NASA Contractor Report 177548

Fluid and Electrolyte Homeostasis During Spaceflight: Elucidation of Mechanisms in a Primate

Susanne Churchill

CONTRACT NAS2-10547
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Fluid and Electrolyte Homeostasis During Spaceflight: Elucidation of Mechanisms in a Primate

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Prepared for
Ames Research Center
CONTRACT NAS2-10547
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FINAL REPORT ON SUPPORTING STUDIES PERFORMED FOR NASA CONTRACT NAS 2-10547 (Period of Performance: 10/1/81 - 3/31/88)

Submitted to: Dr. Henry Leon, Technical Monitor
Drayton Swartz, Contracting Officer

Submitted by: Susanne Churchill, Ph.D., Project Manager and Co-Investigator

This report covers supporting studies performed under contract NAS 2-10547 which has funded science activities for the period 10/1/81 to 3/31/88.

1. Design of LBPP Apparatus Seal (Task 2.3.2.d, Statement of Work 2-28152)

Rationale: Previous work with this model was limited by problems in maintaining a stable LBPP over an extended period of time, due primarily to fluctuations caused by positional changes of the monkey.

Method: 1. Evaluate gasketing materials for a cuff that would provide:
- o biocompatibility with monkey (flexible, non-irritating)
- o good seal with both chair and monkey
- o easy adjustment to fit each monkey

2. Evaluate a design that would use such materials to yield a satisfactory cuff.

3. Evaluate the entire chair design to maximize animal comfort and welfare, thus minimizing twisting and straining.

4. Test the best design in six animals exposed to LBPP for a a period of time equivalent to anticipated ground based studies (7 days).

Results: This first study undertaken with NASA support has provided the means whereby we are currently able to reproducibly and humanely conduct studies of up to 10 days duration. We have designed a cuff which meets all of the above criteria and is now routinely used in 11 of our LBPP studies. During the course of this evaluation and redesign we have devised modifications of the primate chair which have also contributed to pressure stability: a water cushion to minimize pressure sores, an adjustable foot perch to provide a comfortable squatting position, an extended frontspiece to permit the animals to lean forward when sleeping. In addition we redesigned the horizontal back plate and catheter tunnel to better protect the catheters as they are routed away from the animal and outside the isolation chamber. This system is now routinely used in all of our LBPP studies.

In seven adult squirrel monkeys subjected to LBPP at 20 torr for 7 days, LBPP was maintained at 19.3 +/- 0.3 torr.

Conclusions/Recommendations: The modifications detailed above have enabled us to reproducibly and routinely work with the squirrel monkey LBPP model for up to 10 days of study. Data generated from such studies have and will continue to provide an excellent basis by which to evaluate the usefulness of this model as an analogue for weightlessness, as well as to understand the basic physiological mechanisms underlying the renal response to central volume expansion in a primate.

2. Evaluation of implanted catheter design and materials (Task 2.3.2.e and 5.9.b., S.O.W. 2-28152)

Rationale: In previous studies it had not been possible to simultaneously measure both blood pressures and to take blood samples during exposure to LBPP as the rate of infusion required to maintain the catheter patent represented a volume load to the animal; in addition the use of 0.45% saline was apparently associated with undesired hematocrit changes.

Method: 1. A new catheter material (Renathane) which purported to be non-thrombogenic and had been extensively tested in human subjects was evaluated for both arterial and venous catheters. It was anticipated that use of this material might obviate the requirement for a continuous infusion.

2. If, however, these new catheters did clot, we intended to determine the minimum infusion rate required to keep them patent and what infusate would do this with the least impact on the animal's health and physiological state.

3. Evaluate implantation technique and site to provide a consistent system.

4. Optimize the flushing technique to maintain longevity of indwelling lines while not compromising the health of the animal.

Results: 1. Renathane evaluation

Although this material was excellent for internal use, it was found to loose fluids to evaporation where exposed to the external environment; loss of fluid in the external portion drew blood from the vessel lumen to the external portion - loss of fluid from the blood portion rendered flushing and maintenance of the catheters virtually impossible. Therefore we devised a new catheter design using two materials: that portion inserted into the vessel and resting in the abdominal cavity was constructed of renathane which was in turn joined to standard polyvinyl chloride tubing as it exited the body cavity to run

under the skin and subsequently externalized between the scapulae. This model has been used satisfactorily both in our lab and in extensive NASA tests and been found to function well for up to two years if maintained according to the protocol developed as part of Method 4 above. Post mortem examination of these renathane catheters shows them to be free of overgrowth or plaque development on the outside and free of tissue or clot buildup on the inside.

During this same study we determined that it was necessary to reduce the size of the arterial catheter from the standard .025 x .040 inches provided by the supplier as the vessel into which it is inserted was occasionally compromised by occlusion. To do this we evaluated several techniques before determining that drawing out the tubing after exposure to heated peanut oil worked best. Only tubing of a predetermined size was utilized to manufacture the catheters.

We also evaluated means of further securing the catheter in the abdomin to reduce strain during flushing and with normal movement of the animal and to seal off the catheter track as it entered the abdomen. We have met with great success using small discs of Dow Corning sylastic sheet imbedded with nylon fibers. This has significantly prolonged the life of our catheters.

2. Clotting

In studies designed to determine how long these catheters provided accurate pressure readings and for the arterial catheter, good transmission of the pulse pressure waveform, we found that it was possible to obtain a clear signal for mean arterial pressure for frou hours; pulse pressure, however, declined by one hour. Mean venous pressure could be accurately recorded for up to four hours. While this was excellent for short term recordings it was not satisfactory for the many day experiments needed for these protocols. In a series of studies evaluating different infusion rates, we determined that a rate Of 2.7 microliters/min (3.8 ml/day) was adequate to provide the required fidelity without significantly effecting the volume status of the animal. The infusate that worked best was heparinized normal saline. This infusion was so slow as to have no impact on hematocrit even when both lines were infused simultaneously. This technique was used satisfactorily in a study of seven adult monkeys exposed in random order to both LBPP and air not under pressure; arterial and venous pressures were measured and blood samples were taken as required. These data have been presented to the American Physiological Meeting and are being prepared for publication.

3. Implantation Technique

Using external physical landmarks as corroborated by post-surgical x-ray of contrast filled catheters, we have determined the optimal length of insertion of each catheter to insure optimal placement. This is especially important for the venous catheter which must be above

the diaphragm and below the right heart to provide a good central venous pressure recording. Our surgical techniques were evaluated by a vascular primate surgeon chosen by NASA and found to be satisfactory by all criteria.

4. Flushing Technique

We have invested considerable effort in modifying the catheter flushing technique and now have a system that we believe to be excellent by two important criteria: patency and freedom from infection. In addition to carefully defining the individual mechanical steps and the associated sterility, we have worked with M. Gellai (Dartmouth Medical School) to devise a solution which can be left in the catheters to prevent either clotting or growth of bacteria (1:1 heparinized saline 100 u/ml:50% dextrose in water). Culture of each line weekly is used to monitor for infection; these are consistently negative.
We have trained ARC personnel in the use of this technique in our laboratory; they have successfuly transferred our method to ARC and have maintained cathers patent and infection free for the required periods of time. This procedure is detailed in Appendix A.

Conclusions/Recommendations: The catheter installation and maintenance technique developed from these studies is reliable, low in risk and allows collection of the targeted data for both ground based and for the flight experiments. No further modification or study is required.

3. Blood Replacement (Task 5.9.a., S.O.W. 2-228152)

Rationale: Serial blood sampling, as would be required for determination of plasma hormone levels, electrolytes, osmolality, hematocrit and possibly volume space measurements, may stress and/or volume deplete and/or modify the response to LBPP in these small primates without adequate replacement.

Method: 1. Evaluate the best method of replacing red blood cells and plasma. Choices included replacement by pooled blood from a group of monkeys, replacement by the monkey's own blood which had been previously collected and frozen, and replacement of the monkey's own red blood cells following each sampling by resuspending the packed pellet from the centrifuged sample in saline and reinfusing.

2. To reduce the size of the required blood sample it was necessary to develop small volume analytical systems for measurement of osmolality and plasma electrolytes and for radioimmunoassays.

Results: 1. We first evaluated the most practical technique and the one most commonly used in other chronic studies: resuspending the spun red blood cells in saline and reinfusing into the animal. We successfully developed a technique to do this in the ground based laboratory; however, due to the absolute requirement for sterile

technique and the opportunity to introduce infection, the procedure was time consuming and cumbersome. Nonetheless we completed a successful study of six animals exposed to seven days of LBPP and to seven days of air not under pressure. Animals not exposed to LBPP showed no changes in hematocrit or plasma variables as a result of blood sampling compensated for by this technique. In order to develop a more efficient system for the spaceflight experiment, we worked with Dr. Henry Leon and Dr. Bob Phillips to simplify the blood sampling technique. As a result the onboard centrifuge was modified to accept a newly designed centrisyringe device (a blood sampling syringe that can also serve as its own centrifuge tube) to minimize the steps and opportunity for contamination. To further improve the procedure, we next evaluated the reinfusion of the packed red blood cells without resuspension; plasma losses were compensated by a chase of normal saline equivalent to the plasma taken for analyses. This worked very well and is now the technique of choice in both our ground based and flight experiments.

2. Working with Dr. A. Clifford Barger, Harvard Medical School, we have developed microvolume radioimmunoassay techniques for the measurement of plasma aldosterone and atrial natriuretic factor, two of the key hormones implicated in the renal response to central volume expansion. Accuracy and reliability have been shown to be equivalent to results generated with classic macro volume assays. At a later date we also began work with Dr. William North, Dartmouth Medical School, to adapt his small volume radioimmunoassay for arginine vasopressin (AVP) to squirrel monkey plasma and urine. We have also developed a technique for flame photometry that uses microliter volumes and with NASA support purchased a microosmometer that requires only 50 microliters of sample.
As a result of this work we are now able to routinely measure all hormones of interest in a single sample on the order of 1 ml of blood. This represents a significant gain in terms of science yield and offers considerable flexibility in design of additional procedures.

Conclusions/Recommendations: Development of microvolume assay techniques and the centrisyringe/packed red blood cell + saline flush technique has greatly improved the scientific yield from both ground based and flight studies. These techniques are reliable, simple and greatly reduce the risk of infection to the animal.

4. Effect of circadian rhythm on plasma constituents (Task 7.8.a.)

Rationale: In order to accurately distinguish changes in plasma hormones due to true physiological response mechanisms from those occurring as a result of natural (circadian) rhythms, it is important to have baseline data on how the hormones of interest fluctuate normally in the course of twenty four hours.

Method: Six chair-trained monkeys were surgically prepared with catheters for blood sampling and allowed to adequately recover.

Studies were conducted by placing animals in our standard LBPP chair for 36 hours of equilibration followed by 60 hours of study during which period blood samples were taken at various time points with sufficient time in between to prevent an effect due to excessive blood sampling. Samples were taken in all animals in random order at each of the following time points: 600, 800, 1000, 1400, 1800, 2000, 2200, and 200. Blood samples were centrifuged and the plasma removed and frozen for later analysis of sodium and potassium concentration, osmolality, and plasma aldosterone concentration. Statistical analysis was performed using paired t-testing as each animal acted as its own control. Red blood cells were resuspended in normal heparinized (10 units/ml) saline and reinfused.

Results: As shown in Table 1, no significant differences between any time points were observed in plasma sodium and potassium concentration and plasma osmolality. As shown in Figure 1, there were large fluctuations observed in plasma aldosterone concentrations. The increased levels observed at 1000 were significantly elevated ($p < 0.05$) over those measured at 0800 and at 1400.

Conclusions/Recommendations: These data have a significant impact on the design and interpretation of experimental results and emphasize the value of separate control studies. As a result, we have chosen the time when plasma aldosterone is most stable for the majority of our ground based studies where is important to understand whether changes in levels of this critical hormone play a role in the observed renal responses.

5. Engineering Requirements Study (Task 7.8.b)

Rationale: To facilitate the engineering team's efforts to support the stated science goals of our experiment as design and implementation of the flight cage system progressed it was clearly necessary to provide the range of certain critical biological parameters.

Methods: See original reports (Appendix) filed following completion of this task (originally Supporting Study 4). Two such studies were conduced at HMS with the support of the ARc Engineering Team on 11/29/82 and 3/24/83.

6. Feasibility Study (Task 7.8.c)

Rationale: It was clearly critical at an early stage to determine whether it was possible to integrate the fully instrumented squirrel monkey (integration of Experiments 223 and 039) into the current flight cage model and generate all of the required data. This study was designed to do this as well as provide the engineering team with critical input regarding biocompatibility of their systems and the monkey and to

provide the crew with a training and evaluation opportunity. This study occurred on October 19-29, 1984 at ARC.

Methods: Four restraint trained squirrel monkeys were surgically prepared with venous and arterial catheters and a biotelemetry implant at the Ames Research Center where all such integrated studies were performed. Following surgical recovery, these animals were used in a series of tests to evaluate the restraint system, the urine collection system, functioning of the prototype feeder, blood draw system, functioning of the waste tray system, the vascular monitoring system, and skin thermistor system as measured from five externally attached sensors. The specific protocol for the major feasibility study in enclosed in the Appendix.

Results: As above these results were primarily observational in nature and were critical in evaluating the prototype systems. See evaluation letter in Appendix.

Conclusions/Recommendations: Such studies are absolutely critical in the evolution of a complicated experiment such as this. It is especially important that all levels of equipment be tested on real subjects as soon and as often as possible.

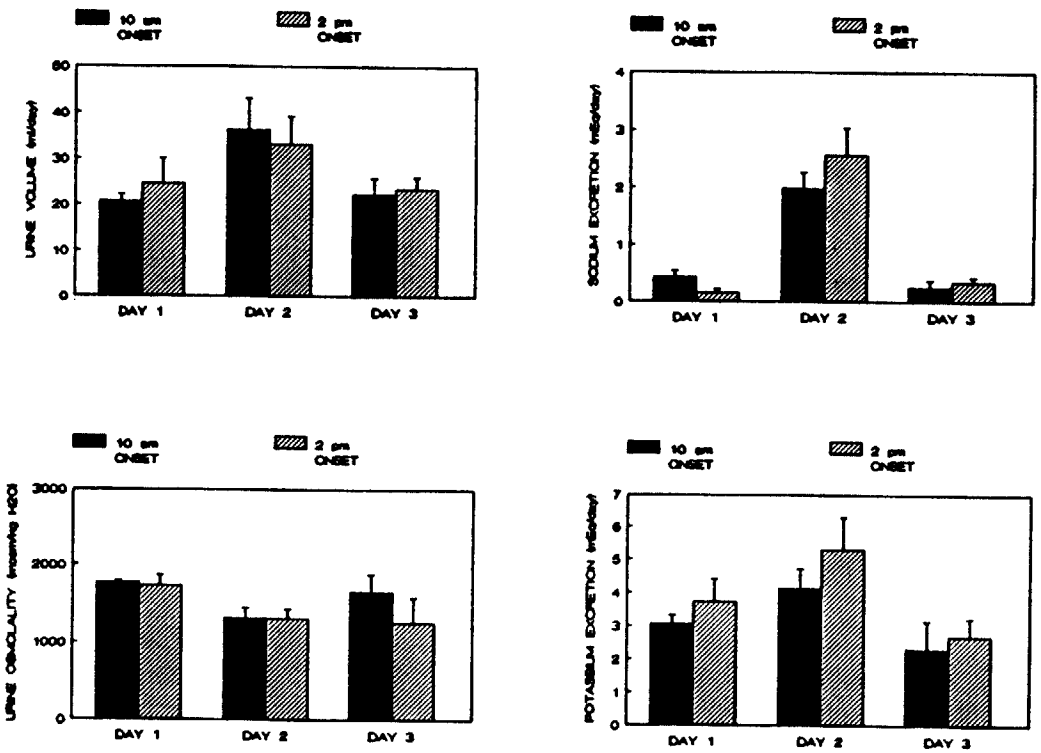
7. Influence of Circadian Timing of Weightlessness Onset (Task 7.8.d)

Rationale: Previous data from this and other laboratories have indicated that the renal response to a volume load is quite different when the load is administered during the day as opposed to at night. To determine whether smaller gradations of response occurred during the daylight hours and thus whether time of launch might influence the renal response to weightlessness, it was important to evaluate the response to LBPP in the morning and in the afternoon.

Methods: Chair-trained squirrel monkeys were surgically implanted with arterial and venous catheters. After appropriate recovery animals were subjected to LBPP at 20 torr at either 10 a.m. or 2 p.m. for a period of 24 hours. Urinary responses, central venous and arterial pressures, and plasma hormones and electrolytes, osmolality and hematocrit were monitored and compared between the two groups.

Results: We observed no discernible influence of the time of LBPP onset and the renal excretory response. Urine volume and the excretion of sodium and potassium were all increased and urine osmolality decreased by 24 hour exposure to LBPP in all animals. As shown in accompanying figure, there were no difference between the 10 am and 2 pm groups for any of these variables.

Renal excretory response to 24 hr LBPP – 10 am vs. 2 pm onset



n = 3 in each group

Conclusions/Recommendations: We conclude that we have at least several hours of latitude within the daylight hours during which the renal response to LBPP is quite consistent. Nonetheless, we propose that large deviations from the time-frame of our protocol be limited since previous experiments demonstrated a dramatic difference in the response to LBPP at night versus day.

8. Determination of Optimum Level of Lower Body Positive Pressure (LBPP) Which Best Simulates the Space Flight Response (Task 7.8.f)

Rationale: In order to optimize the ground based LBPP system as a model for weightlessness it will be necessary to know what level of LBPP best mimics the effect of weightlessness on cardiovascular and renal response. These data were felt to be especially important as the LBPP model has been proposed for ongoing use in post flight studies where specific mechanisms and possible interventions would be evaluated.

Method: We proposed evaluating the response of the squirrel monkey to several steps of LBPP: 20, 25 and 30 torr. In these studies animals prepared as indicated in the previous protocols were to be exposed to control conditions and each level in independent studies for the time course of the renal and/or cardiovascular response. The parameters of interest were urinary volume, osmolality, and electrolyte composition, central venous and arterial pressure response, and plasma hormone levels, electrolytes, osmolality and hematocrit.

Results: Our first study evaluated the response of seven adult male squirrel monkeys exposed to seven days of both LBPP at 20 torr and as a control air not under pressure. These data are presented in Figures 2-6 and in Tables 2-3. After preliminary studies to ascertain that the monkeys and our system would tolerate a stepwise increase to 25 torr we repeated this protocol in a group of new monkeys with no signifiant differences being apparent in any of the parameters measured. Subsequent preliminary studies at 30 and 40 torr suggested that the animals did not easily tolerate this degree of pressure and thus prohibited further study at higher levels.

Conclusions/Recommendations: We believe that 20 torr of LBPP creates a maximal central volume translocation in the squirrel monkey and thus is the optimal level for further studies regarding characterization of the renal response and the efferent limb mechanisms responsible for this response.

9. Baseline Nominal Flight Conditions (Task 7.8.f.)

Rationale: To determine what physiological changes occur secondary to the conditions accompanying launch (noise, vibration and acceleration)

as compared to the responses to weightlessness alone, it was clear that a prior study whereby animals were exposed to launch conditions alone would be of great value in interpreting the data gained from flight.

Methods: As designed, this study would have exposed the four animals selected for EVT (fully instrumented and subjected to the full flight protocol) to the noise, launch and vibration profiles typically encountered during shuttle launch.

Results: Due to logistical problems, these studies were not performed during EVT. Instead, it has been proposed that if possible these studies be performed post flight on the actual animals. Availability and access to a centrifuge are critical.

Conclusions/Recommendations: The rationale is extremely valid and if at all possible, these studies should be performed as designed.

Normal values $P_{osm} = 292$; $P_K = 3.46$, $P_{Na} = 146$.

TABLE 1

Plasma Osmolality

Time	Bo	Fang	Moe	Bubba	Curly	Larry	$\bar{X} \pm SD$
8 AM	294	298	291	288	280	303	292 ± 8
10 AM	283	299	299	291	302	299	296 ± 7
2 PM	291	296	292	291	293	294	293 ± 2
6 PM	292	293	293	294	295	297	294 ± 2
8 PM	293	291	293	288	283	289	290 ± 4
10 PM	292	290	290	292	295	289	291 ± 2
2 AM	288	290	290	286	294	290	290 ± 3
6 AM	261*	287	302	288	286	290	291 ± 7
			Plasma Potassium				
8 AM	3.47	3.58	3.23	3.43	3.58	3.75	3.51 ± .18
10 AM	2.97	3.50	3.29	2.92	3.16	3.30	3.19 ± .22
2 PM	3.63	4.20	3.83	2.91	3.11	3.30	3.50 ± .48
6 PM	3.78	4.29	3.67	3.22	3.24	3.19	3.66 ± .41
8 PM	3.50	4.33	4.05	3.59	2.73	3.44	3.61 ± .5
10 PM	3.67	4.47	3.84	3.58	3.76	3.52	3.81 ± .3
2 AM	3.38	4.33	3.15	3.08	3.07	3.28	3.38 ± .48
6 AM	3.35*	4.06	3.22	3.16	3.25	3.60	3.53 ± .3
	(3.22 ± .92)	(4.10 ± .36)	(3.60 ± .33)	(3.21 ± .33)	(3.24 ± .32)	(3.42 ± .19)	
			Plasma Sodium				
8 AM	149.0	148.3	145.9	150.5	148.5	149.8	148.7 ± 1.6
10 AM	144.4	146.3	143.0	146.4	141.8	142.3	144.0 ± 2.0
2 PM	147.4	148.9	142.3	145.6	144.2	144.7	145.5 ± 2.4
6 PM	148.1	146.1	144.6	149.5	143.2	147.3	146.5 ± 2.3
8 PM	149.0	149.2	143.3	147.0	142.2	143.8	145.8 ± 3.1
10 PM	145.5	145.8	140.8	145.7	142.4	146.1	144.4 ± 2.2
2 AM	148.2	150.2	144.4	147.5	144.9	144.4	146.6 ± 2.4
6 AM	131.8*	150.0	144.3	150.1	143.6	146.8	147.0 ± 3.1

FORM IS THE COOP * discolored

TABLE 2

Response of Plasma Osmolality and Electrolytes
to Seven Days of LBPP and Control Conditions

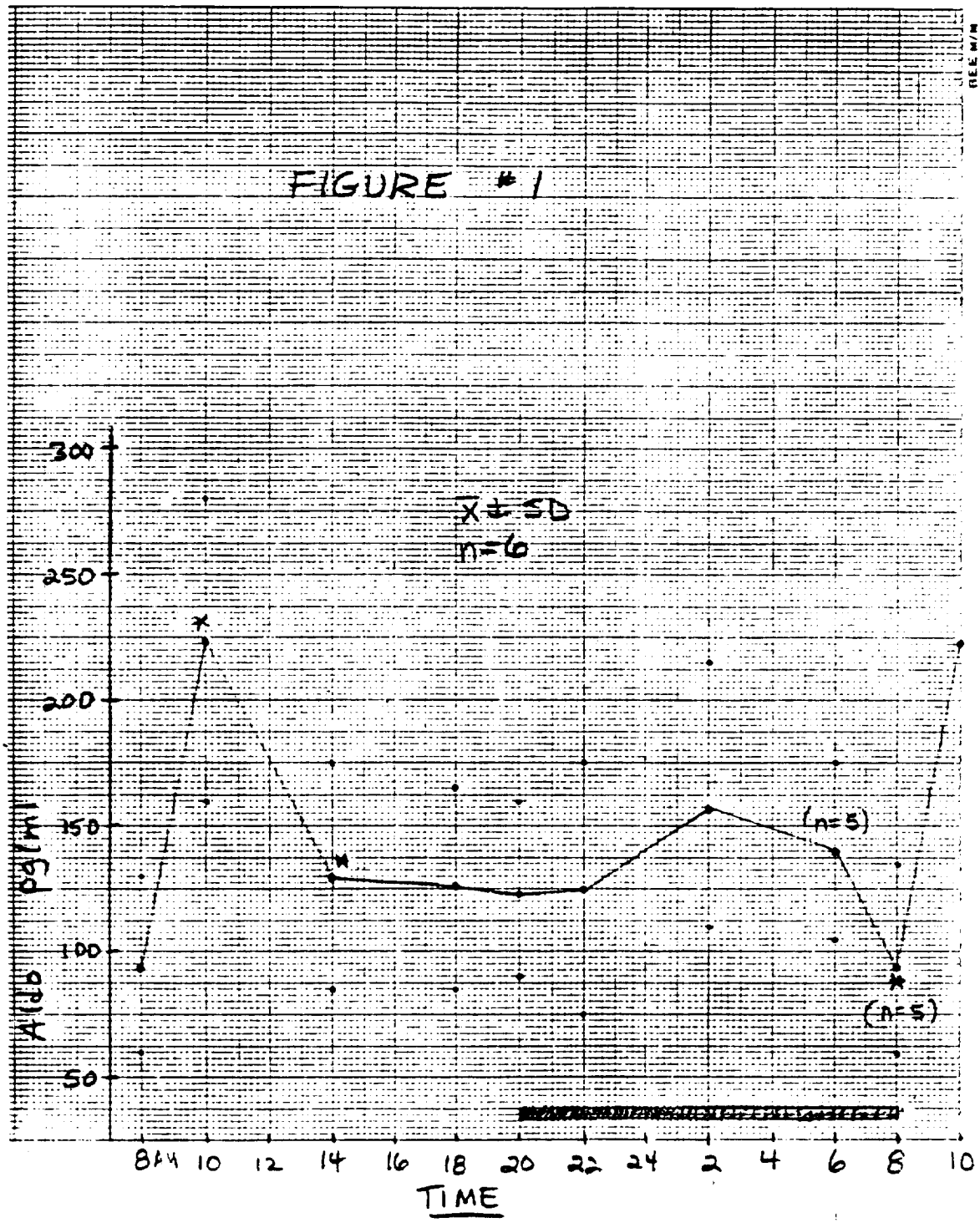
Study	Day of Study								
	0		1	2	3	4	5	6	7	8
	1300	1700								
Osmolality (mOsm/kg)										
LBPP	301 ±3	297 ±2	294 ±3	304 ±3	302 ±4	297 ±2	303 ±3	299^{o} ±3	299 ±2	298 ±2
Control	299^{x} ±3	295 ±2	299 ±2	299^{x} ±1	300 ±2	293^{x} ±2	298^{x} ±5	293^{o} ±3	293^{x} ±3	294^{o} ±2
Plasma Na (mEq/L)										
LBPP	147.4 ±2.4	146.4 ±3.0	145.0 ±1.9	148.9 ±2.3	144.6 ±2.6	148.3 ±3.1	149.0 ±1.5	149.2^{o} ±2.7	150.2 ±2.4	146.7 ±1.7
Control	150.3 ±1.6	148.9 ±2.7	147.2 ±1.7	148.1^{x} ±0.9	149.3 ±0.7	146.9^{o} ±0.9	146.2^{x} ±3.7	146.1^{x} ±2.8	145.7^{x} ±3.7	147.4^{o} ±5.0
Plasma K (mEq/L)										
LBPP	3.62 ±.27	3.26 ±.24	3.08 ±.14	2.89 ±.07	2.82 ±.08	2.97 ±.15	2.87 ±.09	3.04^{o} ±.18	3.05^{x} ±.20	3.16 ±.17
Control	3.43^{x} ±.19	3.59 ±.16	3.07 ±.11	3.05^{x} ±.08	3.05 ±.13	2.81^{x} ±.18	2.99^{x} ±.16	3.00^{x} ±.09	2.93^{x} ±.16	2.83^{o} ±.17

Values are expressed as mean ± S.E.M. for an n=7 except where indicated: ^{o}n=5, ^{x}n=6.

TABLE 3

Free Water and Osmolar Clearances During Chronic Exposure to Lower Body Positive Pressure and Control Conditions

Study	Day of Study								
	0	1	2	3	4	5	6	7	8
C_{H2O}									
LBPP	-.104 ±.033	-.088 ±.051	-.106 ±.041	-.127 ±.043	-.070 ±.099	-.111 ±.021	-.109 ±.038	-.106 ±.034	-.089 ±.034
Control	-.119 ±.032	-.126 ±.037	-.118 ±.031	-.088 ±.127	-.121 ±.056	-.113 ±.042	-.116 ±.045	-.102 ±.027	-.086 ±.030
C_{Osm}									
LBPP	.131 ±.015	.160 ±.017	.153 ±.022	.176 ±.022	.148 ±.022	.151 ±.013	.150 ±.024	.142 ±.018	.113 ±.020
Control	.148 ±.016	.155 ±.015	.150 ±.014	.177 ±.022	.158 ±.023	.148 ±.017	.155 ±.022	.138 ±.011	.115 ±.017



PLASMA ALDOSTERONE LEVELS AS A FUNCTION OF TIME OF DAY

* = significantly different from previous level at $p<0.05$ or less.

FIGURE LEGENDS

Figure 2. Effect of 7 days of lower body positive pressure (Days 1-8) as denoted by black bar on mean arterial blood pressure in 5 squirrel monkeys. Days 0 and 9 are pre- and post-study "control" days. Values are presented as 24-hour means ± S.E.M. When compared with Day 8 by paired t-test, $p<0.05$ or below.

Figure 3. Effect of 7 days (Days 1-8 as denoted by black bars) of lower body positive pressure (LBPP) or no pressure (control) on daily urinary sodium excretion, volume, and osmolality. Days 0 and 9 are pre- and post-study "control" days. Values are presented as 24-hour means ± S.E.M. for 7 animals. When compared by paired t-test, $p<0.05$ or below.

Figure 4. Effect of first 2 days (Days 1 and 2) of lower body positive pressure (LBPP) or no pressure (control) on 2-hourly urinary sodium excretion, volume, and osmolality. Day 0 is a pre-study "control" day. Values are presented as mean ± S.E.M. for each 2-hour increment throughout the day for 7 animals. Black bars denote hours of darkness (8 p.m. to 8 a.m.). When compared by paired t-test, $p<0.05$ or below.

Figure 5. Effect of 7 days (Days 1-8 as denoted by black bars) of lower

body positive pressure (LBPP) and no pressure (control) on water and sodium balance. In each case balance was calculated by comparing oral intake to urinary losses and did not include a factor for insensible loss. Days 0 and 9 are pre- and post-study "control" days. Values are presented as mean ± S.E.M. for 7 animals. When compared by paired t-test, $p<0.05$ or below.

Figure 6. Effect of 7 days (Days 1-8 as denoted by black bar) of lower body positive pressure (LBPP) or no pressure (control) on plasma aldosterone concentration. There were no differences between groups by paired t-test on any day of the study.

FIGURE 2

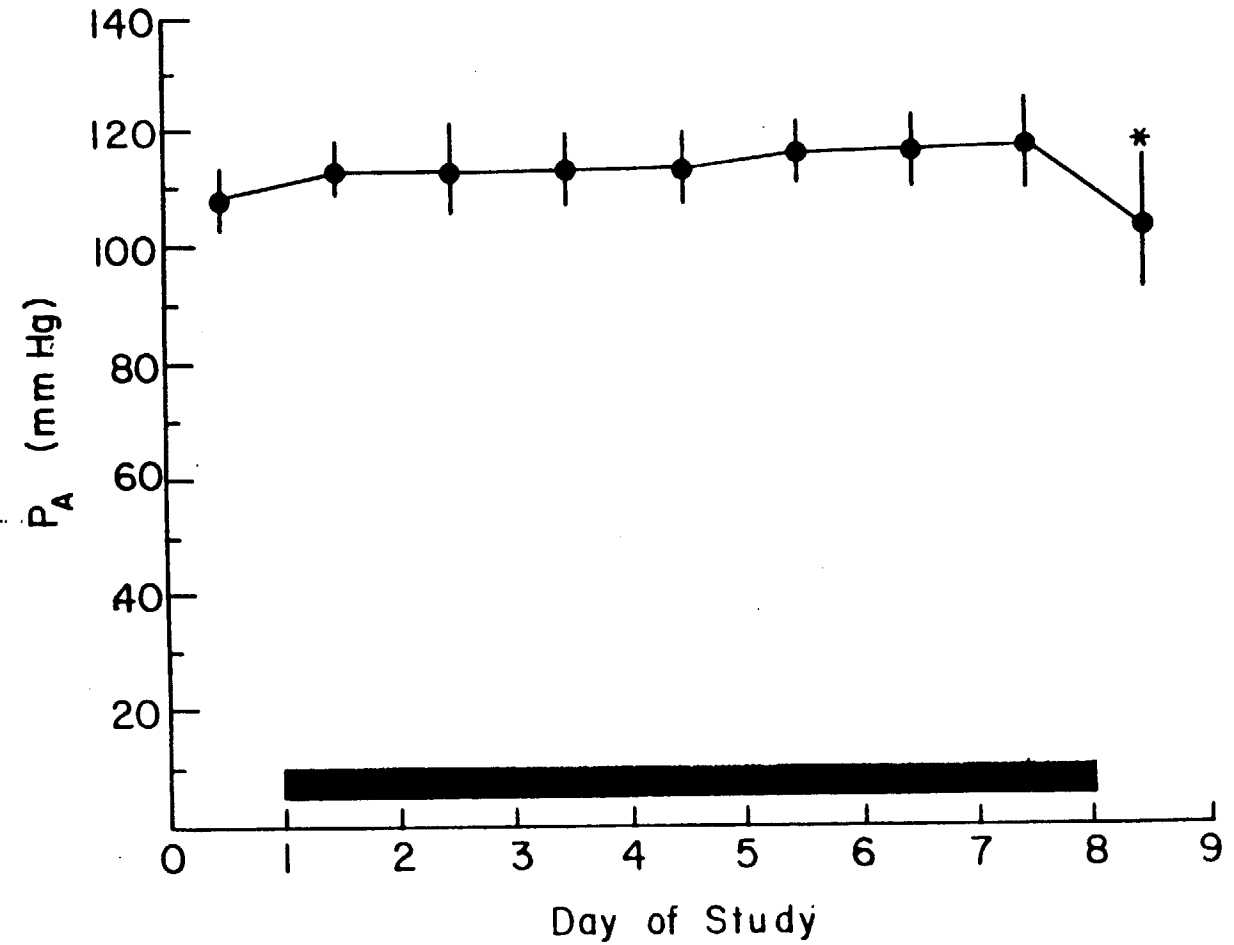


FIGURE 3

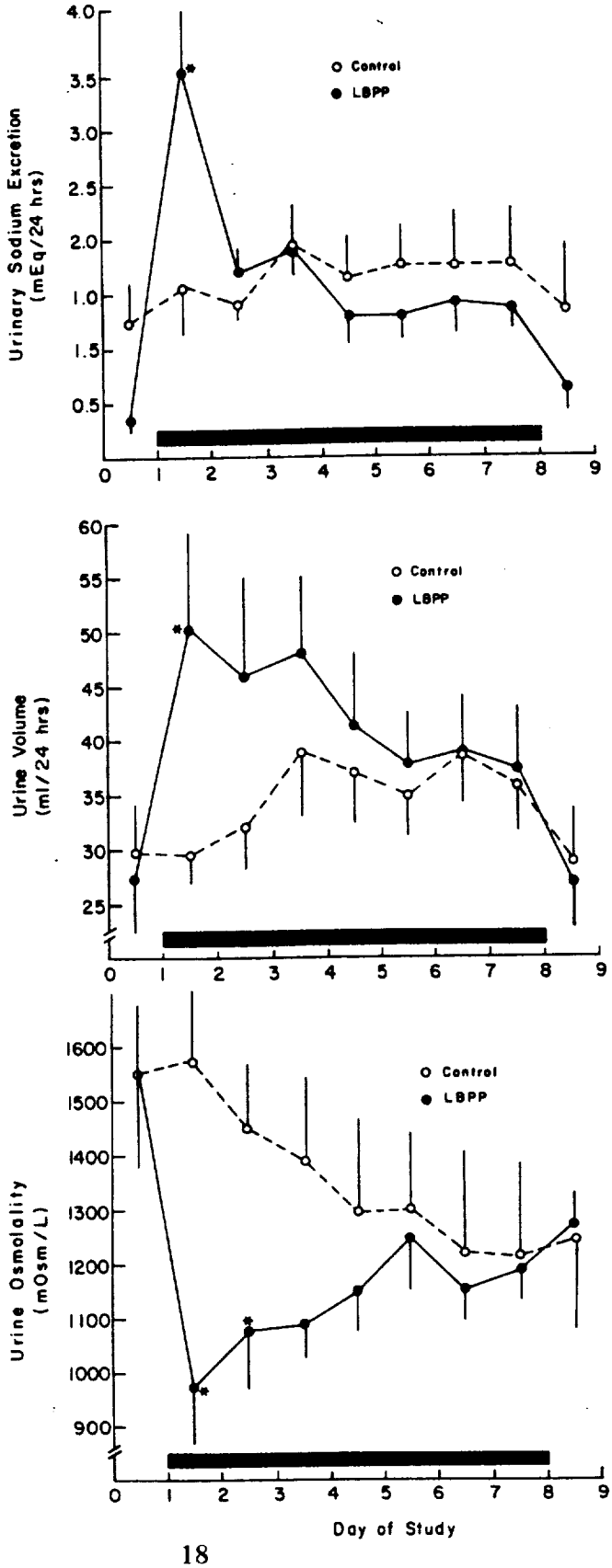

Urinary Sodium Excretion (mEq/24 hrs)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
1
2
3
4
5
6
7
8
9
Control
LBPP
Urine Volume (ml/24 hrs)
60
55
50
45
40
35
30
25
Urine Osmolality (mOsm/L)
1600
1500
1400
1300
1200
1100
1000
900
Day of Study

FIGURE 4

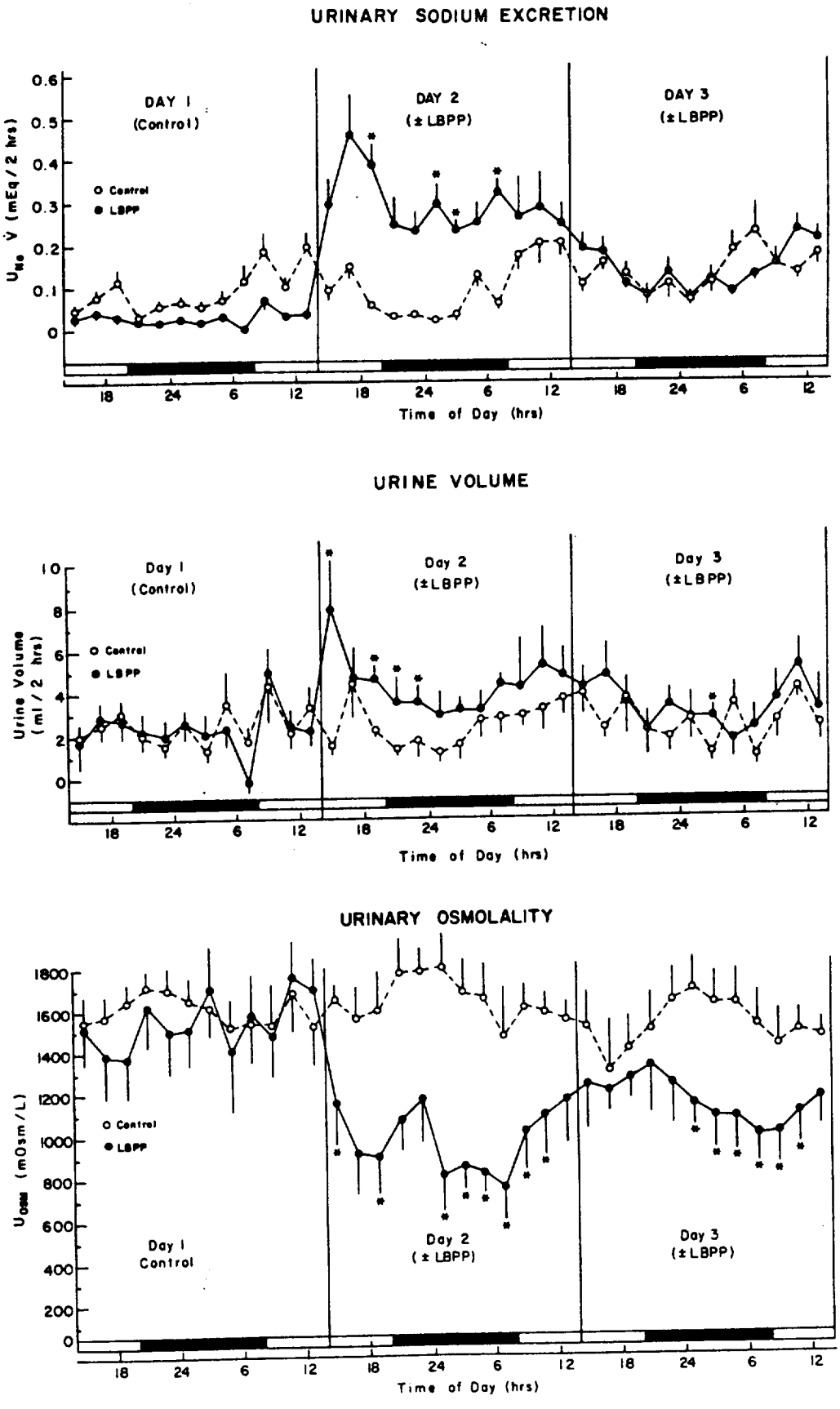

URINARY SODIUM EXCRETION
DAY 1
(Control)
DAY 2
(± LBPP)
DAY 3
(± LBPP)
O Control
● LBPP
Time of Day (hrs)
URINE VOLUME
Day 1
(Control)
Day 2
(±LBPP)
Day 3
(±LBPP)
Urine Volume
(ml / 2 hrs)
Time of Day (hrs)
URINARY OSMOLALITY
Day 1
Control
Day 2
(± LBPP)
Day 3
(±LBPP)
Time of Day (hrs)

FIGURE 5

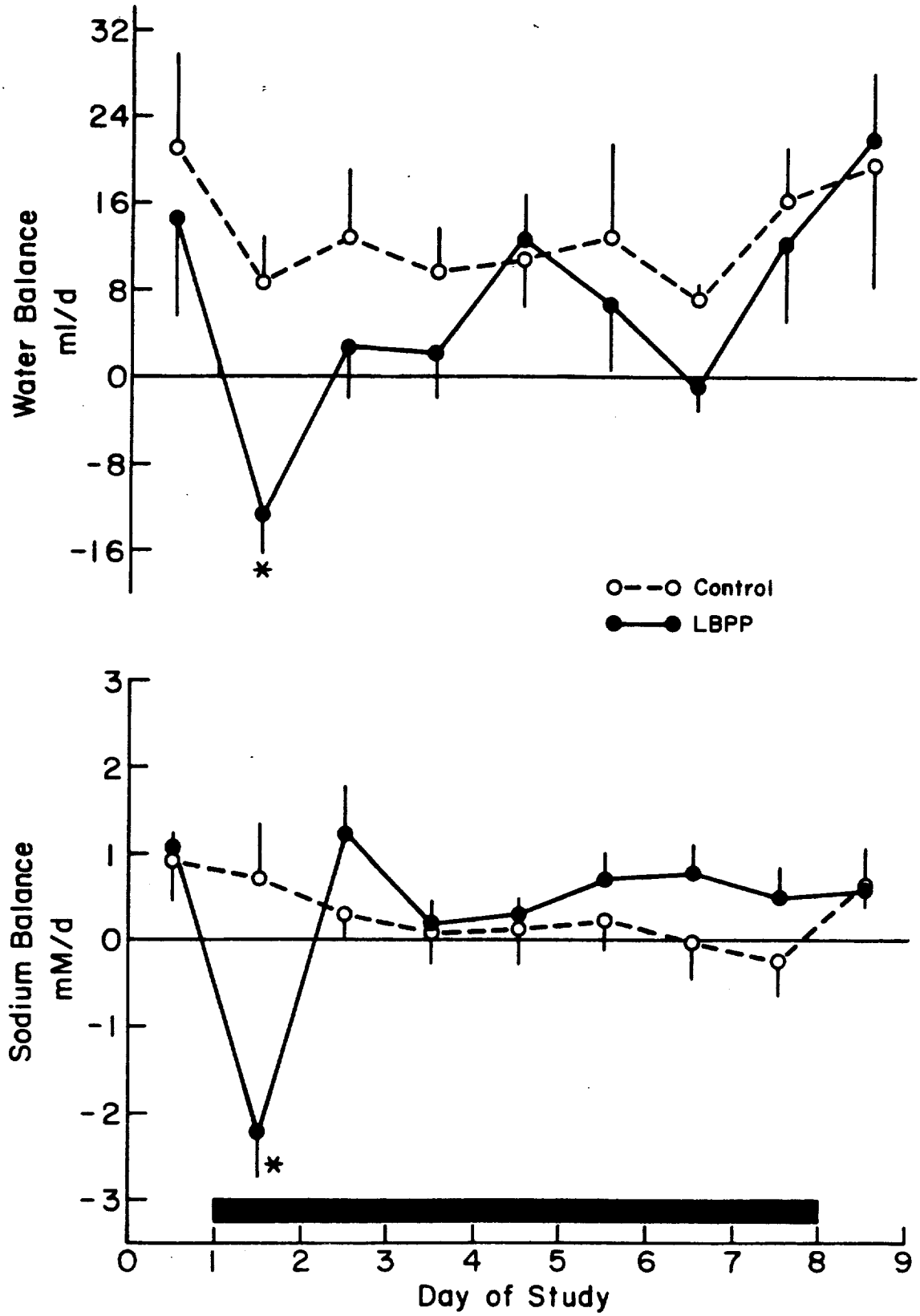
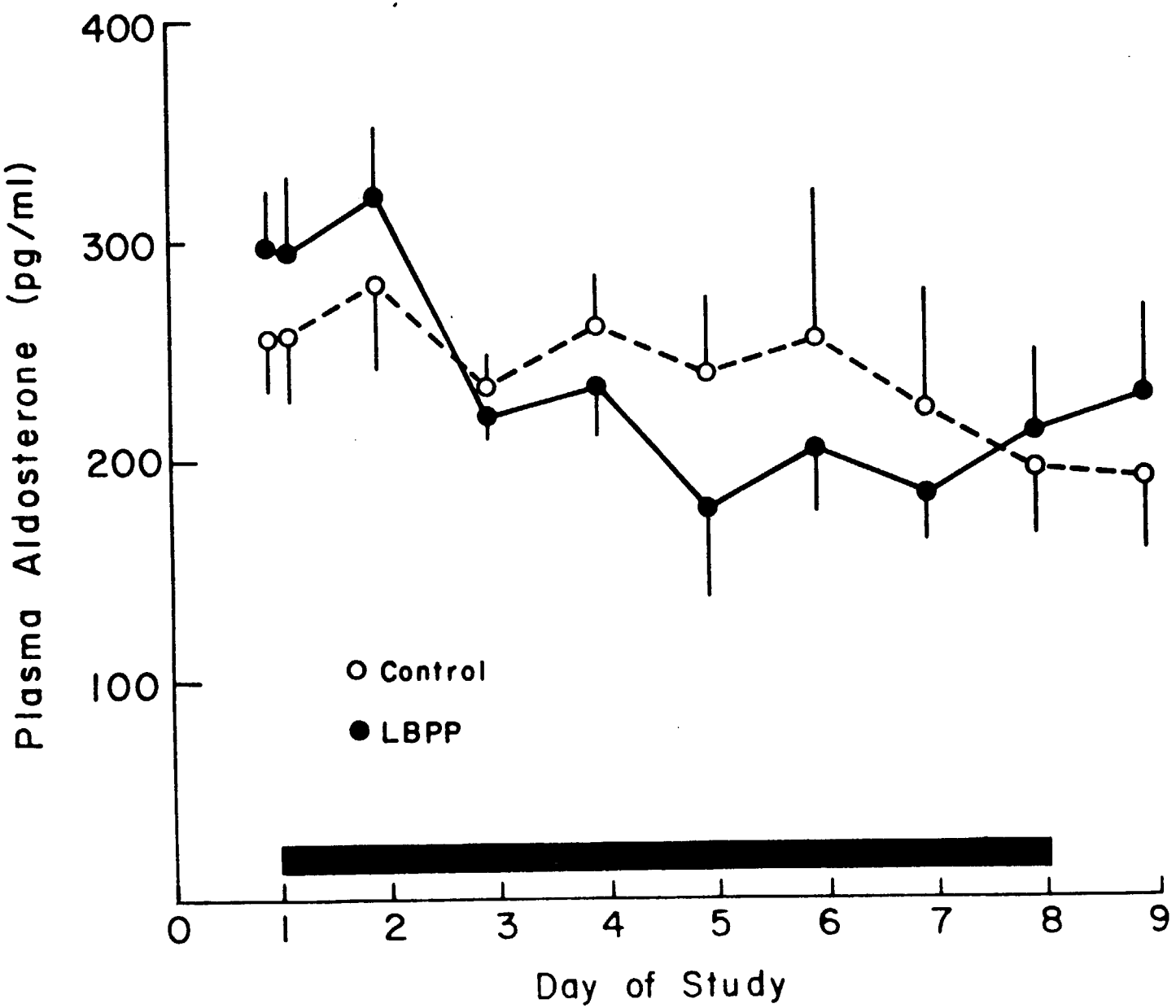

Water Balance
ml/d
32
24
16
8
0
-8
-16
*
Control
LBPP
Sodium Balance
mM/d
3
2
1
0
-1
-2
-3
*
0
1
2
3
4
5
6
7
8
9
Day of Study

FIGURE 6


400
300
200
100
Plasma Aldosterone (pg/ml)
O Control
● LBPP
0
1
2
3
4
5
6
7
8
9
Day of Study

APPENDIX A

PROCEDURE FOR MAINTENANCE OF CATHETERS

1. Steam sterilize instrument tray containing Kalt clamp, gauze 2x2 pads, and needle holder.

2. Prepare and keep sterile necessary syringes:

   a. Wipe top of sterile saline vial with alcohol wipe. Place sterile needle on sterile 1 cc syringe and use to withdraw approximately 1/2 cc of saline from vial. The same needle can be left in place and used for all syringes, but should be removed immediately after this procedure. Open sterile pack containing two 21-gauge needle adaptors and attach syringe to adaptor: leave in pack to maintain sterile. Prepare 1 syringe for each catheter and insert in separate bag. Each bag will end up containing all the necessary syringes (3) for flushing each individual animal.

   b. Use above procedure to fill same number of syringes with 1 cc of sterile saline. Attach to second needle adaptor remaining in each of sterile packs.

   c. Use above procedure to fill same number of sterile syringes with dextrose:heparinized saline mixture.

3. Chair monkey and C-clamp back plate at appropriate angle to permit access to catheters.

4. Unzip jacket far enough for unhindered access to catheters.

5. Remove catheters from velcro pocket, untape and uncoil carefully taking care not to rotate entire catheter.

6. Saturate several 2x2's in sterile pan with 70% ethyl alcohol. Hold obturator with needle holder and wipe catheter from the obturator toward the back of the monkey. Repeat several times.

7. Clamp catheter with Kalt clamp taking care to position clamp such that this can be rested on the chair without any pull on the catheter. Throughout all procedures great care must be taken to prevent the weight of the clamp falling directly on the catheter. The tips of the clamp should be filed down slightly and protected with pieces of tubing to prevent crimping or cutting of the catheter.

8. Using an alcohol or dry 2x2 to hold the catheter tip in one hand, use the other hand to remove the obturator from the catheter by grabbing with alcohol swab or needle holder. Place the obturator in the sterile tray and cover with a puddle of 70% alcohol.

9. Syringe the tip of the catheter with a stream of 70% ethyl alcohol.

10. Using an alcohol or dry wipe to hold the catheter tip, attach the syringe half-filled with saline, release the Kalt clamp, and withdraw the fluid in the catheter deadspace until blood reaches the tip of the catheter. If necessary, a slight inward flush should be used to free the tip of the catheter of clotted blood. Replace the Kalt clamp, remove syringe and needle adaptor and place to one side.

11. Attach second saline-filled syringe to catheter and release Kalt clamp. Flush approximately .7cc of saline (this amount will vary according to the catheter length) into the catheter at a brisk rate. This step is designed to free the catheter lumen from cells and clotted blood. The monkey will normally feel the cool fluid and shake his upper body in response. Replace the Kalt clamp.

12. Remove the syringe to a non-sterile area, leaving the needle adaptor on the catheter. Attach the dextrose:heparinized saline syringe to the needle adaptor after first introducing sufficient fluid into the needle adaptor hub to prevent the occurrence of air bubbles. Release Kalt clamp and carefully fill the deadspace of the catheter with this mixture (deadspace volume is measured at time of surgery and is usually approximately .15 cc - overfill slightly, i.e. to .2 cc).

    The following is very critical:

    a. Maintain positive pressure on the syringe plunger (to prevent back-bleeding) until the Kalt clamp is on the catheter. It is imperative that the Kalt clamp clamp tightly enough to prevent back flow into the catheter lumen. This obviously is more important for the arterial catheter than the venous, as hydrostatic pressure is much greater in the former.

13. Rinse tip of catheter with alcohol stream or dip in beaker of ethyl alcohol. Ideally the catheter tip should be cut back with a sterile blade at each flush (the rough edge of the end will trap

bacteria), but for chronically-maintained animals this is obviously impossible. This should, however, be cut back periodically in order to ensure a tight fit of the obturator.

14. Pick up obturator with sterile needle holder and insert 3/4 of its length into the catheter. Release Kalt clamp and push remainder of obturator into catheter, thereby displacing blood that would have backed up into the tip when the area under the Kalt clamp was freed. Inspect catheter for leaks around the obturator or blood in the line.

15. Repeat for other catheter.

16. Carefully coil catheters and secure coil with adhesive tape taking care to ensure that the catheter is not rotated and that the obturator is safely protected. Insert catheter coils in velcro pocket, taking great care to leave a sufficient loop of catheter outside the pocket for adequate strain release.

17. Inspect entire back area carefully, paying particular attention to the exit holes of the catheter and the skin area over the route of the catheter under the back skin.

18. Close jacket zipper and secure zipper tab with a cable tie which is passed through the zipper handle and the bottom margin of the jacket.

19. Once every 1 to 2 weeks a sterile blood sample should be taken from both catheters with a sterile syringe and cultured on blood agar plates to monitor for line infections. After Step 10, blood should actually be withdrawn into the saline-filled syringe, the syringe and needle adaptor removed and another syringe and needle adaptor carefully inserted, and a small blood sample taken and transferred carefully to the shielded surface of the blood agar plate. The sample should be streaked at a later time with a sterile wooden stick. These plates are incubated at appropriate temperature and time; if bacterial colonies are evident, submit to the veterinary for identification and sensitivity testing.

APPENDIX B

ENGINEERING REQUIREMENTS STUDIES

EXPERIMENT NO. 223
FLUID AND ELECTROLYTE HOMEOSTASIS

REPORT FOR SUPPORTING STUDY #4
ENGINEERING EVALUATION

TO: JIM CONNOLLY, NASA ARC
FROM: SUSANNE CHURCHILL, HMS

This study was conducted in order to gather baseline data in support of engineering design requirements for SL-4 experiment #223. Two adult male flight size squirrel monkeys were studied for a seven day period according to the standard HMS Lower Body Positive Pressure (LBPP) control protocol. These studies were conducted according to a plan previously agreed upon by the NASA Engineering Staff and the HMS Science Staff (Appendix A). Animals were placed in restraining chairs on 11/29/82; data collection commenced the following day (11/30/82) at 10 am and continued until 10 am on 12/7/82 (Day 7). The NASA team, Jim Connolly and Henry Leon, were present for the first four days of this study (11/29 -12/2/82). A preliminary report of this test was made at the PDR meeting held at ARC 12/17/82.

PROTOCOL

Please refer to Figures 1-6 for graphic illustration of the text.

Animals #856 and #861 were selected for their general good health and ability to tolerate extended chair sitting. On Monday, 11/29 (Test Day 0), these two animals were placed in plexiglas restraining chairs located inside isolation chambers with a controlled 12 hour light-dark cycle (8am - 8pm)(Fig. 1,2). Both animals sat on perches padded with water cushions and wore urine collection devices designed and fabricated by Sallie Petrou of this laboratory (Figures 3,4). Connector tubing passing from this device through the isolation chamber floor conveys urine to a programmable fraction collector. The scrotal area had been previously treated with Skin-Prep dressing wipes. All points of contact with the urine device were well padded with soft foam. Animal 856 had a special perch allowing him to squat rather than sit with legs extended. Water was freely available from a ball valve spout mounted on the right side of the chair at mouth level. Food (Bio-Serve Reward Diet #T-130) was available ad lib from a food dish fixed to the left side of the chair at waist level. Both animals had been on this diet for a month prior to the test. Feces were collected in a container filled with sawdust and positioned under the animal's anus.

Both animals had indwelling arterial and venous cathers, implanted as indicated in Table 1 according to the standard HMS protocol. These catheters, which pass through the skin just below the level of the shoulder blades and extend several inches

beyond the animal, extended, were joined to sterile connector tubing (lengths and specifications given in Figure 5) by a hollow stainless steel connector. That part of the catheter which passes under the jacket was protected by a shield of tygon tubing which is in turn fastened in place by ties through the back of the jacket. The connector tubing passes out from under the bottom margin of the jacket (still enclosed by the tygon tubing) and travels under a lucite horizontal waist restraint plate. A thick walled tygon shield carries the catheters from the chair to the chamber wall where they pass through to the transducer domes, mounted at appropriate levels (level with each catheter tip) on the exterior of the isolation chamber (Fig. 6). The transducers (HP#1280C) are interfaced to signal conditioners (HP #8805D) through a computer directed multiplexer system. A Hewlett Packard four channel recording system provides hard-copy display of the pressures.

After the catheters were connected to the transducer domes and the infusion begun (heparinized saline, 10 U/ml, at a rate of 0.027 ul/min into each arterial and venous catheter by means of Harvard Apparatus pump #975), the thermistor probes (YSI #27) were attached to the back according to the protocol set forth in NASA Communication of 6/14/82 R. Mains to J. Connolly, "NASA/ARC Experience with Skin Adhesives". Briefly, a small patch of hair was shaved from the back lateral to the spine and just under the shoulder blades. The skin was scrubbed with a teflon pad and sponged with alcohol. The probe was attached with cyanoacrylate glue and a patch of microfoam tape taped over the thermistor lead for strain relief. The temperature probe lead also ran through the protective tygon tubing through the cage wall to the multiplexer.

The animals were then allowed to adjust to the restraint overnight. At 10 o'clock Tuesday morning (Test Day 1) the transducers were zeroed and data collection was begun by means of our computer driven system. Specifically:

1. Arterial and venous blood pressures were monitored continuously. These data were sampled and recorded by the computer every 15 minutes throughout the experiment. At the same time hard copy was provided by means of the computer activated chart recorder (5 sec sample). With this system data could also be accessed at any moment on on the terminal screen. At 10 am every day all transducers were rezeroed to atmospheric pressure. Pressures were measured and recorded as systolic, diastolic and mean arterial pressure, and mean venous pressure.

2. Heart rate was monitored and recorded as above by means of an HP heart rate signal conditioner (8811) enslaved to the arterial pressure signal conditioner.

3. Skin temperature was monitored and recorded by a direct digital output from the multiplexer to the computer.

4. Urine was collected under oil in hourly samples by the computer activated fraction collector. Samples were measured for volume daily and frozen (4° C) for later analysis.

5. Daily food and water consumption was determined by weighing and recorded in the study log.

6. A daily (10 am) 1cc blood sample was taken (method in Appendix B), spun in a refrigerated centrifuge and the plasma frozen (-30° C) for later analysis. Hematocrit was also measured and recorded.

7. Feces were weighed daily and amounts recorded in the log.

8. Daily health status assessment was made and comments recorded in the computer log file.

On Test Day 7 both animals were removed from the restraint system, bathed in warm water and checked carefully for any signs of abrasion or soreness. Both animals appeared in excellent health. The photographs in Figures 1-3 were taken at this time. Blood culture was also performed on an aliquot of the last sample and found to be negative for both animals.

RESULTS

Mean control values and test data are presented in Tables 2-5 and Figures 7-8 as indicated below:

Body Weight	Table 2
Cardiovascular Measurements	Table 3
Skin Temperature	Figures 7A and B
Urine Output	Figures 8A and B, Appendix C
Food and Water Consumption	Table 4
Feces Output	Table 5

DISCUSSION AND EVALUATION

We feel that this test successfully represented our typical 7-day control study protocol. Both monkeys ate and drank appropriately and experienced no obvious physical or psychological problems due to the confinement. All systems, with the exception of the skin thermistors, operated according to specification. The individual test components are discussed below.

We would like to emphasize, however, that this test is comparable to what should happen during spaceflight only in the basic techniques and methodologies employed and in the parameters which need to be measured. The actual values anticipated will be quite different and can best be estimated only by use of LBPP.

1. Body Weight. As presented in Table 2, neither animal experienced a significant change in body weight during the study. The decrease in weight seen in #856 during the month pre-test reflects this animal's apparent dislike for the BioServe diet as he had to be deprived of the normal TEKlad diet in order to switch him onto the pelleted food. Once accustomed to it, however, he steadily gained weight on this diet.

2. Cardiovascular Measurements. Continuous infusion of heparinized saline into each arterial and venous line at the specified rate keeps these lines patent and enables continuous monitoring of both pressures. Data were presented as ranges (Table 2) to provide reasonable estimations of the limits encountered during our control studies. We anticipate that all values will be higher during launch and re-entry and that the mean venous pressure will be sustained higher for at least the first half of the spaceflight. We find this system ideal because we are able to 1)sample and store specific values every 15 minutes in the computer memory, 2)have a hard copy backup of each sample and 3) have the capacity to evaluate cardiovascular status at any instant on the terminal screen.

Unfortunately, we have just recently identified a number of systems design problems with the HP 1280C pressure transducers that may invalidate certain of the pressure measurements. These transducers have such a prolonged mechanical settling time following activation by the multiplexer that our values may have been sampled (15 seconds after changing channels) before the real pressure value was on line. While I would not expect the arterial values to be very different from those reported, the actual venous values may be somewhat different (same general range, however). In addition, due to a serious negative zero drift problem with these transducers, all venous values are 0 - 5 mm Hg below what they should be. This explains the rather unbelievable and frequent very negative mean venous pressure values. This should only be negative (and then by a few mm Hg) when the monkey lowers his trunk below the level of the zero port on the transducer. The occasional moderately high mean venous pressure occurs whenever the animal speaks, defecates or micturates (Valsalva maneuver). The one very high venous pressure (509 mm Hg) would appear to have occurred as a result of a clot in the line (self correcting due to the pressure build up). We are presently correcting the transducer problem by switching to the HP

#1290 transducer, which after careful evaluation does not have the same problems as the 1280. The 1290 is more sensitive to temperature shifts, however, and as this is not a linear effect will give rise to new problems if not used in a temperature controlled area.

2. Skin Temperature. Fixing the probes with superglue worked quite nicely - the animals seemed to have no awareness of the entire process or the presence of the probe. The microfoam tape stuck well to #85# but would not stick well at all to #861. I had occasion to open the jacket of #85# on day 5 in order to retie the tygon tubing and noticed that the probe had come unglued at that time. The probe of #861 was unglued on day 7 when the animals were removed from the protocol. I do not think this was a fair test of the protocol however, as the tygon shielding and the catheters undoubtedly caused a lot of pushing and tugging on the thermistor probe. Animal #856 continued to show a nice circadian temperature rhythm even after the probe fell off (I left if free floating under the jacket). Animal #861 also demonstrated a temperature rhythm but within a narrower range than that seen for animal #856. This may indicate that the probe came off fairly early into the test. The rhythm is not very apparent in Figure 7B as the scale is compressed. I have indicated the high and low values in the insert.

4. Urine Output. We have combined the hourly values into four hourly sums for purposes of comparison with the flight protocol (Figures 8A-B,Appendix C). While there is some circadian variation in the urine volumes, there does not appear to be a clearcut period during which urine volume is significantly reduced. Once again, however, the definitive test for this information remains the LBPP protocol. The highest volume obtained for any four hour period was 32.8 ml.

5. Food and Water Consumption. Table 4, self-explanatory.

6. Feces Output. As indicated in Table 5, feces were uniformly formed and quite dry throughout the test. Volume varied with intake.

FIGURE 1

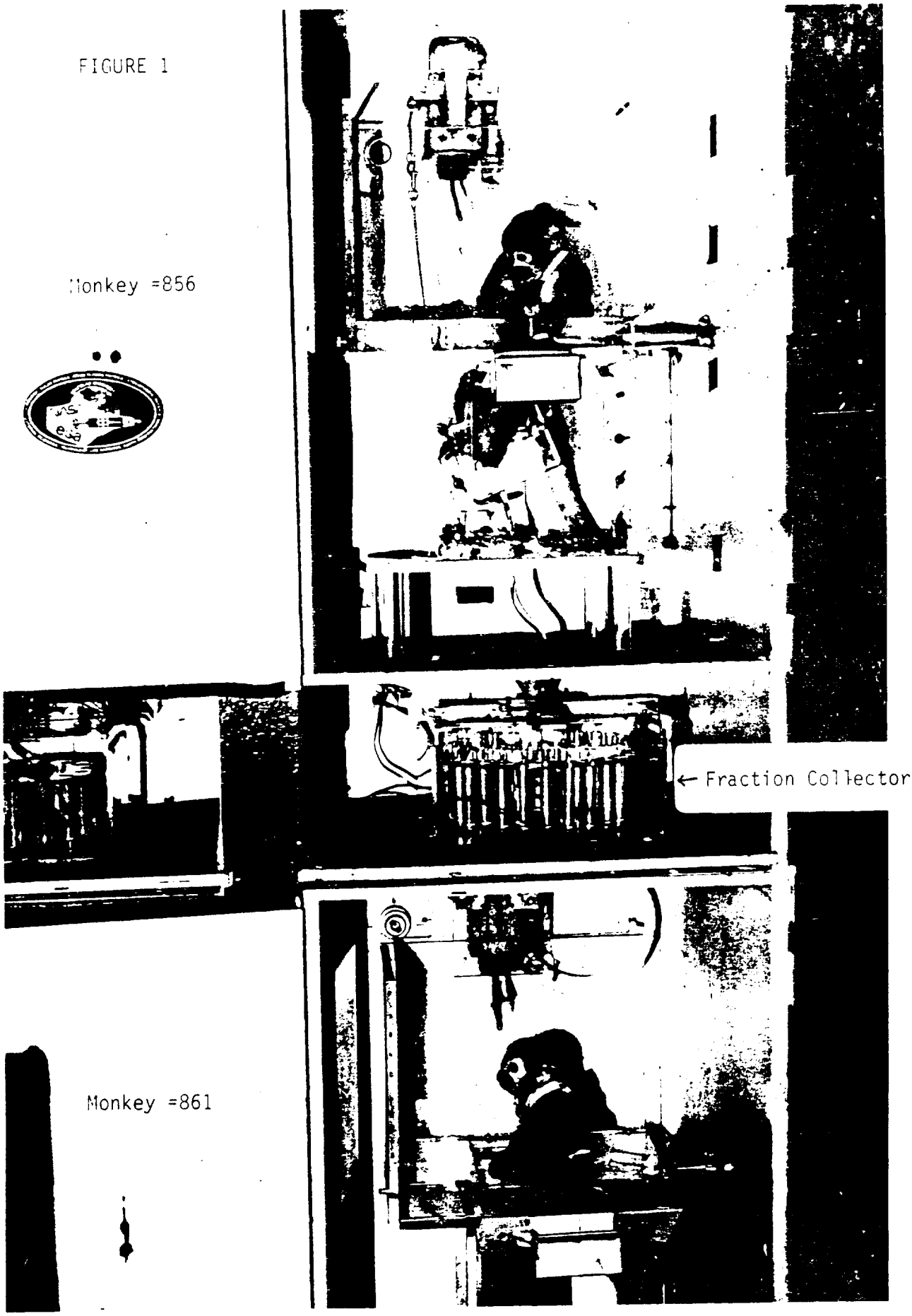
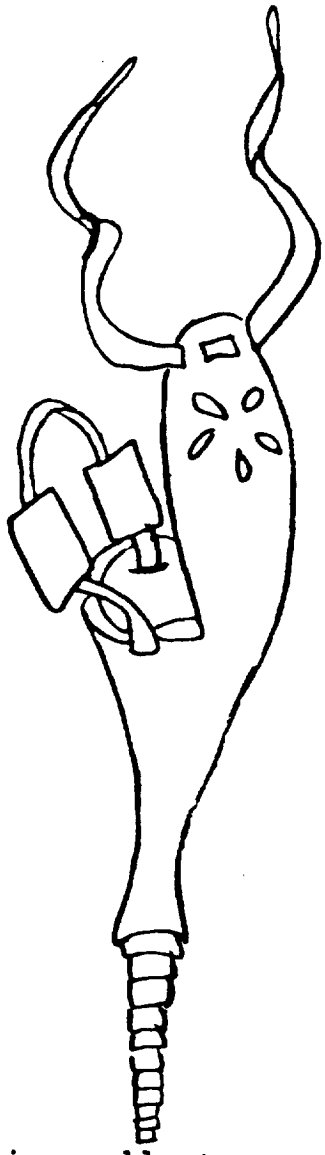


FIGURE 3



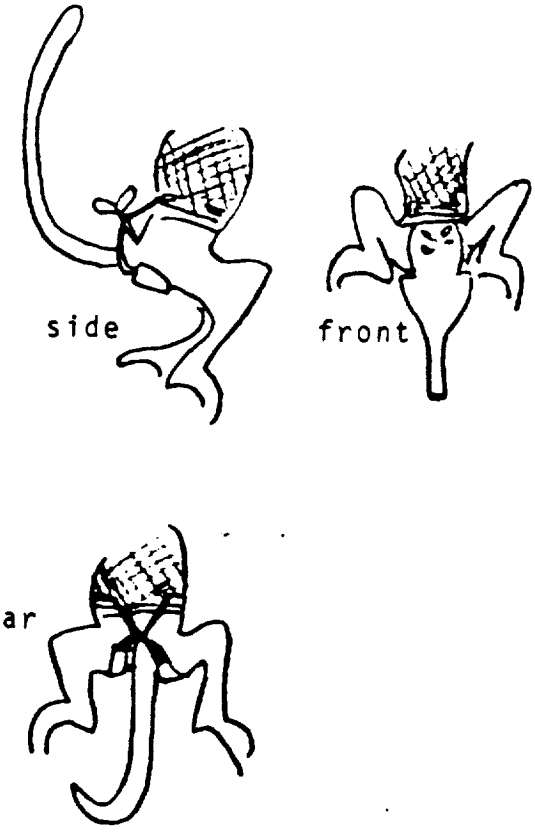


Figure 4 -Urine collector

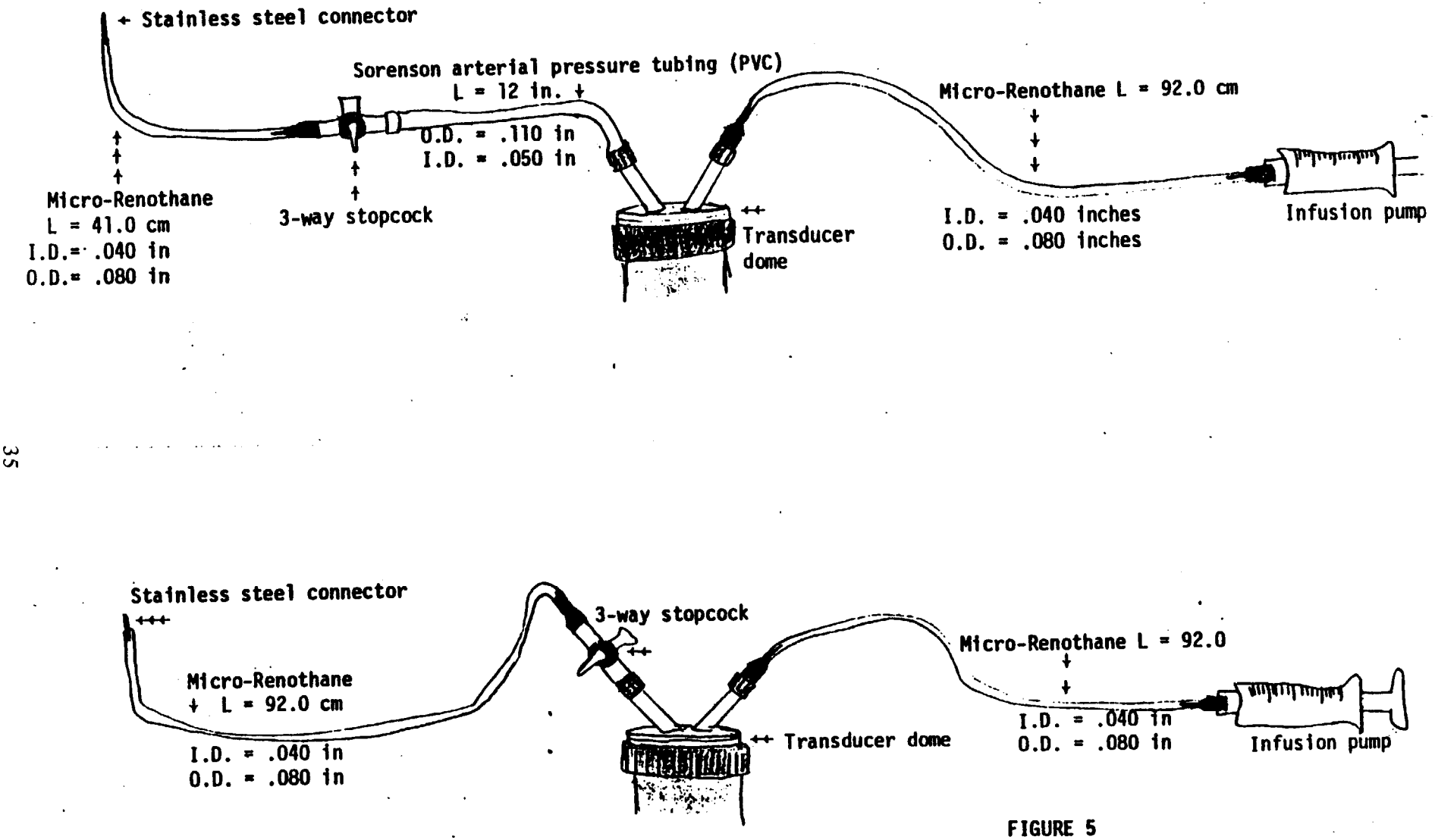
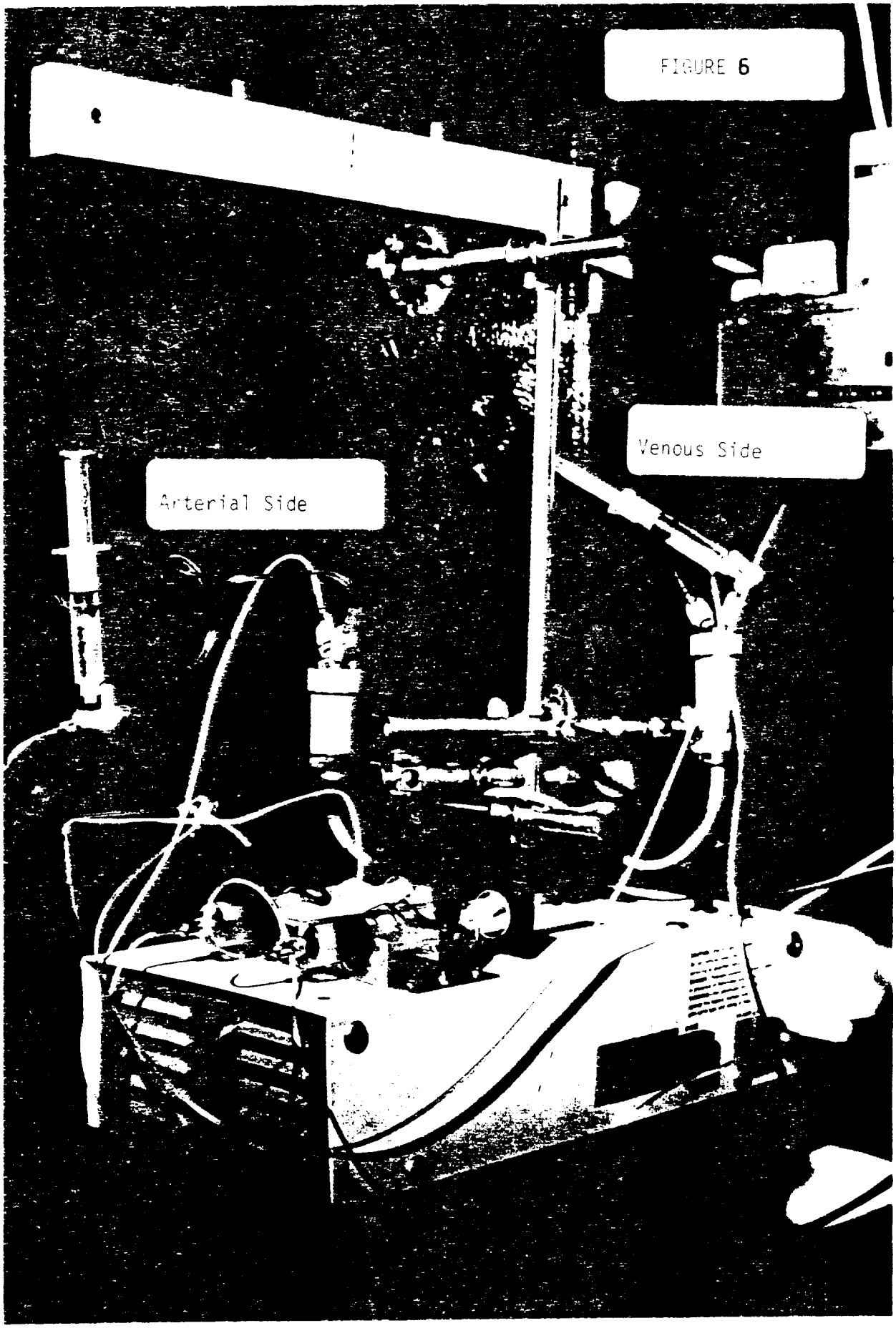
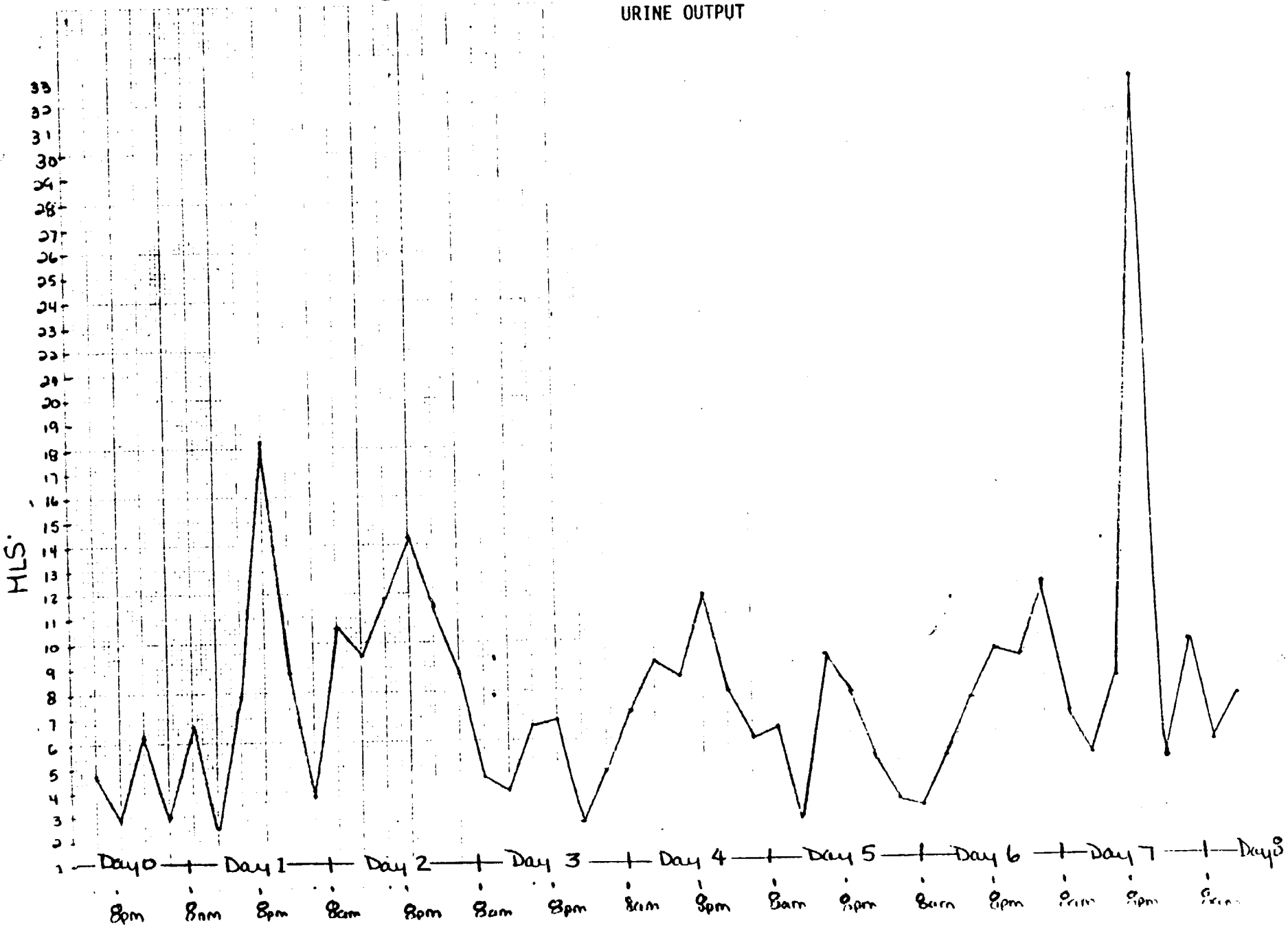


FIGURE 5
INFUSION SYSTEM



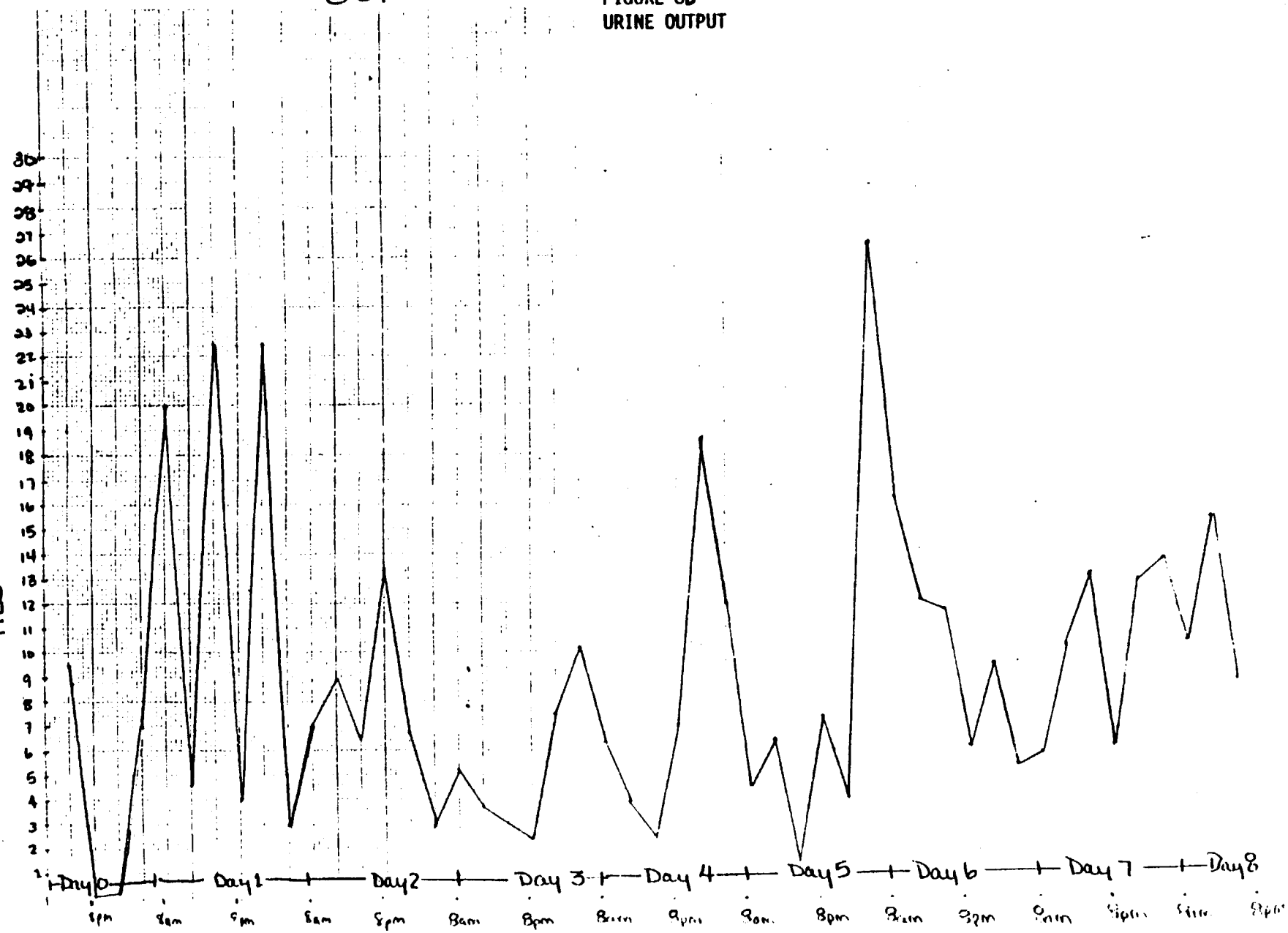
#856

FIGURE 8A
URINE OUTPUT



#861

FIGURE 8B
URINE OUTPUT



APPENDIX A

SL-4 COMBINED SQUIRREL MONKEY EXPERIMENTS TEST PLAN

GOAL

In order to obtain critical design requirement data for the major hardware items to be developed (urine collection, blood withdrawal/infusion/ pressure, and skin thermistors) a combined experiment test should be conducted. The test should be done as soon as possible and minimize new hardware and analytical technique development. The test site should be at HMS because the Moore-Ede experiment preparation is more complicated and there is full-time staff available to provide the intensive support necessary for this effort. The projected test start date is November 29, 1982. The test should produce 72 continuous hours of data on two subjects. Subjects could be installed on Monday and removed on Friday with the 3 days in between as an uninterrupted data collection period.

METHOD

SUBJECTS

Description

Four adult, male, flight size (800-1200 gm) squirrel monkeys should be selected as test candidates. The candidates should be pre-selected for their ability to accept collar and chain handling, chair restraint and consumption of normal caloric amounts of the specified pellet diet. The animals should also be acclimated to the jacket, urine collection system and temperature probes.

Conditioning

The four test candidates should be trained in the colony cage to daily handling using the collar and chain. Also candidates should be gradually conditioned (over a 6-8 week period) to tolerate 4-5 days of seated restraint while wearing a nylon mesh restraint jacket. Subjects should be conditioned to eat the pellet diet and they should be placed exclusively on this diet at least two weeks prior to the start of the test and continuing though one week of the post-test period. After subjects are able to tolerate 24 hours of restraint, they should have the HMS condom-type urine collection system and dummy skin thermistors applied during most of the remaining conditioning trials.

Surgical Implantation

The three most promising test candidates of the group of four should be implanted with the HMS arterial and venous catheter systems a TBD time prior to test initiation. Protective jackets should be applied to the subjects continuously to protect the exteriorized catheters. Catheters should be perfused periodically and maintained according to the standard HMS procedures. Samples of blood should be withdrawn periodically via the catheters and cultured for bacterial growth to ensure that infection is not present.

Test Candidate Selection

Selection of 2 of the 3 implanted subjects will be made on the morning of test day 1 by reviewing each subject's conditioning and clinical records. The rationale for subject selection should be included in the test report prepared by HMS at the end of the test.

HARDWARE

Description

At a minimum, two test hardware assemblies are required for the conditioning and test periods and items for each subsystem are discussed below.

Skin Temperature

Only 2 skin temperature will be measured at thoracic (central) and base of the tail (peripheral) sites. Aa 3/16 in. O.D. Yellow Springs Instrument (YSI) small surface temperature probe should be glued to the above sites during test assembly by the method described in the report "NASA/ARC Experience with Skin Adhesives," dated 6/14/82. The leads should be routed under the jacket to an exit site below the waist divider and then to the HMS data acquisition system. The thermistors should be taped below the lead for strain relief.

Feeder and Control System

This system is under development by NASA/ARC but cannot be supplied to HMS in time for this test. The BioServe small primate pellet diet will be supplied ad lib to the test subjects in a small cup. The schedule for administering the diet is described in this test plan. HMS should order appropriate amounts of this food from BioServe, Inc.

Waterer

Water will be supplied ad lib via a gravity-feed throughout the conditioning and test periods. This system should be installed on the colony cage throughout the pre-test period.

Isolation Chamber

An enclosure with a door will provide sound and visual isolation and environmental control for each subject. The chamber will contain the subject and restraint system, the feed and water systems, urine colleciton system and provide ports for exit of vascular catheters, urine colleciton tube, water line and the thermistor leads. It will control temperature to TBD values and provide a light/dark cycle of 12:12 with the light cycle beginning at 0800 hours. The light level should be 600 lux for the day and less than 1 lux for the night. The chamber should be opened during the test period only for feeding and excreta collection once/day except for emergencies. The chamber should not be opened at night except for removal of the test subject in an emergency.

Restraint Chair

The chair provides restraint at the waist with the subject in the seated position. A leg divider will not be used since leg thermistors will not be used and the present urine collection system does not require it. This chair should allow a feces collection to be made daily.

Restraint Jacket

A nylon mesh jacket will be worn by subjects at all times after exteriorization of the vascular catheters. Also the thoracic thermistor will be protected by the jacket from the subject.

Urine Collection System

The current HMS system includes a condom-type scrotum cup, transport tube and 4 hour fraction collector. The fraction collector is computer controlled and urine will be automatically collected from outside the isolation chamber twice/day.

Blood Withdrawal/Infusion/Pressure System

This system includes arterial and venous catheters, each attached to a pressure transducer, blood withdrawal valve and constant-rate infusion pump. The pressure transducers monitor pressure from fluid-filled catheters. The blood withdrawal valve permits by-passing the infusion pump so a daily arterial blood sample can be taken in a syringe. The infusion system will provide constant low-flow flushing of the catheter lines with heparinized saline to ensure catheter patency and accurate pressure monitoring. The blood withdrawal volume, catheter infusion rate, anti-coagulant concentration and saline fluid replacement (if used) should closely duplicate the methods proposed for spaceflight. Vascular pressures and heart rate (from the arterial pulse) should be measured continuously.

Development

For this test the major items that require development are: (1) the computer-controlled cardiovascular data acquisition system and (2) the skin temperature attachment method and data recording system. Development support should be provided to HMS for item (2) by NASA/ARC.

Testing

In order to ensure that all hardware is functioning properly, a 24 hour dry-run test should be conducted with the complete system and two fully instrumented subjects by November 22nd so that time is allowed for correcting any problems encountered.

DATA

Pre-Test

The following data should be collected on the catheterized test candidates prior to the test;

- Weekly Body Weight (gm) in cage and restraint
- BioServe Pellet Consumption (gm/day) in cage and restraint
- Urine Production (approx. ml), consistency and diet influence on
- Heart Rates and Vascular Pressure during restraint
- Catheter Maintenance Requirements (blood culture, antibiotics, etc.)

Test

The following data should be collected on the two test subjects during the test;

- Body Weight (gm) before eating, prior to chairing
- Continuous Arterial Pressure (mm Hg) with systolic, diastolic and mean values
- Continuous Venous Pressure (mm Hg) with mean values
- Continuous Heart Rate (beats/min) from arterial pulse
- Catheter Infusion Volume (ml/day)
- Heparinized Saline Concentration (mg/ml)
- BioServe Pellet Consumption (gm/day)
- Water Consumption (ml/day)
- Urine Production (ml/4 hr)
- Feces Weight (approx. gm), consistency
- Skin Temperatures (deg. C), 5 seconds duration every 15 minutes
- Daily Blood Sample (ml)
  - Plasma
  - Hematrocrit (ml)

Post-Test

The following data should be collected on the 2 test subjects for 1-4 weeks after the test;

- Body Weight (gm) before eating, after removal from chair and at week intervals for 4 weeks
- BioServe Pellet Consumption (gm/day) for 1 week in cage
- Water Consumption (ml/day) for 1 week in cage
- Feces Weight Consistency for 1 week in cage
- Colony SKF Diet Consumption (gm/day) for 3 weeks in cage
- Photos of Penis and Scrotum
- Evaluation of skin thermistor attachment and lead routing (photos)
- Evaluation of conditioning level of subject from physiological data.

TEST PROCEDURE

Test Initiation

The test should begin sometime on November 29 and end on December 3, 1982. The subjects should be installed in the test without tranquilization. The 3rd subject will serve as a back-up throughout the test but will remain in the colony cage otherwise. A Test Log should be kept by the Test Coordinator (Dr. S. Churchhill) and the NASA Test Monitor. All pertinent science and hardware-related events should be recorded. A copy of this Log should be attached to the 30 day reports.

Daily Protocol

0800 - Lights on
- Urine collection (2000, 2400, 0400 hr samples from previous day), note precipitate and freeze
- Record pellets eaten and refill cup
- Record water consumed and refill
- Collect feces, weigh, take sample and place in freezer, dump pan and refill with shavings
- Take 1 ml arterial blood sample, centrifuge, read hematocrit, remove plasma and freeze

1200 - Urine collection (automatic)
1600 - Urine collection (0800, 1200, 1600 hr samples from this day) note precipitate and freeze
2000 - Lights off

TEST RESULTS

HMS will provide a joint preliminary data analysis summary and a list of recommendations to NASA within 30 days of the end of the test and a final report within 90 days. The final report may not include complete data analysis on the biological samples (blood, urine and feces) but should provide as much information as possible on hardware-related methodology. The data items listed in this report for the Pre-Test, Test and Post-Test periods should be addressed in the reporting of test results. The NASA test monitor will provide a test report with recommendations to the Project within 15 days of the test conclusion.

TABLE 1

CATHETER DATA

Animal No.	Species	Date of Catheter Implantation	Catheter Material
856	Wild Bolivian	5/12/82	Renothane
861	Domestic Columbian	10/20/82	Renothane

TABLE 2

BODY WEIGHT

(g)

DAY	ANIMAL NO. 856	ANIMAL NO. 861
1 Month Pre-Test	1380	1070
Test Day 0	1330	1075
Test Day 7	1355	1035
1 Month Post-Test	1480	1020

TABLE 3

RANGE OF CARDIOVASCULAR PARAMETERS

Animal No.	Test Day	Heart Rate (Beats/Min)		Arterial Pressure (mm Hg)						Venous Pressure (mm Hg) Mean	
				Systolic		Diastolic		Mean			
		Min	Max	Min	Max	Min	Max	Min	Max	Min	Max
856	0	154	296	116	155	116	140	109	146	-3	26
861		122	280	77	161	61	129	88	141	-2	38
856	1	134	295	118	168	90	127	110	143	-3	12
861		120	292	71	152	55	132	85	142	-5	30
856	2	124	264	124	158	109	124	116	143	-3	4
861		130	286	70	146	50	114	64	147	-5	33
856	3	138	291	149	190	123	172	140	181	-9	10
861		120	233	109	179	95	148	107	164	-10	10
856	4	167	263	112	180	95	165	103	166	-6	9
861		141	254	89	181	72	149	83	167	-8	5
856	5	168	302	108	149	89	125	103	137	-6	1
861		149	280	87	150	65	132	81	135	-4	3
856	6	144	247	118	152	103	133	111	138	-5	509
861		164	277	102	158	73	127	88	140	-7	22
856	7	146	245	121	156	98	133	113	144	-9	1
861		145	264	98	149	72	129	85	139	-7	8

TABLE 4

FOOD AND WATER CONSUMPTION

Animal No.	Average For Pre-Test Period	Test Day 0	1	2	3	4	5	6	7	$\bar{x}$ for 1-7	Average for Post-Test Period
		BIO-SERVE DIET CONSUMPTION (g/Day)									
856	33.5	53.6	17.7	86.3	51.6	70.7	32.1	40.0	36.6	47.9	29.6
861*	41.8	12.5	38.9	35.9	5.8	18.6	27.3	44.0	39.9	30.1	36
		WATER INTAKE (ml/Day)									
856	66	60	90	120	83	93	90	93	127	108	65
861	112	175	105	50	60	92	130	125	115	97	68

* This animal tends to throw his food out of reach.

TABLE 5

FECES WEIGHT
(g/Day)

DAY	ANIMAL NO. 856	ANIMAL NO. 861
0	21.2	14.4
1	10.5	13.3
2	40.0	5.0
3	14.1	3.9
4	16.4	10.7
5	10.9	7.3
6	11.3	16.7
7	11.5	11.0

Consistency uniform: fairly dry, well-formed pellets.

APPENDIX B

BLOOD SAMPLE PROCEDURE (as copied from Harvard Medical School protocol book)

Each experimental day a blood sample of 1 cc is taken from the arterial line. The sampling port is the flush syringe port on the 3-way stopcock located 12 inches from the transducer dome (see Figure 5.

To prevent the animal from bleeding back into the line, turn the animal "off" at the stopcock in the arterial line. Then turn off the infusion pump. Remove the flush syringe and place on sterile field. Place a sterile 1 cc syringe on the flush syringe port and withdraw the saline from the line. This step is considered complete once the blood appears non-diluted. At this stage, the fluid entering the syringe will look considerably more viscous and, because it is heavier, will stay in the bottom of the syringe. Close stopcock to the animal; remove this syringe and place on sterile field.

Place a new, chilled, sterile 1 cc syringe on the sample port; open stopcock to the animal and remove 1 cc of blood from the flush port on the arterial line. Once this is complete, close stopcock, remove the syringe and immediately transfer the blood to pre-labeled 1 cc conical polypropylene tubes containing Ca-EDTA. Shake to put the EDTA in solution and fill 2 to 3 micro-hematocrit tubes from the conical tube. Stopper the tube and place it on ice.

Turn the pump back on and allow it to develop a pressure head. Using the first 1 cc syringe, flush the line with the saline and blood which was initially in the line. Reconnect the original flush syringe and flush .1 or .2 cc heparinized saline (10 μ/cc) through the line to clear remaining blood cells. Reconnect the animal to the pump via the stopcock.

Remove conical tube with blood from ice and spin blood down in a cooled centrifuge. Once the blood has separated, pipet plasma from the top and store at -30° in a second pre-labeled polypropylene tube. Discard red blood cells and check lines for air or bleeding back. Centrifuge hematocrit tubes and read.

Materials

(2) 1 cc syringes (sterile)
(2) conical centrifuge tubes
    -1 containing Ca-EDTA (evaporated)
(1) centrifuges (hematocrit and standard)
    (from stock solution)
(1) ice bucket
(3) micro hematocrit tubes
(1) freezer at -30°C

APPENDIX C

URINE VOLUMES

(ml/4 Hr)

ANIMAL NO. 856

Time	Test Day								
	0	1	2	3	4	5	6	7	8
9-13	-	2.5	9.5	4.0	9.1	2.7	5.2	5.2	7.5
13-17	4.7	7.9	11.8	6.6	8.5	9.3	7.5	8.3	-
17-21	2.9	18.3	14.3	6.8	11.8	7.8	9.5	32.8	-
21-1	6.3	8.8	11.4	2.6	7.9	5.2	9.3	5.0	-
1-5	3.0	3.8	8.7	4.7	7.0	3.4	12.2	9.8	-
5-9	6.7	10.6	4.5	7.1	7.4	3.1	6.9	5.7	-

ANIMAL NO. 861

Time	Test Day								
	0	1	2	3	4	5	6	7	8
8-12	-	19.9	7.0	5.2	6.3	4.5	16.2	5.8	10.3
12-16	-	4.6	8.9	3.7	3.9	6.4	12.0	10.1	15.3
16-20	9.5	22.5	6.5	18.2	2.5	1.5	11.7	13.0	8.7
20-24	.1	4.0	13.4	2.4	7.0	7.3	6.1	6.1	-
24-4	.2	22.5	6.7	7.5	18.6	4.1	9.5	12.8	-
4-8	6.9	2.9	3.0	10.2	11.9	26.4	5.3	13.6	-

EXPERIMENT NO. 223

FLUID AND ELECTROLYTE HOMEOSTASIS

REPORT ON

EVALUATION OF NASA URINE COLLECTION DEVICE UNDER LBPP CONDITIONS

TO: JIM CONNOLLY, NASA ARC
FROM: SUSANNE CHURCHILL, HMS
DATE: 21 APRIL 1983

This study was conducted in order to: 1) evaluate the performance and biocompatability of the NASA prototype urine collection system for a 7 - 10 day period; 2) to measure urine output under conditions of LBPP in order to approximate the size requirement for the urine collection tubes. Two adult, male, flight-size squirrel monkeys were studied for a 7-plus day period according to the current HMS protocol for LBPP studies. This study was conducted according to an outline previously agreed upon by the NASA engineering and HMS science staffs (Appendix A). Although vascular pressure data were not required for this experiment, one animal (No. 861) was configured in the full-up mode in order to: a) demonstrate all phases of catheter hookup, the blood draw and reinfusion procedure, catheter protection requirements and other maneuvers critical to the design of flight equipment and procedures; and b) provide the HMS staff with initial data on the fully integrated LBPP system. A protocol study by the HMS staff had been planned prior to the test run, but due to the interaction of NASA scheduling constraints and obligatory down-time due to minor medical problems with each of the two subjects, this had not been possible.

Animals were fitted with a urine device and placed in restraining chairs on 3/24/83. LBPP and data collection commenced the following day (3/25/83) and continued for 7 days until 3/30/83. Animal No. 859 was removed at this time as abrasions were evident. Animal No. 861 remained in his chair through day 9-½ (3/31) and was removed at this time at the request of the NASA staff in order to evaluate the animal's scrotal area. This same animal participated in the previous NASA test (11-12/83). The NASA team, Norm Donnelly and Paul Fusco, were present for the full study.

PROTOCOL

The protocol for this study was essentially identical to that reported previously (see report for Engineering Study No. 4, submitted 12/83, Appendix B), with the exception of the urine device and particulars pertaining to LBPP. Each animal had worn this device at least 24 hours two weeks prior to this test. The urine devices were fitted by Susanne Churchill according to the NASA-ARC protocol and with the guidance of the NASA team. The collection tube was adapted to the HMS solenoid system which, in turn, passed urine to the fraction collector at hourly intervals. On day 2, the waist cuffs were snugged-up and the lower chamber sealed tightly. Air from the standard lab line was warmed to ambient temperature and passed into these chambers until pressure, controlled by balancing air inflow and outflow rates (outflow buffered by passing the exit line into a deep water reservoir) remained steady at 20 mms of mercury. Pressure was maintained at 22-15 mms of mercury for a 7-day period. Animal No. 861 was depressurized briefly on day 1 of LBPP, per request of the NASA team, in order to evaluate apparent misfit of the scrotal device.

Food and water consumption were measured daily. Animal health status was evaluated at least twice daily by opening the chamber door and at will by checking through a peephole. A blood sample was taken at 2:00 P.M. daily from animal No. 861, according to the same requirements as for the flight samples. Plasma was harvested, stored and the red blood cells rediluted and reinfused.

On day 7 of LBPP, both animals were depressurized. Animal No. 859 was removed from the study at this time in order to inspect abrasions in the scrotal area. Animal No. 861 was kept in the chair for an additional day in order to evaluate urine flow after release of LBPP.

RESULTS

1. Urine Collection Device

This system appeared to function very well for the length of time it was on each animal (8 days for No. 859; 9-½ days for No. 861). Both animals seemed comfortable with this system and appeared to void urine frequently. As urine was collected hourly in this test, storage function of the tube over a 4 hour period could not be evaluated. In both animals, a small amount of solid material, presumably ejaculate, was evident in the upper area of the collection tube. It was also evident that in both cases the large O ring would conform better to the body if shaped somewhat differently. Specifically:

a. Animal No. 859

This animal's scrotal support device was one provided earlier by Paul Fusco. Although it tended to rotate down and backward, this did not appear to impair the operation of the urine collector. By day 8, however, small sores were evident in the monkey's groin. This was very likely the product of adapting the system to the LBPP, however, and not a design problem. Because the collection tube length was a little too long, each time the solenoid valve opened and drained this sealed system the "extra" length of reservoir tubing was sucked into the solenoid tubing adaptor and, as a result, the entire urine system was constantly pulled downward. This probably was the chief contributing factor to the small groin abrasion under the large O ring. The animal also sustained a cut in the right thigh from the lower edge of the upper rubber strap. This, too, was aggravated or caused by positioning of the LBPP cuff directly above the strap. On examination, the ventral surface of the penis appeared somewhat irritated, apparently by the inner surface of the flange. There appeared to be no swelling or other problems in the penis or scrotal area. The animal was bathed and the abrasions treated with antibiotic ointment. On examination one day later, the abrasions were healing well, but a large serous scab was loosely attached under the penile sheath. Three days later, healing was complete in all areas.

b. Animal No. 861

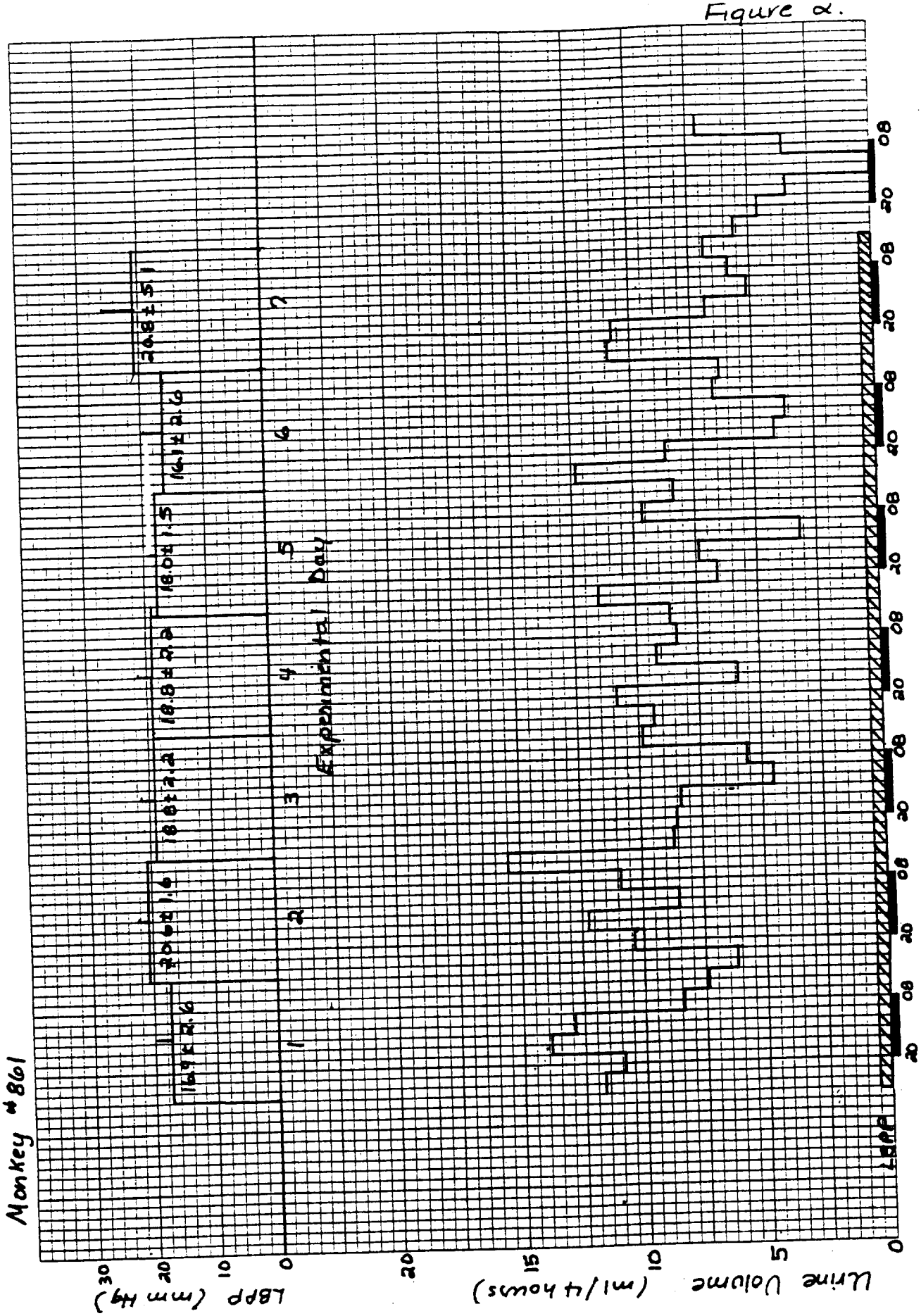
This scrotal device was modified somewhat based on observations made during our trial. The scrotal bag opening was increased to allow easier entry of the scrotal sac. This increased the distance between the O rings and the lower area. The top distance was not changed from the original one. When this animal was removed on day 9, it was noted that the large O ring had broken on the left side at the junction of the scrotal sac. This had not impaired the functioning of the unit, however, which was still firmly attached. Paul Fusco applied a syringe vacuum to the system before it was removed and found it to be nicely sealed. This animal's scrotal area and groin were in excellent condition, with no sign of rubbing or abrasion. The monkey was allowed to remove the tube himself as it was quite firmly attached. He accomplished this with great gusto and promptly ripped the tube to shreds.

2. Urine Volume Under LBPP

Four hour urine outputs and daily mean LBPP's for each animal are presented in Figures 1 and 2 and in Appendix C. The normal circadian variation in day-night urine volumes was present in both animals throughout, with the exception of night 1 in animal 861. The largest 4 hour urine volume was 17.3 ml, observed in animal 859 on day 5. The largest 4 hour

FIGURE 1.

Figure 4.



volume observed in animal 861 was 15.5 ml on day 2. The largest hourly void in either animal was 6.9 ml. As anticipated, animal 861 demonstrated a brisk urine flow in the beginning two days of LBPP, followed by a sustained and somewhat lower urine flow. It is curious that animal 859 failed to demonstrate this initial elevation in urine flow rate, especially in light of his ingestion of large volumes of water on days 1 and 2. A dramatic decrease in total 24 hour urine output was evident in animal 861 after removal of LBPP (mean value for LBPP days 3-7 = 46.6 ml/24 hours vs. 24.3 ml per 24 hours post-removal of pressure). Animal 859 was removed from a chair immediately after the withdrawal of pressure.

3. Food and Water Intake

These data are presented in Table 1.

4. Vascular Pressures

In order to provide the engineering staff with updated pressure information, the limits encountered in this experiment has been determined. These are presented in Table 2. The central venous pressure data are especially relevant given the problems encountered in the previous test with transducer shift and the influence in this test of LBPP. High venous pressure excursions were frequently encountered in excess of 40 mms of mercury; these were presumbably the result of Valsalva maneuvers associated with vocalization, micturition, or defecation. Precise definition of this upper limit was not possible in this study as the Hewlitt Packard chart recorder upper limit for this channel at our current range setting was 40 mms of mercury. I have enclosed several xeroxes of typical excursions in venous pressure as seen on the HP chart record (Appendix C).

5. Discussion/Evaluation

a. Urine Collector

The prototype version of the NASA design appeared to function quite well for the 8 to 9 days of this test. The problems that did occur were few and were most likely due to adaptation of the collection system to the HMS setup. Suggestions for improvement were discussed together and summarized as follows:

1) Smooth the inside angles of the flange

2) Develop a support ring that better conforms to the shape of the scrotal area

3) Adjust the position of the upper straps

TABLE 1

FOOD AND WATER CONSUMPTION

ANIMAL NO.	LBPP TEST DAY 1	2	3	4	5	6	7	$\bar{x}$ for 1-7
	TEK-LAD DIET CONSUMPTION (g/Day)							
859	27.4	16.7	20.3	24.8	-	57.6	35.3	30.4
861	39.6	37.9	27.2	44.9	-	11.2	23.0	30.6
	WATER INTAKE (ml/Day)							
859	115.3	153.9	72.7	74.1	121.3	97.9	95.9	104.4
861	82.8	99.5	76.8	77.2	104.4	103.4	56.3	85.8

TABLE 2

RANGE OF CARDIOVASCULAR PARAMETERS

ANIMAL NO. 859

	HIGH	LOW
Venous Pressure (mm Hg)	>40	0
Arterial Pressure* (mm Hg)		
Systolic	187	155
Diastolic	180	147
Heart Rate (beats/min)	300	228

* This animal was somewhat hypertensive throughout the study.

4) Attach the scrotal sac to the large support ring in such a fashion as to keep the stitches away from the point of contact with the animal

There was some concern urine volumes obtained under LBPP from Monkey No. 859 were not consistently greater than those seen in a control type experiment performed last November. To rule out any negative impact of the collection device on volume output, we will shortly rerun this animal through an LBPP test using our urine collection device.

It would also appear advisable to begin including the rigid inner tube, whenever possible, in the testing procedure in order to evaluate the impact of urinary sediment and/or ejaculate on its function. As we currently do not have the capacity to analyze urinary sediment size or composition, it is advisable to pursue a practical evaluation as soon as possible.

In summary, it is my opinion based on this lg performance that this system, once perfected, will quite adequately support the quantitative collection of urine from the squirrel monkey. It will obviously be quite important to test this system in an integrated RAHF environment that includes the final restraint system. As the design of the restraint system is very critical to the testing and optimal functioning of the urine system, it is important to begin evaluating the interaction of the two systems immediately.

b. Urine Volume Under LBPP

Due to the scheduling constraints discussed previously, this was the first LBPP test performed in this laboratory using the new equipment and was thus a prototype experiment. As a result, the positive air pressure was not ideally stable throughout the experiment. However, given that each mm of mercury rise in central venous pressure is associated with much greater rises in applied body pressure, it is unlikely that the physiologic stimulus caused by 15 mms of mercury was sufficiently different from that of 20 mms of mercury to markedly affect the urine volume output. This variation would obviously be unacceptable in the more formal study which we will be undertaking in the upcoming weeks. We will provide these data as they become available.

Based on the recent data, and that previously published on animals subjected to 4 days of LBPP, collection tube volumes of 30 to 40 ccs would appear to be adequate. There will, of course, be individual voids which may push this limit. In fact, it is my understanding that this was the basis for selecting the upper limit chosen. As I reported to Norm in my letter of 3 May 1982, the largest two-hourly void observed in the data from the published study was 16 ccs (per 2 hours). The highest hourly void

observed in the two animals just studied was 6.9 ccs. These high values are fairly infrequent but unfortunately hard to predict, as the magnitude of the response can be quite varied from animal to animal as well as for any single animal. As we have stated previously, if the volume of an occasional 4 hour void were to exceed the capacity of the collection tube (~30 ccs), this would not invalidate the results.

c. Cardiovascular Parameters

Because of the fairly frequent excursions encountered in central venous pressure, it will be important that the flight sampling procedure be designed to avoid instantaneous samples which, if they occurred during a chirp, etc., would not be representative of true mean pressure. Some form of time average sample would be ideal. We have found the backup chart record provided by HP to be critical to our evaluation of mean pressure. This is a very important issue as the changes anticipated in mean venous pressure (on the order of 1-10-10 mms of mercury) are less than these essentially artifactual excursions.

EXPERIMENT NO. 223
FLUID AND ELECTROLYTE HOMEOSTASIS

REPORT FOR SUPPORTING STUDY #4
ENGINEERING EVALUATION

TO: JIM CONNOLLY, NASA ARC
FROM: SUSANNE CHURCHILL, HMS

This study was conducted in order to gather baseline data in support of engineering design requirements for SL-4 experiment #223. Two adult male flight size squirrel monkeys were studied for a seven day period according to the standard HMS Lower Body Positive Pressure (LBPP) control protocol. These studies were conducted according to a plan previously agreed upon by the NASA Engineering Staff and the HMS Science Staff (Appendix A). Animals were placed in restraining chairs on 11/29/82; data collection commenced the following day (11/30/82) at 10 am and continued until 10 am on 12/7/82 (Day 7). The NASA team, Jim Connolly and Henry Leon, were present for the first four days of this study (11/29 -12/2/82). A preliminary report of this test was made at the PDR meeting held at ARC 12/17/82.

PROTOCOL

Please refer to Figures 1-6 for graphic illustration of the text.

Animals #856 and #861 were selected for their general good health and ability to tolerate extended chair sitting. On Monday, 11/29 (Test Day 0), these two animals were placed in plexiglas restraining chairs located inside isolation chambers with a controlled 12 hour light-dark cycle (8am - 8pm)(Fig. 1,2). Both animals sat on perches padded with water cushions and wore urine collection devices designed and fabricated by Sallie Petrou of this laboratory (Figures 3,4). Connector tubing passing from this device through the isolation chamber floor conveys urine to a programmable fraction collector. The scrotal area had been previously treated with Skin-Prep dressing wipes. All points of contact with the urine device were well padded with soft foam. Animal 856 had a special perch allowing him to squat rather than sit with legs extended. Water was freely available from a ball valve spout mounted on the right side of the chair at mouth level. Food (Bio-Serve Reward Diet #T-130) was available ad lib from a food dish fixed to the left side of the chair at waist level. Both animals had been on this diet for a month prior to the test. Feces were collected in a container filled with sawdust and positioned under the animal's anus.

Both animals had indwelling arterial and venous cathers, implanted as indicated in Table 1 according to the standard HMS protocol. These catheters, which pass through the skin just below the level of the shoulder blades and extend several inches

beyond the animal, extended, were joined to sterile connector tubing (lengths and specifications given in Figure 5) by a hollow stainless steel connector. That part of the catheter which passes under the jacket was protected by a shield of tygon tubing which is in turn fastened in place by ties through the back of the jacket. The connector tubing passes out from under the bottom margin of the jacket (still enclosed by the tygon tubing) and travels under a lucite horizontal waist restraint plate. A thick walled tygon shield carries the catheters from the chair to the chamber wall where they pass through to the transducer domes, mounted at appropriate levels (level with each catheter tip) on the exterior of the isolation chamber (Fig. 6). The transducers (HP#1280C) are interfaced to signal conditioners (HP #8805D) through a computer directed multiplexer system. A Hewlett Packard four channel recording system provides hard-copy display of the pressures.

After the catheters were connected to the transducer domes and the infusion begun (heparinized saline, 10 U/ml, at a rate of 0.027 ul/min into each arterial and venous catheter by means of Harvard Apparatus pump #975), the thermistor probes (YSI #27) were attached to the back according to the protocol set forth in NASA Communication of 6/14/82 R. Mains to J. Connolly, "NASA/ARC Experience with Skin Adhesives". Briefly, a small patch of hair was shaved from the back lateral to the spine and just under the shoulder blades. The skin was scrubbed with a teflon pad and sponged with alcohol. The probe was attached with cyanoacrylate glue and a patch of microfoam tape taped over the thermistor lead for strain relief. The temperature probe lead also ran through the protective tygon tubing through the cage wall to the multiplexer.

The animals were then allowed to adjust to the restraint overnight. At 10 o'clock Tuesday morning (Test Day 1) the transducers were zeroed and data collection was begun by means of our computer driven system. Specifically:

1. Arterial and venous blood pressures were monitored continuously. These data were sampled and recorded by the computer every 15 minutes throughout the experiment. At the same time hard copy was provided by means of the computer activated chart recorder (5 sec sample). With this system data could also be accessed at any moment on on the terminal screen. At 10 am every day all transducers were rezeroed to atmospheric pressure. Pressures were measured and recorded as systolic, diastolic and mean arterial pressure, and mean venous pressure.

2. Heart rate was monitored and recorded as above by means of an HP heart rate signal conditioner (8811) enslaved to the arterial pressure signal conditioner.

3. Skin temperature was monitored and recorded by a direct digital output from the multiplexer to the computer.

4. Urine was collected under oil in hourly samples by the computer activated fraction collector. Samples were measured for volume daily and frozen (4° C) for later analysis.

5. Daily food and water consumption was determined by weighing and recorded in the study log.

6. A daily (10 am) 1cc blood sample was taken (method in Appendix B), spun in a refrigerated centrifuge and the plasma frozen (-30° C) for later analysis. Hematocrit was also measured and recorded.

7. Feces were weighed daily and amounts recorded in the log.

8. Daily health status assessment was made and comments recorded in the computer log file.

On Test Day 7 both animals were removed from the restraint system, bathed in warm water and checked carefully for any signs of abrasion or soreness. Both animals appeared in excellent health. The photographs in Figures 1-3 were taken at this time. Blood culture was also performed on an aliquot of the last sample and found to be negative for both animals.

APPENDIX B

HMS/ARC URINE COLLECTION SYTEM TEST PLAN

I PURPOSE

The urine collection system has been successfully applied on SM's for periods of several hours through four days. This test is designed to: (1) Extend the duration to 7 - 10 days; (2) Determine the effect of LBPP on the system; (3) Collect accurate urine output data on SM's while under the influence of LBPP. The test will also serve to simulate a 7 day mission.

II PROCEDURE

Two SM's who have been restraint trained for at least seven days to the Harvard restraint, will serve as test subjects. Ideally, both SM's will have worn the urine collection tube continuously for 24 hours at some period during the training. Neither animal will be instrumented with thermistors or BTS.

The Harvard isolation chamber and programmable fraction collector will be used. Food and water will be available ad lib. The Harvard LBPP apparatus will be utilized to simulate the effects of zero-g for a seven day period.

Daily food and water consumption will be determined and feces weighed weekly. Urine will be collected in hourly samples using the Harvard fraction collector.

III PROTOCOL

On Monday, 3/21 (Test Day 0), two animals with urine collection tubes and flanges securely attached, will be placed in Harvard restraint chairs located in isolation chambers with a controlled 12 hour light-dark cycle (8 am - 8 pm). The urine collection tubes will pass through the chamber flooring to a programable fraction collector set for hourly aliquots.

Water will be freely available from a ball valve spout mounted at mouth level. Food pellets will be available ad lib from a dish fixed to the chair at waist level. Feces will be collected in a container positioned under the animal's anus. The animals will be allowed to adjust to the restraint overnight.

On Tuesday morning 3/22, (Test Day 1), the LBPP apparatus will be set at TBD mm Hg and turned on. Daily measurements to be made include: (1) Hourly urine samples, (2) Food & water consumption & (3) Health status assessment. Part of the health status assessment will include an examination of the urine collection system for functionality and compatibility with the animals.

On Tuesday morning 3/29, (Test Day 8), the LBPP apparatus will be turned off and the daily measurements will continue. The test will continue for 1-2 more days at the discretion of the PI. On the last test day, an attempt will be made to evacuate the urine tube using a vacuutainer to determine if the seal on the animal's penis is still intact. The animals will be carefully examined at the conclusion of the test to assess the biocompatibility of the urine collection system.

IV TEST RESULTS

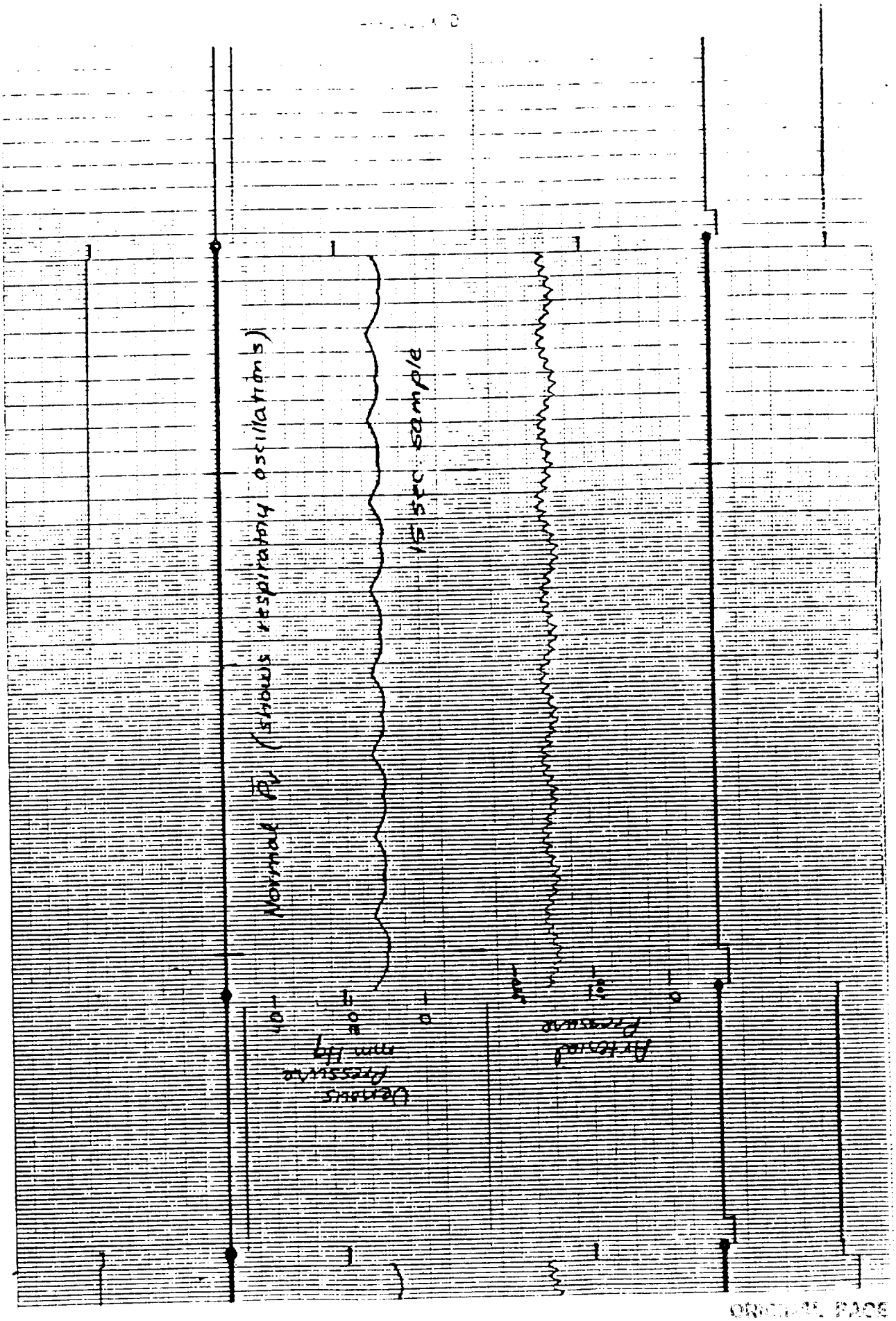
The PI will provide a final report to ARC within 45 days of the completion of the test. In addition to a quantitative analysis of the urine, an examination of the urine samples for particulate matter and ejaculate is very important.

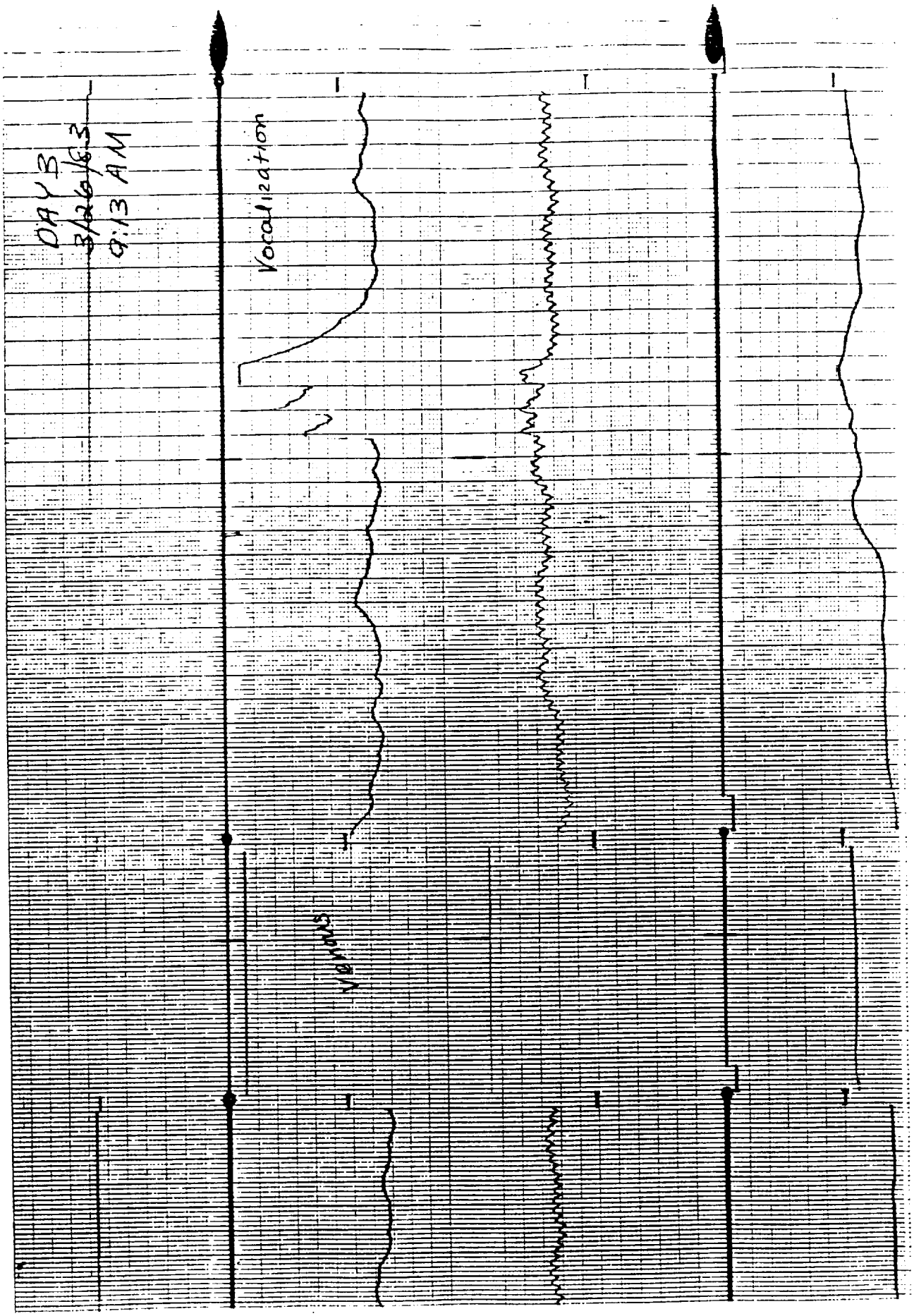
APPENDIX C

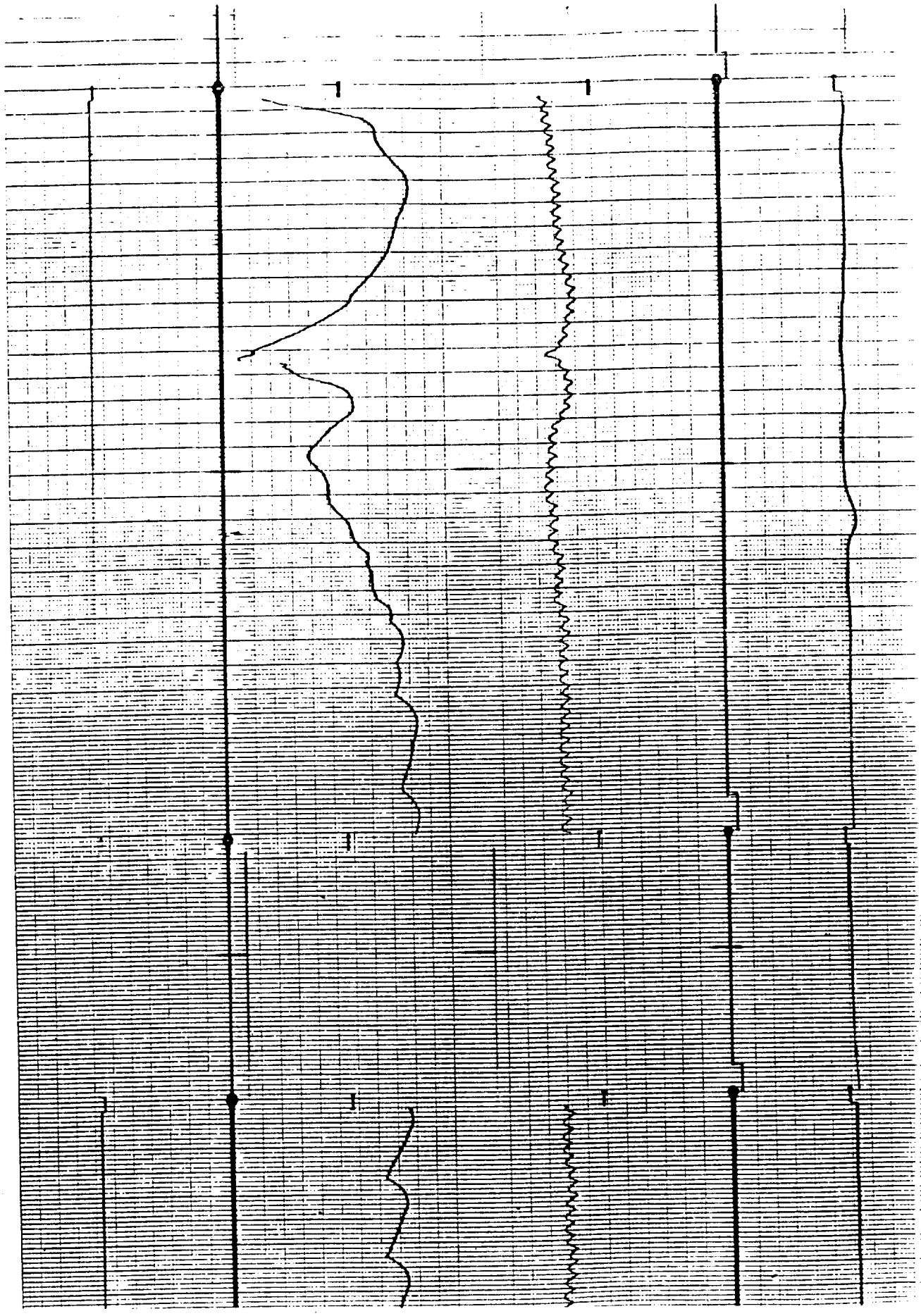
URINE VOLUMES
(ml/4 Hr)

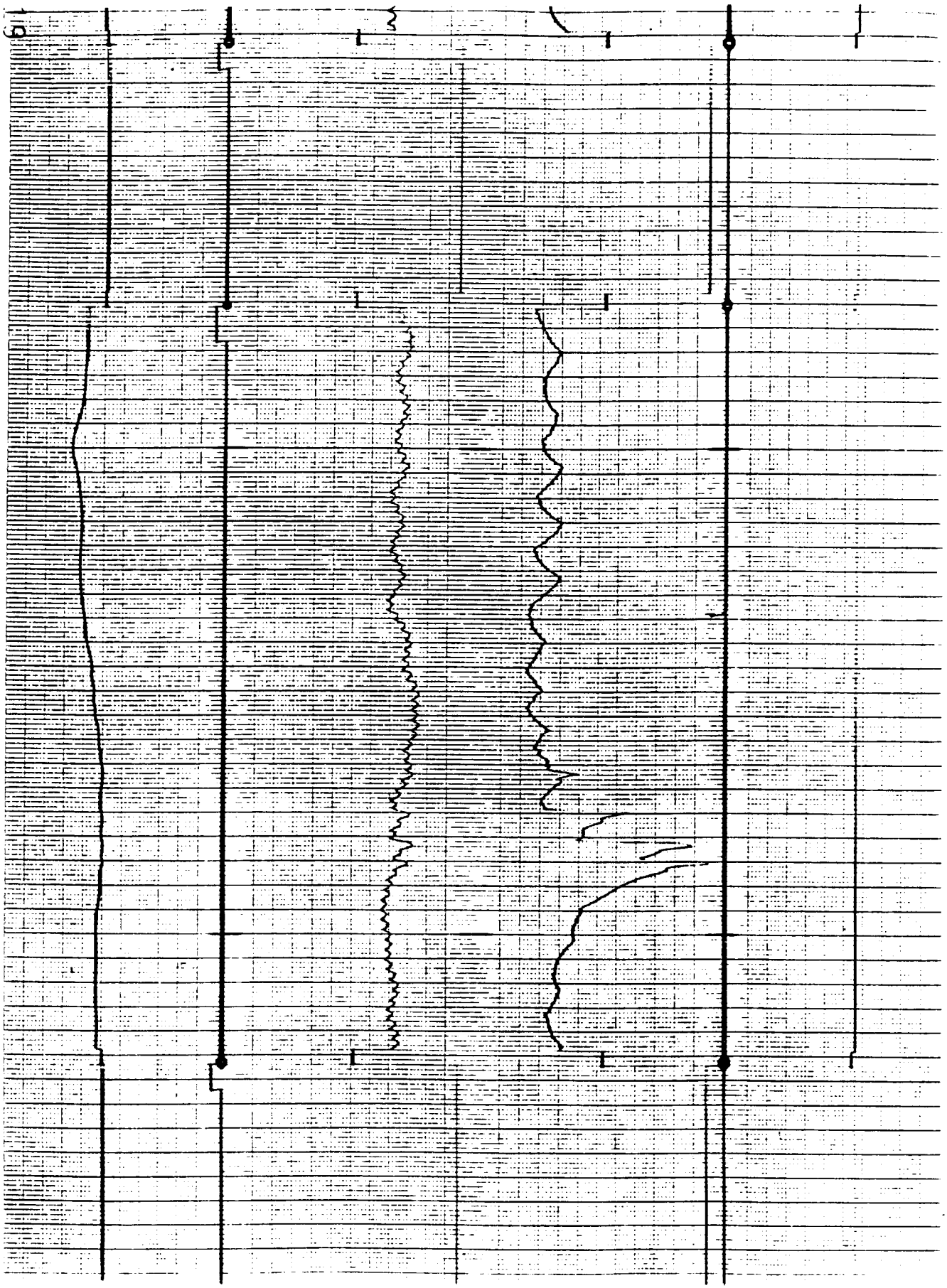
ANIMAL NO. 859	TEST DAY							
TIME	1	2	3	4	5	6	7	8
14-18	Total volume >19.2 ml	1.6	8.3	10.0	17.3	15.8	15.8	Animal removed on Day 7
18-20		3.0	9.5	11.7	8.9	13.6	7.3	
20-02		1.6	7.2	7.7	9.1	9.7	7.2	
02-06		2.7	4.1	7.4	7.6	6.7	8.7	
06-10		9.2	7.2	3.8	13.5	16.7	8.6	
10-14		7.1	6.9	14.5	5.9	14.3	4.4	
TOTALS	19.2+	25.2	43.2	55.1	62.3	76.8	52.0	
ANIMAL NO. 861								
14-18	11.7	6.2	8.7	9.4	11.5	12.3	10.9	5.6
18-20	10.8	10.4	8.5	10.8	6.6	8.7	10.8	4.6
20-02	13.9	12.2	8.3	5.8	7.4	4.2	6.9	3.4
02-06	12.8	8.5	4.5	9.2	3.1	3.6	5.1	0
06-10	8.4	10.8	6.1	8.4	9.7	6.6	5.9	3.5
10-14	7.3	15.5	9.8	8.6	8.3	6.4	6.9	7.2
TOTALS	64.9	63.6	45.9	52.2	46.6	41.8	46.5	24.3

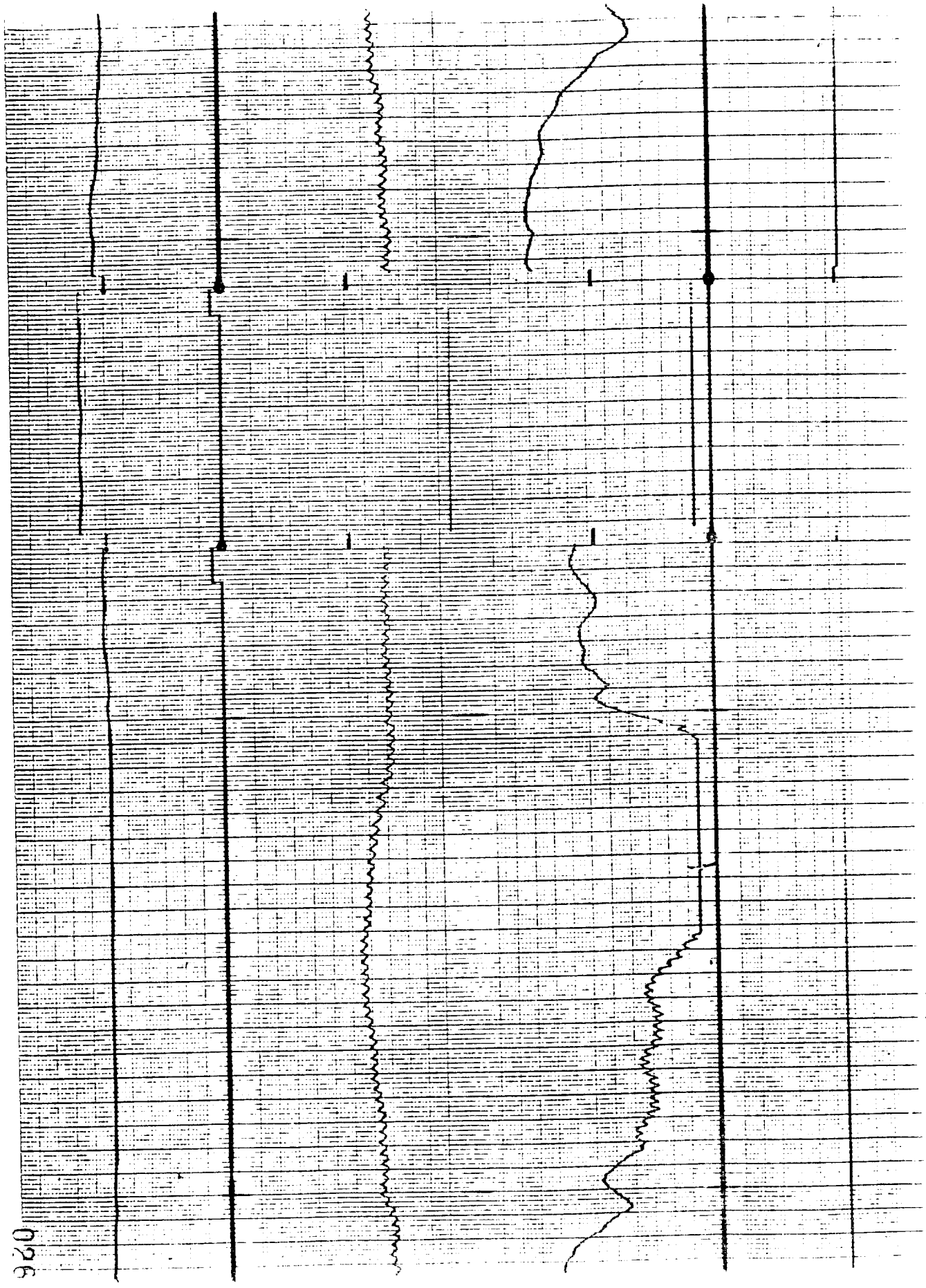
49.2

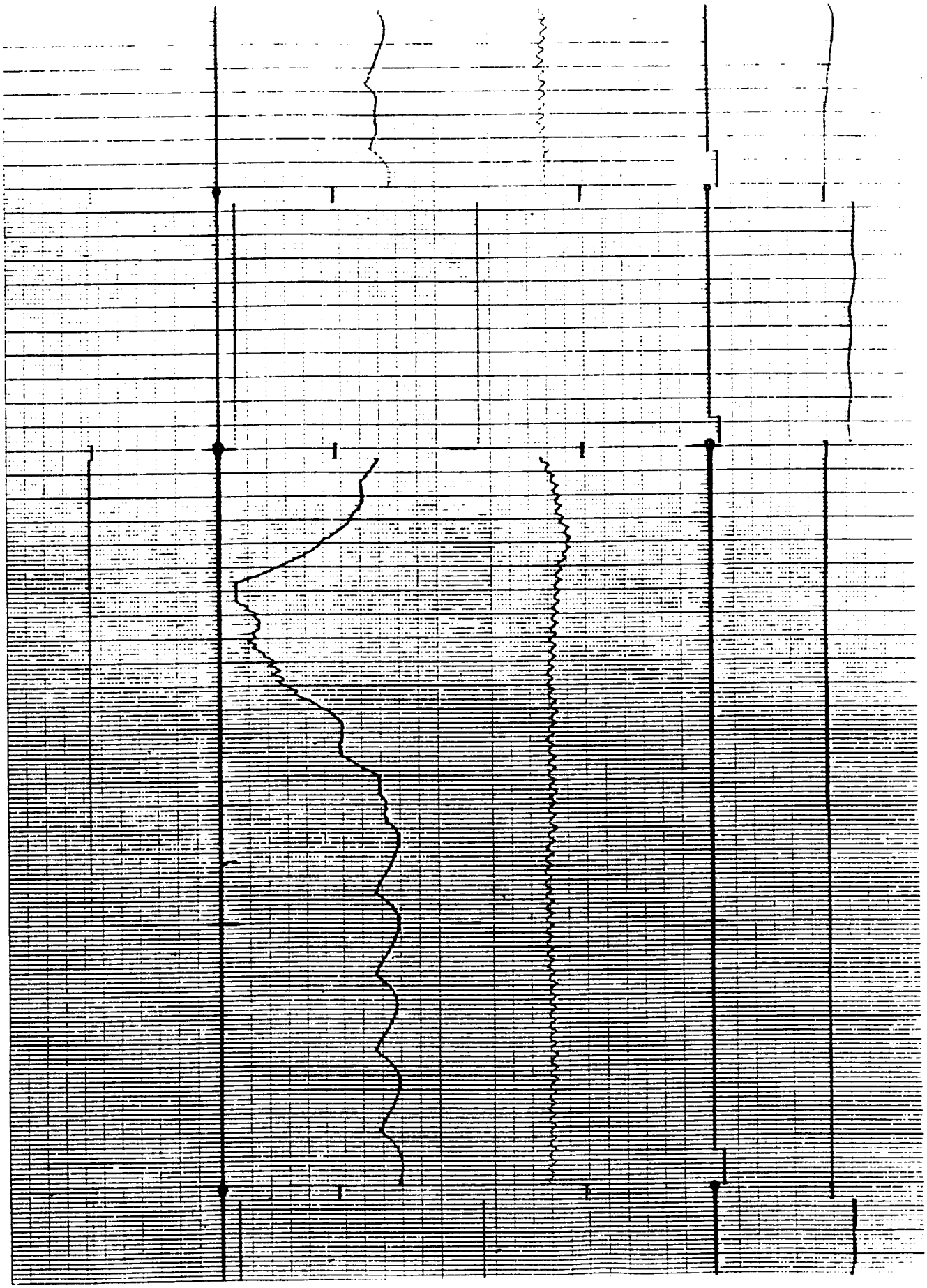












APPENDIX C

FEASIBILITY STUDY:

PRIMATE EXPERIMENT INTEGRATED TEST

TEST PLAN

PRIMATE EXPERIMENT INTEGRATED TEST

OCTOBER 19 -29, 1984

ARC SL-4 PRIMATE EXPERIMENT INTEGRATED HARDWARE TEST PLAN

1.0 BACKGROUND

A major milestone for SL-4 Moore-Ede/Fuller Squirrel Monkey Experiment hardware development will be an integrated test using fligh-like hardware and two monkey subjects for the expected flight experiment duration. The test will provide the first opportunity for the flight crew to engage in training with flight equipment that interfaces with the test subjects under simulated conditions. In order to successfully conduct a test of this complexity, significant coordination must occur among the participants. This plan is meant to provide a basis for that coordination.

2.0 SCOPE

The two hardware test units will consist of SL-4 Primate Experiment flight-like components except for the SIM RAHF and the Ground Support Equipment. A wooden SIM RAHF will simulate the actual flight RAHF (none are available) and the Ground Support Equipment is expected to be undergoing validation during this time period.

The procedures to be used for conducting the test will, in general, follow those listed in the PDR and CDR for the experiments. The preflight and postflight data collection periods will be shortened however and the test will begin at L-2 days, include a 7-day flight period and end at R+12 hours. This will require approximately 10 continuous days of operation for all equipment. Written procedures should be followed during the test so that these can be evaluated as well as the hardware. The major emphasis for the test is experiment hardware verification. It is expected that deviations from the PDR/CDR timelines will have to be made to focus on hardware evaluation, but any procedures which can be readily evaluated should be performed whenever possible. Post-test, deviations from the PDR/CDR timeline should be noted and any possible hardware impact evaluated in anticipation of conducting the upcoming Experiment Verification Test (EVT).

Both monkeys should be configured as as they will be for the flight including ECG inplants, catheters, urine tubes and skin temperature sensors. The monkeys will have been conditioned to at least 10-days of restraint in the SL-4 cage including operation of the feeder and waterer system. In order to maximize the possibility of successful test completion, four monkeys should be surgically prepared and the best two candidates chosen just prior to beginning the test.

All data should be recorded using the data acquisition schedule proposed for flight. Real-time test data will be recorded and will include: ECG, venous pressure, heart rate, body temperature, five skin temperatures and food consumption. The test will focus on hardware performance and methodology, therefore a total data analysis will not be attempted. The same rationale should apply to the biological samples acquired during the test such as blood and urine. Validation of hardware and procedures will be the main goals.

All test-related animal activities will be conducted in a manner which ensures that subjects will be maintained in a SPF (Specific Pathogen Free) condition as specified in the LSFEP Animal Certification Plan. Also, the recommendations of the Animal Verification Review panel will be followed. These include following procedures such as maintaining controlled access to animal test areas and wearing of gowns and masks by test personnel when in those areas.

3.0 PLAN/RESPONSIBILITES

Due to the unavailability of an actual flight RAHF, a wooden SIM RAHF will be used to house both test subjects. The test will be conducted within a controlled access trailer in the Animal Care Facility. The trailer is partitioned in two to maintain SPF conditions in the actual test area containing the animals.

3.1 Hardware Elements

SIM RAHF to contain:

Cage
- Feeder system
- Lixit waterer system (gravity bottle feed)
- Restraint system
- Urine collection system
- Pressure measurement and blood sampling system
- Signal conditioner package
- Motor controller package
- Power distribution box
- Switch box

Data Select Panel to contain:

- Launch/Re-entry recorder
- 400 Hz, 115V inverters
- Carousel timer
- Subject monitoring
- LSLE, RAU buffering *
- Data collection and display system

- Centrifuge
- Refrigerator/Freezer

The checkout of all test hardware elements will be accomplished by the experiment team at ARC prior to the start of the test.

3.2 Animal Preparation and Care

Selection and Training Procedures

Responsibility of the ARC animal trainers with HMS and UCR concurrence.

* Not in use during this test.

Animal Preparation and Care Procedures

Catheter implantation - responsibility of HMS
ECG/DBT implantation - responsibility of UCR with HMS concurrence

Temperature sensor attachment procedure - responsibility of UCR

Post-surgical animal care - ACF with HMS & UCR concurrence.

Catheter maintenance - ARC animal trainers with HMS concurrence.

All animal preparations, protocols and procedures will be subject to review by the ARC Animal Care and Use Committee prior to the start of the test.

4.0 TEST PREPARATION AND SCHEDULE

October 12 Prepare trailer, install SIM RAHF and check out lighting, fans, etc.

October 13 Install 2 cages in SIM RAHF and check out cage subsystems and data collection and display system.

October 18 Select 2 best test candidates. Both test candidates to be shaved for skin sensor attachment and venous catheter lines to be installed in both cages.

October 19 Begin 10-day test. Both test subjects to be fitted with restraint jackets, skin sensors and urine tubes before installation in cage and attachment to venous catheter line.

After cages are installed in SIM RAHF, data system is to be checked out and light/dark cycle begun in trailer.

Begin flight procedures.

October 22 Crew arrives for training on flight procedures.

October 26 Crew departs after participating in debriefing with experiment team and training personnel.

October 29 Remove test subjects, conduct clinical exam and return animals to vivarium cages.

Conduct a post-test review with PIs and experiment team.

5.0 TEST PERSONNEL REQUIREMENTS

Due to the extensive coordination of hardware, animal subjects and test personnel required to prepare for and conduct this test, it will be mandatory that a test coordinator be assigned. The test coordinator will have the responsibility for: (1) ensuring that this plan is followed as closely as possible, (2) that appropriate notes are kept throughout on both science and hardware concerns, (3) that quality data collection is maximized, (4) that the animal subject's well-being is monitored appropriately by Bionetics animal health technicians and

veternarians and (5) that access to the test area is controlled.

Since this test will be the first hands-on exposure of the crew to the equipment, it is likely that some trouble-shooting and problem solving will be required. The test conductor must act to ensure that the appropriate personnel are available when needed and that the presence of other personnel is minimized.

The key test support personnel are:

Test Coordinator	N. Donnelly
Primary Investigators	C. Fuller (UCR); M. Moore-Ede & S. Churchill (HMS)
Project Directors	J. Miller (UCR); S. Churchill (HMS)
Experiment Manger	H. Leon (ARC)
Engineering Manager	J. Connolly (ARC)
Experiment Design Engineer	P. Fusco (ARC)
Veterinarians	J. Goldsboro (ARC); D. Moore (Bionetics)
Animal Care/Support	L. Mullin, M. Williams (Bionetics)
Training Coordinator	A. LaBoy (ARC)

6.0 TEST CONDUCT GUIDELINES

In order to maintain SPF conditions as closely as possible and to ensure compliance with the ACF policy, the following guidelines are to be observed.

6.1 Gowns and masks are to be worn when in the presence of a test subject. In addition, gloves are to be worn when in contact with a test subject.

6.2 The ACF veterinarian has the final word on an animal's health status.

6.3 There will be no admittance to the test room without L. Mullin, M. Williams or the ACF veterinarian present.

6.4 Contingency actions (other than animal health) will be decided by H. Leon, J. Connolly and N. Donnelly.

6.5 Trailer keys will be maintained by H. Leon, J. Connolly, N. Donnelly, L. Mullin and M. Williams.

6.6 Attendance in the animal prep room is to be restricted to active participants, PI's and staff and observers approved by the Test Coordinator.

APPENDIX A

DAILY ACTIVITY SCHEDULES

THRUSDAY 10/18/84

1315 Select two test candidates

1330 Remove each SM from vivarium cage and weigh.

1340 Share upper arms, upper thighs, and base of tail to prepare sites for skin sensor placement.

1400 Return both SM to vivarium cages.

1415 Begin assembly and integration of venous catheter lines, transducers and stopcocks into both cages.

1600 Complete integration/assembly.
Drape both cages with sterile cloth and leave in preparation room.

FRIDAY 10/19/84

FIRST SM

0800 1. Take 1 SM from vivarium cage and remove jacket.
2. Attach temperature sensors to animal's arms.
Room C8 3. Place restraint jacket on SM and zip top half.
4. Place SM on table and attach 2 leg and tail temperature sensors.
5. Anchor strain relief tape.
6. Guide catheter lines, ECG/DBT probe and thermistor leads through jacket opening and zip lower jacket.
7. Place SM on table and attach urine collection system.
8. Install SM in restraint system.
9. Attach urine collection tube to urine valve.
0930 10. Connect Skin sensor leads to plug.

0945 Remove SM/cage to trailer test site.

1000 Connect catheter lines to pressure measurement and blood sampling system.

1030 Checkout data displays for temperature, pressure and heart rate readouts.

1100 1. Install cage in SIM RAHF.
2. Connect waterer tube to cage.

SECOND SM

0945 Repeat 1 - 10 above.

Room C8

1115

1130 Remove SM/cage to trailer test site.

1145 Connect catheter lines to pressure measuremment and blood sampling system.

1200 Checkout data displays for temperature, pressure and heart rate readouts.

1230 1. Install cage in SIM RAHF.
2. Connect waterer tube to cage.

1300 Begin test.

ACTIVITY SCHEDULE - TEST DAYS 2, 3

SATURDAY 10/20/84 - SUNDAY 10/20/84

0930 1. Health check (ACF Vet)

2. Carousel changeout (L. Mullin/M. Williams)

3. Waste tray changeout (Day 3 only) (L. Mullin/M. Williams)

4. Feeder checkout (day 3 only)(L. Mullin/M. Williams)

5. Pressure transducer zeroing and measurement (J. Connolly)

6. Water replenishment (L. Mullin/M. Williams)

1000 Blood draw/re-infusion (S. Churchill)

ACTIVITY SCHEDULE - TEST DAYS 4, 5, 6, 7, 8

MONDAY 10/20/84 - FRIDAY 10/26/84

0930 1. Health check (ACF Vet) (Crew)

2. Carousel changeout (Crew)

3. Waste tray changeout (Crew)

4. Feeder checkout (Crew)

5. Pressure transducer zeroing and measurement (Crew)

6. Water replenishment (L. Mullin/M. Williams)

1000 Blood draw/re-infusion (Crew)

ACTIVITY SCHEDULE - TEST DAYS 9, 10

SATURDAY 10/27/84 - SUNDAY 10/28/84

0930
1. Health check (ACF Vet)
2. Carousel changeout (L. Mullin/M. Williams)
3. Feeder checkout (Day 9) (L. Mullin/M. Williams)
4. Pressure transducer zeroing and measurement (J. Connolly)
5. Water replenishment (L. Mullin/M. Williams)

1000 Blood draw/re-infusion (S. Churchill)

ACTIVITY SCHEDULE - TEST DAY 11

MONDAY 10/29/84

0930 1. Health check (ACF Vet)

2. Carousel changeout (L. Mullin/M. Williams)

3. Waste tray changeout (L. Mullin/M. Williams)

4. Pressure transducer zeroing and measurment (J. Connolly)

1000 Blood draw/re-infusion (S. Churchill)

1045 1. Disconnect water lines, data cables and remove cages from SIM RAHF and place in C8.

2. Remove animals from cages, conduct health inspection and weigh.

1115 Return animals to vivarium cages.

1330 Post-Test review.

HARVARD MEDICAL SCHOOL
DEPARTMENT OF PHYSIOLOGY AND BIOPHYSICS

MEMORANDUM

21 November 1984

To: All Concerned

Re: Integrated Hardware Test/Crew Training
October 19 - 29, 1984

From: Susanne E. Churchill, Ph.D.
Co-Investigator, Primate Experiment

During the hardware integration test and crew training of this past October 19th - 29th, a number of issues came up which I would like to bring to your attention. While I realize this is yet another list documenting concerns, I am doing this for the purposes of my own organization. Should there be any unique items here, so much the better. As you will notice, most of these items pertain to crew training issues.

Item 1 - Urine Collection System

a) Length of the urine tube should be standardized and should be of sufficient length to provide some slack. The tube on one monkey appeared to be stretched a bit too tightly around the restraint pole and may have contributed/caused the penile "sore" observed during take down.

b) It was confirmed by the Specialists and the P.I. that the tubes should be numbered consecutively from 1 to 1xx. At each change-out, only those tubes which have cycled over the needle valve will be removed to stowage. Consequently, it is imperative that the Specialist note what this tube was prior to removing the carousel from the cradle. The first tube in the sequence which has not been punctured will then be moved up to slot no. 1. The remaining empty tubes in the carousel will also be moved forward and the remaining slots filled by fresh tubes from stowage.

c) The Specialists requested that slot no. 1 be clearly marked as with a piece of tape, since this will always note the reinstallation position.

d) It will be important that numbers denoting the position 180 degrees from the forward-facing tube be very clear in order to ensure that all used tubes, whether they contain urine or not, are removed.

e) All urine tubes will be labeled a second time with a Sharpie marker. This will be especially important in the event of an out-of-sequence manoeuvre or an error in the installation.

f) A request was made by the Specialists to mark the tight screw-slot position for each slot on all carousels.

g) It is imperative that we resolve the issue of timing of the urine carousel change. This should occur as nearly as possible in increments of L + 4, in order to capture that all-important first sample. In addition, the Specialist must be aware of whatever this time will be in order not to change out the carousel during a normal change cycle. As the cage door must be closed following removal of the carousel, it is possible that a change command could enter in the absence of a carousel with the result being that 8 hours of urine would collect in the urine tube, posing a serious threat to the competency of the system.

h) The Specialists determined that during carousel change out, the pit pin could most easily be stored in its true slot unless this would be needed to secure the carousel at the GPWS holder.

i) The issue still must be resolved of what to do in the event of a failure of the urine valve. If this occurs, it will most likely be due to either a bent needle or a clogged valve.

Item 2 - Blood Draw System

a) Velcro fastening systems will be needed on the RAHF outer door in two forms:

1. Opposing strips of velcro to serve as pockets, and
2. A one-sided velcro strip from which to hang the various blood draw packets. We will also need these for EVT.

b) The 10 cc syringe should be graduated in tenths in order to ensure precise delivery of the flush volume.

c) The plastic tips used for rinsing the stop cock should be replaced by standard luer hub adaptors, as it was difficult to install the plastic tips. The pockets in each pack which are to be used for the restorage of sterile items should be marked in large letters "STERILE" to avoid accidental deposit of "trash" in these slots.

d) Problems with back-leak from the reactivials were serious enough to occasion a re-work of this system. Ideally, whatever system is used would be as small as possible, have a conical bottom to maximize plasma harvest, and would, perhaps, carry a vacuum sufficient to automatically withdraw most, but not all, of the expected plasma harvest. It is extremely important that this vacuum not be excessive as red blood cells must not be delivered into the storage vial, as would invariably occur with a stronger vacuum.

e) The requirement for a larger air bleed syringe is emphasized again in order to minimize the number of times that this is backfilled from the VSP which tends to introduce red blood cells into this relatively static volume.

f) Although the air bleed syringe was not designed for this purpose, it turns out to be the perfect system for flushing the catheter tip whenever the Specialist has a few extra moments and would like to generate an additional central venous pressure value for our experiment. Unfortunately, while this method worked well on several occasions, it did not always seem to free the catheter tip. There was a question from me that perhaps a stopcock inserted in lieu of the T in the air bleed syringe-transducer-VSP system might cure this problem. This issue may be resolved following EVT when a flight-like cage is available to our laboratory. If this procedure could be made to work reliably, it would result in an enormous data gain for our experiment as the procedure is enormously simplified; in the absence of such a straightforward procedure, it is unlikely that Specialists will take the time and effort required.

g) Centrifuge

1. The absence of a break poses a serious problem to the time-lining of this experiment, as well as serving to greatly increase the time before the sample is cooled.

2. The current red blood cell pack is not optimal. If possible, a spin at higher RPMs is desirable in order to increase the plasma harvest.

h) In order to facilitate hematocrit reading, a hematocrit reading device and a recording log should be provided at some easily accessible location, convenient to the Specialist.

Item 3 - Vascular Transducers

a) The Specialists have requested that the set screw be changed to a knob in order to facilitate zeroing of the transducer. There is no need to bring this knob forward in the cage.

b) There was general concern expressed regarding the zero stability of the transducer as drifts in this would severely limit the collection of accurate CVP data.

c) The positioning of the transducer within the cage must be standardized so that a correction factor for zero offset can be determined and tested.

Memorandum to All Concerned
21 November 1984
From: Susanne E. Churchill, Ph.D.

Item 4 - Miscellaneous

a) Concern was expressed as to whether or not the stainless plenums covering the feeder box and emergency water delivery hole would prevent a clear view of the monkey.

b) The issue of what to do with garbage surfaced on several occasions. As much of the refuse from the blood draw procedure should be considered a biohazard, procedures must be developed to safely dispose of these materials.

c) The RAHF door when open must have a tethering system as the kits will be hung on this door in a secure fashion.

d) The orientation of the Specialist doing the blood sampling has yet to be determined, especially for the lower two cages. It is possible that special restraint devices will be needed to provide comfortable access to the VSP.

e) A request was made by the Specialists to fly an adequate supply of vacutainers in the event that trouble-shooting of the urine system was required.

I would like to take this opportunity to thank all the individuals who worked so hard to make this test and training session such a successful event. In particular, we feel that the Engineering Staff has developed a series of unique and proficient hardware items which will allow us to accomplish our proposed scientific goals during the spaceflight. As before, the feedback from/and interaction with the Payload and Mission Specialists has contributed significantly to the improvement and streamlining of our experimental procedures.

Several of the above items are action items for this laboratory to resolve. We look forward to expediting these issues as soon as a flight-like, fully equipped cage can be made available to us. If the suggested target date of March 1, 1985 can be met, we will make every effort to close out the remaining issues within a 6-month period so that the cage system can be returned to NASA ARC in preparation for KSC ground support studies.

Thanks to all concerned.

With best wishes,

Sincerely,

Susanne

Susanne E. Churchill, Ph.D.
Co-Investigator and Project Director

NASA
National Aeronautics and Space Administration

Report Documentation Page

1. Report No.	2. Government Accession No.	3. Recipient's Catalog No.
NASA CR-177548		

4. Title and Subtitle	5. Report Date
Fluid and Electrolyte Homeostasis During Spaceflight: Elucidation of Mechanisms in a Primate	April 1990
	6. Performing Organization Code

7. Author(s)	8. Performing Organization Report No.
Susanne Churchill	A90095
	10. Work Unit No. 106-30-02-40

9. Performing Organization Name and Address	11. Contract or Grant No.
Institute for Circadian Physiology 677 Beacon Street Boston, MA 02215-3203	NAS2-10547
	13. Type of Report and Period Covered Contractor Report

12. Sponsoring Agency Name and Address	14. Sponsoring Agency Code
National Aeronautics and Space Administration Washington, DC 20546-0001	

15. Supplementary Notes

Point of Contact: Adrian P. Mandel, Ames Research Center, MS 240A-3, Moffett Field, CA 94035-1000
(415) 604-6912 or FTS 464-6912

16. Abstract

Although it is now well accepted that exposure to the hypogravic environment of space induces a shift of fluid from the lower extremities toward the upper body, the actual physiological responses to this central volume expansion have not been well characterized. Because it is likely that the fluid and electrolyte response to hypogravity plays a critical role in the development of Cardiovascular Deconditioning, elucidation of these mechanisms is of critical importance. The goal of flight experiment 223, scheduled to fly on SLS-2, is the definition of the basic renal, fluid and electrolyte response to spaceflight in four instrumented squirrel monkeys, Saimiri sciureus. The studies described in the following text were those required to support the development of flight hardware and optimal inflight procedures, and to evaluate a ground-based model for weightlessness, lower body positive pressure (LBPP).

17. Key Words (Suggested by Author(s))	18. Distribution Statement
Spacecraft, Homeostasis, Squirrel monkey, Renal, Lower body positive pressure	Unclassified—Unlimited Subject Category—55

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	95	A05

For sale by the National Technical Information Service, Springfield, Virginia 22161