A dual-energy CT method has been developed for accurate measurement of spinal mineral content, and phantom validation of the method has been accomplished. A joint U.S. - U.S.S.R. effort was initiated to use CT to measure bone loss in cosmonauts. This effort will continue, and the CT method will be used in bed-rest studies to be conducted in collaboration with U.S. scientists. Normative single energy CT data from 350 male and female control subjects have been obtained to define the normal age-related changes in vertebral mineral content. Normative dual energy data will be acquired on control subjects to compare with the single energy results obtained to date. Vertebral autopsy specimens will be scanned using single and dual energy CT methods and the mineral content so obtained will be compared with that resulting from chemical analysis.

Measurement of Trabecular Bone (199-20-34-04)

Richard B. Mazess (University of Wisconsin)

This task aims to decrease the precision errors of single-(iodine-125) and dual-photon (gadolinium-153) absorptiometry to provide more sensitive measurements of compact and trabecular bone mass. Sensitivity will be increased by decreasing the source-detector distance and by increasing the detector collimation, thereby increasing count rate by a factor of 10 and decreasing precision error from 3% to 1%. Design work has been performed on a fast amplifier, one with a one-microsecond pulse and a high slew rate. The system will now be constructed and tested, with particular attention being given to corrections for deadtime, scatter, and geometric factors. It is also planned to investigate the most effective manner to scan vertebral specimens laterally. By lateral scanning the centrum can be measured without the influence of the anterior processes, which may be slower to change than the centrum.

# APPENDIX B

## INFLIGHT EXPERIMENTS PLANNED FOR SPACELAB MISSIONS

### Vitamin D Metabolites and Bone Demineralization

Heinrich K. Schnoes, Hector F. DeLuca (University of Wisconsin)

Quantitative measurement of the blood levels of biologically active vitamin D metabolites of flight crew members of the Spacelab 2 mission may indicate whether the well known derangements of calcium metabolism experienced in long-term missions reflect themselves in any way in a modulation of vitamin D metabolism to its various metabolites. Since the biosynthesis of vitamin D metabolites is subject to regulation by several physiological parameters, monitoring these metabolites under normal and zero-gravity conditions may yield information about the molecular aspects of the homeostatic mechanism in weightlessness. Metabolite levels will be determined by established methods such as competitive protein binding, high pressure liquid chromatography and gas liquid chromatography, as well as by newly developed techniques.

### Pathophysiology of Mineral Loss During Spaceflight

Claude D. Arnaud, Christopher E. Cann (University of California)

The purpose of these experiments is to evaluate changes in calcium homeostasis occurring during space flight in Spacelab 4 crew members. To determine hormonal effects, the circulating levels of calcitropic hormones, parathyroid hormone (PTH), calcitonin, and the active vitamin D metabolites (25-hydroxycholecalciferol, 1,25-dihydroxycholecalciferol, and 24,25-dihydroxycholecalciferol) will be determined using sensitive assay systems. PTH determination will include analysis of the various circulating forms of this hormone (intact, N-terminal, C-terminal) in an attempt to characterize any flight-induced changes in its secretion and metabolism. Serum ionized calcium will also be determined. Direct measurement of intestinal calcium absorption will be done with the dual-isotope technique, using stable calcium isotopes. This will determine changes in intestinal handling of calcium and identify the source of the increased fecal calcium seen in the Skylab experiments. These changes will be correlated with measurments of 1,25-dihydroxycholecalciferol to determine if there are primary defects in the kidney or intestine. Analysis of the calcium tracer curves will also provide information on rates of total body calcium turnover and, by inference, information about early changes in bone turnover.

## Bone, Calcium, and Space Flight

Emily Morey-Holton (NASA Ames Research Center), Christopher E. Cann (University of California)

Data from rats flown aboard Cosmos biosatellites suggest that diaphyseal bone formation ceases sometime after the eleventh day of flight; thus, bone measurements should be made in the initial flight phase to determine what happens to bone turnover during this critical period. The first objective of the proposed experiment will allow more precise calculation of the length of flight time required to significantly inhibit bone formation in both juvenile and adult rats. Tetracycline labeling of bone, both continuous and pulse labels, will be one technique used to determine bone turnover. Continuous tetracycline labeling is necessary to quantify endosteal bone formation and resorption; intermittent or pulse labels can be used to assess periosteal formation as no resorption occurs at this surface in the bone shaft in rats. Using semi-quantitative measurements such as medullary area and endosteal osteoclast nuclei counts, no changes in bone resorption resulting from exposure to weightlessness have been found to date. Continuous tetracycline labeling, a quantitative technique, should provide definitive answers as to whether any increase in diaphyseal bone resorption does occur. A second objective of this proposal is thus to determine total skeletal formation and resorption and to measure excretion and intestinal absorption of calcium. $^{47}Ca$ will be used for the kinetic studies to be done on days 6-8 of the flight period while 24 hour urine and fecal samples collected during the entire flight will be used to calculate calcium balance. These metabolic studies are expected to show a significant shift toward negative calcium balance, primarily through increased fecal excretion. Kinetic analysis will define the mechanisms behind this loss of calcium. Ground-based information will be obtained using a hypodynamic rat model system developed in this laboratory.