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PART I

A Study of the Role of Extravascular
Dehydration in the Production of
Cardiovascular Deconditioning by
Simulated Weightlessness (Bedrest)

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A. Introduction

For many years the occurrence of orthostatic intolerance following bedrest or weightlessness has been the subject of extensive investigation (1-16). The implications of these studies have been the basis for several review articles (17-23).

A number of potential reasons for post-recumbency or post-weightlessness orthostatism have been considered. These include: the development of autonomic insufficiency; plasma volume depletion; and diminished venous compliance with greater blood pooling in the lower extremities.

In prior studies we have demonstrated an intact neurovascular system following bedrest by: greater orthostatic increases in heart rate; an equal or greater increase in peripheral vascular resistance; unchanged responsiveness to tyramine, a catecholamine releaser; and by an equal or augmented sympathetic response to Valsalva maneuver (1). These studies also failed to show a hemodynamically significant decrease in plasma volume following bedrest. Similarly, Miller did not find good correlation between plasma volume decrease and the occurrence of vasodepressor syncope following bedrest (6). Although our previous studies provided no data on the question of increased venous pooling following bedrest we stated: "Although this cannot be totally dismissed, it has been shown that venous tone is increased minimally and transiently by the stimulus of gravity" (24-26). "Thus, there was little protective effect even before bedrest. Since the hydrostatic column is no greater after bedrest than before, it would be unlikely that its effect would be more pronounced after bedrest" (1).

In our prior studies we demonstrated a net water deficit of 2-3 liters during fourteen days of absolute bedrest. This occurred with a minimal decrease in plasma volume during bedrest. On this basis we postulated that the fluid loss had occurred from extravascular compartments. If this occurred, tissue pressure would be diminished during post-recumbency tilting. Thus, there would be less resistance to capillary filtration with a large and progressive transudation of plasma water into the tissues of the lower extremities during the post-recumbency tilt. The progressive diminution of vascular filling pressure would result in a falling venous return to the right heart. Central blood volume and stroke volume would decrease progressively. Initially, an increase in sympathetic tone would maintain blood pressure as nearly optimal as possible. At later stages the increase in peripheral resistance would tend to further impede blood flow and venous return. Eventually a critical lower limit of central vascular filling would be reached signaling the imminent occurrence of failure of blood flow to the low pressure baroreceptors. The latter would attempt to maintain blood flow by decreasing sympathetic tone. The resultant increase in vascular capacity would result in a dramatic drop in blood pressure while the relative vagotonia would cause the marked bradycardia or sinus arrest seen in severe cases of post-recumbency orthostatism.

From their classic bedrest study of immobilized subjects, Dietrick, Whedon and Shorr suggested that there was a greater loss of fluid into the extravascular compartment in their subjects during post-recumbency tilting (4). Lamb also suggested that redistribution

of fluid volume may play a role in cardiovascular deconditioning (20). A similar speculation was made by Vogt (11). He subsequently demonstrated large changes in extracellular fluid volume (^{35}S) and total body water ($^3\text{H}_2\text{O}$) during ten days of bedrest (28).

The present study was designed to define the alterations in body fluid compartments that occur during four weeks of weightlessness as simulated by absolute bedrest.

B. Experimental Design

1. General

Healthy male volunteers, ages 21 - 29, served as subjects for the present study. All volunteers were obtained from the Federal Correctional Institution, Lompoc, California by permission of the U.S. Bureau of Prisons. All volunteers were screened by the Medical Staff of F.C.I.-Lompoc to exclude major or chronic health defects. Also, potential volunteers were subjected to a 70° passive tilt for twenty minutes to exclude the presence of autonomic insufficiency. A tilt table with English saddle was provided to the F.C.I. staff for this purpose.

On completion of the screening procedure, the volunteer was transported to the U.S. Public Health Service Hospital, San Francisco, California. On arrival the volunteers were admitted to the Metabolic Unit where a complete medical history was taken and a physical examination was performed. A twelve-lead electrocardiogram was done as well as a chest x-ray. A flat film of the abdomen was taken to exclude radiopaque renal stones. Routine laboratory evaluation included C.B.C., urinalysis, serology, fasting blood sugar and blood

urea nitrogen, S.G.O.T. and alkaline phosphatase.

Each subject was placed on a metabolic diet for seven days prior to entering the study and was maintained on this diet throughout the study. The dietetic aspects of the study are covered under B-4, Metabolic Dietetic Program. During the week of equilibration, the volunteers were instructed in urine and stool collection and in intake recording by the metabolic nurses.

The details of the study were explained to each subject verbally and in writing and each subject signed an informed consent permit.

The study consisted of three phases, a fourteen day ambulant control phase, a twenty-eight day bedrest phase, and a fourteen day ambulant recovery phase. During the ambulatory phases the environmental temperatures were maintained as near constant as possible and no exercise except walking was permitted. These measures were necessary in order to avoid large differences in insensible loss between the ambulant and bedrest phases. While at bedrest the horizontal position was required at all times with unrestricted movement in this axis. Arm movement was limited to forearm raising with elbows on the bed. One pillow was permitted for head support. All excretory activities and meal consumption were carried out in this position. Excessive boredom was avoided by the use of reading material, games, radio and television.

Initial total body radiation dose calculation permitted performance of eleven multiple isotope volume studies on the subjects. However, after the initial two subjects it became apparent that

performance of the studies at such close intervals would require dose augmentation beyond permissible limits. Adjustments were made in frequency on Subjects 3 and 4 and it was determined that a maximum of seven studies could be performed. Thereafter, only minor adjustments in scheduling were made. These were necessitated by random and uncontrollable events and were deemed to have no significance to the over-all study. An example would be the shift of a study by one day on non-critical days, i.e., not during bedrest onset or termination.

2. Detailed Protocol .

The protocol to be presented is the final protocol on which all analyses are based.

a) Daily Procedures

- 1) Temperature, pulse and blood pressure recordings, b.i.d.
- 2) Determination of body weight on a metabolic balance immediately after completion of 7:30 AM urine collection.
- 3) Determination of intake and output.

b) Urine Collection and Analyses

- 1) Twenty-four hour collections were made on Days:
C 1-6; C 9,10; C 13,14; B 1-6; B 13,14; B 27,28;
R 1-8; R 13,14. Forty-eight hour collects were made on all other study days.

Each voided sample was placed in a gallon container without preservative. The urinal was rinsed with 50 ml of distilled water. This aliquot was also poured into the container. The sum of rinsing volumes was deducted from the total twenty-four hour volume to determine urine output. Total volume was utilized in calculating chemical data. Pooling of two day samples was done by combining 20%

volume aliquots of the two samples into a single sample. (For example: If Day 1 urine volume was 2000 cc, a 20% aliquot or 400 cc was removed; if Day 2 urine volume was 2500 cc, a 20% aliquot or 500 cc was removed; the 400 and 500 cc aliquots were combined to give a representative 48 hour sample.) All urines were refrigerated until sample aliquots were obtained. The latter were frozen.

2) Urine specimens were analyzed as follows:

- a. All samples were analyzed for Na^+ , K^+ , Cl^- , Ca^{++} , nitrogen and creatinine.
- b. Aldosterone excretion was determined on the following days: C2; C 7-8; C13; C14; B1; B3; B 7-8; B 15-16; B 21-22; B27; R1; R2; R6; R 11-12.
- c. Osmolality was determined on days: C1; C9; B2; B 14; B 28; R7; R 14.

c). Stool Collection and Analysis

Stools were collected in three day pools, stored in an epoxy lined paint can. Stool weight was determined by subtraction of can weight. A known weight of distilled water was added to the fecal material. This mixture was homogenized by a paint can shaker. An aliquot of the homogenate was frozen for subsequent analysis.

Stools were prepared for calcium analysis as follows: 2 ml. of fuming nitric acid were added to a known weight of fecal homogenate (approximately two grams). This was heated until digestion was complete. The digestate was then diluted to 100 ml. for analysis.

d) Blood Chemistry and Hematology

Blood samples were drawn on Days C1, C9, B2, B 14, B 28,

R7, R 14. These were analyzed as follows:

1) All samples were analyzed for Na^+ , K^+ , Cl^- , Ca^{++} , osmolality and creatinine.

2) Red blood count, hemoglobin, hematocrit, white blood count and red blood cell indices were performed on all study days on Subjects 1-12. This study was then discontinued as there appeared to be no change which was not related to phlebotomies.

3) Serum Haptoglobin and Hemoglobin - Initially this study was planned on all samples. Since the analyses were not available in our laboratory, arrangements were made to have them performed by Bioscience Laboratories. After many futile attempts to obtain reliable results, the analysis was abandoned.

4) Serum proteins were determined before and after tilt on Day C 14 and R1 on Subjects 7, 9-18, and 23-24.

e) Isotopic Volume Studies

1) Plasma Volume as determined by ^{125}I -RISA, Extracellular Fluid Volume as determined by ^{82}Br mine, Red Cell Mass as determined by ^{51}Cr mium and Total Body Water as determined by $^3\text{H}_2\text{O}$ were measured on Days C1, C9, B2, B 14, B 28, R7, and R 14.

2) Pre- and post-tilt ^{125}I -RISA plasma volume was determined on Days C14 and R1 on Subjects 7, 9-24.

f) 70° Tilt and Exercise Studies

These studies were performed on Days C 14, R1, R3, R8 and R 15.

g) Pulmonary Function Studies

Performed during Control Week 2 and on Recovery Day 1.

3. Methodology

a) Biochemical Methods - All studies were performed in duplicate.

1) Sodium and Potassium were determined on serum, urine and diet by standard techniques using an Instrumentation Laboratories flame photometer, Model 143.

2) Calcium was determined on serum, urine, diet and feces by means of a Perkin-Elmer atomic absorption spectrophotometer by methods previously described (1).

3) Chloride was determined on serum and urine by means of a Buchler-Cotlove Chloridometer.

4) Creatinine was determined on serum and urine by methods previously described (1).

5) Total urinary nitrogen was determined utilizing methods perviously described (29).

6) Serum proteins were determined by previously described methods (1).

7) Aldosterone excretions were determined by contract with Bioscience Laboratories, Van Nuys, California.

8) Serum and urine osmolality were determined using an Advanced Instruments Osmometer.

b) Isotopic Methods

1) Multiple Isotopic Volume Studies

a. General. The performance of multiple isotope studies on repeated occasions requires that the doses utilized be maintained below a total body radiation dosage of 100 mr/week. In the present investigation it was necessary to perform the studies

at close intervals. Due to the half-lives of three of the four isotopes (^{125}I - 57.4 days, ^{51}Cr - 26.5 days, ^3H - 12.26 years, ^{82}Br - 35.9 hrs.) there was a relatively high residual count at the time of performance of each study beyond the first. Therefore, in order to obtain a sample to residual ratio for each isotope of no less than 2 to 1, it was necessary to augment the dose of ^{125}I , ^{51}Cr , and ^3H with each study. The total dose of each isotope was calculated to give a gross sample count rate of at least 10,000/20 min. counting period. The combination of short study intervals, isotope half-lives, need for good counting statistics and consideration of patient risk resulted in an inability to perform more than seven studies on each subject during an eight week period.

In the preparation of patient doses, sterile technique was maintained at all times. The amount of isotope contained in dose syringe was determined gravimetrically by weighing on a Mettler balance to the nearest 0.05 mgm. Approximately 10% of each dose was expelled into a 100 ml. volumetric flask for standard preparation. Syringes were then re-weighed to determine standard and pre-injection syringe weights. Prior to injection of the isotopes, a blood sample was taken for determination of residual isotope concentration. An indwelling needle was securely placed in a forearm vein of the fasting basal patient and attached to a bottle of 5% D/W. All isotopes were injected through the injection port of the I.V. tubing, the syringe plunger being held firmly to prevent any backwash of fluid from the tubing which would alter the post-injection syringe weight. The system was flushed after delivery of each isotope and

100 ml of 5% D/W was allowed to run in after delivery of the final isotope. Syringes were re-weighed to determine the weight of dose delivered. Heparinized samples were then drawn at hourly intervals for six hours. Subjects were allowed to eat and resume normal fluid consumption one hour after isotope injection. After preparation (see individual isotopes) all samples were counted on a combination Tracerlab Gamma Guard II-Corumatic system. Each sample was counted three times for twenty minutes. The mean of the three values was used in calculations. The total dose of each isotope was determined as follows:

$$\text{Dose} = \frac{\text{Net CPM/ml Standard} \times \text{Dilution Standard (ml)} \times \text{Weight Dose}}{\text{Weight Standard}}$$

b. ^{125}I -RISA Plasma Volume Determination

The initial dose utilized was 2 μc . This was increased by 1 μc increments as subject's residual count increased. The required dose was made up from ^{125}I -RISA stock in a sterile 10 vial to which 0.1 ml. of sterile human albumin was added. The latter was necessary in order to prevent adsorption of the ^{125}I -RISA by glass. The volume was adjusted to allow 2.2 ml. of the dose to be drawn into the injection syringe. One ml. of 25% albumin was also added to the standard flask (see above) for the same reason.

Plasma ^{125}I -RISA levels show a significant decrease over short time periods, and Hoyer has shown multiple sampling and extrapolation of the ^{125}I disappearance curve to T_0 to be the most accurate means of determining plasma volume (30). During the initial phases of the present study, samples were drawn at 10, 20,

30 and 60 minute intervals after injection, as well as at 1, 2, 4 and 6 hour intervals. This allowed a comparison of plasma volumes obtained from a semilog arithmetic regression line derived from the 10, 20, 30 and 60 minute counts with those obtained from regression of the 1, 2, 4 and 6 hour counts. The results of this analysis are shown in Part I, Table A. It is apparent that there is no significant difference between the plasma volume results obtained by the two sampling techniques ($P < .3$). However, the mean correlation coefficient for the regression lines obtained with the long sampling interval was $-.962$ as contrasted to a value of $-.352$ for the short sampling interval. Because plasma volumes were unchanged and the correlation coefficients for the regression lines were much better with longer sampling intervals, sample collection was changed to hourly sampling for the initial six hours after injection. This not only allowed a better portrayal of the ^{125}I -RISA disappearance curve, but also allowed a reduction in sampling frequency and volume. All plasma samples were held for 5-7 days before counting to allow time for decay of ^{82}Br .

It has been observed in this laboratory that external factors such as tourniquet application alter ^{125}I -RISA count levels. This appears to be due to a local concentration change resulting from transudation of plasma water and it correlates closely with changes in hematocrit. This has necessitated the use of a correction factor based on hematocrit. In any series of samples the mean or predominant hematocrit is accepted as being the true hematocrit. Net CPM/ml plasma for each sample are then

corrected by the formula:

$$\text{Corrected CPM} = \frac{\text{Net CPM/ml} \times \text{Mean Hct.}}{\text{Sample Hct.}}$$

After further correction of plasma counts by subtraction of the pre-injection residual count level the hourly counts are regressed semi-logarithmically to the time of injection (T_0). Plasma volume is then calculated by the formula:

$$\text{Plasma Volume} = \frac{\text{Total Dose CPM}}{\text{CPM/ml Plasma } (T_0)}$$

⁵¹Chromium Red Cell Mass (Volume) Determination

The dose utilized on the first study was 8 μ c. This was increased to 31 μ c by the final study. Total dosage was 115 μ c for the seven studies. In the preparation of each dose, 9 ml. of the subject's blood was drawn with a sterile heparinized syringe and injected into a sterile plastic tube capped with a sleeved stopper. The required dose was injected into the tube and the tube was rotated gently for 45 minutes at room temperature. After centrifugation, the plasma was removed and discarded. The cells were then washed three times with 5 ml. sterile saline to remove all ⁵¹Cr which was not bound to red cells. The cells were then re-suspended in saline and withdrawn into a sterile syringe for injection. Calculation of delivered dose and preparation of standards was as noted above. Following an early equilibration ⁵¹Cr count levels remained quite stable over the six hours of study. Therefore, only the pre-injection and first four hourly samples were prepared for ⁵¹Cr counting. Five ml. of whole blood from each

sample were pipetted into glass counting tubes calibrated at 3 ml. The tubes were centrifuged and the plasma removed. Cells were washed three times with 5 ml. normal saline to remove ^{82}Br . The volume of the red cells was then adjusted to the 3 ml. calibration mark. The red cells were then lysed with saponin. Following counting, all results are corrected for decay to the time of injection. The sample counts represent net CPM/ml of whole blood. These are divided by the hematocrit (corrected for trapped plasma) to convert them to net CPM/ml of red cells. The Red Cell Mass is then calculated for each of the first four hourly samples by the formula:

$$\text{Red Cell Mass} = \frac{\text{Total dose CPM}}{\text{Net CPM/ml red cells sample} - \text{Net CPM/ml red cells residual}}$$

The four values were then averaged to determine Red Cell Mass.

d. $^{82}\text{Bromine}$ Extracellular Fluid Volume Determination

The dose utilized for all studies was 20 μc . The distribution of radioactive bromine is similar to that of chloride (31). It is also known to be distributed to areas not considered part of the extracellular compartment, such as red cells and gastric fluid. In addition, it is excreted by the kidneys. Prior studies have differed in their findings regarding red cell uptake. Staffurth and Birchall found uptake to be approximately 5% of the ^{82}Br dose (32). However, Nicholson and Zilva found an uptake of only 2% after 24 hours (33). The question of red cell uptake was investigated in the course of our studies in order to insure valid data. In our experience, red cell uptake reached equilibrium soon after intravenous injection and

remained relatively stable over the six hour sampling period. However, as might be anticipated, the percentage of dose taken up by the red cells varies with red cell mass. When mean red cell uptake, expressed as percentage of ^{82}Br , dose was related to mean red cell mass in ten subjects, the correlation coefficient was found to be 0.948. The mean red cell uptake of ^{82}Br for 63 determinations of extracellular fluid volume on nine subjects was 4.4% of the total dose with a range of 3.2 - 5.77. Since red cell mass consistently shows a decrease with the multiple phlebotomies performed in this study, it has been necessary in all studies to count both whole blood and plasma and correct the dose for the amount of ^{82}Br lost to red cells.

Many authors feel that corrections for red cell uptake and urine loss of ^{82}Br compensate for changes in the observed extracellular space which are reflected by changes in plasma levels of ^{82}Br after equilibration of the dose (32-34). They use a single determination or the mean of several determinations as their value for extracellular space. However, it is our experience that serial determinations of extracellular volume at 2, 4 and 6 hour intervals, with corrections for both red cell uptake and urine loss, show a consistent increase in the observed space. This is shown in Part I, Table B. These findings indicate a progressive loss of ^{82}Br from the extracellular compartment which is not accounted for by these corrections. Since this loss is consistently seen in all cases, it has been corrected for by semi-logarithmic regression of the observed values for extracellular fluid volume to T_0 . As urine

loss of ^{82}Br is also a continuing process; it was reasoned that this loss should also be accounted for by the regression curve of serial extracellular fluid volume determinations. A comparison of values obtained with and without correction for urine loss was made. The results are shown in Part I, Table C. The values shown with correction were derived from three sampling times, the latest at six hours. The values without correction were determined on the basis of five sampling times, the latest at six hours. It is apparent that no difference in the two methods exists. Therefore, collection of urine was discontinued and samples were drawn hourly over the six hour period to give a more complete description of plasma ^{82}Br disappearance.

Howe and Ekins have shown an ^{82}Br loss of 20% into the gastric fluid after 12 hours (35). However, Nicholson and Zilva found only 0.3% of the dose to be lost in feces over a three day period (33). Thus, most of the ^{82}Br secreted with gastric fluid must be reabsorbed into the general pool. Since this represents a continuous process, it should also be accounted for by regression of the plasma disappearance curve.

Calculation of extracellular fluid volume was made in the following manner. The total dose of ^{82}Br delivered was calculated as previously described. The effective dose was then calculated by correcting for RBC loss as follows:

$$\text{RBC Loss} = \frac{\text{Red Cell Mass} \left[\text{CPM/ml Whole Blood} - \text{CPM/ml Plasma} \times (1 - \text{corrHct}) \right]}{\text{Corr. Hct.}^*}$$

* Correction for trapped plasma.

Extracellular fluid volume for each sampling time was then calculated by the formula:

$$\text{Extracellular Fluid Volume} = \frac{\text{Effective } ^{86}\text{Br Dose (CPM)}}{\text{Net Plasma CPM/ml}} \times .9^*$$

* Factor to correct for Gibbs-Donnan equilibrium.

The natural log of the volume at each of the six sampling intervals was obtained. Using linear regression, the log of the volume at T_0 was determined. The extracellular fluid volume at T_0 is the antilog of this number.

e. ^3H Total Body Water Determination

The dose of ^3H utilized on the first study was 700 μc . The total dose was increased by 50 μc increments with each study. It had been noted previously that plasma $^3\text{H}_2\text{O}$ concentration shows a progressive decline after injection and that regression to T_0 is necessary to obtain accurate results (36). In our laboratory the decrease in plasma concentration from the extrapolated value at T_0 to the final sample at six hours varied from 1.1% to 9.9% with a mean decrease of 4.7%. Therefore, the results from the six hourly samples have been used to determine the concentration at T_0 in the present study. This type of analysis corrects for continued distribution and equilibration as well as urine loss. The results of studies using correction for urine loss and using linear regression without urine loss correction are shown in Part I, Table D. It is apparent that results are virtually identical and correlation coefficients are improved when linear regression is utilized without urine correction. Therefore, urine loss correction

was eliminated in the present study. In preparation of the samples and standards for counting, the special lyophilization manifold described by Moss was used (37). Lyophilization is preferable to protein precipitants in the elimination of interfering plasma constituents and other isotopes. This is true because the protein precipitants are strong quenchers. This makes liquid scintillation counting difficult and perhaps less accurate due to lower count rates. In the present study all samples and standards were lyophilized in duplicate. Precaution was taken to avoid contamination of the lyophilized samples with atmospheric moisture.

During the course of study it was noted that repeat analyses of stored samples gave total body water results which were at great variance from initial results. This problem was approached from several viewpoints. The reason for the change was related to the method of plasma storage. It had been our practice to retain the plasma used for gamma counting for any future need. This plasma was frozen in the plastic tube in which it had been counted. The tube was capped with laboratory sealing film. To evaluate the question of loss of radioactivity during storage, known amounts of tritium were added to plasma and frozen in plastic tubes and compared with matching samples frozen in glass tubes. After three weeks of storage the samples were lyophilized and counted. Recoveries from the glass-stored samples ranged from 101.4% to 103.9% while those from the plastic-stored samples ranged from 46.9% to 89.3%. The mechanism of this loss of ^3H is not known. Since $^3\text{H}_2\text{O}$ is fully in equilibrium with H_2O in the samples, any water loss would not change

the concentration of ^3H in plasma water. The results suggest an exchange of ^3H with H^+ from the walls of the plastic tube. After discovery of this problem, all residual plasma was frozen in glass tubes.

Following lyophilization, 0.1 ml. of each sample and standard was placed in a liquid scintillation counting vial with 1 ml. Amersham-Searle NCS solubilizer. After approximately one hour 10 ml. of scintillation fluid (PPO-POPOP in toluene) was added to the vial and the sample was counted. Total body water was calculated by the formula:

$$\text{T.B.W.} = \frac{\text{Total Dose Count}}{\text{Net CPM/ml Plasma H}_2\text{O at T}_0}$$

2) Pre- and Post-Tilt ^{125}I -RISA Plasma Volume Determination

This study was performed in an attempt to define the volume of fluid transudated from the plasma compartment during the 70° tilt. Prior to the pre-bedrest tilt and the first post-bedrest tilt, ^{125}I -RISA was injected and plasma samples were obtained at ten minute intervals for a period of thirty minutes. Pre-tilt plasma volume was calculated as described above (C-2-a-2) by extrapolation of the three plasma results to T_0 .

The patient was then placed in the 70° tilt. Immediately on tilt down (T_d) and at 5 and 10 minutes after T_d additional plasma samples were obtained. Predicted post-tilt plasma CPM/ml were calculated by extrapolating the pre-tilt plasma ^{125}I -RISA disappearance curve to T_d (Pre CPM T_d). The post-tilt

plasma disappearance curve was also extrapolated to T_d to obtain the actual plasma CPM/ml at T_d (Post CPM T_d). Post tilt plasma volume was then calculated as follows:

$$\text{Post-Tilt Plasma Volume} = \text{Pre-Tilt Plasma Volume} \times \frac{\text{Pre CPM } T_d}{\text{Post CPM } T_d}$$

c) 70° Tilt Study

On all study days the subjects were brought to the laboratory in a fasting basal state. All measurements were made by non-invasive techniques and were recorded on an oscilloscopic recorder.* EKG was measured by a modified Lead I utilizing three thoracic leads. Heart rate was measured beat-by-beat by a tachometer triggered by the R wave of the QRS complex. Blood pressure was measured by a standard manual sphygmomanometer. Lower limb volume was measured by a single strand mercury-in-rubber strain gauge. This gauge was calibrated at zero impedance with a 20 gram weight. The device was then placed 10 cm. below the right tibial tubercle and tightened to the zero point. Oxygen consumption was measured in duplicate by previously described techniques prior to tilting (1). Basal heart rate and blood pressure were obtained on at least two occasions prior to tilt. Following baseline measurements the subject was placed in a 70° foot down tilt using a tilt table equipped with an English saddle. The tilt was maintained for 20 minutes or until syncope or pre-syncope (symptomatic hypotension) occurred. During the tilt changes in heart rate and limb volume were recorded continuously. Blood pressure was measured every thirty seconds. Following termination of the tilt, these measurements were continued

* Electronics for Medicine

for five minutes with an additional spot check at ten minutes.

d) Exercise Study

This was performed following recovery from the tilt study. Instrumentation was the same except for discontinuation of limb volume measurements. Exercise was performed in the supine position utilizing a bicycle ergometer* at a pedaling rate of 50 RPM. For Subjects 1-17 a standard protocol was used. The subject pedaled for six minutes each at work levels of 50 watts, 75 watts and 100 watts. Rest was allowed between each work load. Blood pressure and heart rate were measured at three and six minutes of each level. Oxygen consumption was measured in the steady state between the fourth and sixth minute of exercise. On completion of the 100 watt exercise period, heart rate recovery was monitored continuously for five minutes with a spot check at ten minutes. The only variation in Subjects 18-24 consisted of a modification in work loads. The subjects warmed up for two minutes at 50 watts and then exercised for six minute periods without rest at work loads starting at 100 watts, increasing by 25 watt increments and terminating with exhaustion.

e) Pulmonary Function Study

This consisted of measurement of vital capacity (VC), one second forced expiratory volume (FEV-1), residual volume (RV), total lung capacity (TLC) and carbon monoxide diffusion capacity (DCO). RV and TLC were done by the helium dilution method. DCO was performed by the single breath method. All techniques were standard (38).

*. Lanooy Ergometer

4. Metabolic Dietetic Program

a) Formula Balance Diet

Subjects 1-4 were fed a diet which consisted primarily of a formula prepared in the Metabolic Kitchen. Two basic diets were used, a 2000 calorie diet and a 2500 calorie diet, with the hope that each subject would maintain his weight within a kilogram for the duration of the study. Both diets were calculated according to the following prescription: 80 grams protein, 40% of the total calories as fat, 3% of the total calories as linoleic acid, 1 gram calcium, 115 milliequivalents of sodium, and a starch-sugar ratio of 1::3. Each subject was interviewed upon admission by the Research Dietitian who decided which of the two caloric levels to use on the basis of the individual's height, weight, and body build. In addition to the diet, all subjects received two hexavitamin pills daily.

Three of the subjects remained on the diet for 63 days, the first 7 days of which served as an equilibration period. The fourth subject, volunteer number 3, remained on the diet for an extra week. They were instructed to eat all food served to them, to rinse containers afterward with distilled water, and to report any spillage so that it could be replaced.

The details of the constituents of the formula, method of preparation, sample menus, and calculated composition of these diets has been described previously (1).

b) Solid Food Balance Diet

The remaining volunteers who participated in this study were fed a balance diet consisting mainly of solid foods. The

conversion from the formula diet to solid foods was made when a new Metabolic Kitchen with complete cooking and dishwashing facilities was opened.

The principal aspects of this type of metabolic food service were as follows:

1. Subjects were on a balance diet for an average of 63 days, which included 7-day equilibration period.¹

2. Each subject selected one menu of his own choice, consisting of three meals and an evening snack, from the foods available. (Part I, Table E)

3. The Research Dietitian then calculated the nutrient content of the chosen menu, adjusting the weights of foods so that the diet was isocaloric and contained 162-163 milliequivalents of sodium.² Those diets which were inadequate in Vitamin C were supplemented with one 50 mg. tablet of Vitamin C per day, dispensed by the nursing staff. No hexavitamins were used.

4. A daily weighed amount of reagent grade sodium chloride was given in labeled individual salt shakers. The subjects had to use all of their salt by the end of the dinner meal.

5. A maximum of three individual pepper packets per day were given and were not calculated into the diet.

¹During the latter part of this study, subjects were allowed to make minor changes in their menus on the second day of the equilibration period after having tried the menu once. Thereafter, no changes in the diet were made unless the subject was losing too much weight.

²It should be noted that there was a calculated difference of 47-48 milliequivalents of sodium between the formula diets and the solid food diets. The reason for this is that a solid food balance diet with only 115 milliequivalents of sodium would have been too unpalatable, since it would have necessitated the use of low sodium foods.

6. No fresh foods were used. Vegetables and fruits were canned, milk was canned or powdered, and eggs and meat frozen.

7. Foods for each subject's study were derived from common lots to insure as much constancy in mineral content as possible.

8. Foods were weighed on Mettler top-loading balance scales with an accuracy to 0.1 gram.

9. Distilled water was used for cooking.

10. The subjects were expected to eat all food served to them. They were instructed to clean their dishes thoroughly and to rinse glassware with distilled water. (Part I, Table F)

11. Foods that were spilled or dropped on the floor were replaced.

12. Four diet aliquots per subject were sent to the laboratory for mineral analysis.

A sample menu with the total calculated nutrient composition follows. When it was necessary to increase the calories because of weight loss, Kool-Aid was added to the diet which did not change the mineral content appreciably.

SAMPLE MENU FOR A SOLID FOOD BALANCE DIET

<u>Grams</u>	<u>Food</u>		<u>Menu</u>
150	Orange juice	<u>Breakfast</u>	Orange juice
25	Wheaties		Wheaties
80	Eggs		French Toast
161	Bread, white		Butter
25	Butter, salted		Jelly
10	Jelly		Milk
21	Sugar		Coffee
540	Milk, whole		Sugar
2.5	Coffee, instant		
120	Rosé wine	<u>Lunch</u>	Rosé wine
150	Beef tenderloin		Beef tenderloin - broiled
20	Instant potatoes (dry wt.)		Mashed potatoes
100	Peas, canned		Peas
15	Jello (dry wt.)		Bread
150	Ground beef		Butter
100	New potatoes, canned		Jello
100	Green beans, canned		Milk
160	Baked custard (home recipe)		
360	Coca Cola	<u>Dinner</u>	Ground beef patty - broiled
60	Tuna, canned dietetic		New potatoes
25	Mayonnaise		Green beans
1.8	Sodium chloride		Bread
			Butter
			Baked custard
			Milk
		<u>Evening</u>	Tuna Sandwich
		<u>Snack</u>	Coca Cola

Salt - 1.8 grams/day

CALCULATED NUTRIENT COMPOSITION OF THE

SAMPLE MENU

<u>Nutrient</u>	<u>Unit</u>	<u>Amount</u>
Calories	----	2932
Protein	grams	144.7
Nitrogen	gms.	23.15
Fat	gms.	116.3
Carbohydrate	gms.	306.0
Alcohol	gms.	11.8
Sodium	mgs.	3744.0
	mEq.	162.78
Potassium	mgs.	4099.0
	mEq.	105.10
Calcium	mgs.	1106.0
	mEq.	55.18
Phosphorus	mgs.	2005.0
Magnesium	mgs.	342.0
	mEq.	28.12
Iron	mgs.	23.4
Vitamin A	IU	4704.0
Thiamine	mgs.	1.43
Riboflavin	mgs.	2.63
Niacin	mgs.	33.1
Ascorbic Acid	mgs.	103.0
Cholesterol	mgs.	1147.0

DIET INFORMATION ON NASA VOLUNTEERS STUDIED FROM AUGUST, 1967 TO JUNE, 1970

<u>Volunteer</u>	<u>Diet</u>	<u>Dates on Diet</u>	<u>Weight Status While on Diet</u>	<u>Notes</u>
1	2000 cal. Formula 2500 cal. Formula	8/15 thru 8/24/67 8/25 thru 10/16/67	Gained 0.8 kg.	Calories increased because of wt. loss. Suspect cheating on diet.
2	2000 cal. Formula	8/15 thru 10/16/67	Lost 0.5 kg.	Very uncooperative. Suspect cheating on diet.
3	2500 cal. Formula	9/26 thru 12/4/67	Gained 2.1 kg.	Extra week on balance diet.
4	2500 cal. Formula	10/31/67 thru 1/1/68	Lost 2.1 kg.	
5	2000 cal., constant sodium, SOLID FOOD	1/11 thru 3/11/68	Lost 0.2 kg.	Diet error -2/27 Received Amphojel.
6	2000 cal., constant sodium	1/11 thru 3/11/68	Lost 1.1 kg.	Diet errors- 1/26, 2/10, 2/20, 2/21.
7	2000 cal., constant sodium	3/27 thru 5/28/68	Gained 0.2 kg.	Diet error - 4/8.
8	2600 cal., constant sodium	3/27 thru 5/28/68	No change	Diet errors- 4/8, 5/5.
9	2300 cal., const. Na 2400 cal., const. Na	6/6 thru 6/18/68 6/19 thru 8/6/68	Lost 2.9 kg.	Calories increased because of wt. loss. Diet error- 6/21.

* Start of solid food balance diets.

DIET INFORMATION ON NASA VOLUNTEERS STUDIED FROM AUGUST, 1967 TO JUNE, 1970 (cont.)

<u>Volunteer</u>	<u>Diet</u>	<u>Dates on Diet</u>	<u>Weight Status While on Diet</u>	<u>Notes</u>
10	2800 cal., constant sodium	6/6 thru 8/6/68	Gained 1.6 kg.	
11	2400 cal., constant sodium	8/14 thru 10/15/68	Lost 0.8 kg.	
12	2200 cal., const. Na 2465 cal., const. Na	8/14 thru 8/19/68 8/20 thru 10/15/68	Lost 0.2 kg.	Calories increased because of wt. loss. Diet errors- 9/13, 10/14.
13	2500 cal., constant sodium	1/22 thru 3/25/69	Lost 0.9 kg.	Diet errors- 2/25, 2/26.
14	2350 cal., const. Na 2590 cal., const. Na	1/22 thru 2/3/69 2/4 thru 3/25/69	Lost 2 kg.	Calories increased because of wt. loss.
15	2500 cal., constant sodium	4/16/ thru 6/20/69	No change	Diet errors- 6/1, 6/2, 6/3, 6/4. Suspect cheating on diet. Did not complete study.
16	2500 cal., constant sodium	4/16 thru 6/24/69	Lost 2.9 kg.	Diet errors- 5/13, 6/1, 6/2, 6/3, 6/4, 6/8, 6/16.
17	2100 cal., constant sodium	7/16 thru 9/16/69	Gained 0.6 kg.	
18	2500 cal., constant sodium	7/16 thru 9/16/69	Lost 1.5 kg.	
19	2255 cal., const. Na 2365 cal., const. Na 2635 cal., const. Na	10/8 thru 10/13/69 10/14 thru 10/28/69 10/29 thru 12/10/69	Lost 0.9 kg.	

DIET INFORMATION ON NASA VOLUNTEERS STUDIED FROM AUGUST, 1967 TO JUNE, 1970 (cont.)

<u>Volunteer</u>	<u>Diet</u>	<u>Dates on Diet</u>	<u>Weight Status While on Diet</u>	<u>Notes</u>
20	2635 cal., constant sodium	10/8 thru 12/10/69	No change	
21	2570 cal., constant sodium	11/6/69 thru 1/6/70	Gained 0.7 kg.	
22	2750 cal., constant sodium	11/6/69 thru 1/6/70	Lost 3.4 kg.	
23	2600 cal., constant sodium	1/15 thru 3/17/70	Lost 1 kg.	Suspect cheating on diet.
24	2930 cal., constant sodium	4/1 thru 8/19/70	Gained 0.8 kg.	

CALCULATED TOTALS OF PERTINENT NUTRIENTS IN BALANCE DIETS OF VOLUNTEERS

STUDIED FROM AUGUST, 1967 TO JUNE, 1970

Volunteer	Cal.	Grams				mg.	Na		mg.	K		mg.	Ca		mg.	Mg	
		PRO	FAT	CHO	ALC *		mEq.			mEq.			mEq.			mEq.	
1	8/15-8/24																
	1996	78.9	88.8	220.2		2640	114.78		2598	66.61		1039	51.84		242	19.90	
	8/25-10/16																
	2497	79.4	110.9	295.2		2646	115.04		2811	72.07		1052	52.49		261	21.46	
2	1996	78.9	88.8	220.2		2642	114.86		2680	68.71		1043	52.04		254	20.88	
3	2500	79.4	110.9	296.0		2642	114.86		2693	69.05		1043	52.04		238	19.57	
4	2497	79.4	110.9	295.2		2646	115.04		2811	72.07		1052	52.49		261	21.46	
5	2024	82.7	67.8	270.7		3726	162.00		3280	84.10		955	47.65		331	27.22	
6	1999	100.5	52.2	281.7		3752	163.13		3569	91.51		933	46.56		270	22.20	
7	2029	102.0	54.7	282.1		3734	162.34		3336	85.53		1012	50.49		248	20.39	
8	2604	104.8	104.9	310.1		3751	163.08		3471	89.00		1067	53.24		287	23.60	
9	6/6-6/18																
	2290	109.0	90.2	260.5		3728	162.08		3006	77.07		908	45.31		241	19.82	
	6/19-8/6																
	2401	109.0	90.5	287.7		3737	162.48		3007	77.10		914	45.61		241	19.82	
10	2806	104.2	108.8	352.5		3745	162.82		3584	91.89		1027	51.25		290	23.85	
11	2386	115.5	69.9	323.8		3744	162.78		3735	95.77		1077	53.74		329	27.06	

* Alc = Alcohol

** Start of solid food balance diets.

CALCULATED TOTALS OF PERTINENT NUTRIENTS IN BALANCE DIETS OF VOLUNTEERS

STUDIED FROM AUGUST, 1967 TO JUNE, 1970

<u>Volunteer</u>	<u>Cal.</u>	Grams				<u>Na</u>	<u>K</u>	<u>Ca</u>	<u>Mg</u>	<u>Fe</u>	<u>Mn</u>	<u>Zn</u>
		<u>PRO</u>	<u>FAT</u>	<u>CHO</u>	<u>ALC.</u>							
						mg.	mg.	mg.	mg.	mg.	mg.	mg.
12	<u>8/14-8/19</u>											
	2225	94.4	81.0	279.7		3722	161.83	2892	74.15	1165	58.13	247
	<u>8/20-10/15</u>											
	2465	94.4	81.0	339.6		3725	161.95	2896	74.25	1166	58.18	247
13	2556	88.0	69.1	395.2		3738	162.52	3336	85.53	639	31.88	266
14	<u>1/22-2/3</u>											
	2349	86.1	92.7	292.6		3746	162.86	2774	71.12	1064	53.09	272
	<u>2/4-3/25</u>											
	2589	86.1	92.7	352.6		3746	162.86	2779	71.25	1064	53.09	272
15	2493	81.8	104.4	306.6		3748	162.95	2493	63.92	696	34.73	221
16	2544	126.1	103.6	276.7		3734	162.34	3611	92.58	878	43.81	259
17	2138	114.0	67.8	268.0		3725	161.95	3827	98.12	1067	53.24	313
18	2510	118.8	99.4	285.1		3728	162.08	3532	90.56	781	38.97	266
19	2634	109.2	89.1	348.9		3748	162.95	3358	85.97	945	47.15	268
20	2637	124.9	116.0	273.4		3726	162.00	3201	82.07	989	49.35	254
21	2571	104.3	92.4	309.8	11.8	3749	163.00	3313	84.94	869	43.36	249

* ALC. = Alcohol

CALCULATED TOTALS OF PERTINENT NUTRIENTS IN BALANCE DIETS OF VOLUNTEERS

STUDIED FROM AUGUST, 1967 TO JUNE, 1970

<u>Volunteer</u>	<u>Grams</u>												
	<u>Cal.</u>	<u>PRO</u>	<u>FAT</u>	<u>CHO</u>	<u>ALC.*</u>	<u>Na</u> mg.	<u>Na</u> mEq.	<u>K</u> mg.	<u>K</u> mEq.	<u>Ca</u> mg.	<u>Ca</u> mEq.	<u>Mg</u> mg.	<u>Mg</u> mEq.
22	2757	103.2	85.3	373.5	11.8	3736	162.43	2719	69.71	863	43.06	227	18.66
23	2614	117.0	108.3	271.9	11.8	3740	162.60	3291	84.38	963	48.05	292	24.09
24	2932	144.7	116.3	306.0	11.8	3744	162.78	4099	105.10	1106	55.18	342	28.12

* ALC. = Alcohol

SOURCES FOR DIET CALCULATIONS

1. Composition of Foods, Raw, Processed, Prepared. Agricultural Handbook No. 8, U. S. Department of Agriculture, Wash., D.C., 1963.
2. Davidson, C.S., P.A. Clifcorn, G.J. Gabuzda, and C. Robinson, "Sodium-Restricted Diets, The Rationale, Complications, and Practical Aspects of Their Use", Publ. 325, National Academy of Sciences - National Research Council, Wash., D.C., 1954.
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4. McCance, R.A. and E.M. Widdowson, The Composition of Foods, Medical Research Council Special Report Series No. 297, London: Her Majesty's Stationery Office, 1960.
5. "Nutritive Composition of Products", Campbell Soup Co., 1969.
6. "Nutritive Values and Ingredients of Beech-Nut Baby Foods", Beech-Nut Baby Foods, Beech-Nut Life Savers, Inc., New York, 1964.
7. Information on the nutritive value of products from a number of food companies.

C. Data Analysis

1. General

Variation of the conditions to which a single subject and his responses are exposed represents an ideal experimental situation as it allows evaluation of the effect of the change in condition in the same person. This evaluation is not so subject to individual variation as would be the case if one population were to be studied under one set of conditions and a second under another.

In the present study a single subject was evaluated in three states: control, bedrest, and recovery. Thus, in the analysis of data, comparison could be made by comparing each subject's data under one circumstance with his data under a different circumstance. In all calculations of significance, a paired "t" test was utilized (39). Significant change had occurred if $P < 0.05$ by two-tailed distribution. If paired data was not present, then neither point in a subject's data was used in that particular comparison. In most cases group means have been calculated using only that data which was complete for the entire study. Where a standard error is expressed, this is universally true. All standard errors represent 95% confidence limits whether so stated or not. The use of data from only those subjects with no missing data points in the calculation of mean results does not preclude including specific portions of their data in significance calculations. For example, if Control Day 1 and Bedrest Day 1 data were present and Bedrest Day 7 data was missing, then none of the data was used in calculation of the mean. However, in calculation of the P

value, that subject's data was included in the Control Day vs. Bedrest Day 1 comparison.

In some situations it was important to show a group trend with relation to time and without regard for totality of data. An example is the tilt heart rate data. We have previously shown the significance of the change in paired data (1). However, this excludes the subject who has syncope before 20 minutes, the very subject of interest in this study. Therefore, in the present study, all data was included in heart rate mean change. However, no standard error or P value is expressed. Other results were so obviously erratic (e.g., limb volume change) or unchanged as to preclude detailed statistical analysis.

2. Specific Data Analysis

a) Response to 70° Tilt

Results prior to tilting and at 5, 10, 15 and 20 minutes (or terminal, if tilt-down occurred earlier) and at 1, 5 and 10 minutes of recovery were considered as representative of the entire tilt period. In the case of heart rate the mean of all results was determined without further statistical analysis. Pulse pressure was determined by subtraction of diastolic from systolic pressure and the mean for the group determined for each time interval. No further statistical analysis was attempted. Review of each individual's limb volume data for all tilts yielded the impression that the methodology used is totally unreliable as a means of comparing day to day change. The change from day to day was totally random. Therefore, no attempt was made to perform statistical analysis on this data.

b) Response to Exercise

Complete statistical analysis was made on heart rate response to and recovery from exercise, oxygen consumption and minute ventilation. Ventilatory equivalent results were calculated as previously described (1) and analyzed statistically. Maximal oxygen uptake was determined from submaximal work data. The result was based on extrapolation to a heart rate of 180/minute. This technique probably underestimates the true maximal oxygen uptake (40). However, since an individual's own data is used for comparison, it undoubtedly represents a true index of change. Maximal oxygen uptake values were corrected further for weight. Both the uncorrected and corrected data were analyzed statistically.

c) Pulmonary Function

All paired results were analyzed statistically.

d) Urine Excretion and Balance

In the analysis of data, each subject's daily results during a given week for sodium, potassium, chloride, calcium and nitrogen were used to determine each subject's mean daily excretion for the week of study under consideration. Statistical comparisons were made between the Control Weeks and the Bedrest and Recovery Weeks, Bedrest Week 4 and the Recovery Weeks, Bedrest Day 1 and Day 2 with Control Week 2 and Bedrest Week 1, and Recovery Day 1 and Day 2 with Bedrest Week 4 and Recovery Week 1.

Calcium balance was calculated using the mean result of dietary analyses as the daily calcium intake. Mean daily urinary and fecal excretion were utilized as daily calcium output.

Results were analyzed statistically.

Mean daily aldosterone excretion was determined on the basis of results available during each week of study. In the analysis of results, the Control Weeks were compared with the Bedrest and Recovery Weeks and with Control Day 14 and Bedrest Day 1. Recovery Day 1 was compared with Bedrest Week 4, Recovery Week 1 and Control Day 14. Bedrest Day 1 was compared with Bedrest Week 1.

Fluid balance was calculated from intake-output data. Analysis was based on the mean daily balance for each week of study and was similar to that for sodium balance.

Osmotic and free water clearance were calculated by standard techniques (41).

e) Isotopic Body Fluid Compartment Data

The data of several subjects was excluded from the statistical analysis for a variety of reasons. These included the absence of some data point(s) as stated previously. Also, the frequency of studies early in the investigation led to questionable counting statistics in some cases. Further, modifications in technique also precluded use of certain of the early studies.

In the statistical analysis those subjects not used for the following determinations were:

Plasma Volume - Subjects 1-6, 15, 23, 24

Red Cell Mass - Subjects 1-4, 7, 8, 15, 23, 24

Extracellular Fluid Volume - Subjects 1-6, 13-15, 23, 24

Total Body Water - Subjects 1-4, 8, 15, 23, 24

In the analysis, the control mean result was compared with Bedrest Day 2, 14 and 28 and with Recovery Day 7 and 14. Recovery Day 7 and 14 were also compared with Bedrest Day 2, 14 and 28.

f) Tilt Plasma Volume and Protein Alterations

The mean pre- and post-tilt plasma volume was determined for the pre- and post-bedrest tilt. The change during tilt and the difference in change between pre-bedrest and post-bedrest results was analyzed statistically. Although mean and standard error were calculated for pre- and post-tilt proteins on the two study days, P values were not calculated as results were virtually identical.

D. Results

1. General

The tables and figures referred to in this section are all contained in Part II of this report.

Table 1 contains the vital statistics and smoking habits of the study population. In spite of their incarcerated state, most of the subjects were in relatively good physical trim. This was true because of the physical recreation program available to them at Lompoc. However, none were trained athletes and no attempt was made to train them prior to the study. The great majority were smokers and no attempt was made to alter this during the course of the study. The subjects were generally cooperative. However, one subject (Subject 15) deserted prior to completing the Recovery Phase. No major health problems arose during the course of the investigation.

2. Response to 70° Tilt

Five of the twenty-four subjects experienced syncope or pre-syncope during the first post-recumbency tilt. Subject 5 had a severe and sudden syncopal episode with a short period of sinus arrest without ventricular activity. This resulted in mild convulsive activity. This subject experienced a milder pre-syncopal episode on Day R15 after successfully tolerating the study on Day R 3 and R 8.

Table 2 shows the individual tilt tolerance time (Terminal Time). If not otherwise indicated, this was 20 minutes. This table gives the heart rate response to tilt and recovery for the individual subjects on each study day. The terminal heart rate was that existent at 20 minutes or at the time of tilt termination. The average heart

rates at the selected times in each tilt are shown in Table 3. Heart rate was higher at all tilt and recovery times during the first post-recumbency tilt. The response had returned to pre-bedrest levels by 48 hours after re-ambulation (Day R 3).

Individual blood pressure data is depicted in Table 4. It should be noted that a number of subjects developed asymptomatic hypotension during tilt. The pulse pressures derived from this data are shown in Table 5 and the group means in Table 6. There was no distinct difference in pulse pressure response after bedrest nor was syncope predictable by pulse pressure alteration. In many cases pulse pressure fell to lower levels in non-syncope subjects than was the case in subjects with syncope.

Changes in limb volume are illustrated in Table 7. This was a very disappointing aspect of the study. Results followed no pattern that was discernible, and the differences seen in an individual's tilt response appeared to be due either to the basic methodology or to random volume changes unrelated to experimental conditions. No attempt was made to approach this data statistically because of its great variability.

3. Response To Exercise

Table 8 contains the individual heart rate response to exercise and recovery. The group means for heart rate response to exercise are presented in Table 9. As is apparent in Table 10, heart rate was significantly higher on the first day of recovery at all levels of exercise. This remained true at the 75 watt and 100 watt levels even 48 hours after resumption of ambulation. Heart rate

response to 100 watt exercise did not return to pre-bedrest levels until some time between seven and fourteen days of recovery. Group means for heart rate recovery from 100 watt exercise are compiled in Table 11. Heart rate was significantly higher at all time intervals immediately following bedrest. Recovery appeared to have returned to normal by 48 hours of re-ambulation.

Individual values for oxygen consumption at rest and during exercise are shown in Table 13 and group means are presented in Table 14. Corresponding minute ventilation data are contained in Tables 15 and 16. Although there was no significant difference in oxygen consumption during post-recumbency exercise, minute ventilation was significantly increased during the first post-recumbency study. The results of this comparison are compiled in Table 17. In an attempt to show this difference more exactly, ventilatory equivalent was calculated for each exercise level. The individual values are shown in Table 18 and the group means in Table 19. Contrary to expectation this calculation tended to mask the increase in minute ventilation. Although mean values for ventilatory equivalent were higher immediately after bedrest, the differences were not significant.

Maximal oxygen uptake values derived from submaximal exercise responses are shown for each subject in Table 20. Results obtained by correction of this data for body weight are shown in Table 21. In both analyses maximal oxygen uptake was significantly less even after one week of re-ambulation. The change was no longer significant after two weeks of re-ambulation.

4. Pulmonary Function

Table 22 shows results of pre- and immediate post-bedrest evaluation of pulmonary function. Vital capacity (VC) was significantly increased by approximately 200 ml after bedrest. Only three of seventeen subjects failed to follow the trend. One second forced expiratory volume (FEV-1) was also significantly increased with eleven of seventeen subjects following the trend. Total lung capacity (TLC) was also increased significantly by approximately 200 ml. Six of nine subjects showed this pattern. Residual volume (RV) was unaltered as was diffusion capacity (DCO).

5. Urine Excretion Studies

The results of individual daily analyses for creatinine, sodium, potassium, chloride and calcium in urine are tabulated in Table 23. Individual data on daily nitrogen excretion are presented in Table 24. Tables 25-29 contain the average daily excretion of sodium, potassium, chloride, calcium and nitrogen by week of study for each subject. Mean values for each week of study are also depicted. As shown by Table 30 excretion of all of the aforementioned was increased throughout bedrest. In the case of sodium and chloride the increased excretion was maximal during the first week of bedrest. Excretion of calcium, potassium and nitrogen increased later during bedrest. However, all except potassium were increased significantly above pre-bedrest levels on the first day of bedrest. Potassium excretion still had not increased significantly by the second day of bedrest. Excretion of sodium and chloride were particularly striking during the first day of bedrest. The excretion of

186.5 $\pm$ 17.6 meq of sodium and 176.6 $\pm$ 17.7 meq of chloride were significantly higher than the daily averages for the first week of bedrest.

Following resumption of ambulation sodium and chloride excretion fell significantly below even pre-recumbency levels for the initial week. This was particularly true during the initial 48 hours. The excretion of sodium during the initial 24 hours averaged 75.9 $\pm$ 13.8 meq. That of chloride averaged 85.1 $\pm$ 14.4 meq. Potassium excretion during the recovery weeks was insignificantly decreased from pre-bedrest levels. Calcium excretion remained slightly, but significantly, above pre-bedrest levels for the initial week of re-ambulation; but fell significantly below these levels during the second week. Nitrogen excretion returned to pre-bedrest levels on the first day of recovery and this remained true during the initial week. However, nitrogen excretion fell to levels which were significantly less than pre-bedrest levels during the second week of recovery.

Individual results for aldosterone excretion are depicted in Table 34. Average daily excretion by week of study for each subject and group means are shown in Table 35. The significant changes in aldosterone excretion occurred on Day 1 of bedrest when levels fell significantly to 10.1 $\pm$ 1.6 μ gm/24 hours and on Day 1 of recovery when levels rose significantly to 17.8 $\pm$ 1.6 μ gm/24 hrs. Interestingly, Day 1 of recovery was a tilt study day and levels significantly exceeded those on Day 14 of the Control Phase, also a tilt study day. On the other hand, the mean level of 11.9 $\pm$ 1.7

$\mu\text{gm}/24 \text{ hrs}$ on Day 14C was not significantly different from Control Week 2. Excretion remained at significantly higher levels throughout the first week of recovery. Statistical results are shown in Table 36.

Table 37 shows the day to day fluid balance for each subject and Table 38 contains the average daily fluid balance by week of study for each subject as well as the group means. Fluid balance became distinctly less positive on Day 1 of Bedrest and remained so throughout bedrest. During recovery there was a significant retention of water above pre-bedrest levels for the initial week. This was quite prominent on the first two days of recovery, the levels significantly exceeding the mean daily balance for the first week. Statistical analysis is shown in Table 39.

The overall results for fluid balances and sodium and aldosterone excretion are depicted in Figure 1.

6. Calcium Balance

As noted above, average daily calcium excretion by week of study is contained in Table 28. The results of analysis of diets for electrolytes including calcium are contained in Table 31. The average calcium content of the diets analyzed was used in the calculation of calcium balance. The average daily fecal excretion of calcium by week of study is compiled in Table 32. There was no significant increase in fecal excretion until the fourth week of bedrest. This change was still evident during the second week of recovery although the trend was toward pre-bedrest levels. Individual and mean calcium balance data was calculated from urinary and fecal

excretion and dietary intake for each week of study. These results are shown in Table 33. Calcium balance became significantly negative during the first week of bedrest and remained so through the first week of re-ambulation. The post-recumbency negative balance was largely due to the continued high fecal output of calcium.

7. Body Fluid Compartments

Each subject's results for plasma volume, red cell mass (volume), extracellular fluid volume, total body water and weight are tabulated in Table 40. The group means for those complete studies considered valid are contained in Table 41. Statistical analyses are shown in Table 42. In the determination of mean weight, the weight of all subjects used for calculation of any mean volume were included. Plasma volume decreased significantly by approximately 200 - 250 ml. during bedrest, while extracellular fluid volume diminished significantly by approximately 500 ml. Whereas the decrease in plasma and extracellular fluid volume appeared to occur quite early, the decrease in total body water appeared to be a slower process, averaging approximately 1500 ml. at the end of bedrest, the only mean change of significance. Red cell mass decreased progressively and significantly during bedrest, tending to level off after bedrest, but remaining approximately 150 ml. lower than pre-bedrest levels. Following bedrest, plasma volume rose to a level approximately 150 ml. above pre-bedrest levels by seven days, and this change remained significant even after two weeks of recovery. Results from study of Subjects 23 and 24 shown in Table 40 suggest that plasma volume is partially restored to pre-bedrest levels by

24 hours after re-ambulation. Extracellular fluid volume and total body water returned to pre-bedrest levels. Weight remained relatively constant throughout bedrest, the only significant decrease occurring on the last day of bedrest.

The above results are presented graphically in Figure 2. An additional feature of this figure is the correction of red cell mass by the volume of red cells removed by phlebotomy to each point in the study. The corrected curve appears to rise prior to bedrest, level off during bedrest and again rise after bedrest, suggesting diminished red cell production in response to phlebotomy during bedrest.

8. Pre- and Post-Tilt Plasma Volume

Individual data for pre- and post-tilt plasma volume and change (ml) in plasma volume during these tilts before and after bedrest is shown in Table 43. Group means and results of statistical analysis are shown in Table 44. Although there was a 500 ml. loss of plasma water during the tilt, the change was no greater after bedrest. The possibility of leakage of protein from the vascular compartment was considered. It was feared that the leakage of protein, some carrying ¹²⁵I might be greater after bedrest. However, as shown in Table 45, the increase in total protein concentration following tilt was the same before and after bedrest, whereas, if a difference existed, albumin became concentrated to a greater extent after bedrest. The data suggest that protein leakage is not greater after bedrest.

9. Serum Chemistries

Table 46 contains results from analysis for each subject at prescribed points throughout the study for serum creatinine, sodium, potassium, chloride, calcium and osmolality. All results were within normal limits and showed no trend related to the study phase.

10. Osmotic and Free Water Clearance

Table 47 compiles urine osmolalities for the various subjects performed on the same day as the serum osmolalities. Table 48 shows the urine output in ml/min on these days along with the calculated osmotic and free water clearance. From the results shown it is apparant that urine output was primarily obligatory.

11. Hematologic Data

Table 49 is a compilation of hematologic data including red blood cell count (RBC), hemoglobin (Hgb), hematocrit (Hct), white blood count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and reticulocyte count (RC).on the initial twelve subjects. The data show no change which cannot be related to phlebotomy.

E. Discussion

As stated in the introduction to this report, the premise on which the study was based was that extravascular dehydration occurred during bedrest with resultant loss of plasma water to the extravascular tissues of the lower extremities during the post-recumbency tilt. This would cause the abnormal orthostatic responses seen following

bedrest and weightlessness. The likelihood of this hypothesis being correct seemed strengthened by the appearance of a report regarding changes in ^{125}I -RISA plasma volume, ^{51}Cr red cell mass, ^{35}S extracellular fluid volume and ^3H total body water during ten days of bedrest (28). These authors studied eleven subjects during three separate ten day bedrest periods, two of which involved intervention procedures, leg cuffs and exercise, respectively. No difference occurred with therapy and results of all three periods were analyzed in composite. The authors observed decreases in plasma volume of 400 ml., in extracellular fluid volume of 2200 ml., and in total body water a loss of 700 ml. A decrease of 200 ml. was noted in red cell mass. The total body water change was not statistically significant and the authors commented that: "The sensitivity of the method to determine total body water may not be great enough to detect the changes which could be expected under experimental conditions."

Although the details of Vogt's study are not clearly delineated, several differences from the current study are apparent. In the present study, subjects were on exacting metabolic diets with a fixed sodium intake. The present investigation provides two control points as well as two recovery points for comparison with bedrest results, whereas Vogt's control point was taken 12 hours after onset of bedrest. Further differences exist such as 28 days of bedrest vs 10 days, and the use of ^{82}Br rather than ^{35}S . Because of these differences, direct comparison in results is not possible. However, at 14 days of bedrest in the present

study the following changes had occurred: plasma volume had decreased 200 to 250 ml. from ambulant values; extracellular fluid volume had diminished by 300 - 450 ml.; total body water had decreased 600 - 750 ml.; and red cell mass had declined approximately 100 ml. In general, the changes tended to be less in the present study where the same isotopes were used (^{125}I , ^{51}Cr), except in the case (^3H) total body water where results were similar. The changes in extracellular fluid volume seen in this study fall far short of those reported by Vogt. This is true even after 28 days. Even though different isotopes with somewhat different distributions were used, one would hardly expect results to be at such extreme variance. In the present study ^{82}Br was chosen because of its ease of handling and virtual constancy of distribution after equilibration. Both ^{35}S and ^{82}Br tend to overestimate the "true" extracellular space. The complexity of distribution of sulfate and its involvement in metabolic processes complicate interpretation of its plasma disappearance (42). Further, although not reported here, each ^{82}Br volume determination was paired with a simultaneous ^{35}S extracellular fluid volume determination by the method of Jeffay in our initial fourteen subjects (43). The study was discontinued because of the extreme complexity of sample preparation which appeared to contribute largely to the erratic results that were obtained. In Vogt's report, the decrease of 2200 ml. in extracellular fluid volume also seems inconsistent with a decrease in total body water of only 700 ml. Such a change would mean movement of 1500 ml. into body cells, an unlikely event.

Stated in the simplest of terms, the present study does not confirm the hypothesis of extravascular dehydration as a cause for post-recumbency orthostatism (15). On the other hand, it delineates quite clearly the sequence of events relating to body fluid alterations during bedrest (and by inference, during weightlessness).

The loss of hydrostatic effects in the supine posture results in a shift of blood from the peripheral venous beds into the thoracic compartment. The increased filling of this compartment appears to exert its effect on the central low pressure baroreceptors, presumably causing inhibition of A.D.H. and aldosterone with resultant sodium and water diuresis (44-47). The alteration in aldosterone has never been confirmed prior to the present study.

It is apparent from the data presented that the adjustments to changes in gravitational stress are quite rapid. On the first day of bedrest, aldosterone excretion drops significantly. This is associated with a large saluresis with obligatory water loss during the initial 48 hours of bedrest. After 48 hours of bedrest plasma volume is virtually as low as after 28 days. This is also true for extracellular fluid volume. Although no remarkable change occurs in these measurements after two days, the average subject continues to have an excess excretion of approximately 10 meq of sodium and 100 ml. of water throughout bedrest. This may be accounted for by either or both of two mechanisms. Firstly, the supine resting subject is less likely to lose as much sodium and water insensibly as he did while ambulant. The difference being accounted for by

renal excretion. Secondly, total body water showed a continuing slow decrease during bedrest. Some of the excess sodium and water may be accounted for by this mechanism. However, mean weight decreased by only 0.43 kilograms during bedrest. This is roughly equivalent to 430 ml. of water which is less than the combined decrease in extracellular fluid volume and red cell mass alone and far less than the 1500 ml. decrease in total body water. Although we agree with Vogt that the ^3H method for total body water is less than ideal (28), this discrepancy is probably more apparent than real. For years it has been known that bedrest and inactivity result in a loss of muscle mass and an increase in adipose tissue. The increase in urinary nitrogen seen in the present study is confirmatory of this fact. The replacement of muscle by fat, a tissue with a lesser water content would present exactly the picture seen here, a decrease in total body water without a corresponding decrease in weight. Many refinements were made in the total body water technique in the course of this study, and the decrease observed is a consistent one. The loss of potassium during bedrest is probably another reflection of muscle breakdown.

Thus, the results of the study do show a large alteration in body fluid compartments as a consequence of bedrest. However, the decrease in fluid available for vascular filling, i.e., the net change in red cell mass and extracellular fluid volume, amounted to only 500 - 700 ml. When this loss is coupled with the 500 ml. loss of plasma water during both the pre-recumbency and post-recumbency tilt, a situation exists which could readily produce

the hemodynamic responses to 70° tilt seen in the present study and in greater depth in our previous investigation (1). The absence of a contributory factor produced by extravascular dehydration is reconfirmed by the failure of plasma volume to decrease by a larger magnitude during the post-recumbency tilt.

The current study was also designed to evaluate the question of change in limb volume during tilt before and after bedrest.

That an increase in limb volume does occur during tilting is unquestionable even when our impedance plethysmography results are interpreted in the most conservative manner. However, our data does not allow a comparison of post-recumbency results with pre-bedrest data for reasons stated previously. Nevertheless, a recent report by Menninger and an unpublished study by McCally showed that the post-recumbency increase in limb volume response to hydrostatic stress was no greater than the pre-recumbency increase (27). Thus, it would appear that not only is there no more extravasation of fluid after bedrest, there is also no increase in venous pooling.

The electrolyte, aldosterone, water balance and fluid compartment data relating to the recovery period represent another manifestation of the rapidity of response of the volume regulating mechanisms. The increase in aldosterone output with resultant profound sodium and water retention produce a full restoration of body fluid volumes during the interval between two days and seven days of re-ambulation. It should be noted that heart rate response to tilt also returned to normal between two and seven days after

re-ambulation. The significant rise of plasma volume above pre-recumbency levels undoubtedly represents a replacement of the depleted red cell mass. The volume of red cell decrease (150 ml.) is identical to the amount of plasma volume excess.

The results of the red cell mass determinations and their correction for red cells withdrawn to each point in the study are most interesting. The leveling off of the corrected results strongly suggests that bone marrow production of red cells is diminished during bedrest and that the bone marrow is incapable of responding to the stimulus of phlebotomy. An as yet unpublished study by Lancaster yielded similar and more detailed results (48).

In our prior study we had found an inability of the subjects to augment stroke volume during supine bicycle exercise following fourteen days of bedrest (1). Cardiac output was maintained at near pre-bedrest levels by cardioacceleration. The duration of this effect was not studied in the prior investigation. In the present study, exercise responses were observed for fourteen days after the termination of bedrest. In the most gross analysis, the subjects who exercised to exhaustion were able to do less exercise after bedrest (Subjects 18 and 21-24). Perhaps the increase in minute ventilation after bedrest played a role in the more rapid onset of exhaustion, as this would increase the work performed in oxygen delivery to the lungs. The decrease in muscle mass suggested above may have weakened the subjects as well. However, it is most likely that the principal factor in the changes seen in exercise tolerance in this study and in our prior study was a decrease in

myocardial function. This is suggested by the decrease in maximal oxygen uptake and its return to normal between seven and fourteen days, the same time interval in which heart rate response to 100 watt exercise returned to normal. Maximal oxygen uptake is dependent on delivery of oxygen to the blood by the lungs, delivery of blood to the tissues by the heart, and on the ability of the tissues to extract oxygen (49). In the presence of normal pulmonary function, it is generally considered to be a measurement of cardiac capacity. In our subjects pulmonary function remained normal after bedrest. In fact, there was an increase in total lung volume, vital capacity and in one-second forced expiratory volume. No alteration in residual volume or diffusion capacity was noted. The increases in total lung capacity and vital capacity were probably related to the decrease in circulating blood volume, and, therefore, in pulmonary blood volume in the supine position. This is the reverse of the situation seen in states associated with pulmonary congestion where total lung volume and vital capacity are reduced (38). In the present study, A-V oxygen difference was not measured. However, this measurement was made in a recently reported study of five subjects placed at bedrest for 20 days (50). A-V oxygen difference was found to be increased at all exercise levels after bedrest and it was concluded that the fall in maximum oxygen uptake seen in that study was probably due to a decrease in myocardial function (50).

In the present study, the loss of calcium during bedrest was quite significant. These results correlate very well with

those obtained in other studies designed specifically to evaluate the problem of disuse osteopenia and its prevention (29,51).

Conclusions

The present study and its collation with our prior studies as well as with other recently published observations on the effects of bedrest (27,29,48,50,51) leads to the following conclusions:

1. The decrease in orthostatic tolerance seen following bedrest is not caused by autonomic insufficiency, extravascular dehydration, or increased venous pooling. Nor is it caused by an increase in plasma water transudation after bedrest.
2. At least two factors play a role in the occurrence of post-recumbency orthostatism, and one of these is unrelated to bedrest. The first factor is the decrease in extracellular (and plasma) volume during bedrest which when coupled with the second factor, an unchanged plasma water transudation during post-recumbency tilting, results in vascular filling which is inadequate.
3. The initial large saluresis and diuresis seen on assuming the recumbent posture and the rapid restitution of plasma volume and extracellular fluid volume to normal after bedrest have been clearly shown to be related to alterations in aldosterone production.
4. A decrease in myocardial function very likely does occur during extended periods of bedrest. Whether this plays a role in the diminished orthostatic tolerance or not is debatable. The return of orthostatic tolerance to normal in conjunction with the return of plasma and extracellular fluid volume to normal suggests

that diminished myocardial function is not a contributing factor in post-recumbency orthostatic intolerance. On the other hand, the more prolonged period for return of exercise tolerance to pre-bedrest levels suggests that diminished myocardial function rather than diminished effective vascular volume is the predominant factor in the alterations seen in exercise responses following bedrest.

5. Red cell production by bone marrow is depressed during bedrest.

6. Calcium loss during bedrest is significant and presents a potential source of difficulty for astronauts involved in spaceflights of long duration.

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G. TABLES

TABLE A
COMPARISON OF SHORT* AND LONG** INTERVAL
SAMPLING FOR PLASMA VOLUME DETERMINATION

Plasma Volume Number	Plasma Volume		Correlation Coefficient	
	Short Interval	Long Interval	Short Interval	Long Interval
1	3036	3057	-.345	-.923
2	3415	3402	-.501	-.989
3	3118	3123	-.198	-.967
4	3523	3230	+.843	-.977
5	3089	3097	-.853	-.998
6	3022	2987	-.429	-.976
7	2659	2649	-.235	-.958
8	2972	3028	-.570	-.983
9	2794	2768	-.893	-.999
10	2936	2954	-.399	-.938
11	2805	2814	-.565	-.989
12	3205	3147	-.112	-.963
13	2975	3012	-.812	-.968
14	3143	3090	+.146	-.751
15	2949	2908	-.352	-.993
Mean	3043	3018	-.352	-.962
SE (95%)	±124	±103	±.240	±.034
P		<.3		

*10, 20, 30 and 60 minute samples used.

**1, 2, 4 and 6 hour samples used.

TABLE B

OBSERVED EXTRACELLULAR FLUID VOLUME
WITH RED CELL AND URINE LOSS CORRECTIONS

Study	2 Hours	4 Hours	6 Hours
1	17.17	17.77	17.98
2	18.98	19.69	19.93
3	17.12	17.88	18.35
4	19.77	20.19	20.66
5	17.50	17.87	18.46
6	19.07	19.37	20.50
7	16.97	17.47	18.32
8	18.75	19.62	20.34
9	16.61	16.89	17.71
10	18.78	19.79	20.27
11	17.22	17.68	17.97
12	18.13	18.92	19.34
Mean	18.01	18.60	19.15
SE (95%)	±.66	±.71	±.72

TABLE C

COMPARISON OF EXTRACELLULAR FLUID VOLUME DETERMINATION
WITH AND WITHOUT CORRECTION FOR URINARY ^{82}Br LOSS

Study	ECFV With Urine Correction		ECFV Without Urine Correction		r
1	15.16	.971	14.85		.983
2	16.74	.965	16.70		.972
3	14.92	.992	14.92		.983
4	17.40	1.005	17.84		1.005
5	15.31	.996	15.31		.984
6	16.44	.953	16.61		.962
7	14.65	.992	14.53		.979
8	16.23	1.000	16.14		.970
9	14.40	.967	14.31		.955
10	16.34	.980	16.41		.983
11	15.19	.994	15.13		.996
Mean	15.71	.983	15.67		.979
SE (95%)	$\pm .65$	$\pm .011$	$\pm .69$		$\pm .009$

TABLE D
TOTAL BODY WATER DETERMINATION

Study	Corrected For Urine Loss	r	Linear Regression	r
1	39.83	.719	39.91	.766
2	36.01	-.065	32.96	.999
3	37.64	.228	37.42	.603
4	34.68	.792	34.47	.923
5	41.25	1.049	40.98	.992
6	37.84	.479	37.51	.844
7	37.15	-.494	37.75	.714
8	28.24	-.611	28.10	.971

TABLE E

FOODS AVAILABLE FOR METABOLIC STUDIES

CEREALS:

Cornflakes
Rice Krispies
Wheaties
Oatmeal

EGGS:

Scrambled
Omelet
French To

BAC-O-Bits (Imitation bacon)

WHITE BREAD (Plain or Toasted)

BUTTER
JELLY
PEANUT BUTTER

FRUIT JUICES:

Apple juice
Cranberry juice
Grape juice
Grapefruit juice
Orange juice
Pineapple jui
Tomato juice

CANNED FRUITS:

Applesauce
Apricot halves
Royal Anne Cherries
Grapefruit sections
Peach slices
Pear halves
Pineapple slices

MISCELLANEOUS:

Salt
Pepper packets (3/day maximum)
Sugar

MEAT & SUBSTITUTES:

Beef tenderloin (steak)
Ground beef
Chicken breast (boned and skinned)
American cheese
Tunafish

STARCHES:

Mashed potatoes (instant)
New potatoes (canned)
Rice
Macaroni

CANNED VEGETABLES:

Beets
Carrots
Corn, whole kernel
Green beans
Peas
Stewed tomatoes

TOMATO SOUP

CRACKERS, ETC.:

Graham crackers
Premium crackers (unsalted tops)
Vanilla wafers
Shortbread cookies (homemade)

BAKED CUSTARD

JELLO

SPONGE CAKE

BEVERAGES:

Milk (whole or nonfat)
Coffee, instant
Tea, instant
Coca Cola
Gingerale
Orange soda
Lemonade
Root Bear.
Seven Up
Wine- red or wh

INSTRUCTIONS FOR SUBJECTS ON METABOLIC BALANCE DIETS

1. You must eat all food and drink that is served to you.
 2. Do not eat or drink anything other than what is served to you by a member of the Metabolic Staff.
 3. Do not chew gum.
 4. Drink only distilled water from your water pitcher.
 5. Clean dishes well and rinse glassware with distilled water from your water pitcher. Drink the rinsings.
 6. The salt shaker which you will receive at the breakfast meal is your supply for the day; therefore, you should use it accordingly. All salt should be used by the end of the dinner meal.
 7. One pepper packet will be given at each meal. The pepper may be used or not as you so choose.
 8. If you should drop or spill any food, report it immediately to a member of the Metabolic Staff. Do not wipe up any liquid which may be spilled. The amount must be estimated and replaced.
 9. Mealtimes are as follows:

Breakfast	8:00 AM
Lunch	12:00 NOON
Dinner	5:00 PM
Snack	9:00 PM
- Breakfast may be delayed when blood work or tilts are being done.
10. Eat your meals when they are served to you. You may not save food for "later" unless you are ill, in which case the kitchen should be notified. Do not remove food from your tray and place it elsewhere.
 11. Visitors are not permitted in the room when either you or they are eating.
 12. Please inform your visitors that you are on a carefully controlled diet, and that they should not bring you anything edible until you have completed the study.

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