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LOVELACE MEDICAL FOUNDATION

ALBUQUERQUE, NEW MEXICO 87108

RESEARCH REPORT ON:

SPECIALIZED PHYSIOLOGICAL STUDIES IN SUPPORT OF

MANNED SPACE FLIGHT

TO: NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

     LYNDON B. JOHNSON SPACE CENTER; HOUSTON, TEXAS

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I

THE EFFECTS OF ACUTE DIRUESIS WITH LASIX

ON THE VOLUME AND COMPOSITION OF BODY FLUIDS

AND THE RESPONSES TO LOWER BODY NEGATIVE PRESSURE

ABSTRACT

This study was designed to analyze the effects of a diuretic (Lasix) induced dehydration on the cardiovascular and hematological responses to lower body negative pressure (LBNP) and to compare them to previous observations on dehydration following exercise in the heat (EH). Ten male subjects, five trained runners and five non-athletes, were exposed to LBNP before (LBNP I) and after dehydration (LBNP II) by Lasix. During both LBNP runs the subjects were monitored for changes in blood volume, heart rate, blood pressure, and variations in the volume of the left calf. Each LBNP test consisted of consecutive stages of 5 min at 0, -20, -30, -40, -50 and -60 Torr. Tests were terminated when syncope was imminent or the full sequence was completed. Tolerance was expressed as a cumulative stress (Torr x min). Average Lasix doses of 0.86 mg/kg resulted in an augmented urinary output and a marked reduction in plasma volume (16.8%). Total plasma electrolytes in the circulation were decreased, however, the plasma electrolyte concentrations in the remaining plasma volume were normal. Urinary electrolyte excretions were elevated and the loss in plasma electrolytes accounted for only a portion of the total loss. Measurements of heart rate, blood pressure and rectal temperature during Lasix dehydration failed to indicate any significant variation from control values. Increases in leg volume under LBNP evidenced a fast and slow filling component and were significantly less during LBNP II. An exponential

decline in leg volume after LBNP release was also noted, but the rate

of volume return was greater following LBNP II. LBNP tolerance was

significantly decreased following acute dehydration, however, no

correlation was found between this decrement and the plasma volume

loss due to diruesis. Leg compliance (Δ% leg volume/Torr x min)

correlated with LBNP tolerance in both LBNP runs. While dehydration

tended to increase average leg compliance, marked individual variabliity

was noted. Heart rate was always significantly increased by LBNP exposure

and maximal percent increases in heart rate correlated well with LBNP

tolerance. Inspite of similar maximal percent heart rate elevations,

the rise in heart rate per unit of stress (Torr x min) was greater

during LBNP II than LBNP I. Pulse pressure was significantly reduced

during both LBNP runs but pulse pressure changes were not correlated

with LBNP tolerance and the decline in pulse pressure per unit of

stress was similar in both LBNP tests. There was a mean plasma volume

loss due to LBNP of 12.2% in euhydrated subjects (LBNP I) but only

an average loss of 4.8% in dehydrated subjects (LBNP II). Plasma

volume lost under LBNP and LBNP tolerated correlated well only during

LBNP I. This was also true for the correlation of plasma volume and leg

swelling. The magnitude of this volume loss also correlated well with

the volume remaining in the leg 40 seconds after LBNP cessation (edema).

It was concluded that Lasix dehydration produced a depletion of

the body electrolytes at the expense of both the plasma and extravascular

compartments. This type of dehydration also resulted in a larger

plasma volume loss than did EH dehydration, although the reduction in

total body weight was nearly equal in both cases. The main reason

for the greater loss in plasma fluid was that more electrolytes were

excreted in the urine after Lasix than were lost in an equal amount

of sweat. Since a considerable portion of these electrolytes came out

of the plasma, correspondingly more water was lost from it. Both modes

of dehydration led to a significant reduction in LBNP tolerance, but

the decrement was greater after Lasix than after EH. A more marked

depletion of blood volume with Lasix accounted for part of this difference,

but the manner in which this depletion was compensated for was also a

factor. Average leg compliance was greater following Lasix dehydration

suggesting less vasoconstrictive activity. This greater propensity

to accommodate fluid in the legs hastened the depletion of the central

blood volume.

Less plasma volume was lost under LBNP after either mode of

dehydration, but more plasma was lost after Lasix than after EH, where

it was minimal. This discrepancy is plausible considering that diuresis

was continuing during the second LBNP test, so that plasma water was

being lost via the kidneys concomitantly with the LBNP stress. After

EH, on the other hand, dehydration ceased after EH immediately followed

by plasma replenishment from the extravascular space, thus counteracting

the plasma loss due to LBNP. This may account for the greater loss

in LBNP tolerance after dehydration with Lasix.

Striking differences were found between those subjects who were

physically active (Runners: R) and those who did not engage in any regular

physical activity (Non-runners: NR). Tolerance to LBNP (Torr x min)

was significantly lower in the R's than the NR's before and after

dehydration, however the R's lost more of their tolerance after dehydra-

tion with Lasix than after exercise in the heat for about the same

fluid loss. The opposite was true for the NR's. Two factors appear

to be responsible for the lower LBNP tolerance in R's: parasympathetic

inhibition of cardiac activity during LBNP and a greater propensity

to pool blood in the lower extremities.

INTRODUCTION

Upon ascending into space, man's cardiovascular system undergoes a deconditioning process. Subsequently, the return to earth's normal gravitational field results in marked orthostatic hypotension. This hypotension has, to date, been transitory in nature, but did appear to become more prolonged with increased flight durations. Therefore, there is no guarantee that with either greatly prolonged space flights or repeated ascents into space, the hypotensive response would remain transitory. Even transitory hypotension poses special problems post-flight and could have a major impact upon the effectiveness of the Space Shuttle operations.

An attempt has been made to prevent the cardiovascular decon-ditioning in space by exposing the astronauts to lower body negative pressure (LBNP) periodically in flight, however this met with limited success (Johnson and Dietlein, 1975). Duplication of the weightless state is not possible on earth. Therefore, prolonged postural changes (Waterfield, 1931a), head-up (Beetham and Buskirk, 1968) and head-down (Kakurin et al., 1976) tilt, centrifugal acceleration (Allan and Crossley, 1972) and LBNP (Venters, 1976) have been used to simulate the effects of returning to earth's gravity after being weightless.

An integral part of all earth-bound studies has been the simulation of the plasma volume loss experienced by astronauts. Bedrest (Van Beaumont et al., 1974), thermal indifferent immersion (Kaiser et al.,

1969 and Stegemann et al., 1975), and thermal stress combined with exercise (Luft et al., 1976) were used extensively to simulate the hypovolemia encountered in space. However, the loss of tolerance observed after dehydration by exercise in the heat might have been attributable to the concurrent hyperthermia as well as loss of plasma fluid. In order to distinguish between these two factors an alternative method for dehydration was sought which did not involve any alterations in body temperature. Diuresis induced with Lasix appeared to be the most suitable method to achieve this end. According to Claremont et al. (1976) dehydration by diuresis and by exercise in the heat produced nearly equivalent total body water losses. However, the plasma volume loss associated with Lasix dehydration was considerably greater than that found after heat and exercise dehydration. It is possible that this may have an important influence on the responses to gravitational stress.

Therefore, the purpose of the present study was threefold: (i) to assess the subject's cardiovascular and hematological responses to LBNP when euhydrated; (ii) to evaluate the effects of fluid and electrolyte losses induced by Lasix diuresis in reference to their influence on LBNP responses; and (iii) to compare the responses of a Lasix dehydrated subject to those who have lost an equivalent amount of fluid by exercising in the heat (Luft et al., 1976) following a similar experimental protocol.

SUBJECTS AND EXPERIMENTAL PROCEDURES

Selection of Subjects

Ten male subjects between 23 and 40 years old volunteered for the study. All were in good health but their physical condition varied, because some of them were physically more active than others. Since a previous study had shown that endurance runners tend to have a lower LBNP tolerance than people of sedentary habits, only 5 subjects were chosen who engaged regularly in running or other strenuous activities while the remainder were not particularly physically active in their occupational or recreational pursuits.

The purpose of the study, the experimental procedures and protocol were explained to the subjects in detail prior to their giving consent to participate. Those who had not been previously exposed to LBNP were given a short familiarization run in order to minimize possible apprehension.

Lower Body Negative Pressure (LBNP) Tests

The LBNP device

The LBNP box was constructed of 1.91 cm plywood and was rectangular in shape. The box measured 122 cm long, 41 cm high and 66 cm wide. A semi-circular opening was cut in one end and a sliding masonite panel allowed the opening to be adjusted to any subject's girth at the iliac crests. The paneled opening was padded with foam rubber and bubble plastic. The entire box was covered with mylar (clear) plastic, which extended 91 cm past each end of the box. One end of

the plastic encased the subject's body just above the panel closure and was cinched tightly with a wide velcro belt. The other end cinched the lead-in tubes for the vacuum and bleed-off lines. An adjustable, well-padded crotch support attached to the box floor prevented the subject from bracing his feet against the box bottom while under negative pressure. Further support was given to the subject's left leg with foam rubber thigh and heel pads.

The negative pressure was created with a shop vacuum pump (Sears, model 315.169613). This pump was able to achieve negative pressures in the box down to -100 Torr in 5-10 sec, and the pressure could be held at any level with the use of a mercury manometer (Emil Greiner Co.) and an aluminum stopcock inserted in the bleed-off line. A pressure of -60 Torr was the maximum used in the series and the above system was always able to maintain this level.

To prevent any rise in interior box temperature and the resulting vasodilatory response, sufficient leaks were maintained to allow air to enter the box during the test. Internal box temperature was monitored with a thermometer inserted into the box under the plastic cover. Room temperature was also maintained at 29°C to minimize the subject's thermoregulatory response.

Test procedure

An LBNP run consisted of six stages: 0 Torr, -20 Torr, -30 Torr, -40 Torr, -50 Torr and -60 Torr, each of 5 min duration. The subjects were allowed to continue only until they began to experience syncope or up to a maximum of 1000 Torr x min (-60 Torr x 5 min). Imminent syncope was assessed from objective signs (pulse and blood pressure) and/or complaints of dizziness or nausea by the subject. Ambient

pressure was re-established immediately upon opening the stopcock
and shutting off the pump, whereupon all subjects, syncopal or not,
recovered rapidly. The -60 Torr level was chosen as a maximum cut-off
point to prevent any undue discomfort from influencing the test results.
In order to specify the individual LBNP tolerance the test duration
as well as the levels of negative pressure tolerated were taken into
account. Adding the products of negative pressure multiplied by the
time at each stage resulted in a curvilinear function of cumulative
stress in terms of Torr x min (Figure 1). This parameter allowed a
uniform assessment of each subject's ability to tolerate a gravitational
stress and lent itself well to correlation with the measured physiological
changes. One disadvantage of limiting the maximal exposure to 1000
Torr x min was that the tolerance of those who were able to reach this
level was probably underestimated, thus biasing the results in favor
of those who were less tolerant. However, only two out of ten subjects
reached the 1000 Torr x min limit in both LBNP I and II, so the effect
of this bias on the mean values was probably minimal.

Two separate LBNP runs were performed on each subject on the
same day. These runs were designed to evaluate the subject's response
to a gravitational stress before and after acute dehydration. The
evaluation was mainly concerned with variations in overall tolerance,
and the associated changes in heart rate (HR) blood pressure (B/P),
leg volume (LV) and plasma volume (PV).

Run LBNP I was conducted in the morning with all subjects in a
euhydrated state. The data from this run represented the subject's
response to gravitational stress exposure in his baseline state was
used as control data to evaluate the effects of dehydration.

Run LBNP II was performed on the same day three hours after
taking a measured amount of an oral diuretic. Data from this run
was used to assess changes in the aforementioned parameters with
respect to the effects of acute dehydration.

A typical test sequence starting at about 8:00 a.m. was as
follows:

Episode	Duration
Report to lab, attach sensors, enter box, cannulate vein.	1 hour
Test LBNP I	30 min
Exit box followed by rest interval to restore plasma volume	30-45 min
Ingest diuretic, collect urine, re-enter box.	3 hours
Test LBNP II	30 min
TOTAL	5½-6 hours

Monitoring

During each LBNP run the subject's B/P and HR were monitored
each min via a cuff placed on the right arm. Changes in the left calf
circumference were recorded during the second and fifth minutes of
each stage. These circumference changes were monitored with a
precalibrated mercury strain gauge (Parks Electronic Laboratory,
model 207 Loosco). The gauge was attached around the left calf
at its largest circumference with a tension equal to 15 g. The
method of attachment, calibration and reading of record was the same
as previously described (Venters, 1976). Graphic records of
limb volume changes were displayed on a Visicorder (Honeywell, model
1508 A). This system utilized the Hg-strain gauge as one side of a
wheatstone bridge and the Visicorder as an alternating current bridge

to permit amplification and recording of changes in gauge length.
The gauge was firmly attached to the limb with moisture resistant
tape (3M Company, No. 1525). HR was also monitored continuously with
a three lead electrocardiogram (ECG) which utilized disposable electrodes
(NDM, 01-1030) and an amplification system coupled with the Visi-
corder for graphic readout.

Limb compliance

Limb or leg compliance, in this text, refers to the leg's ability
to gain or lose volume both intravascularly and extravascularly
relative to the cumulative LBNP stress in Torr x min. This value was
calculated from a linear regression analysis relating the percent
volume shift during the second and fifth minutes of each LBNP stage
to its corresponding Torr x min value. The regression formula yielded
the manner in which a change in leg volume was related to Torr x min
of stress as follows:

$$y = a + b(x)$$

where y: percent change in leg volume

      x: Torr x min of stress at the point where leg volume was

        measured

      a: intercept of the regression line

      b: slope of the regression line

The slope (b), was indicative of the rate at which volume was shifted
from an initial point per Torr x min of LBNP stress exposure. This
slope was then multiplied by 10^5 to obtain whole numbers and gave a
quantitative expression of a subject's propensity to shift a given
volume to his legs for a given LBNP stress (Δ% volume/Torr x min).

Dehydration

Between LBNP runs I and II, each subject underwent acute dehydration by diuresis. This was achieved through the use of oral Lasix (4-chloro-N-furfuryl-5-sulfamoyl-anthranilic acid, Hoechst-Roussel Pharmaceuticles). Preliminary studies indicated that a dose of 50-70 mg would, in three hours, produce a weight loss of 2.0 - 2.5% of body weight through increased urine output. The relative subject dose in mg was based upon the results of these preliminary tests. Subjects were asked to void immediately prior to taking the Lasix, which was accompanied by 20 cc distilled water. This was to insure that all urine eliminated was indicative of only the Lasix effects. No fluid replacement was allowed during this period of diuresis. All urine eliminated during this time was measured for volume and collected for pooled analysis of electrolytes and osmolality. Subject weight was taken just prior to Lasix ingestion (baseline) and each time the subject voided after the Lasix dose. The repeated weights were compared with their respective urine outputs to insure that all weight loss was accounted for by the urinary output. Every 30 min the subject's HR and B/P were measured both supine and upright. The measurement was done in both positions to anticipate possible orthostatic hypotension due to the rapid fluid loss. Rectal temperature (Tre) was also measured before and after LBNP I and LBNP II. This was to insure that there was no change in core temperature due to either LBNP or Lasix dehydration which might affect the peripheral circulation.

Blood volume and constituents

To analyze the direct effects of LBNP and Lasix dehydration on blood volume and composition, venous samples were drawn at various

points during the LBNP tests. Total hemoglobin (THb) and blood volume
(BV) were determined with a carbon monoxide (CO) rebreathing method
(Myhre, et al., 1968).

With the subject resting supine in the LBNP box an indwelling
catheter (B-D, 6743) was placed in a peripheral arm vein under local
anesthetic (1 cc, 2% Xylocaine). Utilizing a 3-way stopcock (Pharmaseal,
K-75), a syringe filled with heparinized saline and a vacutainer system
(B-D), samples could be taken rapidly at any time and the system could
be kept open. The use of this system allowed the subjects to undergo
a single needle placement for all blood sampling.

After catheter placement the subjects breathed, via mouthpiece,
100% O_2 for 5 min on an open system. At this time a baseline sample
was drawn. After switching to a closed system with CO_2 absorber,
a measured amount of 99% CO was then injected into the system.
Subjects then continued to breath on the closed system for 10 min
and a second sample was drawn. An infrared method was used to measure
carboxyhemoglobin saturation (Coburn, et al., 1964). From this meas-
urement the THb was calculated as follows:

$$THb = \frac{V_{CO} \times 0.985 \times 100}{1.34 \times S_{CO}}$$

where THb: total circulating hemoglobin

     V_{CO}: volume of CO in ml (STPD) injected into rebreathing

          system

   0.985: average fraction of CO taken up by the blood at the

          end of 10 min rebreathing, the remainder being bound

          to myoglobin

    1.34: CO capacity of 1 g hemoglobin (Hb)

S_{CO}: measured carboxyhemoglobin saturation

Blood volume was calculated from Hb and THb as follows:

$$BV = \frac{THb}{Hb \times 10}$$

Plasma volume (PV) was then calculated from BV and hematocrit (Hct) as follows:

$$PV = \frac{BV \times (100 - Hct)}{100}$$

Red blood cell volume (RCV) was calculated as follows:

$$RCV = BV \; 1 - \frac{Hct}{100}$$

Based on previous studies in this laboratory it was assumed that THb did not change significantly during the LBNP runs or Lasix dehydration (Luft, et al., 1976; Myhre and Robinson, 1977). By making this assumption, the effects of LBNP and dehydration on PV, BV and RCV could be evaluated by the measurement of Hb and Hct at various times. The values were subsequently entered into the above equations to calculate the desired PV, BV, and RCV. Hb concentration was measured by the cyanmethemoglobin method using a Beckman spectrophotometer (Beckman, model DU). Hct was determined with a microhematocrit centrifuge (Chicago Surgical and Electrical Company, model 33).

To evaluate the separate effects of LBNP and Lasix dehydration upon BV and constituents samples were drawn as follows:

Time drawn	Amount	Purpose
Before LBNP I	35 cc	THb analysis; plasma electrolyte and osmolality
2 min after LBNP I	18 cc	Hb and Hct; plasma electrolyte and osmolality
30-45 min after LBNP I	3 cc	Hb and Hct

Time drawn	Amount	Purpose
Immediately before LBNP II	18 cc	Hb and Hct; plasma electrolyte and osmolality
2 min after LBNP II	18 cc	Hb and Hct; plasma electrolyte and osmolality
Total Draw	92 cc	

Comparison of these samples allowed: (i) solely LBNP effects;

(ii) solely Lasix dehydration effects, or (iii) combined LBNP and

Lasix dehydration effects to be evaluated. The additional sample

drawn 30-45 min after LBNP I was to insure that blood parameters had

been returned to before LBNP I values prior to Lasix administration.

At least 60 cc of the 92 cc blood drawn was replaced with heparinized

saline during the drawing and flushing procedure involved in obtaining

a sample. Therefore, each subject's total volume loss was on the order

of 30 cc. The plasma electrolyte and osmolality samples were allowed

to separate and were then centrifuged for 20 min (IEC, model SBC)

to insure maximal plasma sample volume. This plasma was then analyzed

for sodium (Na^+) and potassium (K^+) on a flame photometer (IL-142),

for chloride (Cl^-) via coulometric-amperometric titration (Radiometer)

and osmolality from freezing point depression (Advanced Instruments

Osmometer). These same instruments and analyses were used to evaluate

the pooled urine sample for electrolyte concentration and osmolality.*

Electrolyte concentrations were expressed in milliequivalents per liter

of volume (mEq/L) and osmolality was expressed in milliosmoles per kg

(mOsm/kg). From these measurements the absolute values in total

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*For these analyses we are indebted to S.E.D. Medical Laboratories, Albuquerque, New Mexico.

mEq and mOsm were calculated, with knowledge of the plasma and urine volumes.

Ancillary measurements

All but one subject (#8) was evaluated in reference to his maximal aerobic power ($\dot{V}_{O2_{max}}$) under work stress on a bicycle ergometer. $\dot{V}_{O2_{max}}$ was obtained by increasing the break load 75 mKg/min from a 3 min baseline of 300 mKg/min until the subject was unable to maintain the pedalling rhythm of 50 cpm (Luft, et al., 1963). ECG, B/P and HR were monitored continuously throughout the test. Douglas bags were collected at given intervals and analyzed for $\%O_2$, CO_2 and volume. These values were used to calculate $\dot{V}_{O2}$, $\dot{V}_{CO2}$, volume inspired ($\dot{V}_I$) and respiratory exchange ratio with methods commonly used in this laboratory. This test was used to insure that the selection of the subjects with reference to their physical condition was valid.

Statistical Analysis

Relationships between LBNP tolerances and the cardiovascular responses associated with LBNP exposure were evaluated by linear regression (Popham and Sirotnik, 1973a), paired "t" and separate "t" models of analysis (Popham and Sirotnik, 1973b). These same analyses were used to evaluate the relationship of LBNP induced cardiovascular responses and direct blood volume and leg volume variations. Analyses of differences in all parameters between euhydrated (LBNP I) and dehydrated (LBNP II) runs were also performed with these analyses. The 95% confidence limit was selected to represent significant changes or relationships among the parameters measured.

RESULTS

Subjects

Ten subjects were recruited from staff members and their personal acquaintances. Mean data on physical characteristics (Table 1) indicates: (i) a mean age of 31 years, (ii) a mean height (Ht) of 178 cm, and (iii) a mean weight (Wt) of 73.1 kg. Maximal aerobic power ($\dot{V}O_{2max}$) under work stress was evaluated using a bicycle ergometer. Mean maximal oxygen comsumption per kg ($\dot{V}O_2$/weight) was 42.3 ml/min/kg. Close inspection of this data (Table 1) indicates that the subjects fall into two physically distinct groups. No statistically significant differences existed between subjects 1 through 5 and subjects 6 through 10, with respect to age, Ht, or Wt. However, the first five had a significantly greater (p<.001) average $\dot{V}O_{2max}$, $\dot{V}O_2$/weight, and O_2 pulse.

Effects of Lasix Dehydration

Administration of oral Lasix induced diuresis within approximately 50 min to 1 hr. This diuresis resulted in a mean urinary output of 1.58 liters, representing a mean body weight loss of 1.6 kg or -2.3% within 3 hr (Table 2). At the same time, plasma volume (PV) was reduced by approximately 530 ml (16.8%) and this was a highly significant decrease (p<.001). The Lasix dose for all the subjects averaged 0.86 mg/kg, but a comparison of subjects 1 through 5 and 6 through 10

indicated a significantly (p<.001) smaller average dose per kg in the former (0.79 mg/kg) than in the latter (0.92 mg/kg).

Despite the appreciable fluid loss, plasma electrolyte concentrations and osmolality remained within normal limits (Table 3). Plasma sodium (Na^+) levels decreased 3 mEq/L, potassium (K^+) levels were constant, chloride (Cl^-) levels decreased 4 mEq/L and osmolality decreased 7 mOsm/kg. However, calculation of total plasma electrolytes in the ciruclation did indicate a genuine loss (Table 4). This was calculated by subtracting the product of electrolyte concentrations (Table 3) and plasma volume (Table 2) after dehydration from the corresponding products before dehydration. Mean loss of Na^+ was 84 mEq, of K^+ was 2.2 mEq, and of Cl^- was 70 mEq. By dividing these figures by the fluid volume lost from the plasma, it would appear that the extravasate had a higher electrolyte concentration than the plasma had in the first place, namely 158 mEq/L Na^+, 4.2 mEq/L K^+, and 132 mEq/L Cl^-.

Urinary output increased with Lasix administration and the urine outflow was accompanied by electrolytes from the plasma and other body fluids. Mean urine electrolyte concentrations of 125 mEq/L Na^+, 18 mEq/L K^+ and 144 mEq/L Cl^- were observed (Table 5). Osmolality of the urine averaged 326 mOsm/kg. Multiplying urinary electrolyte concentrations by the urine volume loss (Table 5) gave the total urinary electrolyte and osmolality losses due to Lasix (Table 6). The excreted fluid contained an average of 198 mEq Na^+, 28.2 mEq K^+ and 229 mEq Cl^-, resulting in an average of 511 mOsm of solutes. This represents a greater mEq loss in Na^+ than K^+, and a mEq Cl^- loss nearl· equal to the sum of the Na^+ and K^+ losses. The Na^+/K^+ excretion ratio

was raised from a normal of 2/1 to 7/1 by Lasix induced diuresis.
While these values were within the normal ranges for urinary losses
during 24 hr, the time period in this case was only 3 hr. Therefore,
the observed values were compared with an average normal 3 hr output,
using values from Table 6:

	Lasix Losses	Normal Losses
Na^+ (mEq)	198	24.4
K^+ (mEq)	28.2	7.8
Cl^- (mEq)	229	22.5
Osmolality (mOsm)	511	113
Urine volume (ml)	1575	138

Lasix induced losses, obviously, represent 811%, 326% and 1018% of
the respective Na^+, K^+ and Cl^- losses normally expected during 3 hr.
Urinary volume and osmolality are similarly greater than expected
(1141% and 452%, respectively).

It was also of interest to assess the amount of urinary electrolyte
losses accounted for by losses in plasma electrolytes by comparing
the mEq plasma electrolyte losses (Table 4) with the mEq urine electro-
lyte losses (Table 6). On the average, 114 mEq more Na^+, 26 mEq more
K^+ and 159 mEq more Cl^- were lost in the urine than came from the
plasma. This means that plasma electrolyte losses accounted for only
42% of the Na^+, 8% of the K^+ and 31% of the Cl^- losses in the urine,
with the remainder coming from extravascular sources.

Measurements of supine and upright HR and B/P during the Lasix
dehydration failed to show any significant increases or decreases with
respect to their baseline values. Rectal temperatures also failed
to evidence any statistically significant variation during this time.
Observed average values were, in fact, only $0.1^\circ C$ different ($37.4^\circ C$
before Lasix and $37.5^\circ C$ after Lasix).

Effects of LBNP Stress

The LV began to increase immediately upon LBNP onset and continued to a maximal increase just prior to LBNP termination. Increases were apparent not only with each progressive LBNP step, but also during constant negative pressure within the LBNP stages (Table 7, LBNP I and Table 9, LBNP II). During the initial 2 min of LBNP (-20 Torr) the greatest LV increase occurred (First column, Table 7 and Table 9). Regardless of subject hydration the average increase during this time was nearly the same (0.92% LBNP I and 0.95% LBNP II). Successive negative pressure increases produced lesser LV increases than did the onset of negative pressure. However, progression from one LBNP stage to another always elicited a greater LV increase than that observed within each stage. The average amount of LV increase within each stage and upon progression to a higher stage was, in most cases, remarkably constant comparing LBNP I and LBNP II. In euhydrated subjects (LBNP I) total leg volume increases averaged 3.85% while in dehydrated subjects (LBNP II) total leg volume increases averaged only 2.34% (Figure 2). This difference was highly significant statistically (p<.001).

Upon releasing LBNP, LV initially declined rapidly towards baseline, then progressively declined at a slower rate. The largest volume decrease always occurred within the first 10 sec after LBNP release. During LBNP I, leg volume decreased by 1.77% within 10 sec (compare Final and 10 sec columns, Table 8). Leg volume decreased by 1.69% within 10 sec of LBNP II release (compare Final and 10 sec columns, Table 10). While the percent decreases are similar, the amount of total leg volume increase they represent is greatly different. A 1.77% decrease after LBNP I represents a 46% volume return, but a

1.69 % decrease after LBNP II represents a 72% volume return. The more rapid volume return after LBNP II was evidenced throughout the measured period. This propensity was evaluated by measuring the leg volume change that persisted 40 sec after LBNP release. It was assumed that the initial rapid drop was due to capacity vessel emptying and the 40 sec value was representative of extravascular volume (edema). Expressing this residual volume as a fraction of total leg volume increase (RLV_{40}/TLV) the following mean edema indices were derived:

	Total leg volume increase %	Residual leg volume 40 %	$\Delta\%$
LBNP I	3.85	1.43	37.1
LBNP II	2.34	0.18	7.7

With the exception of two subjects (No. 3 and No. 9), who completed both tests, LBNP tolerance (Torr x min) was consistently lower following dehydration (Table 11). Mean tolerance averaged 829 Torr x min before and 496 Torr x min after dehydration, a difference of 40% ($p<.01$). Dividing the total leg volume change by tolerance indicates that leg volume increased 0.47% per 100 Torr x min. Obviously, this is an oversimplification since leg volume increased more with changes in LBNP than with constant LBNP (i.e., leg volume did not increase in an arithmetic fashion). Therefore, limb compliance ($\Delta\%$ volume/ Torr x min x 10^5) was calculated (Table 11). The mean leg compliance (LC) during LBNP I (533) and during LBNP II (850) differed by 59%; however, this was not statistically significant. The reason for the lack of significance appeared to be in the great intra-individual variations. The data revealed that while some individuals greatly increased their LC with dehydration, others actually decreased.

Closer inspection of LC and tolerance values revealed a trend towards greater LC being associated with lesser tolerance. Individuals whose LC increased with dehydration also found their maximal tolerance greatly decreased, but those whose LC decreased, had lesser or no tolerance decreases (Table 11). These observations indicated a relationship between LC and a subject's ability to tolerate LBNP, which was evaluated by linear regression as follows:

$$y = a + b(x)$$

where   y: LBNP tolerance in Torr x min

        x: Individual LC value

        a: Intercept of regression line

        b: Slope of regression line

Regressions calculated for both LBNP I and LBNP II indicated a highly significant ($p < .01$) correlation between LC and tolerance in the form of greatest LC being associated with least tolerance. However, the slopes and intercepts of these regression lines are obviously different (Figure 3).

The characteristic tachycardia associated with orthostasis and LBNP was also observed in this study. In order to evaluate HR responses to LBNP before and after dehydration, the average HR's during the final min of the test were compared to the average HR's during the 5 min control period before LBNP began (Table 12). Regardless of hydration state, HR always increased significantly ($p < .001$) during LBNP. Control and final HR values were not significantly different between LBNP I and LBNP II and neither were the average % increases (43% and 46%, respectively). Despite these similar % increases, differences do exist.

Tolerance was significantly different between LBNP I and LBNP II

(Table 11) and a linear regression analysis indicated a significant

(p<.01) correlation between tolerance and %ΔHR (Figure 4). The slope

of the regression line for LBNP II is, however, greater than that for

LBNP I and indicates greater %ΔHR for a given LBNP stress.

Pulse pressure (PP) decreased significantly (p<.001) during

both LBNP runs (Table 13). On the average PP decreased 43% during

LBNP I and 45% during LBNP II, and the difference was not significant.

Control and final PP were both lower in dehydrated subjects (26% and

32% respectively), but again this difference was not statistically

significant. Unlike HR increases, PP decreases are not correlated

with cumulative stress tolerance.

A linear regression analysis comparing maximal %ΔHR and maximal %ΔPP

under LBNP was performed (Figure 5). A highly significant (p<.01)

correlation existed during LBNP I, in the form of greatest %ΔHR being

associated with greatest %ΔPP. Even though the same trend existed

during LBNP II, the correlation was not significant (p>.10). Pooling

values for LBNP I and LBNP II again resulted in a significant correlation

(p<.01) of the same form.

Average THb was 862 g for the subjects in this test series. LBNP

induced hemoconcentration was evidenced by increases in Hb and Hct

after LBNP release (Table 14). Hb increased an average of 1.1 g/100 ml

and Hct increased an average of 3.1% during LBNP I, but only 0.4 g/100 ml

and 1.4%, respectively, during LBNP II. Part of this difference was

accounted for by the hemoconcentration due to Lasix dehydration and

part by the difference in LBNP exposure. On the average Lasix dehydration

resulted in a 1.7 g/100 ml Hb increase and a 4.3% Hct increase (comparing

before LBNP I and LBNP II values, Table 14). Both of these were statistically significant increases (.02<p<.05, Hb and p<.01, Hct).

The average Hb content of the red cells did slightly increase during LBNP I and slightly decrease during LBNP II, but these changes were not significant (Table 15). Lasix dehydration also increased the average Hb content of the red cells but again this was not significant (compare before LBNP I and LBNP II values, Table 15).

Red cell volume (RCV) tended to become smaller during LBNP I and Lasix dehydration (Table 16). In contrast, RCV tended to increase during LBNP II. This would suggest that some fluid was extracted from the cells as well as the plasma in two situations (LBNP I and Lasix dehydration) and that some fluid was absorbed by the cells in LBNP II. These RCV changes were consistent with those observed in the Hb content of red cells (Table 15). However, none of the RCV changes was statistically significant.

The marked changes in BV during LBNP stress (Table 16) were presumably due to fluid shifts in or out of the vascular system and these were more directly reflected in the alterations in PV (Table 16). Euhydrated LBNP exposure resulted in a significant (p<.001) average PV loss of 365 ml. The PV loss associated with dehydrated LBNP exposure was also significant (p<.01), but amounted to only 126 ml. Not only was there less ml PV lost during LBNP II, but the respective percent of initial PV it represented was less (Figure 2). An average of 12.2% of initial PV was lost during LBNP I, but PV loss during LBNP II was only 4.8% of initial and the difference was significant (p<.001). Prior to Lasix administration the subject's PV was allowed to return to its pre-LBNP I level. Therefore, the control PV was used

to calculate the average 16.8% PV loss due to Lasix (Figure 2). The average 21.6% PV loss seen after LBNP II was merely the sum of the PV loss due to Lasix and the PV loss due to LBNP II.

A high correlation (p<.01) was found between % PV loss induced by LBNP and cumulative stress tolerated (Torr x min) in euhydrated subjects (Figure 6). However, when dehydrated this correlation was not significant, but the trend towards greatest stress tolerance being associated with largest % PV losses was maintained. Regressions were also computed comparing % PV loss due to Lasix and the change in tolerance between LBNP I and LBNP II, but no significant correlation was found.

A high correlation (p<.01) was indicated between PV loss (ml) and % LV increase in euhydrated but not dehydrated subjects (Figure 7). However, in both cases a trend towards greatest PV loss being associated with largest % LV increase was evidenced. Since the increase in LV during LBNP is attributable to the accumulation of fluid outside as well as inside the blood vessels, the former should correspond more closely to the observed loss of PV. An approximation of the amount of fluid extravasated was obtained from the increase in LV remaining 40 sec after release of LBNP (%RLV40), when most of the blood pooling had subsided (Tables 8 and 10). A linear regression of ΔPV versus %RLV40 on the data from LBNP I and II gave a highly significant correlation between these two variables to the effect that the larger %RLV40, the greater the loss of PV.

DISCUSSION

Lasix Dehydration

Previous studies revealed that Lasix administration always resulted

in an increased urinary output and subsequently caused a PV loss

(Kleinfelder, 1963; Robson et al., 1964 and Stason et al., 1966).

In the present study Lasix administration also elicited similar responses.

The 3 hr diuresis resulted in an average urinary output of 1.58 liters and

consequently a highly significant (p<.001) PV loss of 16.8% (Table 2).

Since Lasix primarily inhibits Na^+ reabsorption without altering

bicarbonate excretion (Kleinfelder, 1963; Stason et al., 1966 and

Davidson, 1969), it was not surprising to have an increased urinary

output. Unfortunately, the experimental protocol in most previous

studies usually called for multiple Lasix doses and utilized patients

with various maladies. Therefore, a direct comparison of urinary output

and PV loss between these and the present study was not possible.

Two recent studies did address the effects of a single oral or

a single intravenous Lasix dose on normal subjects. Claremont et al.

(1976) orally administered 60-80 mg of Lasix and produced a mean 3 hr

urine output of 2.18 liters (-2.3 to -3.2% of initial body weight).

At the same time PV decreased an average of 15 to 16%. Kimura et al.

(1976) intravenously injected 60 mg of Lasix and noted an average

PV loss of only 11.2%. However, prior to Lasix injection their subjects

stood erect for 1 hr and PV was somewhat depleted by this procedure.

Therefore, the actual PV loss was more on the order of 17%. Presently,

an attempt was made to arrive at a Lasix dose which led to approximately

the same degree of fluid loss as had been attained in the previous

(Luft et al., 1976) exercise and heat study, namely an average of

2.3% body weight. Preliminary tests with the drug indicated that a

dose of 0.9 mg/kg would be appropriate and this actually produced an

average weight loss of 2.3% in the main study (Table 2). Inspite of

fairly well balanced effects of both modes of dehydration as far as

total body water loss, the PV loss with Lasix (16.8%) was considerably

greater than after exercise and heat dehydration (12.8%). Following

exercise and/or thermal dehydration Costill and Fink (1974), and Myhre

and Robinson (1977) also noticed lesser PV losses (6 to 7%) with nearly

equivalent weight losses. A possible explanation for these discrepancies

may be that with diruesis considerably more Na^+, K^+ and Cl^- were lost

in the urine than were lost in an equal volume of sweat. In both thermal

and diuretic dehydration Na^+ and Cl^- constituted large ionic depletions

of the extracellular fluid (Woodbury, 1974). During thermal dehydration,

Costill, Coté and Fink (1976) found an increased total body fluid

osmolality and a decreased volume of the intracellular and extracellular

fluid compartments in proportion to their original volumes. In contrast,

Woodbury (1974) proposed a reduction only in extracellular volume with

little or no change in intracellular volume if Na^+ and water were lost

in such a manner that plasma Na^+ and osmolality were not altered. He

also postulated a decreased sweating rate during prolonged exercise and/or

thermal dehydration in an attempt to reduce Na^+ loss, when its intake was

inadequate or absent. However, during Lasix diuresis Na^+, K^+, and Cl^-

losses continued unchecked throughout the dehydration (Claremont et al.,

1976; Kimura et al., 1976). This was primarily the result of the drug's

mode of action (i.e., blocking Na^+ reabsorption). Therefore, it is not surprising that substantially more plasma water might be lost than would be anticipated with thermal and/or exercise dehydration.

A major condition of the above explanation is that plasma electrolyte concentrations and osmolality do not change. This phenomena was previously observed in other studies (Stason et al., 1966 and Claremont et al., 1976) and data from the present study was consistent with this contention (Table 3). However, electrolytes were apparently lost from somewhere because the total plasma electrolyte (mEq) levels were reduced as the PV decreased (Table 4), but the electrolyte concentrations in reference to the volume remaining stayed the same. The estimated electrolyte concentration of the extravasate was, in fact, higher than the plasma initially. This indicates that some of the electrolytes must have come from some other source than the plasma fluid. An extravascular electrolyte source would allow the replenishment of plasma electrolyte concentrations and account for the higher concentrations in the extravasate. Garrett (1971) previously proposed a similar utilization of body reserves in individuals with altered renal function.

Increased urinary output was always accompanied by enhanced urinary electrolyte losses (Kleinfelder, 1963; Robson et al., 1965; Davidson, 1969; and Claremont et al., 1976). The present study also evidenced this augmented electrolyte loss (Table 5). While these losses were within the normal values for a 24 hr period, they were greater than those expected within a normal 3 hr period. As previously proposed (Kleinfelder, 1963 and Davidson, 1969) Na^+ excretion was increased more than K^+. Excretion of Cl^- also exceeded that of sodium, in fact, it was nearly equal to the sum of Na^+ and K^+ excretion. Favorable

contributed by the plasma and 66% by extravascular sources. The electrolyte distribution relative to the total loss indicates that, whereas the Cl^- distribution was about the same as that for water, slightly more Na^+ was contributed by the extravascular space for the same amount of water. The greatest discrepancy was found in K^+, where 92% of the total K^+ loss came from the extravascular space. Since the K^+ concentration of interstitial fluid is usually in fairly close equilibrium with the plasma and no marked depletion of K^+ was seen in the latter, it is most likely that the major part of the K^+ excreted from the extravascular space originated within the cells, presumably in the muscles, where 90% of the intracellular K^+ is located (Woodbury, 1974).

Few studies have addressed the effect of a diuretic induced fluid and electrolyte loss on the total exchangeable electrolyte content. The total amount of the electrolytes discussed here found in exchangeable form in adult man is on the average: 41 mEq/kg Na^+, 52.8 mEq/kg K^+ and 33 mEq/kg Cl^- (Woodbury, 1974). Using these values for the average weight of our subjects (73.1 kg) one can compare the electrolyte loss observed in this study with the total exchangeable amounts available:

	Estimated Total Exchangeable mEq	Electrolyte Lost mEq	% of Total
Na^+	2997	198	6.6
K^+	3860	28.2	0.73
Cl^-	2412	229	9.5

Similar losses were observed by Claremont et al. (1976), and they proposed that these losses were easily handled by the body under normal conditions but could have some effect during strenuous exertion. They also surmised that the major K^+ came from muscle tissue, and therefore, some alteration in resting membrane potential was also possible.

The alteration in resting membrane potential should have been offset by the decreased exchangeable Na^+ concentrations. However, under any type of stress even a slight variation could have shown up as an increased cardiac excitability or altered sympathetic neural function.

An interesting point in considering the PV and electrolyte losses in this study was that the Lasix dose per kg body weight differed. Referring to Table 2, only 0.79 mg/kg of Lasix elicited an average 17.5% PV loss in subjects 1 through 5, but a 0.92 mg/kg Lasix dose gave only a 16.1% PV loss in subjects 6 through 10. While the difference in PV losses were not significant, the mg/kg dose was ($p < .001$). Similarly the electrolyte losses associated with the greater fluid loss were slightly greater in relation to total exchangeable electrolytes. This indicates a marked variability in Lasix susceptibility which must be taken into account when using this diuretic.

The Effects of Dehydration by Diuresis (Lasix)

and by Exercise in the Heat on Responses to LBNP

The experimental design of the present study involved the use of an oral diuretic to induce acute dehydration. This type of dehydration has not previously been investigated in reference to its effects on responses to LBNP. However, Luft et al. (1976) have reported the effects of exercise and heat dehydration (EH) on LBNP responses. This study also employed identical fluid shift measurements and cumulative stress protocol to those in the present study. Therefore, the following discussion will be divided into two parts: (i) a direct comparison between the current study and that of Luft et al. (1976), designated as EH and (ii) a summary section evaluating those differences with reference to studies that employed other procedures for manipulating plasma volume.

The increase in LV under LBNP was not as great after, as before either Lasix or EH dehydration, however, the difference between LBNP I and LBNP II was greater after Lasix (Figure 2). Differences in the loss of PV by dehydration may have influenced the magnitude of the LV shift. The greater PV loss after Lasix apparently allowed a critical reduction in central blood volume to occur before LV had increased as far as in the euhydrated state.

Upon releasing LBNP, LV exhibited an initial rapid decline followed by a progressively slower rate of decline, regardless of subject hydration. However, the actual rate of volume return was greatly different comparing LBNP I and LBNP II (Table 8 and Table 10, respectively). Similar LV declines were noticed in the EH study, but edema indices

(as defined above) did indicate a definite difference. Equivalent
edema indices were noted in both Lasix and EH studies following
euhydrated LBNP (37.1% and 38%, respectively). In contrast, the
edema index following a dehydrated LBNP in the EH study was much
greater than in the present study (18% and 7.7%, respectively).

Both modes of dehydration led to a significant loss (p<.01) in
LBNP tolerance, however the decrement was greater after Lasix (Table 11)
than after EH (40% and 31%, respectively). Despite the significant PV
loss associated with both types of dehydration no correlation was found
between the PV deficits caused thereby and the lower tolerance in LBNP II.
This indicated that even though the PV loss influenced the tolerance,
it was not the sole factor responsible for the decreased tolerance.

Dehydration of either form was always associated with a greater
average LC but marked individual variations were noted with both
Lasix (Table 11) and EH. In each study, vasoconstriction was assumed
to be reflected by low LC. The greater LC of a dehydrated subject
indicated a decreased vasoconstrictive response. However, the observa-
tion of an increased LC in some dehydrated subjects and a decreased
LC in others demonstrated a marked variability in the direction and
magnitude of the vasoconstrictive response. A most surprising aspect
of the LC comparisons was the 59% elevation found after Lasix (Table 11)
and only a 14% greater LC following EH. As to whether this difference
reflects a direct effect of Lasix or merely a general effect of diruesis
is not clear. Nevertheless the main conclusion is that the mechanism
responsible for controlling LC was affected differently by the two
modes of dehydration. The difference in euhydrated and dehydrated LC
with Lasix (Table 11) and EH was not significant because of intra-
individual variations.

Despite the wide scatter between individuals (Table 11), there was a high inverse correlation between tolerance and LC both before (LBNP I) and after dehydration (LBNP II). However, the regression line for LBNP II is lower and the angle of the slope is less than in LBNP I (Figure 3), reflecting the reduced tolerance associated with increased compliance, as compared to LBNP I. Augmented compliance was also a characteristic of the LBNP test after EH, but to a lesser degree. After exposure to EH rectal temperature rose to 38.4^{o}C and one might attribute the greater LC to peripheral vasodilation. This argument can not be applied to explain the much larger increment in compliance after Lasix, so other factors must be involved here which will be discussed later.

Without exception HR increased during all LBNP tests. During the control runs (LBNP I) the initial HR was very similar in both groups (61 bpm before Lasix and 64 bpm before EH) and the increase in percent of control was also close (44% and 41%). After dehydration with Lasix the initial HR was only slightly elevated and the percent increase was the same but the maximum HR was reached at a significantly lower level of stress. After dehydration by EH, the control HR was 18 beats higher than before, while the final value was 23 beats more than prior to dehydration and 21 beats higher than the corresponding value in the Lasix group at exactly the same amount of cumulative stress. The higher HR before and throughout LBNP II after EH are no doubt attributable to the fact that their T_{re} was 1°C higher than in LBNP I due to the heat exposure, while no difference in temperature was observed between the two tests with Lasix. It is noteworthy that while hyperthermia markedly affected the HR response to LBNP, it apparently

had no deleterious effect on the tolerance of this stress itself.
Inspite of the appreciable differences in actual HR during LBNP
after Lasix and EH, the percent increase in frequency was similar
in both studies (44% and 47% respectively). The regression lines in
Figure 4, where maximal Δ%HR is plotted against LBNP stress before and
after dehydration with Lasix, show that approximately the same maximal
Δ%HR was reached on the average before and after dehydration, but
at a considerably lower end point of tolerance in the latter. These
observations imply that a limit may exist to the effectiveness of
the HR response in compensating for a dwindling stroke volume in this
particular form of stress.

LBNP exposure dramatically reduced pulse pressure (PP) regardless
of the method of dehydration. Regressions indicating the percent
fall in PP per unit of stress in the present study were as follows:

LBNP I   y = 10.18 + 0.0391(x),   r = .788 p<.01

LBNP II   y = 21.30 + 0.0369(x),   r = .554 p<.01

where x: amount of stress expressed as Torr x min

y: percent decrease in PP over control

The PP response to LBNP appears no more pronounced (slope) after Lasix
than before. Despite lower control and final PP in the Lasix dehydrated
subjects, the average maximal Δ%PP was only 1% lower (Table 13).
In contrast, euhydrated and dehydrated control PP were identical in
the EH study, but final PP was lower in the dehydrated subjects.
This resulted in a 15% greater average maximal Δ%PP. The greater PP
decrease in EH subjects was probably accounted for by vasodilation
and increased blood pooling in exercised muscles.

Another pertinent finding of the present study was a high inverse correlation between maximal $\Delta\%HR$ and maximal $\Delta\%PP$, which was significant ($p<.01$) only in euhydrated subjects (Figure 5). While the correlation was not significant in LBNP II, the trend of greatest maximal $\Delta\%HR$ being associated with largest maximal $\Delta\%PP$ was maintained. In general, the increase in HR associated with an equivalent decrease in PP appears greater after Lasix dehydration. This is consistent with a dehydrated subject having a smaller stroke volume as the result of a lower total blood volume and a depressed venous return. A smaller stroke volume elicits an augmented HR response to prevent a fall in PP.

Plasma volume losses induced by LBNP were less following either form of dehydration than before. A lesser PV loss in a dehydrated subject was to be expected because overall stress duration was less and the total PV was decreased to begin with. However, the 4.8% PV loss associated with the Lasix study (Figure 2) was considerably greater than that in the EH study (1%), while LBNP tolerance was nearly identical in both (496 Torr x min, Lasix and 495 Torr x min, EH). Therefore, more PV was lost under an equivalent LBNP stress in a Lasix dehydrated subject. On the other hand, the discrepancy in euhydrated PV loss between Lasix and EH (12.2% and 8.7%, respectively) may be accounted for in part by the differences in average tolerance (829 Torr x min, Lasix and 720 Torr x min, EH).

Another interesting variation between the two studies was the correlation of this PV loss with LBNP tolerance. LBNP tolerance always correlated well with the PV loss in the EH study but only in euhydrated subjects in the present study (Figure 6). Even though ΔPV

and LBNP tolerated were always correlated in the EH study, the correlations were lower in a dehydrated subject. Why dehydrated subjects evidenced a lower correlation with EH and no correlation with Lasix is not clear, but may be due to the interaction of various other factors with tolerance.

A highly significant (p<.01) correlation was presently found between maximal leg swelling and PV loss (ml) under LBNP in euhydrated subjects (Figure 7). An equally good correlation was demonstrated when euhydrated and dehydrated data were pooled in the Lasix study (n = 10) and in the EH study (n = 20). In contrast, no significant correlation was indicated during LBNP II, but the trend toward greatest PV loss being associated with largest %LV increase was maintained. Since the LV increase is attributable to both intravascular and extravascular accumulation, the latter should correspond more closely to the observed PV loss. This concept is supported by a highly significant (p<.01) correlation between PV loss and residual leg volume (%RLV40) in both studies.

The only situation where there was a discrepancy in the change in plasma volume and the edema formation between the two modes of dehydration was in LBNP II. With Lasix there was less edema after the second test (RLV = 0.18%) than after EH (RLV = 0.24%), whereas Δ%PV was greater (4.8%) in the former than in the latter (1.0%).

These inconsistencies in the movement of intravascular and extravascular fluids can be reconciled in view of the different methods of dehydration employed in the two studies. Preliminary studies had shown that a period of three hours after ingestion was sufficient to attain a total body water loss equivalent to that produced by

2 hours exercise in the heat, but it was also apparent from these tests that diuresis and water loss continued for several hours longer. Thus, as our subjects were exposed to LBNP II, both diuresis and LBNP stress were depleting the plasma volume. This would explain why the usual relationship seen between ΔPV and %RLV was not seen in the Lasix study, because the fluid lost by diuresis did not show up as edema in spite of a greater overall plasma loss. Dehydration by EH, on the other hand, ceased immediately after leaving the hot room, followed by a fairly rapid replenishment of PV even without ingestion of any fluid (Myhre, unpublished data). This process probably continued during the subsequent LBNP II test, where the negative pressure tended to reduce PV again, as evidenced by the substantial residual swelling of the legs (%RLV). Thus the antagonistic effects of the two processes resulted in a minimal net loss in PV (1.0%).

The differences in %RLV and Δ%PV also point out a surprising effect of dehydration with both Lasix and EH. Less PV was lost and less edema was formed after dehydration (LBNP II) than during LBNP I in the euhydrated state (Table 16 and page 23, results). One reason for this is apparent, namely the lesser duration of LBNP after dehydration. However, this is not sufficient to account for the much smaller amount of extravasation in the second test. Dehydration both by Lasix and EH led to a considerable increase in plasma protein concentration, which was measured in the EH study (+17%) but not in the Lasix study, where it probably was even greater corresponding to the larger loss in plasma water (ΔPV). The higher protein concentration would elevate the oncotic activity of the plasma and tend to counteract the hydrostatic pressure gradient across the capillary walls, resulting in a

smaller amount of fluid loss to the tissues during LBNP after dehydration (%RLV). This might also explain the weaker correlation between ΔPV and tolerance to LBNP in dehydrated subjects mentioned above.

Another difference between dehydration by EH and with Lasix was that the former was inevitably associated with an elevated body temperature (T_{re}) at the time of LBNP II, while there was no significant differenct in T_{re} between LBNP I and II after taking Lasix. Experiments on a human centrifuge by Allen and Crossley (1972), where the subjects were made hyperthermic by immersion in hot water, but were not dehydrated, showed that hyperthermia had a deleterious effect on tolerance for gravitational stress. This was attributed to increased skin blood-flow in response to hyperthermia compounding the loss of central blood volume on the centrifuge ($+G_z$). With this in mind we suspected that the hyperthermia of our subjects in the EH series might have contributed similarly to the loss of LBNP tolerance after dehydration and this was the main reason for repeating the same experiments using diuretic rather than thermal dehydration. But the results presented here demonstrate an even greater loss in LBNP tolerance after dehydration with Lasix than with EH for approximately the same loss of water in percent body weight. The implications are that the elevated core temperature in the EH series did not contribute substantively to the observed loss in tolerance. Indeed, when a correlation was tested between the difference in tolerance between LBNP I and II versus the increase in body temperature, the coefficient was not negative as expected, but positive with acceptable statistical significance (p<.05). At the time it was conjectured that those subjects with higher T_{re} had not perspired as profusely as the others, who had maintained a

better thermal balance, and were, therefore, less dehydrated. But this was not verified statistically.

Two major points in the preceding discussion deserve further consideration: (i) the lack of correlation between PV loss due to dehydration and LBNP tolerance in both studies; and (ii) the more marked LC increase associated with Lasix dehydration.

Previous studies revealed that a PV loss and/or vasodilation resulted in a decreased orthostatic or LBNP tolerance (Eichna and Bean, 1944; Beetham and Buskirk, 1958; Lind, Leithead and McNicol, 1968; and Greenleaf, Bosco and Matter, 1974). These authors also alluded to the existence of a relationship between the magnitude of the PV loss and the ability to tolerate stress. Miller, Johnson and Lamb (1965) and Van Beaumont (1974) made a similar postulation about bedrest induced PV losses and stress tolerance. The general conclusion of these investigations was that the greater the PV loss, the lower the effective blood volume and hence less stress was necessary to achieve a critical blood volume shift.

Support for this concept came from studies where the PV depletion was decreased or normalized prior to stress exposure. The use of LBNP during bedrest decreased PV loss and tilt table tolerance improved according to Stevens et al. (1966). Hyatt and West (1977) transiently returned PV and LBNP responses to near pre-bedrest values with combined LBNP and volume replacement with saline during bedrest. Intermittent exercise during thermal indifferent immersion decreased diuresis and post-immersion tilt table tolerance improved (Stegemann et al., 1975). Unfortunately, stress tolerance was not always fully normalized and even the improvements in tolerance were generally transitory. These

results demonstrate a major effect of PV loss on tolerance alterations, but do not establish it as the sole factor governing orthostatic or LBNP tolerance.

Greenleaf et al. (1977) contended that bed confinement and a subsequent decrease in physical activity resulted in cardiovascular deconditioning. While a marked PV reduction was found, the cardiovascular deconditioning contributed to a decreased tolerance as much as the PV loss. This conclusion is consistent with the lack of correlation between dehydration induced PV decreases and LBNP tolerance alterations in the present and EH studies. Therefore, it seems reasonable to conclude that even though the magnitude of the PV loss influences LBNP tolerance, the manner in which this loss is compensated for is of equal importance.

The marked differences in LC augmentation in the Lasix and EH study may have been the direct result of the dehydration method employed. Thermoregulatory vasodilation was a consistent response to heat stress in several previous studies (Abramson, 1967a; Lind, Leithead and McNicol, 1968 and Allan and Crossley, 1972). Ardill and Fentem (1965) and Gilbert and Stevens (1966) proposed a diminished venous tone in response to rising body temperature. Therefore, the increased rectal temperature ($38.4^{\circ}C$) and the thermoregulatory vasodilation associated with EH may explain the increased LC. The level of LC alteration (+14%) after dehydration was consistent with retention of some vasoconstrictive ability, but an inability to override thermoregulatory vasodilation, as proposed by Johnson et al. (1973).

An explanation for the even greater increment in LC following Lasix is less clear. Hoche and Graybiel (1974) and Kimura et al. (1976)

proposed an interaction between increasing peripheral renin activity
and decreasing venous tone, that was controlled by the magnitude of
the PV loss. Therefore, the larger PV reduction associated with Lasix
dehydration may have depressed venous tone more than with EH. On the
other hand, the variations in vasoconstrictive responses (LC) may have
been related to an altered sympathetic neural activity. Previously,
Stegemann, Framing and Shiefeling (1969) and Stegemen et al. (1975)
proposed that decreased tilt table tolerance following immersion was
due to an incomplete vasoconstrictive compensation for the PV loss.
This blunted compensation was also evidenced in head-down tilt studies
(Kakurin et al., 1976 and Volicer, Jean-Charles and Chobanian, 1976).
They concluded that a depressed vasoconstrictive response was the
result of a decrease in sympathetic neural activity. However, the
lower neural activity was not due to a lack of sympathetic innervation
but to a diminished neural responsiveness. Skipka, Deck and Böning
(1976) estimated sympathetic neural activity by vanillylmandelic acid
excretion and noted that while excretion was always increased during
a PV loss, the magnitude was less in subjects with low tilt tolerance.
Lower baseline and exercise vanillylmandelic acid excretion levels were
previously documented in highly conditioned athletes (Letunow et al.,
1965; Hartley et al., 1973 and Jarsumbeek, Wirth and Haase, 1975).
Venters (1976) also demonstrated that distance runners were less
tolerant of LBNP stress and had higher LC values than did non-athletes.
In both the Lasix and EH studies vasoconstriction was assumed to be
reflected by LC. A plausible speculation might be that the observed
differences in LC are the result of variations in sympathetic neural

activity between subjects. In other words, an altered neural activity in some subjects blunted the ability of vasoconstriction to compensate for the PV loss more than in others and was reflected by higher LC values.

Differences Between Runners and Non-runners

in Their Response to LBNP

Before and After Dehydration

In the present investigation dealing with dehydration and LBNP
tolerance after diuresis with Lasix as well as in a similar study
where dehydration was induced by exercising in the heat (EH) (Luft
et al., 1976) half of the subjects (No. 1-5 in this report) were
endurance runners (R) who exercised regularly while the other 5 (No.
6-10) did not pursue any vigorous activity (NR). This selection of
subjects was necessary because there was always a greater number of
volunteers who were runners, but it was deemed advisable to limit
their number in order to avoid any bias in the results due to possible
differences in physical condition. As it turned out, there were a
number of striking differences in the responses of the two groups,
not only in the effects of the two different modes of dehydration but
also in their responses to LBNP stress thereafter. As was to be
expected, the better physical condition of the R's was well demonstrated
in their performance in the maximal exercise test (Table 1), where
subjects 1-5 had a mean $\dot{V}_{O_{2}max}$/kg of 51.2 ml/kg and the NR's (6-10)
only 31.1 ml/kg. The corresponding values in the previous EH experi-
ments were 45.0 ml/kg and 34.0 ml/kg respectively. Incidentally,
the subjects in the two studies were not the same.

After ingestion of Lasix the R's lost 1.86 liters of fluid and
the NR's only 1.29, a difference of 44%, even though the former were
purposely given a smaller dose per kg than the latter, (Table 1)
based on previous experience. The greater diuretic response of the R's

to Lasix is even more impressive when expressed in terms of diuresis

in liters per mg/kg of the drug. This gives 2.35 for the R's and only

1.40 for the NR's a 68% greater effect on the former. Part of the

difference may be due to a difference in body composition between the

two groups, whereby it can be assumed that the R's had a relatively

greater fat-free weight and consequently more body water to start with

than the others. But, at best this could only account for 10% of

the difference. Unfortunately, body composition was not determined

in the Lasix study. In the previous study using EH for dehydration

(Luft et al., 1976), the R's lost 2.0 liters of fluid and the NR's

1.9 liters. However because of a difference in average weight between

the two groups the percentage weight loss was 2.8% in the R's and 2.2%

in the NR's. When the fluid loss was related to fat-free weight

(immersion method) in these subjects, the difference was much less,

3.1% for the R's and 2.9% for the NR's.

For the following discussion one should bear in mind that the

degree of dehydration relative to body weight was similar for the R's

in the Lasix study (2.7%) and after EH (2.8%), but the NR's were less

dehydrated in both studies (1.8% with Lasix, 2.2% with EH). At

present we have no explanation for the striking difference in the

diuretic response to Lasix between the R's and NR's. Unfortunately,

there is a paucity of data on the dose response relationships of Lasix

in healthy individuals either physically active or not.

Despite the disparity in the dehydration of the two groups the

losses in plasma volume (PV) were not that far apart, namely 17.4%

for the R's and 16.5% for the NR's. This was more than observed

in the EH study where the values were 12.3 for the former and 13.3 for

the latter. As pointed out earlier in this report, dehydration by Lasix tends to tax the PV relatively more than EH for approximately the same degree of overall dehydration, and this was common to the R's and NR's. Furthermore, the fact that the R's lost barely one percent more PV than the others, despite a much greater total fluid loss, suggests that they have a greater ability to conserve PV in the face of dehydration, possibly in consequence of their frequent exposure to dehydration in endurance running, particularly in the summer when these tests were done.

While the average tolerance for LBNP was always significantly lower in R's than NR's, in the euhydrated state, the difference was even greater (-42%) in the subjects involved in the Lasix study than in the EH series (-30%) before dehydration. Both modes of the latter led to a decrement in LBNP tolerance, but the two groups were affected differently in that the R's lost more (40%) of their initial tolerance after EH than after Lasix (36%), while the NR's lost only 26% after EH but 43% with diuresis, and this inspite of the fact that they were less dehydrated than the R's. Apparently LBNP tolerance is more adversely affected by EH in the R's than by diuresis, whereas the opposite is true for NR's. These differences may be related to the observed changes in the rate of pooling of blood in the legs expressed as leg compliance (Table 11). The latter was much greater after Lasix than EH for both groups, but the NR's showed a larger increase during LBNP II after Lasix than after EH which is compatible with their greater loss in tolerance than the R's.

The resting control heart rates (HR) were consistently lower in the R's than the NR's both before and after dehydration, although

they were slightly higher after the latter in both groups. The maximal heart rate during LBNP was on the average 25 bpm less with the R's than the others regardless of their state of hydration. Frick et al. (1967) have demonstrated that the bradycardia of endurance athletes at rest is due to parasympathetic inhibition. It is well known that athletes maintain the same resting cardiac output as other people, despite bradycardia with a larger stroke volume. No doubt this is an advantage during exercise, but may well represent a handicap in the LBNP situation with a dwindling stroke volume which is not as well compensated for by the HR response as in the NR's.

Thus, the parasympathetic inhibition of cardiac activity combined with the greater propensity for blood pooling in the lower extremities appear to be the principal reasons for the significantly lower LBNP tolerance of the endurance athletes in this study.

Whether the greater susceptibility to orthostatic stress observed in this investigation is peculiar to athletes whose activities involve the lower extremities predominantly, or whether other types of vigorous exercise have similar effects is currently being studied.

REFERENCES

Abramson, D.I. Cardiovascular responses in thermoregulation. In:
Circulation in the Extremities, edited by David I. Abramson.
New York: Academic Press, 1967a, p. 229-259.

Allan, J.R. and R.J. Crossley. Effect of controlled elevation of body
temperature on human talerance to +Gz acceleration. J. Appl.
Physiol. 38:418-420, 1972.

Ardill, B.L. and P.H. Fentem. Emptying of the capacity vessels of the
forearm against pressure during simulated gravitational shifts of blood.
J. Physiol. (London). 181:46P-47P, 1965.

Beetham, W.P. and E.R. Buskirk. Effects of dehydration, physical
conditioning and heat acclimatization on the response to passive
tilting. J. Appl. Physiol. 13:465-468, 1958.

Claremont, A.D., D.L. Costill, W. Fink and P. VanHandel. Heat tolerance
following diuretic induced dehydration. Med. and Science in Sports.
8:239-243, 1976.

Coburn, R.F., G.K. Danielson, W.S. Blakeniovc and R.E. Forster.
Carbon monoxide in blood: Analytical method and sources of error.
J. Appl. Physiol. 19:510-515, 1964.

Costill, D.L., R. Coté and W. Fink. Muscle water and electrolytes
following varied levels of dehydration in man. J. Appl. Physiol.
40:6-11, 1976.

Costill, D.L. and W. Fink. Plasma volume changes following exercise and thermal dehydration. J. Appl. Physiol. 37:521-525, 1974.

Davidson, I. Fluid and electrolyte balance. In: Clinical Diagnosis by Laboratory Method, edited by I. Davidson. New York: W.B. Saunders Co, 1969, p. 645-672, 1267-1270.

Eichna, L.W., and W.B. Bean. Orthostatic hypotension in normal young men following physical exertion, environmental thermal loads or both. J. Clin. Invest. 23:942, 1944.

Frick, M.H., R.O. Elovainio and T. Somer. The mechanism of bradycardia evoked by physical training. Cardiologia. 51:46-54, 1967.

Garrett, T.A. Fundamentals of body water and electrolytes. Morton Grove, Illinois: Baxter Laboratories Inc., 1971, p. 48.

Gilbert, C.A. and P.M. Stevens. Forearm vascular responses to lower body negative pressure and orthostasis. J. Appl. Physiol. 21:1265-1272, 1966.

Greenleaf, J.E., J.S. Bosco and M. Matter, Jr. Orthostatic tolerance in dehydrated, heat acclimatized men following exercise in the heat. Aerospace Med. 45:491-497, 1974.

Greenleaf, J.E., H.O. Stinnett, G.L. Davis, J. Kollias and E. Bernauer. Fluid and electrolyte shifts in women during +Gz acceleration after 15 days of bed rest. J. Appl. Physiol. 42(1):67-73, 1977.

Hartley, L.H., Y.W. Mason, R.D. Hogen, L.G. Jones, T.A. Kotchen, E.H. Mougey, F.E. Wherry, L.L. Pennington and P.T. Ricketts. Multiple hormonal response to graded exercise in relation to training. J. Appl. Physiol. 33:602-606, 1972.

Hoche, J. and A. Graybiel. Value of exercise at ½ earth gravity in preventing the deconditioning effects of simulated weightlessness. Aerospace Med. 45:386-392, 1974.

Hyatt, K.H. and D.A. West. Reversal of bedrest-induced orthostatic intolerance by LBNP and saline. Aviat. Space and Environ. Med. 48:120-124, 1977.

Jarsumbeek, B., D. Wirth and H. Haase. Vanillinmandelsäureausscheidung bei ergometrischer Vita-maxima-Belastung. Med. Sport. 15:111-117, 1975.

Johnson, J.M., M. Niederberger, L.B. Rowell, M.M. Eisman, and G.L. Brengelman. Competition between cutaneous vasodilator and vaso-constrictor reflexes in man. J. Appl. Physiol. 35:798-803, 1973.

Johnson, R.S. and L.F. Dietlein. Cardiovascular studies. NASA Technical Memorandum TM-X-58160. Lyndon B. Johnson Space Center, Houston, Texas, 1975, p. 21-26.

Kaiser, D., H.U. Linkenback and O.H. Gauer. Änderung des Plasma Volumens bei Immersion in ein thermoindifferentes Wasserbad. Pflügers Arch. 308:166-173, 1969.

Kakurin, L.I., V.I. Lobachik, V.M. Mikhailov and Y.A. Semkevich. Antiorthostatic hypokenesia as a method of weightlessness simulation. Aviat., Space and Environ. Med. 47:1083-1086, 1976.

Kimura, T., K. Minai, K. Matsui, T. Mouri, T. Sato, K. Yoshinaga and T. Hoshi. Effect of various states of hydration on plasma ADH and renin in man. J. Clin. Endocrin. and Metab. 42:79-87, 1976.

Kleinfelder, H. Experimental investigations and clinical trials of Fursemide, a new diuretic. German Med. Monthly. 8(11):459-465, 1963.

Letunow, S.P., P.M. Barbarin, O.R. Nemirowitsch-Dantschenko and R.A. Dshuganjan. Untersuchung der Funkitonen des Nebennierenmarks bei Sportlern unter dem Einfluss körperlicher Belastung. Med. Sport. 5:77-80, 1965.

Lind, A.R., C.S. Leithead, and G.W. McNicol. Cardiovascular changes during syncope induced by tilting man in the heat. J. Appl. Physiol. 25:268-276, 1968.

Luft, U.C., D. Cardus, T.P.K. Lim, E.C. Anderson and J.L. Howarth. Physical performance in relation to body size and composition. Annals of the New York Academy of Sicence. 110, Part II:795-808, 1963.

Luft, U.C. A study of factors affecting tolerance of gravitational stress simulated by lower body negative pressure. In: Specialized Physiological Studies in Support of Manned Space Flight. Final Report: NASA Contract 9-14472, February, 1976.

Miller, P.B., R.L. Johnson, and L.E. Lamb. Effects of moderate physical exercise during four weeks of bedrest on circulatory functions in man. Aerospace Med. 36:1077-1082, 1965.

Myhre, L.G., D.K. Brown, F.G. Hall, and D.B. Dill. The use of carbon monoxide and T-1824 for determinig blood volume. Clin. Chem. 14:1197-1205, 1968.

Myhre, L.G. and S. Robinson. Fluid shifts during thermal stress with and without fluid replacement. J. Appl. Physiol. 42(2):252-256, 1977.

Popham, W.J. and K.A. Sirotnik. Regression. In: Educational Statistics: Use and Interpretation, edited by W. James Popham. New York: Harper and Row, Co., 1973a, p. 95-122.

Popham, W.J. and K.A. Sirotnik. The t-test. In: Educational Statistics:

    Use and Interpretation, edited by W. James Popham. New York:

    Harper and Row, Co., 1973b, p. 123-149.

Robson, A.O., D.N.S. Kerr, R. Ashcroft and G. Teasdale. The diuretic

    response to Frusemide. Lancet. 2:1085-1088, 1964.

Skipka, W., K.A. Deck, and D. Böning. Effect of physical fitness on

    vanillylmandelic acid excretion during immersion. Europ. J.

    Appl. Physiol. and Occupat. Physiol. 35:271-276, 1976.

Stason, W.B., P.J. Cannon, H.O. Heinemann, and J.H. Laragh. Furosemide,

    a clinical evaluation of its diuretic action. Circulation. 34:

    910-920, 1966.

Stegemann, J., H.D. Framing, and M. Schiefeling. Der Einfluss einer

    6-stüdigen Immersion in thermoindifferenten Wasser auf die

    Regulation des Kreislaufs und die Leistungsfahigkeit bei Trainerten

    and Untrainerten. Pflügers Arch. 312:129-139, 1969.

Stegemann, J., U. Meier, W. Skipka, W. Hartlieb, B. Hemmer, and

    U. Tibes. Effects of multi-hour immersion with intermittent

    exercise on urinary excretion and tilt-table tolerance in athletes

    and non-athletes. Aerospace Med. 46:26-29, 1975.

Stevens, P.M., P.B. Miller, T.H. Lynch, C.A. Gilbert, R.L. Johnson,

    and L.E. Lamb. Effects of lower body negative pressure on physical

    changes due to four weeks of hypoxic bedrest. Aerospace Med.

    37:466-474, 1966.

Van Beaumont, W., J.E. Greenleaf, H.L. Young and L. Juhos. Plasma

    volume and blood constituent shifts during +Gz acceleration after

    bedrest with exercise conditioning. Aerospace Med. 45(4):

    425-430, 1974.

Venters, M.D. Effects of acute dehydration, heat acclimatization and physical fitness on fluid shifts induced by lower body negative pressure. Albuquerque, NM: University of New Mexico, 1976, Thesis.

Volicer, L., R. Jean-Charles, and A.V. Chobanian. Effects of head-down tilt on fluid and electrolyte balance. Aviat. Space and Environ. Med. 47:1065-1068, 1976.

Waterfield, R.L. The effects of posture on the circulating blood volume. J. Physiol. (London). 72:110-120, 1931a.

Woodbury, D.M. Physiology of body fluids. In: Physiology and Bio-physics, Vol. II. edited by Ruch and Patton. Philadelphia: W.B. Saunders Co., 1974, p. 450-479.

TABLE 1

PHYSICAL CHARACTERISTICS AND PERFORMANCE

Subj.	Age	Ht cm	Wt kg	$\dot{V}O_2max$ L/min	$\dot{V}O_2/$ Weight ml/min/kg	Max Pulse bpm	O_2 Pulse ml/beat	Lasix Dose mg
1	23	174	60.3	3.72	62.5	168	22.1	50
2	29	178	73.9	3.27	44.6	179	18.3	60
3	40	171	68.8	3.33	48.5	155	21.5	50
4	31	183	75.0	3.78	50.5	173	21.9	60
5	34	178	74.5	3.74	50.1	174	21.5	60
6	35	175	76.0	2.29	30.1	169	13.5	70
7	30	180	72.8	2.50	34.3	183	13.6	70
8	29	173	79.4					70
9	26	183	75.0	2.58	34.4	190	13.6	70
10	33	183	75.7	1.93	25.5	187	10.3	70
Mean	31	178	73.1	3.02	42.3	175	17.4	63
SD	±4.8	±4.4	±5.2	±0.70	±11.9	±10.8	±4.6	±8.2

TABLE 2

EFFECTS OF LASIX DEHYDRATION ON PLASMA VOLUME, URINE OUTPUT, AND BODY WEIGHT

Subj.	Lasix mg/kg	Urine Output Liters	Weight Loss kg	Weight Loss %	Initial Plasma Volume (ml)	Plasma Volume Loss (ml)	Plasma Loss %
1	0.83	1.84	1.9	-3.2	2,918	-462	-15.8
2	0.81	2.10	2.1	-2.9	3,018	-632	-20.9
3	0.73	1.09	1.2	-1.7	2,694	-438	-16.3
4	0.80	1.72	1.8	-2.4	4,317	-587	-13.6
5	0.79	2.57	2.6	-3.5	3,924	-824	-21.0
6	0.92	1.13	1.2	-1.5	2,375	-386	-16.3
7	0.96	1.66	1.7	-2.4	3,180	-802	-25.2
8	0.88	0.75	0.8	-1.2	2,453	-259	-10.6
9	0.93	1.24	1.3	-1.7	2,865	-348	-12.1
10	0.92	1.67	1.7	-2.3	3,397	-562	-16.5
Mean	0.86	1.58	1.6	-2.3	3,114	-530	-16.8 ·
SD	±0.07	±0.54	±0.52	±0.76	±0.62	±187.2	±4.44

· Significant decrease (p<.001)

TABLE 3

PLASMA ELECTROLYTE CONCENTRATION AND OSMOLALITY

	Pre-Lasix				Post-Lasix			
Subj.	Na^+ mEq/L	K^+ mEq/L	Cl^- mEq/L	Osm mOsm/kg	Na^+ mEq/L	K^+ mEq/L	Cl^- mEq/L	Osm mOsm/kg
1	145	4.1	110	300	144	3.8	104	287
2	143	3.8	107	285	139	3.8	102	280
3	143	4.1	107	288	141	3.8	103	284
4	142	4.0	110	291	137	3.7	104	276
5	156	4.1	122	307	149	3.8	112	303
6	142	3.8	105	290	139	4.3	101	283
7	145	4.0	106	299	144	4.5	102	294
8	140	3.0	108	286	137	3.5	106	282
9	145	4.2	108	296	144	3.9	108	287
10	140	3.7	108	284	140	3.6	105	284
Mean	144	3.9	109	293	141	3.9	105	286
SD	±4.6	±0.35	±4.8	±7.6	±3.8	±0.35	±3.3	±7.6

TABLE 4

TOTAL PLASMA ELECTROLYTE CHANGES AFTER 3 HOUR LASIX DEHYDRATION

Subj.	Na^+ Pre-Lasix mEq	Post-Lasix mEq	Δ mEq	K^+ Pre-Lasix mEq	Post-Lasix mEq	Δ mEq	Cl^- Pre-Lasix mEq	Post-Lasix mEq	Δ mEq
1	423	354	- 69	12.0	9.3	-2.7	321	255	- 66
2	432	332	-100	11.5	9.1	-2.4	323	243	- 80
3	485	318	- 67	11.1	8.6	-2.5	288	232	- 56
4	613	511	-102	17.3	13.8	-3.5	475	388	- 87
5	612	462	-150	16.1	11.8	-4.3	479	347	-132
6	337	276	- 61	9.0	8.6	-0.4	249	201	- 48
7	461	343	-118	12.7	10.7	-2.0	337	243	- 94
8	343	301	- 42	7.4	7.7	+0.3	265	233	- 32
9	415	362	- 53	12.0	9.8	-2.2	309	272	- 37
10	476	397	- 79	12.6	10.2	-2.4	367	298	- 69
Mean	450	366	- 84	12.2	10.0	-2.2	341	271	- 70
SD	±96.8	±72.8	±33.4	±2.9	±1.8	±1.3	±79.2	±57.6	±29.9

TABLE 5

URINE OUTPUT, ELECTROLYTE CONCENTRATION
AND OSMOLALITY AFTER LASIX DEHYDRATION

Subj.	Output Liters	Na^+ mEq/L	K^+ mEq/L	Cl^- mEq/L	Osmolality mOsm/kg
1	1.835	124	22	145	323
2	2.095	127	14	143	312
3	1.094	121	23	136	325
4	1.715	138	21	156	337
5	2.570	130	17	155	335
6	1.125	125	13	142	309
7	1.660	127	20	147	333
8	0.754	117	14	136	369
9	1.240	131	19	153	345
10	1.665	108	17	127	275
Mean	1.575	125	18	144	326
SD	±0.54	±8.2	±3.6	±9.3	±24.9
Normal Range (24 hr)	0.6-1.6	40-90	20-60	40-120	300-1000

TABLE 6

TOTAL ELECTROLYTES AND OSMOLALITY
LOST IN URINE IN 3 HOURS

Subj.	Na^+ mEq	K^+ mEq	Cl^- mEq	Osmolality mOsm
1	228	40.4	266	593
2	266	29.3	300	654
3	132	25.2	149	356
4	237	36.0	268	578
5	334	43.7	398	861
6	141	14.6	160	348
7	211	33.2	244	553
8	88	10.7	103	277
9	162	20.2	190	428
10	180	28.3	211	458
Mean	198	28.2	229	511
SD	±72.3	±13.1	±85.4	±173.5
Normal Range (24 hr)	130-260	25-100	110-250	800-1000

TABLE 7

LBNP I CHANGES IN LEG VOLUME DURING LBNP
IN PERCENT OF INITIAL VOLUME

Torr:	-20		-30		-40		-50		-60		
Min :	2	5	2	5	2	5	2	5	2	5	Final
Subj. 1	1.05	1.08	2.21	2.52	3.10	3.41	4.03	4.28	4.98	5.46	5.46
2	1.38	1.41	2.05								2.26
3	0.68	0.76	1.15	1.31	1.74	1.93	2.35	2.66	3.20	3.60	3.60
4	1.40	1.66	2.36								2.42
5	1.18	1.42	2.26	2.62	3.30	3.70	4.39	4.70	5.30	5.81	5.81
6	0.63	0.59	1.23	1.45	2.26	2.58	2.95	3.22	4.14	4.27	4.27
7	0.25	0.29	0.46	0.63	1.02	1.22	1.51	1.85	2.45	2.67	2.67
8	0.86	0.68	1.22	1.30	1.75	2.03	2.63	2.95	3.36	3.70	3.70
9	0.89	0.83	1.34	1.58	1.92	2.19	2.73	3.11	3.61	4.01	4.01
10	0.92	0.88	1.27	1.43	2.03	2.33	3.29	3.65	4.11		4.33
Mean	0.92	0.96	1.56	1.61	2.14	2.42	2.99	3.30	3.89	4.22	3.85
SD	±0.35	±0.43	±0.63	±0.66	±0.75	±0.81	±0.92	±0.90	±0.94	±1.09	±1.20

TABLE 8

LBNP I   CHANGES IN LEG VOLUME AFTER RELEASE OF LBNP
IN PERCENT OF INITIAL VOLUME

Subj.	Final	10 sec	20 sec	30 sec	40 sec	50 sec	60 sec	120 sec	180 sec
1	5.46	2.25	1.97	1.68	1.52	1.35	1.31	1.12	1.09
2	2.26	1.42	0.87	0.72	0.58	0.58	0.67	0.82	0.78
3	3.60	2.33	1.88	1.64	1.47	1.36	1.28	1.34	1.31
4	2.42	1.05	0.75	0.75	0.79	0.66	0.56	±0	±0
5	5.81	3.68	3.32	3.16	2.91	2.83	2.71	2.54	2.46
6	4.27	1.38	1.47	1.33	1.22	1.12	0.98	0.94	0.81
7	2.67	1.63	1.45	1.28	1.14	1.07	1.00	0.95	0.88
8	3.70	1.60	1.31	1.04	0.84	0.66	0.57	0.38	0.35
9	4.01	2.51	1.90	1.61	1.38	1.19	1.13	1.05	1.01
10	4.33	2.97	2.74	2.67	2.40	2.12	2.01	1.68	1.28
Mean	3.85	2.08	1.77	1.59	1.43	1.29	1.22	1.08	1.00
SD	±1.20	±0.82	±0.79	±0.79	±0.73	±0.70	±0.68	±0.69	±0.65

TABLE 9

LBNP II   CHANGES IN LEG VOLUME DURING LBNP
IN PERCENT OF INITIAL VOLUME

Torr:	-20		-30		-40		-50		-60		
Min :	2	5	2	5	2	5	2	5	2	5	Final
Subj. 1	1.20	1.53	2.10	2.40	2.85	2.91	3.40				3.56
2	0.65										0.72
3	0.50	0.35	0.78	0.84	1.32	1.54	1.94	2.21	2.82	3.40	3.40
4	1.20										1.24
5	1.16	1.49	2.43	2.84	3.42						3.69
6	0.79	0.88	1.59	1.59	2.32	2.47					2.84
7	1.00	0.96	1.20	1.26	1.48	1.53	1.80	1.97			2.22
8	1.36	1.13	1.53	1.74	2.07						2.15
9	0.64	0.50	0.88	1.02	1.25	1.39	1.70	1.86	2.18	2.33	2.33
10	0.98										1.26
Mean	0.95	0.98	1.50	1.67	2.10	1.97	2.21	2.01	2.50	2.87	2.34
SD	±0.29	±0.45	±0.61	±0.73	±0.82	±0.68	±0.80	±0.18	±0.45	±0.76	±1.04

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TABLE 10

LBNP II CHANGES IN LEG VOLUME AFTER RELEASE OF LBNP
IN PERCENT OF INITIAL VOLUME

Subj.	Final	10 sec	20 sec	30 sec	40 sec	50 sec	60 sec	120 sec	180 sec
1	3.56	1.07	0.67	0.21	0.08	±0	-0.14	-0.59	-0.75
2	0.72	0.23	-0.04	-0.25	-0.25	-0.32	-0.34	-0.63	
3	3.40	1.18	0.93	0.80	0.73	0.58	0.46	±0	-0.18
4	1.24	0.53	0.37	0.30	0.30	0.30	0.17	-0.08	
5	3.69	1.70	1.51	1.36	1.29	1.20	1.05	0.91	0.86
6	2.84	-0.22	-0.22	-0.04	-0.11	-0.22	-0.38		
7	2.22	0.55	0.47	0.41	0.34	0.18	0.10	-0.11	-0.11
8	2.15	0.36	0.22	±0	-0.16	-0.29	-0.40	-0.57	-0.45
9	2.33	0.69	0.27	±0	-0.33	-0.42	-0.56	-0.58	-0.58
10	1.26	0.37	0.02	0.02	-0.10	-0.14	-0.14		
Mean	2.34	0.65	0.42	0.28	0.18	0.09	-0.02	-0.21	-0.20
SD	±1.04	±0.55	±0.51	±0.48	±0.51	±0.50	±0.49	±0.52	±0.57

TABLE 11

| | LBNP TOLERANCE | | LEG COMPLIANCE | |
| | LBNP I | LBNP II | LBNP I | LBNP II |
Subj.	Torr x min	Torr x min	*	*
1	1000	640	502	466
2	238	54	828	1403
3	1000	1000	330	319
4	163	67	1282	1936
5	1000	397	532	860
6	1000	546	427	471
7	1000	785	271	206
8	1000	413	348	441
9	1000	1000	369	212
10	887	59	441	2184
Mean	829·	496·	533	850
SD	±333	±365	±305	±732

* Leg Compliance: $\dfrac{\Delta\% \text{ Leg Volume}}{\text{Torr x min}} \times 10^5$

· Difference is significant (p<.01)

TABLE 12

HEART RATE UNDER LBNP

Subj.	LBNP I Control bpm	Final bpm	Δ %	LBNP II Control bpm	Final bpm	Δ %
1	52	73	+40	49	77	+57
2	53	63	+19	53	63	+19
3	54	75	+39	54	87	+61
4	48	60	+25	49	77	+57
5	65	95	+46	75	93	+24
6	64	101	+58	67	93	+39
7	58	86	+48	57	103	+81
8	57	98	+72	66	93	+41
9	64	79	+23	65	104	+60
10	94	151	+61	101	125	+24
Mean	61	88	+43··	64	92	+46···
SD	±13	±26.2		±15.7	±17.2	

·· Significant increase (p<.001)
··· Significant increase (p<.001)

TABLE 13

PULSE PRESSURE UNDER LBNP

	LBNP I			LBNP II		
Subj.	Control Torr	Final Torr	Δ %	Control Torr	Final Torr	Δ %
1	53	25	-33	41	15	-63
2	36	27	-25	21	15	-29
3	23	15	-35	21	10	-52
4	48	33	-31	34	26	-24
5	48	28	-42	32	20	-38
6	42	24	-43	25	17	-32
7	39	18	-54	27		
8	42	16	-62	35	13	-63
9	61	46	-25	49	24	-51
10	32	13	-59	20	9	-55
Mean	42	25	-43··	31	17	-45···
SD	±10.8	±10.0		±9.6	±5.9	

·· Significant decrease (p<.001)
··· Significant decrease (p<.001)

TABLE 14

TOTAL HEMOGLOBIN (THb), HEMOGLOBIN CONCENTRATION (Hb) AND HEMATOCRIT (Hct)

		LBNP I				LBNP II			
Subj.	THb g	Hb g/100ml		Hct %		Hb g/100ml		Hct %	
		Before	After	Before	After	Before	After	Before	After
1	819	15.4	16.9	45.0	48.7	16.9	18.1	49.4	52.5
2	812	14.9	15.6	44.6	46.3	17.0	17.1	50.1	50.9
3	800	15.9	17.4	46.6	51.4	17.6	18.0	50.4	52.3
4	907	12.8	13.3	39.2	40.6	13.9	14.6	42.7	44.3
5	1096	15.6	17.2	44.2	47.6	17.9	18.5	49.3	51.0
6	678	15.6	16.6	45.5	48.4	17.3	17.7	49.2	50.5
7	905	16.2	16.7	43.2	45.2	18.7	18.4	50.9	51.6
8	778	17.4	19.3	45.3	50.1	18.6	19.1	47.5	48.1
9	866	16.9	18.3	44.1	48.1	18.0	19.0	47.6	50.0
10	959	16.0	17.0	43.8	46.8	17.7	17.0	47.8	47.3
Mean	862	15.7	16.8	44.2	47.3	17.4	17.8	48.5	49.9
SD	±114	±1.24	±1.59	±1.99	±2.96	±1.35	±1.31	±2.35	±2.57

TABLE 15

HEMOGLOBIN CONTENT OF RED CELLS

	LBNP I		LBNP II	
Subj.	Before g/ml	After g/ml	Before g/ml	After g/ml
1	0.343	0.347	0.342	0.345
2	0.334	0.336	0.339	0.337
3	0.340	0.339	0.349	0.345
4	0.326	0.329	0.326	0.329
5	0.352	0.360	0.363	0.364
6	0.342	0.342	0.352	0.351
7	0.374	0.369	0.367	0.357
8	0.383	0.384	0.392	0.397
9	0.383	0.380	0.379	0.380
10	0.364	0.364	0.369	0.360
Mean	0.354	0.355	0.358	0.357
SD	±0.021	±0.019	±0.020	±0.020

TABLE 16

BLOOD VOLUME (BV), PLASMA VOLUME (PV) AND RED CELL VOLUME (RCV)

	LBNP I						LBNP II					
	BV Liters		PV Liters		RCV Liters		BV Liters		PV Liters		RCV Liters	
Subj.	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
1	5.305	4.841	2.918	2.484	2.387	2.357	4.852	4.525	2.456	2.150	2.396	2.375
2	5.447	5.223	3.018	2.805	2.429	2.418	4.780	4.739	2.386	2.327	2.394	2.412
3	5.045	4.585	2.694	2.228	2.351	2.357	4.549	4.435	2.256	2.115	2.293	2.320
4	7.100	6.797	4.317	4.037	2.783	2.760	6.509	6.215	3.730	3.462	2.779	2.753
5	7.033	6.390	3.924	3.348	3.109	3.042	6.115	5.910	3.100	2.896	3.015	3.014
6	4.358	4.097	2.375	2.114	1.983	1.953	3.919	3.825	1.989	1.893	1.926	1.932
7	5.599	5.412	3.180	2.955	2.419	2.457	4.844	4.913	2.378	2.377	2.466	2.536
8	4.484	4.041	2.453	2.071	2.031	1.970	4.178	4.073	2.194	2.114	1.984	1.959
9	5.126	4.736	2.865	2.458	2.261	2.278	4.805	4.555	2.517	2.277	2.228	2.278
10	6.013	5.628	3.397	2.994	2.634	2.684	5.403	5.628	2.835	2.966	2.595	2.662
Mean	5.551	5.175	3.114·	2.749·	2.439	2.428	4.998	4.882	2.584··	2.458··	2.408	2.424
SD	±0.94	±0.91	±0.62	±0.62	±0.34	±0.34	±0.81	±0.79	±0.51	±0.49	±0.33	±0.34

· and · difference is significant (p<.001)
·· and ·· difference is significant (p<.01)

TEST PROFILE
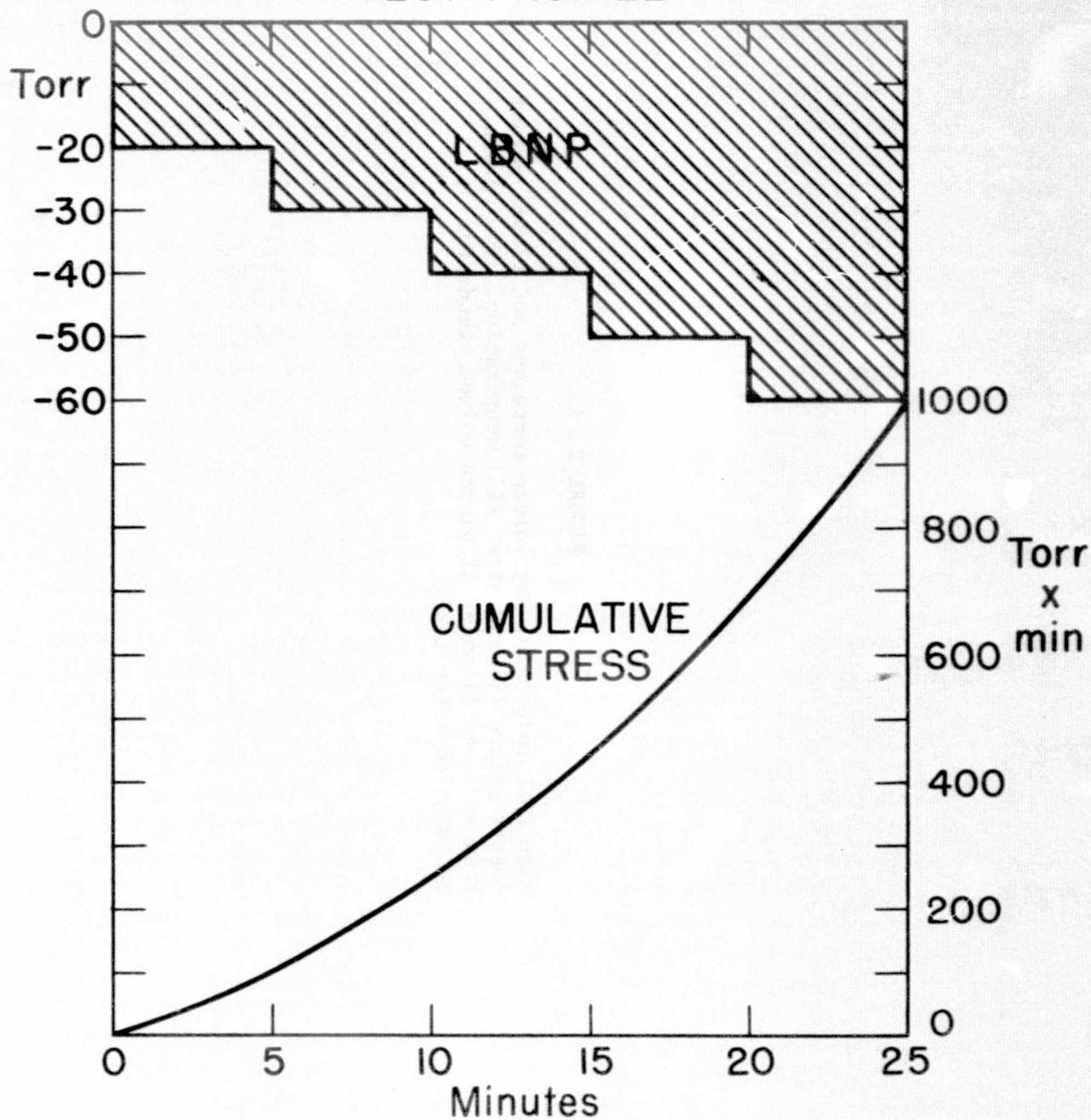
FIGURE 1

FIGURE 2

Mean LBNP tolerance, leg volume increases and plasma volume
losses before (I) and after (II) dehydration. The column
between I and II under Δ% plasma volume stands for
dehydration after Lasix.
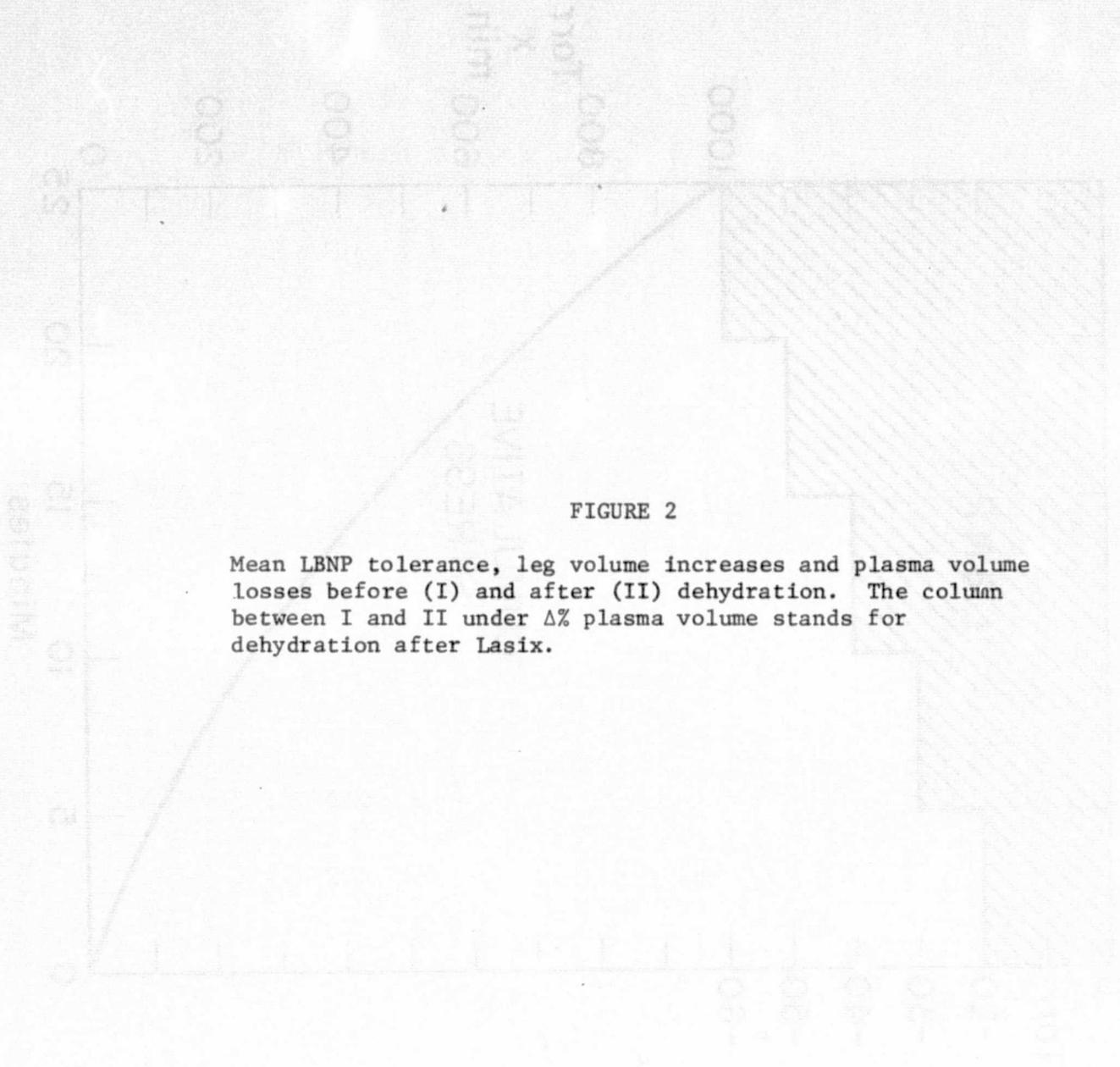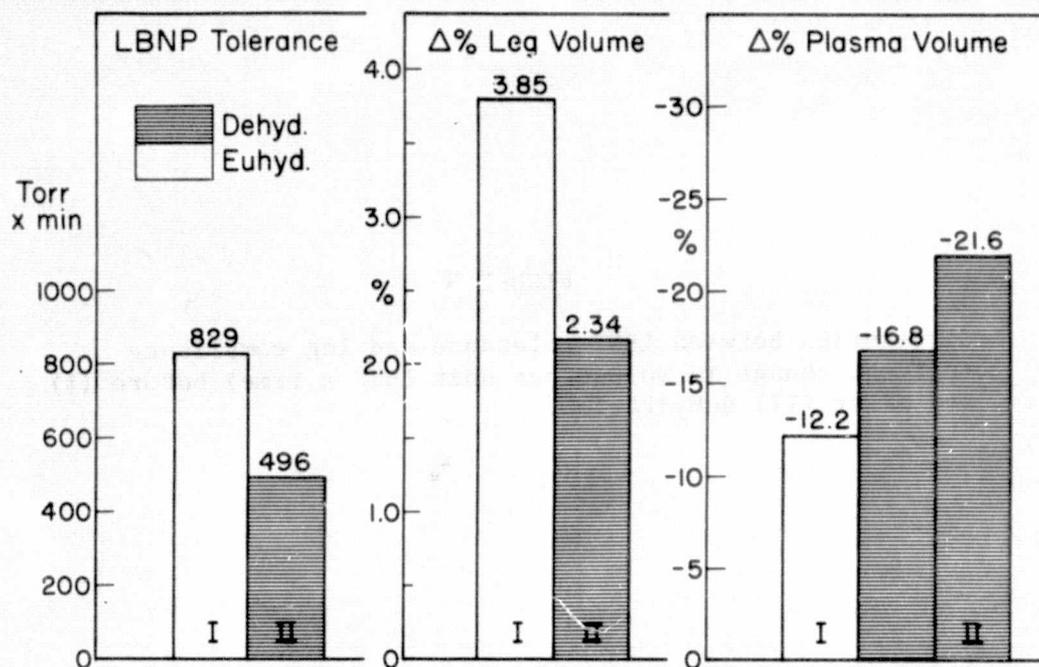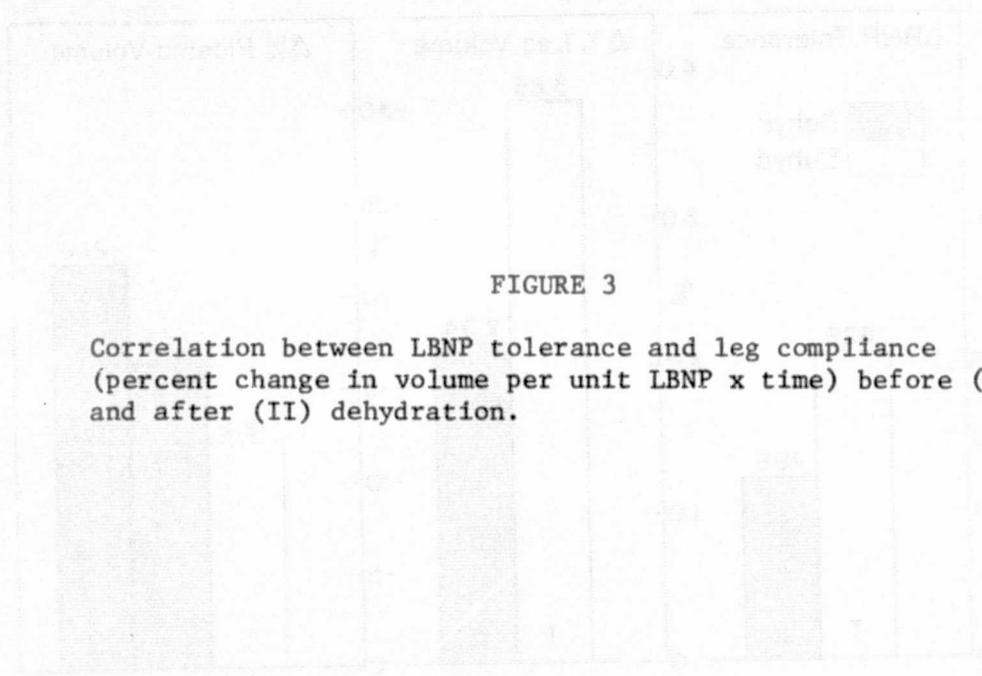
FIGURE 2

FIGURE 3

Correlation between LBNP tolerance and leg compliance
(percent change in volume per unit LBNP x time) before (I)
and after (II) dehydration.
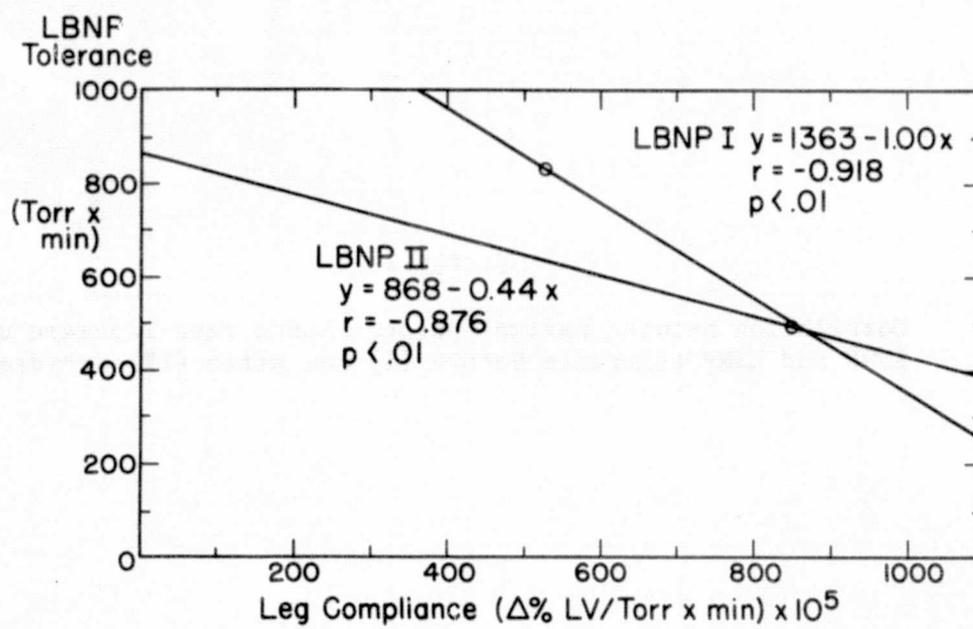
FIGURE 3
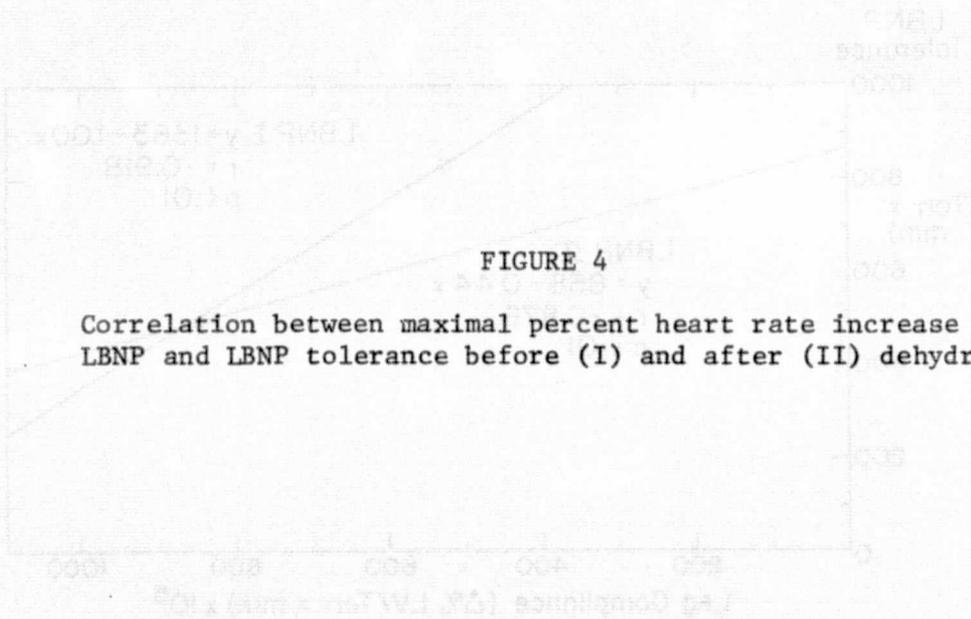
FIGURE 4

Correlation between maximal percent heart rate increase under
LBNP and LBNP tolerance before (I) and after (II) dehydration.
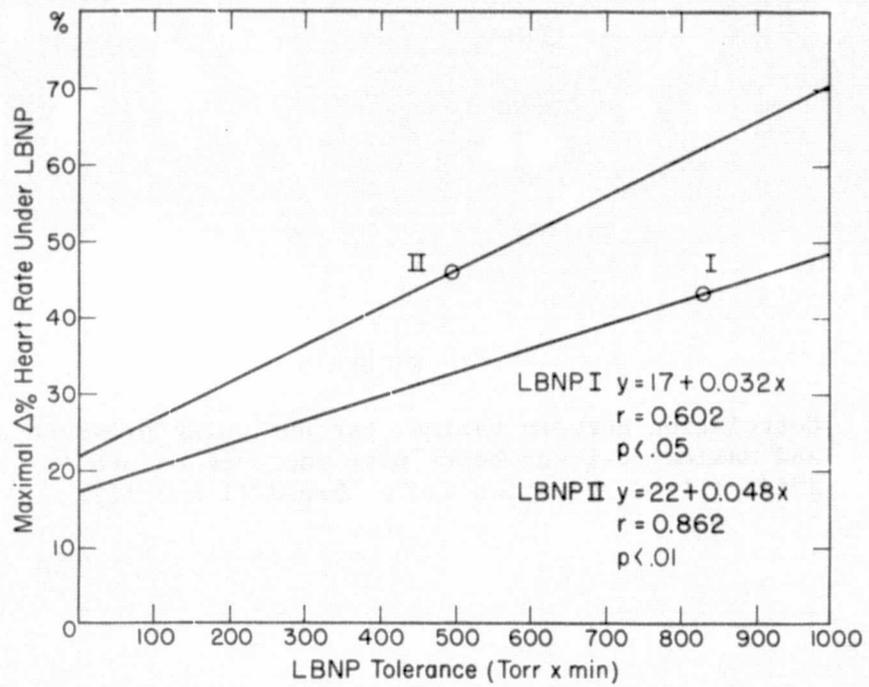
FIGURE 4
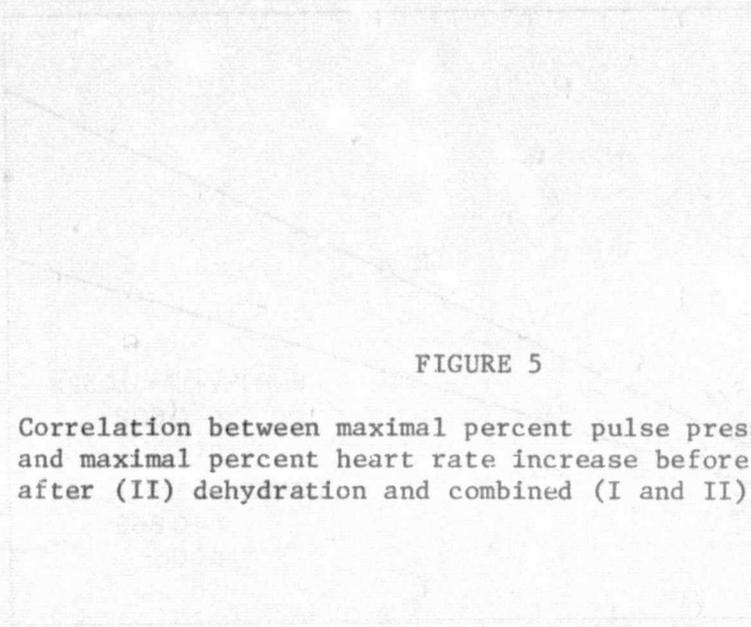
FIGURE 5

Correlation between maximal percent pulse pressure decrease
and maximal percent heart rate increase before (I) and
after (II) dehydration and combined (I and II).
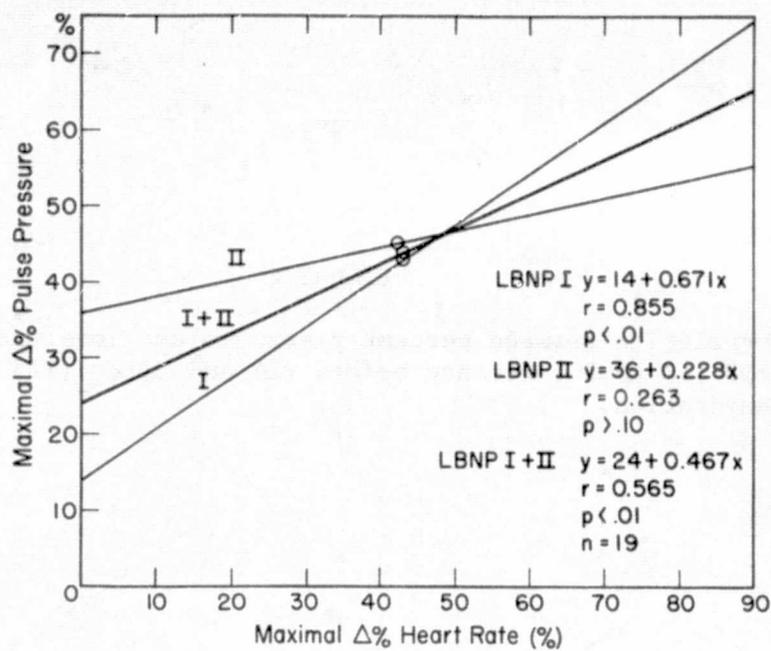
FIGURE 5

FIGURE 6

Correlation between percent plasma volume loss under
LBNP and LBNP tolerance before (I) and after (II)
dehydration.
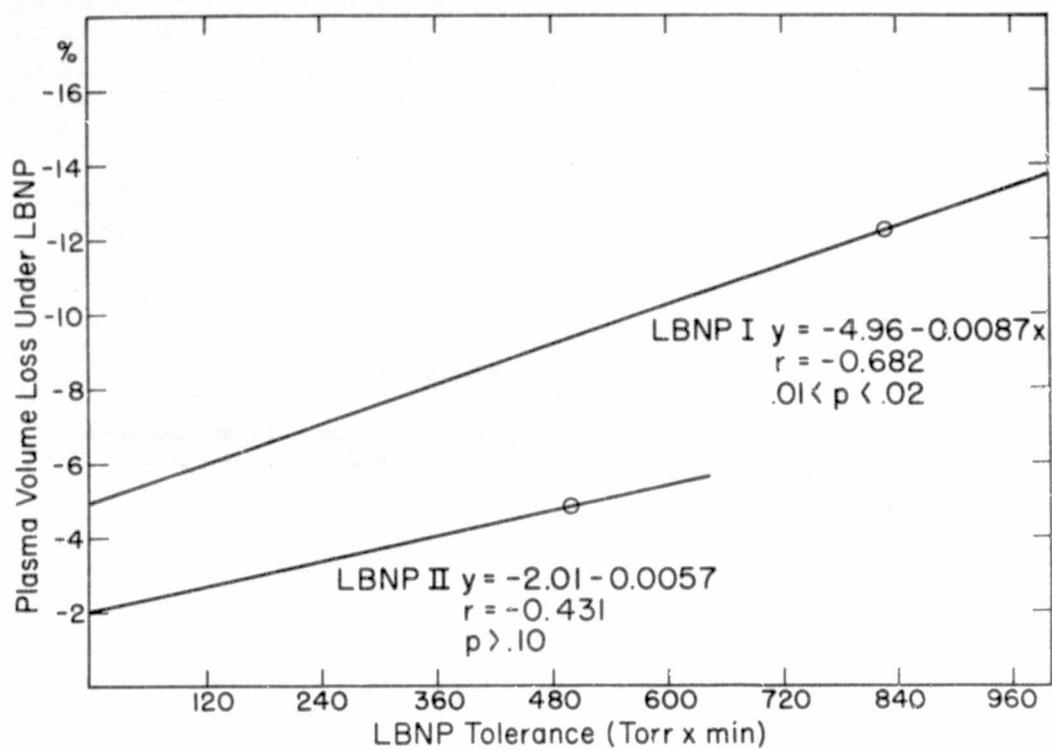
FIGURE 6

FIGURE 7

Correlation between percent leg volume increase and
plasma volume loss under LBNP before (I) and after (II)
dehydration.
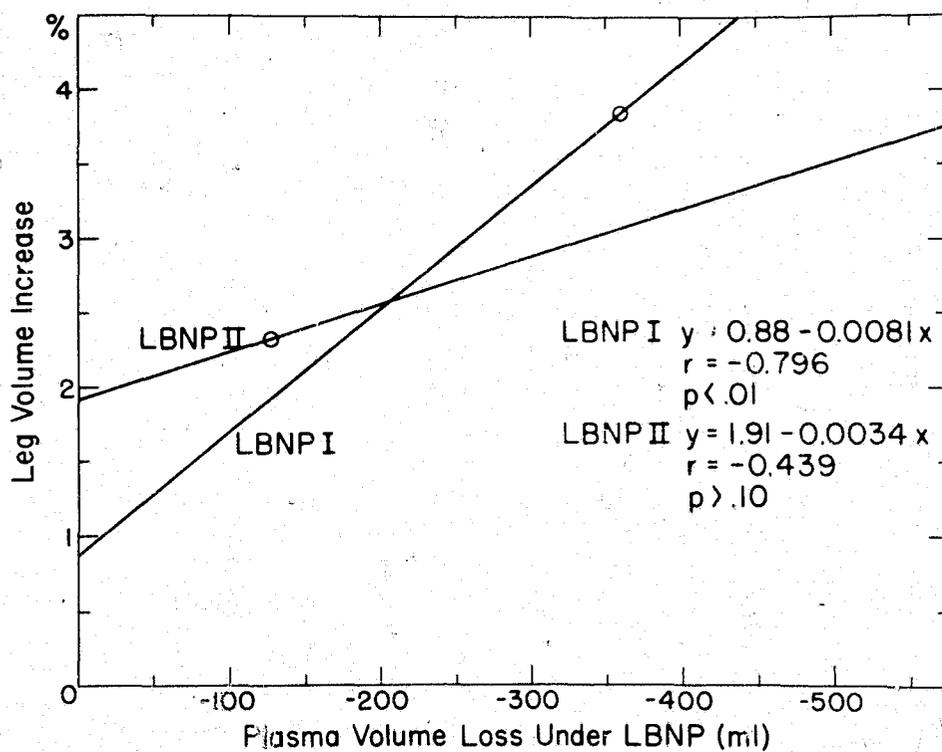
FIGURE 7

II

EFFECTS OF PERIPHERAL BLOOD SEQUESTRATION

DURING LBNP ON PLASMA VOLUME DETERMINATIONS

ABSTRACT

Blood samples were obtained from peripheral forearm vessels with indwelling needle before, during the last min and after lower body negative pressure (LBNP) in 16 experiments to determine whether plasma volume (PV) estimates would be affected by significant amounts of blood sequestration in the lower body. Total hemoglobin (THb) was estimated prior to LBNP with a 10 min CO rebreathing procedure and hemoglobin (Hb) and hematocrit (Hct) baselines established. When PV loss due to LBNP was calculated with Hb and Hct values from the circulating compartment (C_c) during the last min of LBNP, a 3% (87ml) loss was obtained assuming no change in THb. However, the Hb and Hct values from the mixed compartment drawn 2 min after LBNP, showed an 11% loss in PV (313 ml). This 72% underestimation of PV loss with the C_c sample must have resulted from the sequestration of blood with a higher than average Hct during LBNP which was mixed with C_c when LBNP ended. Subsequent sampling of Hb in 6 experiments showed that meaningful estimates of PV can only be obtained by sampling between one and 3 min after LBNP, after mixing has taken place, but before an appreciable amount of fluid has returned to the circulation from the tissues.

INTRODUCTION

The estimation of changes in total circulating plasma volume is important in studying the effects of dehydration, exercise and gravitational stress on the dynamics of the body fluid compartments. The commonly employed technique is to measure the control or baseline absolute volume compartments of the blood (BV), plasma (PV) and red cells (RCV) by indicator dilution techniques. The indicator is usually some form of a tag for red cells, either radio-active, protein dye or carbon monoxide (CO), the latter now commonly used (11). The total mass of circulating hemoglobin (THb) is thus determined and BV, PV and RCV can be calculated with simultaneous measurements of hemoglobin concentration (Hb) and hematocrit (Hct) obtained from a peripheral vein or artery. Subsequent hemoconcentration or hemodilution during or after experimental procedures is then estimated from Hb and Hct measurements alone, assuming that THb does not change. This so-called "intrinsic method" has proven validity in resting man (9).

Since most studies are concerned with relative changes in PV, the absolute accuracy of the baseline value is not of prime importance. The crucial assumption that remains is that the blood sampling site chosen for subsequent measurements of Hb and Hct must be representative of the true values for Hb and Hct in the total circulatory system. This must also be true for the baseline sample, requiring that the subject must have attained a circulatory steady state before experimental

variables are introduced (5). The sequestration of red cells in any part of the circulatory system during the time between control and subsequent samples will result in a relative hemodilution in the remaining circulatory compartment which is sampled and PV will thus be over-estimated and any plasma loss underestimated. Sequestration does take place in small vessels in animals when arterial pressure is lowered (6), but the effect of gravitational stress on sequestration in man has not been clearly documented. If sequestration does occur, the amount of hemoglobin in the red cells in the compartment excluded from effective circulation will be reflected in the difference in Hb and Hct in samples obtained from the circulating compartment while sequestration is present and another drawn an appropriate time after the sequestered and circulating compartment have been mixed, provided that no change in total intravascular plasma has taken place between the two measurements.

The purpose of this study was to determine whether an appreciable amount of blood is sequestered during lower body negative pressure (LBNP) by comparing Hb and Hct just prior to and just after the release of LBNP (LBR). The latter blood sample was assumed to be representative of the mixed compartment (M_c) and the sample during the latter stages of LBNP was assumed to represent the circulating compartment (C_c), which would differ from M_c according to the magnitude of the sequestered compartment (S_c).

METHODS

Eleven males with a mean age of 34 yr were subjects in the study.
Three of the subjects took part in the experiments twice and another
was studied 3 times (n=16). Eight of the subjects (n=12) of the present
investigation were part of a comprehensive study to determine the effects
on LBNP tolerance of dehydration by ingestion of a diuretic. The LBNP
protocol of these 12 runs was a progressive one where LBNP was increased
stepwise every 5 min and terminated after 5 min at -60 Torr or when-
ever the subject showed signs of imminent syncope (8). LBNP stress
was expressed in Torr x min to take into account both the time and the
negative pressure tolerated. In these experiments control and subsequent
blood samples were obtained with a vacutainer through an indwelling
catheter previously placed in a peripheral vein in the forearm. In
another experiment (8c in Table 1) the subject was exposed to LBNP
for 20 min at -40 Torr. In this case blood was obtained as above but
was drawn with a syringe through an indwelling needle. In these 13
experiments THb was measured with the CO rebreathing method (11),
following a 5-min lung washout with O_2 and the injection of 30 cc of
CO into the rebreathing apparatus. Blood CO concentration was obtained
with the Coburn technique (3). The results of 3 other subjects with a
constant LBNP exposure for 10 min at -60 Torr were also included in
this study (7). In these experiments arterial blood was sampled with

a syringe from an indwelling needle in the brachial artery. Blood
gases and Hb only were measured in these subjects while THb was estimated
from body weight and Hct from the Hb/Hct values of the other 13 experi-
ments (see notes to Table 1). In all 16 experiments the sampling catheter
or needle was kept patent with a heparinized saline solution and the first
portion of each sample was discarded. Control and subsequent blood
samples were analyzed for Hb in duplicate, with the cyanmethemoglobin
technique (Hycel) utilizing a Backman spectrophotometer (Model DU),
and Hct was measured in duplicate samples after being spun for 3 min
in a Microhematocrit centrifuge (Model 33). During each LBNP exposure
the increase in leg volume (LV) was estimated as a percentage increase
as described previously (7) with a mercury strain gauge. The final
LV increase achieved just prior to LBR was divided by the LBNP tolerated
to give an index of leg compliance. The LBNP apparatus and procedure
have been described elsewhere (7).

The control blood samples for THb, Hb and Hct were obtained after
the subjects had been in the supine position for at least 20 min. In
all but 2 cases the last blood sample during LBNP (C_c) was drawn during
the last min of LBNP, just prior to LBR, and in cases 10 and 11 it was
drawn 2 min before LBR. After LBR the first sample (M_c) was taken
on the average about 2 min after LBR (Table 1). It was not taken during
the first 30 sec to avoid any "bolus" sampling of the blood returning
from the lower body (7). In 6 cases periodic samples were taken up
to 10 min after LBR to follow the course of hemodilution during recovery
as plasma returned from the extravascular to the intravascular compartment.

Calculations for PV loss during LBNP were made with the following formula:

$$\text{PV loss (ml)} = \text{THb(g)}\left[\left(\frac{100 - \text{Hct}_C(\%)}{\text{Hb}_C(\text{g\%})}\right) - \left(\frac{100 - \text{Hct}_{M_C}(\%)}{\text{HbM}_C(\text{g\%})}\right)\right] \quad (1)$$

where C = control value and

M_C = value after LBR (mixed compartment)

The loss in PV was also calculated with the value for Hb and Hct obtained just prior to LBR, assumed to represent the circulating compartment (C_C), by substituting Hct_{C_C} and Hb_{C_C} for Hct_{M_C} and HbM_C in equation 1.

The results were analyzed using a paired t-test and standard least squares linear regressions to test for significant differences or correlation coefficients (p<.05).

RESULTS AND DISCUSSION

All results and calculated values are summarized in Table 1.
The mean value for Hb obtained during the last min of LBNP was 0.3 g%
higher than the control value. This represented a significant change
(p<.05) reflecting the hemoconcentration resulting from the removal
of plasma from C_c. A corresponding increase in Hct of 0.6% was noted
which was of the same order of statistical significance. After the
removal of LBNP, Hb increased another 0.8 g% after 2 min while Hct
increased an additional 2.2%, both of these values for M_c were signifi-
cantly higher than those for C_c (p<.00001). None of the values for
Hb/Hct ratio in C, C_c or M_c (see notes to Table 1) were significantly
different from each other, indicating that the red cells did not
undergo any significant changes in size during the course of LBNP.

When PV loss was calculated from Hb and Hct obtained after LBR
(M_c) with equation 1, a mean loss of 313 ml (11%) was obtained which
correlated with the amount of LBNP stress (r = +.58, p<.02) and un-
doubtedly reflects plasma water lost from the circulation due to
extravasation in the lower extremities during LBNP (7, 8). On the
other hand, when PV loss was calculated with C_c values in equation 1
a much smaller value of 87 ml resulted, 72% less than that computed from
M_c. In fact, 5 of the experiments showed a gain in PV during LBNP
using this sample. These individual values were not as well correlated
with LBNP stress or tolerance as were those calculated from the M_c

sample ($r = +.46$, $p<.10$). The two calculations of PV loss were related

to each other as shown by a positive correlation coefficient (+.70,

$p<.005$). However, as the regression equation for this relationship

(equation 2) demonstrated, if one saw no change in PV using the C_c

sample, a 245 ml loss in PV would have taken place if calculated with

the properly mixed sample (M_c).

$$PV \text{ loss } (M_c) = 245 + 0.78 \times PV \text{ loss } (C_c) \qquad (2)$$

The discrepancy in PV loss calculated by the two samples must be related

to the fact that the samples drawn while the subjects were still exposed

to LBNP were not representative of the total circulation and some red

cells and Hb were effectively removed from the sampled compartment

(sequestered). It stands to reason that the amount of blood sequestered

must be proportional to the difference in PV loss (ΔPV loss) calculated

from the two samples. It would also seem reasonable that the amount

of blood sequestered should have some relationship to the amount of

LBNP tolerated by the subjects, yet the relationship between ΔPV loss

and LBNP tolerance was not significant. However, when ΔPV loss per

stress (ml/Torr x min) was compared with leg compliance the relationship

was very close ($r = +.91$, $p<.00001$). Furthermore, ΔPV loss was related

to true PV loss as computed from M_c ($r = +.50$, $p<.05$). This leads

one to suspect that individuals with large venous distensibility in

the dependent vessels also tend to sequester more blood, presumably

because of relatively poor circulation resulting from lack of vaso-

motor tone. The correlation of ΔPV loss with true PV loss may indicate

that with the relatively slower circulation and more sequestration

more plasma is induced to leave the circulation when exposed to the

greater hydrostatic pressure gradient across the blood vessels in the lower body. The latter relationship also demonstates that under conditions of greater true PV loss the underestimation of PV loss using the C_c sample will be larger in absolute terms.

These results demonstrate that the amount of apparent blood sequestration during an average exposure of some 700 Torr x min of LBNP is very significant and produces a marked 72% underestimation of true PV loss if blood samples are obtained prior to proper mixing in the circulatory system. The situation thus exists where the measurement of a true reduction in PV can be effectively nullified by sequestration of red cells if proper sampling procedures are not carried out.

The characteristics of the sequestered compartment (S_c) are of interest, but can not be directly discerned from the mean data in Table 1. The mean value for ΔPV loss was 226 ml which must bear some relationship to the amount of red cells removed from the circulation. If one assumes that C_c contained all the plasma then one can calculate from the mean data that 202 ml of red cells were effectively sequestered. However, some plasma must also be contained in S_c and RCV_{S_c} would then be proportionately greater. The fact that a sequestered compartment was visualized does not necessarily imply that red cells in this compartment do not exchange at all with the central circulation. A comparable situation was created by Brown et al. (2) who measured blood volume and THb (CO method) with and without venous congestion of the legs (thigh cuffs). With the cuffs inflated BV and THb were grossly underestimated if the rebreathing period was less than 20 min. However, if rebreathing was continued longer, blood CO levels gradually returned to control levels without congestion. This would indicate

that the exchange of RCV_{C_c} with RCV_{S_c} is reduced during congestion

producing the same phenomenon as in our experiments. Similar conclusions

can be drawn from the results of Balakhovsky et al. (1) who measured

a reduction of 35% in circulating blood volume with the CO method

with 10 min exposure to -80 Torr LBNP in 12 subjects. They noted

a marked fall in blood CO concentration during LBNP, presumably because

of some CO being trapped by the sequestered red-cells. But the control

value of CO was restored shortly after release of LBNP when mixing was

complete.

The effects of apparent blood sequestration on blood volume measure-

ments in the standing position may not be as notable as during LBNP

or venous congestion with cuffs because of the activity of postural

muscles. Waterfield (12) compared circulating RCV in 8 subjects with

a 20 min CO rebreathing method after 40 min standing to supine values

and found a 4% or 85 ml reduction which he attributed to red cell

shrinkage. However, this difference could as easily have been the

result of sequestration since he did not measure Hb to confirm a change

in the Hb/Hct ratio. Hagan et al. (5) recently reported no change in

red cell water content after 35 min of standing, nor did they report

any loss in RCV with a 10 min CO rebreathing method after 25 min of

standing. From these few studies it would seem that blood sequestration

is less likely to influence estimates of PV during standing than during

LBNP, especially since the last two studies (5, 12) both reported 15%

reductions in PV which exceeds our value of 11% and any sequestration

evident during sampling should reduce the PV loss estimate. One study

which did estimate PV with Hb and Hct values obtained prior to the

release of LBNP does tend to support our evidence for blood sequestration.

Murray and co-workers (10) estimated PV with samples during a progressive LBNP procedure in 4 subjects. They noted a 1.2% and 3.5% reduction after 150 and 500 Torr x min, which compares with our 3% value for PV loss after 700 Torr x min when using the C_c sample. However, in their protocol they then reduced LBNP in a stepwise manner and noted a 9% reduction in PV after the subjects were returned to -20 Torr from -40 Torr. This larger PV loss showing up later at a reduced negative pressure was probably indicative of better mixing of previously seques-tered blood even though LBNP had not been completely terminated. The 9% loss in PV probably represented the loss incurred during the preceding more intense stress and compared favorably with our 11% value at a slightly greater stress.

The other question that remains is whether the effect of blood sequestration in the dependent part of the body during gravitational stress is noted equally at various sampling sites. Eisenberg (4) has determined that the total body hematocrit change with gravitational stress (standing) is best measured by sampling arterial or central venous blood because of congestion at the sampling site in peripheral venous sampling while upright. During LBNP, where the relationship of venous or arterial sampling site to the atrial level is constant, this is not a factor and no statistically significant differences were noticed in the present study.

In summary, we have shown that serious overestimations of PV and underestimations of plasma loss during LBNP resulted when estimates were made from blood samples taken during LBNP. This is the result of apparent sequestration of a substantial volume of red cells in the lower body. Valid measures of PV fluctuations can only be made from

blood samples drawn after LBNP, when the intravascular blood compartments have been thoroughly mixed, but before the extravascular fluid re-enters the circulation.

In view of the importance of the time factor in sampling for plasma volume after any kind of gravitational stress, a series of samples was taken at 2-min intervals after release of LBNP (Fig 1). Most of the first samples after LBR (n=16) were drawn between one and 3 minutes (average 1 min and 52 sec) and this seemed to coincide with the maximum increase in Hb as a result of complete mixing of C_c and S_c. One might speculate that a higher peak value for Hb might have been obtained if samples had been drawn during the first min. But it is questionable whether mixing would be complete during that time and samples with spuriously high or low Hb might be obtained, depending on the relative contribution from S_c and C_c. In any event the time for sampling after stress of this kind should be consistent to obtain meaningful results. It is obvious from Fig 1 that samples taken after 4 and 6 min would already give considerable underestimates of PV loss due to the progressive replenishment of plasma water from the tissues which is nearly 80% complete after 10 min and finally becomes asymptotic according to observations of Hagan et al. (5) during changes in posture.

C - 2

REFERENCES

1. Balakhovsky, I.S., O.A. Virovets and V.G. Voloshin. Changes in

   the circulating blood volume during lower body negative pressure

   exposure. Kosmicheskaia Biologiia i Meditsina. 4:27-30, 1970.

2. Brown, E., J. Hopper, Jr., J.J. Sampson and C. Mudrick. Venous

   congestion of the extremities in relation to blood volume determinations

   and to mixing curves of carbon monoxide and T-1824 in normal human

   subjects. J. Clin. Invest. 30:1441-1450, 1951.

3. Coburn, R.F., G.K. Danielson, W.S. Blakemore and R.E. Forster, II.

   Carbon monoxide in blood: analytical method and sources of error.

   J. Appl. Physiol. 19:510-515, 1964.

4. Eisenberg, S. Effect of posture and position of the venous sampling

   site on the hematocrit and serum protein concentration. J. Lab.

   Clin. Med. 61:755-760, 1963.

5. Hagan, R.D., F.J. Diaz and S.M. Horvath. Plasma volume changes with

   movement to supine and standing positions. J. Appl. Physiol.

   45:414-418, 1978.

6. Lawson, H.C. The volume of blood--a critical examination of methods

   for its measurement. In: Handbook of Physiology, Section 2:

   Circulation, Vol. 1. Edited by W.F. Hamilton. Washington:

   American Physiological Society, 1962, p. 23-49.

7. Loeppky, J.A., M.D. Venters and U.C. Luft. Blood volume and cardio-

   respiratory responses to lower body negative pressure. Aviat. Space

   Environ. Med. 49(11):1297-1307, 1978.

8. Luft, U.C., L.G. Myhre and M.D. Venters. Tolerance to lower body negative pressure before and after acute dehydration in the heat. Fed. Proceedings. 35:725, 1976.

9. Metcalf, W. The intrinsic method for serial plasma volume determinations. J. Lab. Clin. Med. 58:704-714, 1961.

10. Murray, R.H., J. Krog, L.D. Carlson and J.A. Bowers. Cumulative effects of venesection and lower body negative pressure. Aerospace Med. 38:243-247, 1967.

11. Myhre, L.G., D.K. Brown, F.G. Hall and D.B. Dill. The use of carbon monoxide and T-1824 for determining blood volume. Clin. Chem. 14:1197-1205, 1968.

12. Waterfield, R.L. The effects of posture on the circulating blood volume. J. Physiol. (London). 72:110-120, 1931.

TABLE 1

LEG VOLUME (LV) CHANGES WITH LBNP AND PV LOSS CALCULATIONS
WITH THb FROM Hb AND Hct VALUES OBTAINED DURING AND AFTER LBNP

Experiment	LBNP Tolerance Torr x min	ΔLV %	THb g	Hb (g%) C	C_C	M_C	Time	Hct (%) C	C_C	M_C	M_C PV loss ml	C_C PV loss ml	ΔPV loss ml
1	1000	5.46	819	15.4	16.0	16.9	2:35	45.0	46.9	48.7	439	207	232
2*	67	1.24	907	13.9	14.2	14.6	2:40	42.7	42.9	44.3	279	92	187
3*	54	0.72	812	17.0	16.7	17.1	3:48	50.1	49.4	50.9	52	−77	129
4	1000	4.27	678	15.6	16.1	16.6	2:10	45.5	46.8	48.4	261	128	133
5a	1000	3.60	800	15.9	16.0	17.4	2:10	46.6	47.3	51.4	452	52	400
5b*	1000	3.40	800	17.6	17.1	18.0	2:15	50.4	49.4	52.3	135	−113	248
6a	1000	3.70	778	17.4	18.0	19.3	2:05	45.3	47.5	50.1	434	177	257
6b*	413	2.15	778	18.6	18.4	19.1	2:35	47.5	47.2	48.1	82	−37	119
7a	1000	4.01	866	16.9	17.9	18.3	2:10	44.1	45.6	48.1	408	233	175
7b*	1000	2.33	866	18.0	18.4	19.1	1:20	47.6	48.5	50.8	290	97	193
8a	1000	5.81	1096	15.6	16.6	17.2	2:10	44.2	45.6	47.6	581	329	252
8b*	397	3.69	1096	17.9	18.2	18.5	1:15	49.3	49.9	50.2	154	87	67
8c	800	3.67	880	14.9	15.7	16.0	0:45	43.8	45.9	47.0	404	287	117
9	523	3.71	841•	16.1√	16.3√	17.2√	0:43	45.2	45.5†	48.0†	320	51	269
10	600	2.74	819•	16.0√	15.7√	16.9√	0:36	44.9	43.9†	47.2†	262	−106	368
11	600	4.04	954•	17.2√	17.2√	18.8√	0:40	48.3	48.0†	52.5†	457	−17	474
Mean	716	3.41	862	16.5	16.8	17.6	1:52	46.3	46.9	49.1	313	87	226
SD	±343	±1.34	±110	±1.3	±1.2	±1.3	±:54	±2.3	±2.0	±2.2	±152	±136	±112

Notes

C: Control; C_c: Circulating compartment; M_c: Mixed compartment (post LBR)

*: Subjects dehydrated, having lost 16% PV prior to LBNP

√: Hb values obtained from arterial samples

•: THb calculated from THb/Wt for n=13 (11.7 g/kg)

†: Hct calculated from Hb/Hct for n=13 (.356, .358 and .358 g/ml for C, C_c and M_c respectively

Time: Time of M_c sample after LBR

FIGURE 1

Mean percent changes in Hb concentration during
and after LBNP with number of subjects and
standard deviations. Abscissa on left: Mean
and 1 SD of LBNP stress. Point at 0 time:
Last sample before LBNP release.
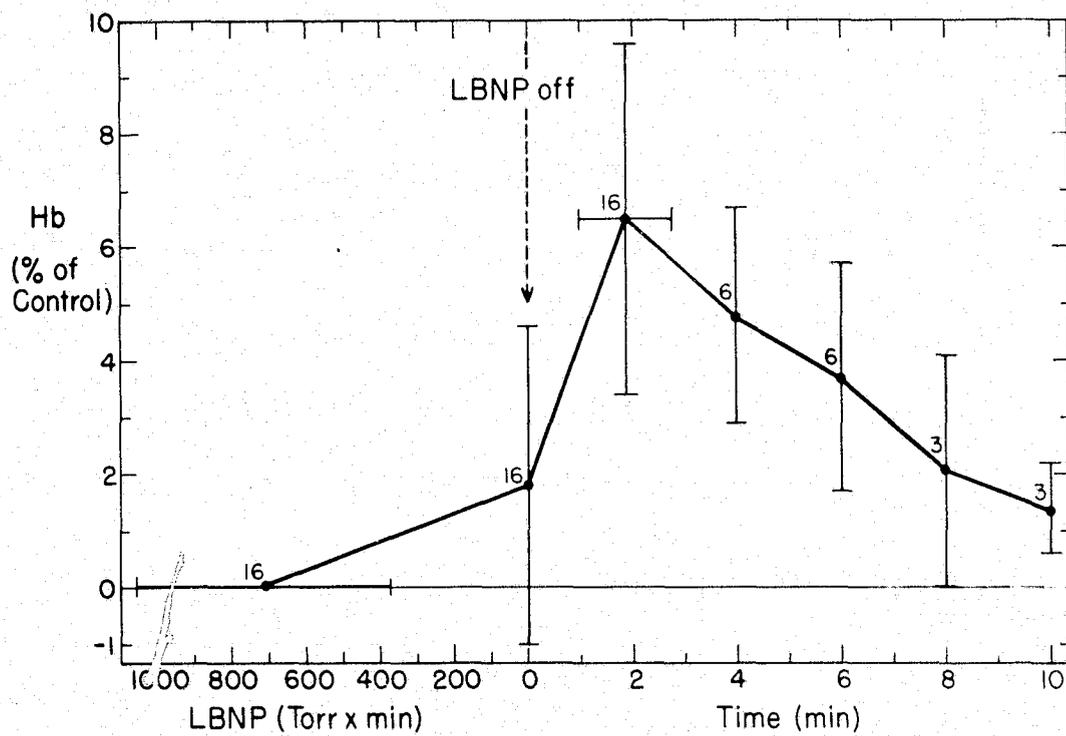
FIGURE 1

III

EFFECT OF LOWER BODY NEGATIVE PRESSURE RELEASE

ON TRANSIENT HYPERPNEA INDUCED BY INHALED GAS MIXTURE

ABSTRACT

Transient breath-by-breath ventilation ($\dot{V}_I$) and end-tidal gases were measured on 6 human subjects in response to 3 ventilatory stimuli: (1) the release of lower body negative pressure (LBR) after 10 min at -40 mmHg; (2) during the one-min inhalation of a gas mixture (GAS) containing 4.4% CO_2, 14% O_2, balance N_2; (3) a combination of LBR and GAS with LBR preceding onset of GAS by 5 sec. After LBR a 29% peak response in $\dot{V}_I$ occurred after 20 sec whereas with GAS and combined stimuli $\dot{V}_I$ levelled off after 25 sec, having risen 100% and 180% respectively. At the same times $P_{ET}CO_2$ had risen 2, 5 and 8 mmHg and $P_{ET}O_2$ had fallen 7, 14 and 19 mmHg respectively. LBR combined with GAS accelerated the ventilatory response by reducing the half-times to 10 sec from 18 sec with GAS alone. Leg volume measurements showed that 50% of the blood pooled prior to LBR had returned from the legs after 4 sec, presumably with a high P_{CO_2} and low P_{O_2}, thus contributing an endogenous respiratory stimulus to the exogenous one of GAS. With the two stimuli combined $\dot{V}_I$ was greater than the sum of each individually, suggesting a priming effect of the returning pooled blood on the chemoreceptors.

INTRODUCTION

When ventilation is stimulated by the inspiration of hypercapnic or hypoxic gas mixtures the response is always preceded by changes in arterial blood gas pressures which serve as the initial stimulus via the peripheral chemoreflex. The ventilatory response thus has a finite time lag, consisting primarily of the lung-to-chemoreceptor stimulus transit time. After ventilation has stabilized at a higher value, while continuing to breathe the gas mixture, the ventilation is never sufficient to maintain alveolar or blood gas concentrations at control values. Therefore, it is possible to objectively define CO_2 or hypoxic sensitivity as the increment in ventilation per unit rise in end-tidal or arterial P_{CO_2} or fall in P_{O_2}. On the other hand when ventilation is increased by a more physiological stimulus, such as the addition to the mixed venous blood of hypercapnic or O_2 depleted blood resulting from alterations in metabolism, the response is usually fast enough and of sufficient magnitude to prevent an appreciable alteration in blood gas concentrations in the peripheral arteries. This dilemma in ventilatory control was well-described 15 years ago by Tenney (28). The latter response characteristics predominate after the onset of exercise in man where ventilation rises immediately with little change in arterial or alveolar gas pressures to account for the stimulus. Fenn has stated that perhaps all the experiments with inhaled CO_2 have nothing to do with the true physiological

regulation of CO_2 and that the real stimuli for the ventilation response to changes in endogenous O_2 and CO_2 are largely unknown (8).

The purpose of the present study was to compare the characteristics of the transient ventilatory response of man under three conditions: 1) the inhalation of a hypoxic and hypercapnic gas mixture (GAS), 2) the simultaneous transient rise in P_{CO_2} and fall in P_{O_2} in mixed venous blood and, 3) a combination of these two conditions. Although the loading of CO_2 into mixed venous blood can be carefully controlled in animal studies with infusion techniques (13) this is not feasible in man. We utilized instead the release of lower body negative pressure (LBNP) which produces a marked transient change in the concentration of blood gases in pulmonary blood as a result of some of it having been pooled in the legs and pelvic region during LBNP. We have previously shown how the release of LBNP (LBR) produces a rapid and marked rise in tidal volume and O_2 uptake corresponding to a drop in arterial and alveolar P_{O_2} and rise in P_{CO_2} which are related to the severity of the preceding LBNP (17). The comparison of the responses might shed further light on the potential existence of intrapulmonary chemoreceptors which has recently again been suggested as a result of investigations by Wasserman's group (24, 29) and Filley and Heineken (10). Another reason for performing these experiments was to verify the results of Filley's group (9), who have recent evidence to show that ventilation decreases during the first breath after a reduction in the mixed venous to alveolar CO_2 gradient (breathing of hypercapnic gas mixture) and that ventilation should increase when the gradient is enlarged (via metabolically

induced mixed-venous hypercapnia). In extending their hypothesis,

the rise in ventilation would be less when these two situations are

combined than during mixed venous loading alone since the venous to

alveolar gradient would be altered less. The results of these experi-

ments would also be interesting in light of a prediction made by

Grodins 15 years ago. He said that if the respiratory control system

were presented with a metabolic and inspired CO_2 load simultaneously

"it might develop a neurosis trying to decide which control mode to

use" (15).

METHODS

Six healthy males were subjects in the study. Their physical characteristics and "CO_2 sensitivity" as measured by a modification (23) of Read's rebreathing test (20) are shown in Table 1. Each subject was exposed to GAS, LBR and the two procedures combined (LBR + GAS), as well as a placebo (air) during one session with about 10 min allowed for recovery between each of the four procedures. The gas mixture consisted of 5.42% CO_2, 13.90% O_2 and balance N_2. This mixture was chosen because at this elevation (P_B = 630 mmHg) it approximated the mean alveolar (end-tidal) P_{O_2} and P_{CO_2} of the subjects, was well-tolerated by them and resulted in stable end-tidal gas values in about 4 breaths. A smaller rise in the mixed venous to alveolar P_{O_2} and P_{CO_2} gradients would theoretically result when it was inspired after LBR than that produced by LBR alone. The first procedure for each subject consisted of either GAS or air (placebo) alone. The gas or air was administered for one min from a plastic bag, controlled by a stopcock on the inspiratory line which was manually controlled. The switch-over during an exhalation was not seen by the subject nor did he know the contents of the bag. Ventilatory measurements were made for one min before, one min during and for two min after inspiring from the bag. In the second procedure the subject was exposed to LBNP for 10 min at -40 mmHg. The equipment and procedure have been described in detail elsewhere (17). The negative pressure was then abruptly removed during an inspiration and during the ensuing exhalation he

was switched to the bag which contained either gas (LBR + GAS) or air (LBR alone). Thus the subject began inspiring from the bag about 5 sec after LBR. The third phase of the experiment was to repeat the first procedure with the alternate bag contents (air or gas) and finally, after another exposure to LBNP, LBR was combined with air or gas. The sequence of air or gas administration was randomly varied for the second and fourth, and first and third procedures.

End-tidal O_2 and CO_2 ($P_{ET}O_2$ and $P_{ET}CO_2$) were recorded continuously with a respiratory mass spectrometer (SRI-MEDSPECT) from a Rudolph (Model 2600) respiratory valve. Inspiratory and expiratory flows were measured with a heated pneumotach (Fleisch, No. 2) and pressure transducer (Validyne, model MP 45-1), mounted between mouthpiece and Rudolph valve. Inspired flow was converted to inspired tidal volume (V_T) with a Validyne integrater (Model TV 102-782). The breathing frequency (f) was obtained from the end-tidal record and ventilation ($\dot{V}_I$) calculated on a breath-by-breath basis from the product of f and V_T (BTPS). Mean inspiratory flow rate was obtained from V_T and inspiratory time. The total deadspace of the apparatus was 100 cc (mouthpiece, valve, pneumotach and connecting tubing). Gas flows and V_T output were corrected for viscosity differences (about 2%) when the gas mixture was inspired. The signal for V_T was electronically calibrated and converted to BTPS with a calibration factor empirically obtained by forcing a known volume of room air through the pneumotach with a spirometer and bag-in-box. Leg volume (LV) was estimated with a Hg-in-silastic strain gauge, placed on the left calf as described elsewhere (17). This was used to estimate the amount and rate of blood returning from the lower body to the central circulation when

LBNP was terminated. All electrical signals were recorded on a Honeywell (Model 1508A) visicorder.

Respiratory variables and LV for each subject were plotted on a breath-by-breath basis against time and then averaged at 5 sec intervals in most cases. A paired t-test was used to test differences between variables for statistical significance ($p < .05$) between procedures.

RESULTS

Figure 1 shows the time-averaged values for the end-tidal gases, LV and the estimated transient increase in blood flow to the lungs and central circulation.

There was no significant difference in the curves for LV after LBR with and without CO_2 so the mean curve is shown. From this curve it is apparent that 5 sec after LBR over one-half of the previously pooled blood had left the legs (55%) and after one min 87% of the pooled blood had returned to the central circulation as LV plateaued slightly above pre-LBNP baseline, indicating an increase in tissue volume during LBNP. As sequestered blood leaves the legs it must show up nearly as rapidly as an augmented flow of blood to the right heart and pulmonary circulation ($\dot{Q}$) and could be represented by a mirror image of the LV curve. The response of $\dot{Q}$ was calculated with the assumption that 630 cc of blood (shaded area) were returned from the legs in a pattern depicted by the mirror image of the LV curve (17). The curve demonstrates in a simplified manner the "bolus" characteristic of this blood flow which undoubtedly is damped to some degree in its passage through the pulmonary circulation.

Following LBR, it is apparent that $P_{ET}O_2$ fell (2.8 mmHg) and $P_{ET}CO_2$ rose (1.6 mmHg) during the 5 sec interval between LBR and the next inspiration. Both of these transients were statistically significant (p<.002) when the runs for LBR and LBR + GAS were combined. With LBR

alone, these trends continued for 15 sec after LBR during which time $P_{ET}CO_2$ had risen 2.7 mmHg and $P_{ET}O_2$ had fallen 7.7 mmHg. The mean values then began returning toward baseline and levelled off after about 60 sec with $P_{ET}CO_2$ being higher and $P_{ET}O_2$ lower than baseline by about 1.5 mmHg. With GAS alone the wash-in curves of the inspired mixture of CO_2 and O_2 were essentially completed after 20 sec or 4 to 5 breaths. The gas mixture inhalation served to raise $P_{ET}CO_2$ by 5 mmHg and drop $P_{ET}O_2$ by 14 mmHg. With the combination of LBR and GAS the transients in gases were more pronounced and approximated a summation of the curves for the individual procedures. The transients were most marked 15 sec after the onset of GAS (20 sec after LBR). At this point the differences from the values for GAS alone (1.3 mmHg for P_{CO_2} and 6.5 mmHg for P_{O_2}), were both statistically significant ($p < .005$). However, towards the end of the minute the two end-tidal gas curves converged.

With the resumption of air breathing after GAS and LBR + GAS the recovery transients for both gases were similar for the first 10 sec, followed by an overshoot of the baseline in both cases during the first min of recovery. The overshoot was more pronounced for both gases after LBR + GAS than after GAS alone. This difference in gas values during recovery was greatest after 15 sec of recovery and amounted to 2.7 mmHg for P_{O_2} and 1.5 mmHg for P_{CO_2} but was not statistically significant. Both of these transients were completed between one and two min of recovery as both gases returned to baseline.

The ventilatory responses of f and V_T to the three procedures are shown in Fig 2. With LBR there was a 20% (0.18 L) rise in V_T after 20 sec, about the same time that $P_{ET}O_2$ and $P_{ET}CO_2$ showed maximum

changes in Fig 1. The rise in V_T as shown is somewhat damped because of time-averaging since the subjects showed a peak response anywhere between 10 and 30 sec. Using individual values the overall mean transient increment in V_T was close to 30%. Nevertheless the transient rise shown on the figure was statistically significant (p<.02) with the transient being virtually completed in 45 sec. There was no clear-cut pattern in f after the removal of LBNP and the rise seen with the first breath after LBR in Fig 2 was not significant. With GAS alone V_T did not show a response until 10 sec or the third inspiration. During the second breath V_T was actually reduced by 0.06 L, however this was not significant. After 25 sec V_T had levelled off, similar to the end-tidal gases, at a value about 70% above baseline. However, f continued to climb. When LBR and GAS were combined, V_T showed a similar pattern to GAS alone except that the 10 sec time lag seen for the latter was removed. The V_T then levelled off at a value similar to that for GAS alone. With f it was apparent that LBR produced marked potentiation to the GAS response, as a faster rise was followed by a significantly higher plateau value. After the subjects were switched to air f fell more markedly when preceded by GAS alone, falling below baseline in 10 sec whereas after LBR + GAS, f remained above baseline for a full min. The recovery for V_T was similar in both cases, with the peak after GAS alone at 120 sec not being significant.

Figure 3 shows $\dot{V}_I$ computed from f and V_T in Fig 2 and also the mean inspiratory flow rate ($\dot{V}_I$). The peak $\dot{V}_I$ following LBR occurred after 20 sec (29% increase). The mean peak, taken individually and peaking anywhere between 5 and 20 sec amounted to 52%. The $\dot{V}_I$ transient was completed after 40 sec. The response following GAS alone was

delayed by 10 sec, similar to V_T, and did not reach a plateau value
before the subjects resumed air breathing (peak: 134%). The $\dot{V}_I$
response after LBR + GAS was also similar to V_T in Fig 2 and did reach
a plateau after about 20 sec at 191% above the control value. Thus LBR
again removed the time lag from the response to GAS. The pattern of
$\overline{\dot{V}_I}$ or "inspiratory drive" (22) was almost identical to $\dot{V}_I$, confirming
that the latter was a true representation of inspiratory drive. The
recovery curves for $\dot{V}_I$ indicate that in all cases the curves returned
to baseline within one min, returning 5 to 10 sec sooner after GAS than
LBR + GAS.

In Fig 4 the transients of end-tidal gases for the run with LBR
and GAS alone have been summed and plotted along with the $P_{ET}CO_2$ and
$P_{ET}O_2$ values obtained when the two procedures were combined. In
general they are very similar. The largest difference occurred at
20 sec (1.2 mmHg P_{CO_2} and 1.3 mmHg P_{O_2}) where the combined procedure
resulted in this slightly larger change in the gases, however these
differences were not significant. The largest differences during
recovery while breathing air occurred at 15 sec, both for P_{CO_2} (1.7 mmHg)
and P_{O_2} (4.2 mmHg), but were again not significantly different. On
the other hand, the differences in $\dot{V}_I$ between the two curves shown in
Fig 4 were statistically significant from the 2nd breath through the
period where GAS was breathed and for the first 20 sec of recovery.
This shows that the summed responses of the end-tidal gases for LBR and
GAS were not different from those resulting from the combined procedure
and yet $\dot{V}_I$ responded more than the summated response to the individual
procedures.

In Fig 5 the points for $P_{ET}CO_2$ shown in Fig 1 have been plotted
against the corresponding points for $\dot{V}_I$ (Fig 3). Points were chosen

every 5 sec and show the rise of $\dot{V}_I$ in relation to $P_{ET}CO_2$. The initial

part of the curves for LBR and LBR + GAS for the first 10-15 sec are

nearly identical, while that for GAS alone shows the lag in $\dot{V}_I$ that

was pointed out earlier (Fig 3). However, after the first 10 to 15

sec the curves for LBR + GAS and GAS show a similar pattern to the peak

value with the former rising to a higher value at about the same P_{CO_2}

after one min. The LBR loop is completed in about 20 sec after the peak

response, with $P_{ET}CO_2$ stabilizing at a higher value than before LBR.

For the other 2 curves it took about 45 sec for the curves to approach

the prior resting value while breathing air, with $\dot{V}_I$ being higher for

LBR + GAS for a given P_{CO_2} during recovery than for GAS alone. This

is indicative of a greater CO2 output for the former during recovery.

The "CO$_2$ sensitivity" slopes shown in Table 1 correlated well with the

slopes obtained between the baseline and peak $\dot{V}_I$ and $P_{ET}CO_2$ points

from Fig 5 for GAS (r = +.78, p<.10) and for LBR + GAS (r = +.92, p<.01).

However, there was no correlation between slopes in Table 1 and the peak

rise slope for LBR alone (r = +.07) indicating that the subject's

$\dot{V}_I$ response to LBR was not related to CO$_2$ sensitivity measured with

the rebreathing technique.

The relationship between ventilation and inspiratory drive and

end-tidal gases are shown in Fig 6. The values are averaged for the

first 3 breaths irrespective of time (f). The end-tidal values are

the ones prevailing at the start of the corresponding V_T and $\overline{\dot{V}_I}$. For

GAS alone significant changes in $P_{ET}CO_2$ and $P_{ET}O_2$ were apparent at

the beginning of inspiration of the second and third breath. These

correspond to the reduced mean V_T and $\overline{\dot{V}_I}$ during the second breath

noted in Fig 2 and 3. With LBR alone the end-tidal gases had signi-

ficantly changed during the course of the first expiration after LBR

although V_T and $\dot{V}_I$ did not increase until the second breath. With LBR + GAS the change in end-tidal gases was greater than with either procedure alone, approximating the sum (Fig 4). However, in this case the ventilatory response showed an immediate and continuous rise and the lag that showed up with GAS alone was overridden by LBR. Therefore, on a breath-by-breath basis, the early ventilatory response was not a reflection of the sum of the individual responses, similar to the findings on a time basis (Fig 4).

The observations from Fig 4 and 6, that the superimposition of LBR on GAS potentiated the ventilatory response, was also borne out by the $\dot{V}_I$ half-times for the three procedures. These have been tabulated in Table 2. With GAS the half-time was 17.8 sec whereas with LBR + GAS it was 9.6 sec (zero time = beginning of inspiration of gas). These values were significantly different (p<.0001). Even taking zero time = LBR, the 15.0 sec half-time for LBR + GAS was still significantly less than 17.8 sec and yet the response at this time was greater (110%) than with GAS alone (73%). The half-time of the $\dot{V}_I$ response to LBR was 9.5 sec which was not significantly different from the other half-times because of the greater variability of the $\dot{V}_I$ responses to LBR alone.

DISCUSSION

The predominant finding in this study was that the response to
the gas mixture was greatly enhanced when preceded by LBNP release.
This was evidenced by the potentiated response in f and V_T (Fig 2)
and $\dot{V}_I$ and $\overline{\dot{V}_I}$ or "inspiratory drive" seen in Fig 3. Therefore, it
seems of prime importance to consider carefully the changes in the
end-tidal gases during the breath that intervened between LBR and the
first inspiration of the gas mixture, an average time interval of
5.0 sec. As noted in Fig 1, $P_{ET}CO_2$ rose some 1.6 mmHg and $P_{ET}O2$
fell by 2.8 mmHg during this time interval. Theoretically, the extent
of these gas pressure changes would be affected by a number of events.
They would be, a) attenuated by an increase in V_T of the breath,
b) magnified by an increase in CO_2 content or reduction in O_2 content
of blood arriving in the lungs that had been previously pooled and
c) potentiated by a decrease in alveolar deadspace which would
reduced the a-A gradients for P_{CO_2} and P_{O2} and increase $P_{ET}CO_2$ and
reduce $P_{ET}O_2$. When the one-breath changes in end-tidal gases were
considered in relation to V_T an inverse relationship was noted be-
tween the two ($r = -0.81$, $p<.001$). Since the mean V_T did increase
by 0.08 L this served to attenuate the P_{CO2} and P_{O2} changes and
indicated that the theoretical change in $P_{ET}CO_2$ with no change in
V_T would have been 2.0 mmHg during the 5 sec compared to the actual
value of 1.6 mmHg. Similar considerations for P_{O2} showed a theoretical
fall in $P_{ET_{O2}}$ of 3.7 mmHg compared to the actual value of 2.8 mmHg.

Therefore, after correcting for V_T there was a relatively greater increment in $P_{ET}CO_2$ and fall in $P_{ET}O_2$ than was actually observed. An estimate of the contribution of a shrinking FRC to these values of 2.0 and 3.7 mmHg for $P_{ET}CO_2$ and $P_{ET}O_2$, respectively, is probably negligible since an earlier study indicated that FRC only fell 100 cc (17) in 5 sec after LBR and this would be reflected as a prolonged alveolar plateau and would not necessarily alter the mean end-tidal values from the previous expiration. A more likely cause of the change in end-tidal gases after 5 sec is the increased hypercapnia and hypoxia in the blood which was delivered to the lungs during this time. Calculations based on the mean end-tidal values, and the mean V_T and FRC volumes during this breath showed that if this altered gas exchange was solely responsible for the end-tidal gases changes it would mean that an additional 9 cc of CO_2 were added to alveolar gas by pulmonary capillary blood and an extra 17 cc of O_2 were removed from the alveolar gas to resaturate the pulmonary capillary blood on its first passage through the alveoli. Although the exact addition of CO_2 and reduction of O_2 content that occurred in the venous blood with pooling in the lower body is debatable it seems not unreasonable from femoral blood measurements showing an $AvDO_2$ of 12 vol% in the upright posture (21) and an estimated lower body blood volume shift to the lungs of 630 cc (17) that the above values account for about 1/5 of the transients required in gas exchange to convert this venous blood to arterial blood. This augmented gas exchange continues for about one min after LBNP although the peak rise occurs after 10 sec (17). It is also noteworthy that the largest fall in LV was noted during the first breath after LBR.

With this rapid change in end-tidal gases being noted one would expect that the arterial blood gas values affecting aortic and carotid chemoreceptors are also rapidly altered after LBR. The magnitude of arterial changes are conceivably less than the end-tidal changes since the a-A gradient is attenuated due to the transient reduction in overall $\dot{V}/\dot{Q}$ ratio due to the relative hyperperfusion. However, in an earlier study a 13 mmHg and 3 mmHg change in PaO_2 and $PaCO_2$ after LBNP was still evident 40 sec after LBR (17). It seems likely therefore that the arterial blood gases are undergoing a change at the time that the gas mixture is first inspired with the combined procedure some 5 to 7 sec after LBR. If fast responding intra-pulmonary chemoreceptors did exist one would expect the $\dot{V}_I$ pattern with LBR + GAS shown in Fig 4 to be similar to the sum of the individual procedures since the end-tidal gases which reflect the stimulus were essentially equal. The fact that the response to GAS was enhanced by LBR seems more likely to indicate that an interaction phenomenon was taking place in the receptor(s) and the sensitivity or gain of the response to GAS was increased by LBR. Whatever the cause, $\dot{V}_I$ was potentiated for a full min after LBR and not just during the LBR transient. Previous reports have shown that the rate of change of $PaCO_2$ during $PaCO_2$ oscillations as well as the mean value can be a potent ventilatory stimulus (5) and that these oscillations are diminished when CO_2 is inhaled (30). In speculating about the presence of intrapulmonary chemoreceptors the stimulus-to-response time lag is crucial. Our study showed a potentiating effect on $\dot{V}_I$ that was first seen 5 to 7 sec (the average time from LBR to beginning and end of inspiration) after sequestered blood arrived in the pulmonary artery. In studying the $\dot{V}_I$ response to CO_2 infusion in

dogs, Sylvester et al. (27) noted a response latency of 6.6 sec after aortic arch infusion and 17.1 sec after SVC infusion. The fast ventilatory component being delayed but not eliminated with the removal of aortic arch and carotid chemoreceptors. Studies in man utilyzing the sudden addition and removal of CO_2 and O_2 in the inspired gas have indicated that the fast $\dot{V}_I$ response can begin between 5 and 6 sec (4, 12). In view of the response times we noted and findings by other investigators it does not seem necessary to invoke the presence of fast-acting intrapulmonary chemoreceptors, especially in view of the large body of evidence which exists to refute their presence (2).

The chemical disequilibrium theory that Filley invoked to account for his finding of a rapid $\dot{V}_I$ response which was inversely related to the $\overline{v}$-A gradient (10), was not borne out by this study. As shown in Fig 6 we did find a reduction in V_T and $\dot{V}_I$ during the second breath on GAS which was not significant, and a non-significant increase during the first breath of the off-response (Fig 2 and 3) which does not disprove his theory. However, when the two procedures were combined (gradients smaller than with LBR alone) the response was very greatly enhanced on the first breath and statistically significant by the second. The depression in $\dot{V}_I$ that he noted consistently with the reduction in $\overline{v}$-A gradients may be related to a vagal CO_2 reflex, which has been documented in the dog (1).

The overshoot of end-tidal gases beyond the pre-GAS baseline, following the inspiration of a gas mixture has been often noted by other investigators and presumably results from the fact that the end-tidal gases are a result of, as well as a stimulus to, ventilation (via arterial gases). With the return to air breathing the reduced

stimulus takes an appreciable time to show up at peripheral and central chemoreceptors, at least 30 sec for the latter (12). Meanwhile $\dot{V}_I$ is still responding to gas values at the receptor site which lag behind the end-tidal values. This phenomenon was accurately predicted by Grodins et al. some 25 years ago (14).

The distinct possibility exists that "CO_2 response" as commonly determined by noting the ventilatory response to inspired CO_2 has little relation to physiological CO_2 sensitivity. We saw no relationship between rebreathing "CO_2 sensitivity" and that computed from the peak LBR response. When $\dot{V}_I$ increases in response to inspired CO_2 it results in an even greater amount of CO_2 being inhaled, a truly non-physiological situation where the inspired CO_2 effectively prevents its elimination by an increase in $\dot{V}_I$ (3, 7, 25). The focus in the literature has been on the study of P_{CO_2} as a ventilatory stimulus, primarily because it has been known for many years that inspired CO_2 produced a much more marked response than alveolar hypoxia. The lesser response to hypoxia than to hypercapnia induced by breathing a low O_2 gas mixture is due to the hypocapnia which always goes parallel with simple alveolar hypoxia. When end-tidal CO_2 is held constant during progressive hypoxia, the response is much greater (18). However, in the physiologic sense the response to mixed venous loads of hypoxic and hypercapnic blood may be more realistic than CO_2 loading by inhaled gases. No studies of hypoxic or hypercapnic and hypoxic blood infusion have apparently been done, whereas with LBR we do have a combination of the two and their effects cannot be separated. The interaction between O_2 and CO_2 in the peripheral chemoreceptors has been extensively studied and has been shown

to be multiplicative (11) as well as additive (26) depending on the range of stimulus values employed.

One possibility that remains is that a transient increase in pulmonary blood flow (Fig 1) and pressure which is seen by the carotid sinus or aortic baroreceptors could affect the sensitivity of the peripheral chemoreceptors during the first few sec after LBR or might stimulate them directly via chemoreceptor efferent pathways of the sinus nerve. Chemoreceptor discharge has been found to increase with a rise in systemic blood pressure in the cat (19). That the baroreceptor reflex is activated with LBR is certainly evident from the immediate and rapid reduction in HR which takes place after LBR. The significance of the efferent fibers to the carotid bodies has recently been explored and it has been shown that many alterations in the physiological state can change chemoreceptor responsiveness via this pathway (19). It is conceivable that the augmentation of blood pressure with LBR is responsible for the initial V_T response (1 or 2 breaths) which is then followed by activation of the systemic arterial control system and when gas is given the arterial chemoreceptor response is enhanced. Towards the end of the first min on GAS after LBR, $\dot{V}_I$ was higher than with GAS alone because LBR had added extra CO_2 which could not be eliminated because of the back-pressure resulting from the inspired CO_2. This seems plausible since CO_2 output was greater during recovery after LBR + GAS.

Whatever the mechanism, the potentiated response to inspired gas mixture is very similar to the unexplained fast component of exercise hyperpnea. Here a sudden shift of pooled venous blood from the legs is presented to the pulmonary circulation at exercise onset and a

"priming" of peripheral chemoreceptors might well take place. A

rapid fall in $P\bar{v}_{CO_2}$ has been documented in man within 15 sec after

the onset of upright leg exercise (6) which coincides with the effects

of LBR. The slower rate of rise in O_2 uptake and $\dot{V}_I$ during supine

exercise compared to upright exercise in the same subjects has also

been noted (16) and could relate to the fact that no shift of pooled

venous blood from the legs occurred in the supine posture.

REFERENCES

1. Bartoli, A., B.A. Cross, A. Guz, S.K. Jain, M.I.M. Noble and D.W. Trenchard. The effect of carbon dioxide in the airways and alveoli on ventilation; a vagal reflex studied in the dog. J. Physiol. (London). 240:91-109, 1974.

2. Dejours, P. Chemoreflexes in breathing. Physiol. Reviews. 42: 335-358, 1962.

3. Dempsey, J.A. CO_2 response: Stimulus definition and limitations. Chest. 70(Suppl. July):114-118, 1976.

4. Downes, J.J. and C.J. Lambertsen. Dynamic characteristics of ventilatory depression in man on abrupt administration of O_2. J. Appl. Physiol. 21:447-453, 1966.

5. Dutton, R.E., V. Cherniak, H. Moses, B. Bromberger-Barnea, S. Permutt and R.L. Riley. Ventilatory response to intermittent inspired carbon dioxide. J. Appl. Physiol. 19:931-936, 1964.

6. Edwards, R.H.T., D.M. Denison, G. Jones, C.T.M. Davies and E.J.M. Campbell. Changes in mixed venous gas tensions at start of exercise in man. J. Appl. Physiol. 32:165-169, 1972.

7. Fencl, V. Ventilatory response to carbon dioxide in humans. Chest. 70(Suppl. July):113-114, 1976.

8. Fenn, W.O. Introductory remarks. Annals of the New York Academy of Sciences. 109(2):415-417, 1963.

9. Filley, G.F., R.C. Hale, J. Kartochvil and D.E. Olson. The hyperpnea of exercise and chemical disequilibria. _Chest._ 73 (Suppl. Feb):267-269, 1978.

10. Filley, G.F., F.G. Heineken. A blood gas disequilibrium theory. _Br. J. Dis. Chest._ 70:223-245, 1976.

11. Fitzgerald, R.S. Single fiber chemoreceptor responses of carotid and aortic bodies. In: _Morphology and Mechanisms of Chemoreceptors._ edited by A.S. Paintal. Delhi: Vallabhbhai Patel Chest Institute, 1976, p. 27-33.

12. Gelfand, R. and C.J. Lambertson. Dynamic respiratory response to abrupt change of inspired CO_2 at normal and high P_{O_2}. _J. Appl. Physiol._ 35:903-913, 1973.

13. Greco, E.C. Jr., W.E. Fordyce, F. Gonzalez, Jr., P. Reischl and F.S. Grodins. Respiratory responses to intravenous and intra-pulmonary CO_2 in awake dogs. _J. Appl. Physiol._ 45:109-114, 1978.

14. Grodins, F.S., J.S. Gray, K.R. Schroeder, A.L. Norins and R.W. Jones. Respiratory responses to CO_2 inhalation. A theoretical study of a nonlinear biological regulation. _J. Appl. Physiol._ 7:283-308, 1954.

15. Grodins, F.S. and G. James. Mathematical models of respiratory regulation. _Annals of the New York Academy of Sciences._ 109(2): 852-868, 1963.

16. Loeppky, J.A., M.D. Venters and U.C. Luft. Gravitational effects on blood distribution, ventilation, and gas exchange at the onset and termination of exercise. In: _Environmental Stress: Individual Human Adaptations._ edited by L.J. Folinsbee, et al. New York: Academic Press, 1978, p. 225-245.

17. Loeppky, J.A., M.D. Venters and U.C. Luft. Blood volume and cardiorespiratory responses to lower body negative pressure. Aviat. Space Environ. Med. 49:1297-1307, 1978.

18. Loeschcke, H.H. and K.H. Gertz. Einflusz des O_2-druckes in der Einatmungsluft auf die Atemtätigkeit des Menschen, geprüft unter Konstant-haltung des alveolaren CO_2-druckes. Pflügers Arch. ges. Physiol. 267:460, 1958.

19. O'Regan, R.G. Efferent control of chemoreceptors. In: Morphology and Mechanisms of Chemoreceptors. Edited by A.S. Paintal. Delhi: Vallabhbhai Patel Chest Institute, 1976, p. 229-246.

20. Read, D.J.C. A clinical method for assessing the ventilatory response to carbon dixoide. Australasian J. of Med. 16:20-32, 1967.

21. Reeves, J.T., R.F. Grover, S.G. Blount, Jr. and G.F. Filley. Cardiac output response to standing and treadmill walking. J. Appl. Physiol. 16:283-288, 1961.

22. Remmers, J.E. Analysis of ventilatory response. Chest. 70 (Suppl. July):134-137, 1976.

23. Strachova, Z. and F. Plum. Reproducibility of the rebreathing carbon dioxide response test using an improved method. Am. Rev. Respir. Dis. 107:864-869, 1973.

24. Stremel, R.W., D.J. Huntsman, R. Casaburi, B.J. Whipp and K. Wasserman. Control of ventilation during intravenous CO_2 loading in the awake dog. J. Appl. Physiol. 44:311-316, 1978.

25. Swanson, G.D. The exercise hyperpnea dilemma. Chest. 73(Suppl. Feb):277-279, 1978.

26. Swanson, G.D. and J.W. Bellville. CO_2-O_2 interaction in man. In: _Morphology and Mechanisms of Chemoreceptors_. Edited by A.S. Paintal. Delhi: Vallabhbhai Patel Chest Institute. 1976, p. 48-63.

27. Sylvester, J.T., B.J. Whipp and K. Wasserman. Ventilatory control during brief infusions of CO_2-laden blood in the awake dog. _J. Appl. Physiol._ 35:178-186, 1973.

28. Tenney, S.M. Concepts of threshold and sensitivity of ventilatory control. _Annals of the New York Academy of Sciences_. 109(2): 634-648, 1963.

29. Wasserman, K., B.J. Whipp and J. Castagna. Cardiodynamic hyperpnea: hyperpnea secondary to cardiac output increase. _J. Appl. Physiol._ 36:457-464, 1974.

30. Yamamoto, W.S. Mathematical analysis of the time course of alveolar CO_2. _J. Appl. Physiol._ 15:215-219, 1960.

TABLE 1

SUBJECTS' PHYSICAL CHARACTERISTICS AND "CO_2 SENSITIVITY"

VALUES OBTAINED BY A REBREATHING TEST (20)

Subj.	Age (yr)	Ht (cm)	Wt (kg)	Slope (L/min/mmHg)	Intercept (mmHg)	$P_{ET}CO_2$ (mmHg)
1	31	183	75.0	2.15	38.0	32.2
2	26	183	76.0	5.09	42.7	38.4
3	30	183	63.9	3.08	37.5	33.4
4	67	180	81.5	2.62	32.3	33.6
5	42	165	60.4	1.66	40.3	38.1
6	31	183	78.2	3.29	40.6	36.7
Mean	38	180	72.5	2.98	38.6	35.4
SD	15	7	8.4	1.19	3.6	2.7

TABLE 2

MEAN PEAK VALUES AND HALF-TIMES OF $\dot{V}_I$ RESPONSE

OF 6 SUBJECTS TO GAS MIXTURE (GAS), LBNP RELEASE (LBR)

AND THE COMBINED PROCEDURE (LBR + GAS)

Procedure	Peak Response	Zero Time	Half-Time	Difference
GAS	145%	First Inspiration	17.8 sec (a)	--
LBR + GAS	220%	LBR	15.0 sec (b)	vs 17.8, p<.02
		First Inspiration After LBR	9.6 sec (c)	vs 17.8, p<.0001
LBR	52%	LBR	9.5 sec (d)	vs (a), (b) or (c) NS

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FIGURE 1

Mean breath-by-breath and end tidal O_2 and CO_2, leg
volume (LV) and pulmonary blood flow (Q̇) response of 6
subjects to LBNP release (LBR), gas mixture (GAS) and
combined procedure (LBR + GAS). Values for gases were
averaged at 5 sec intervals on a time basis. Circles
represent beginning of inspiration for last breath before
and first breath after switching inspiratory stopcock.
LV was recorded continuously and Q̇ calculated from
inverse of LV curve assuming 630 cc of blood was
shifted from the lower body to pulmonary blood (17).
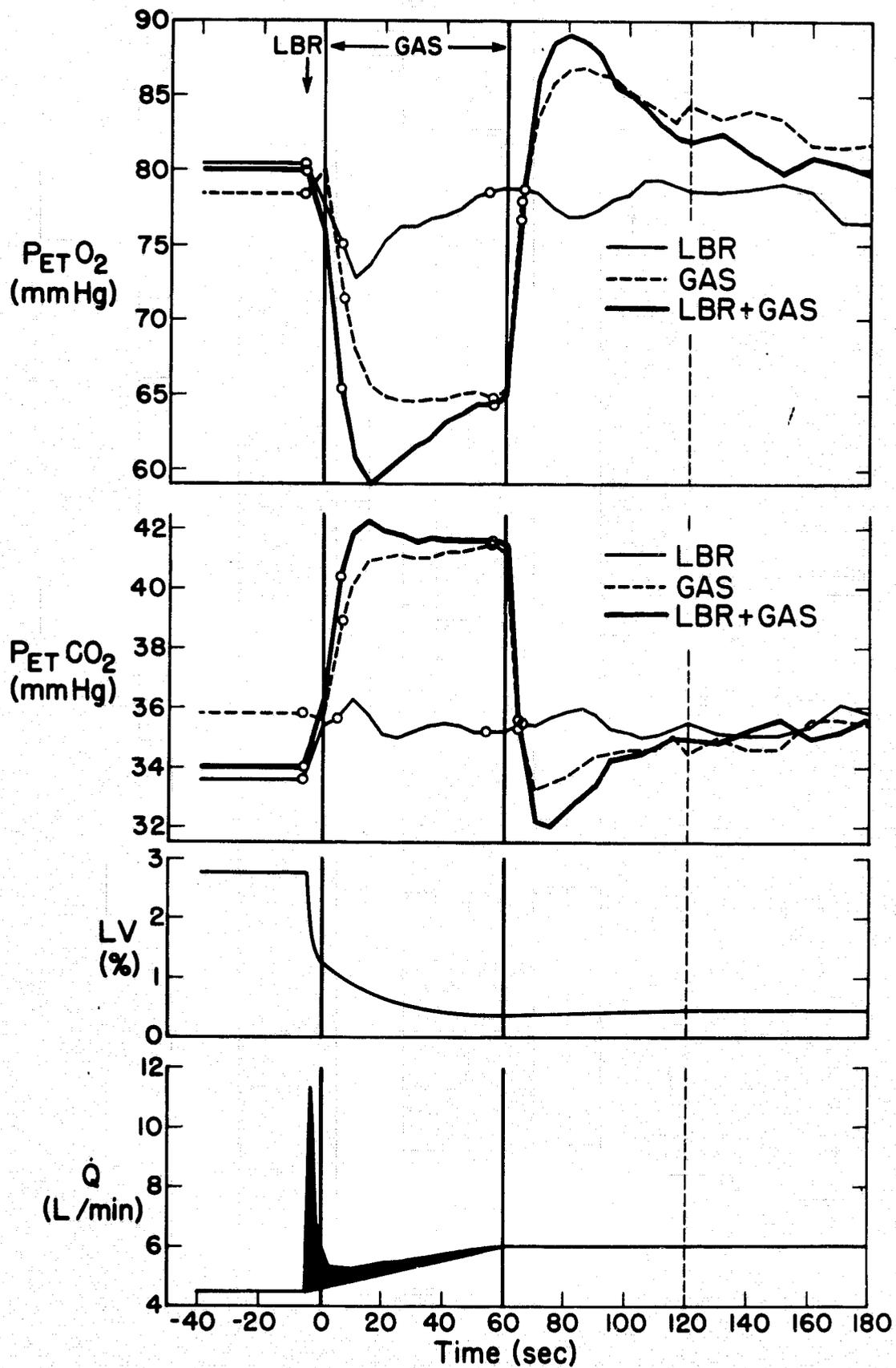
FIGURE 2

Mean respiratory frequency (f) and tidal volume (V_T)
response to 3 procedures. See legend to Fig 1.

FIGURE 2

FIGURE 3

Average breath-by-breath mean inspiratory flow ($\overline{\dot{V}_I}$) and ventilation ($\dot{V}_I$) in response to 3 procedures. See legend to Fig 1.
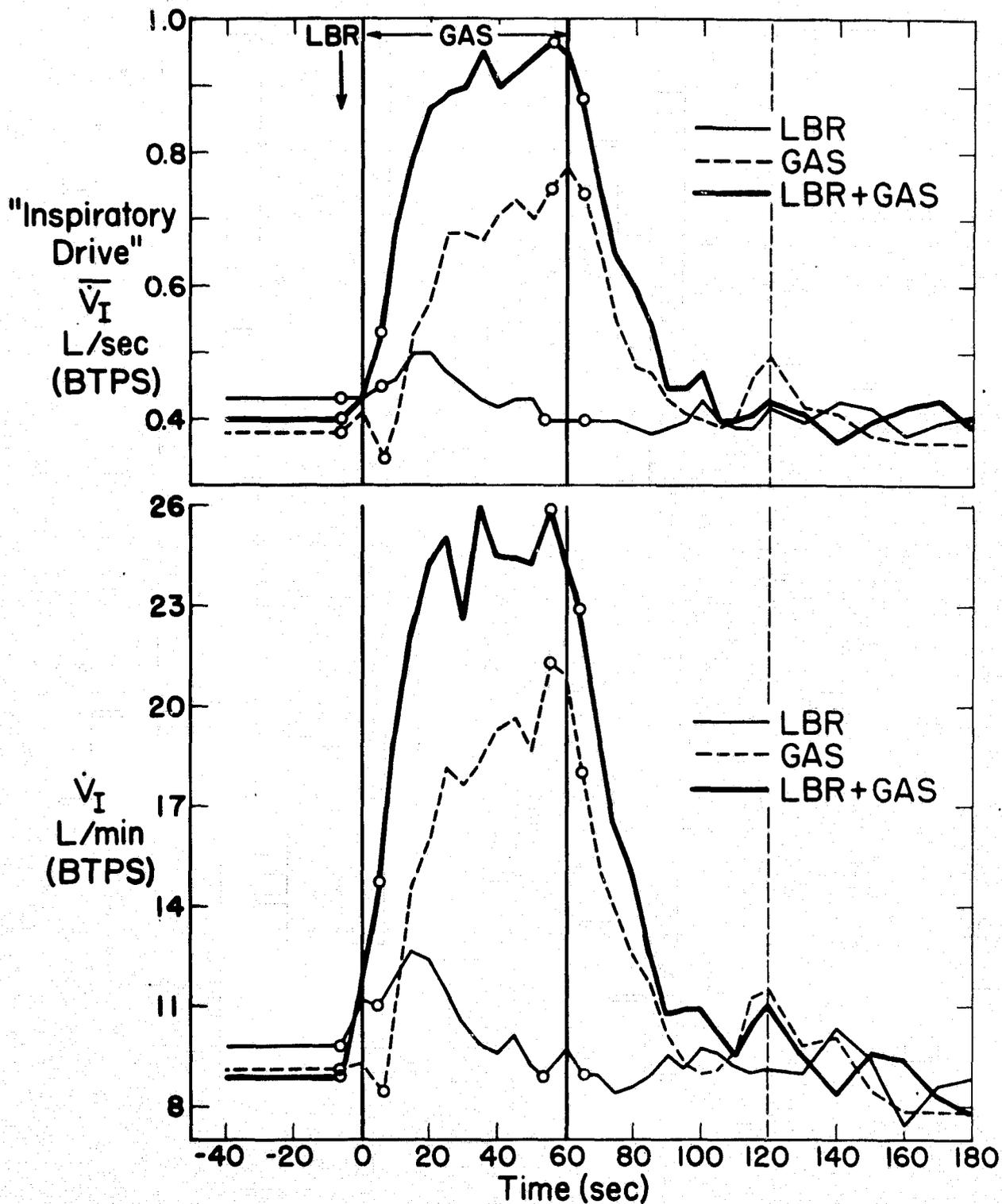
FIGURE 4

Mean end-tidal gas and ventilation ($\dot{V}_I$) responses to
LBR + GAS (Actual) compared to the added response to
LBR alone and GAS alone (sum). See legend to Fig 1.
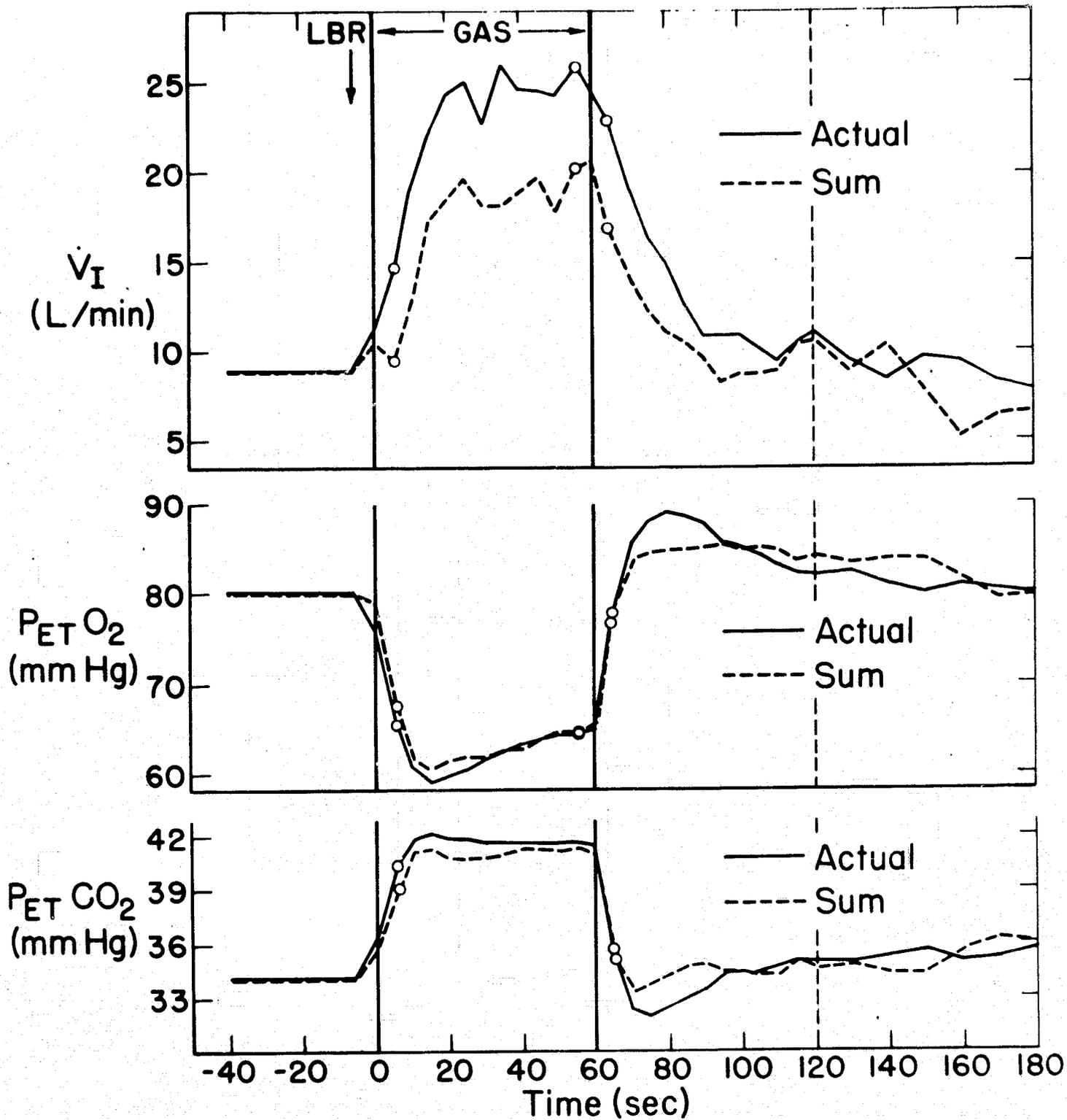
FIGURE 5

Ventilation ($\dot{V}_I$) in response to change in $P_{ET}CO_2$ during 3 procedures. Circles represent mean values prior to procedure. Points plotted at 5 sec intervals. Values for $P_{ET}CO_2$ are the same as in Fig 1 and for $\dot{V}_I$ they are the same as in Fig 3.

FIGURE 5
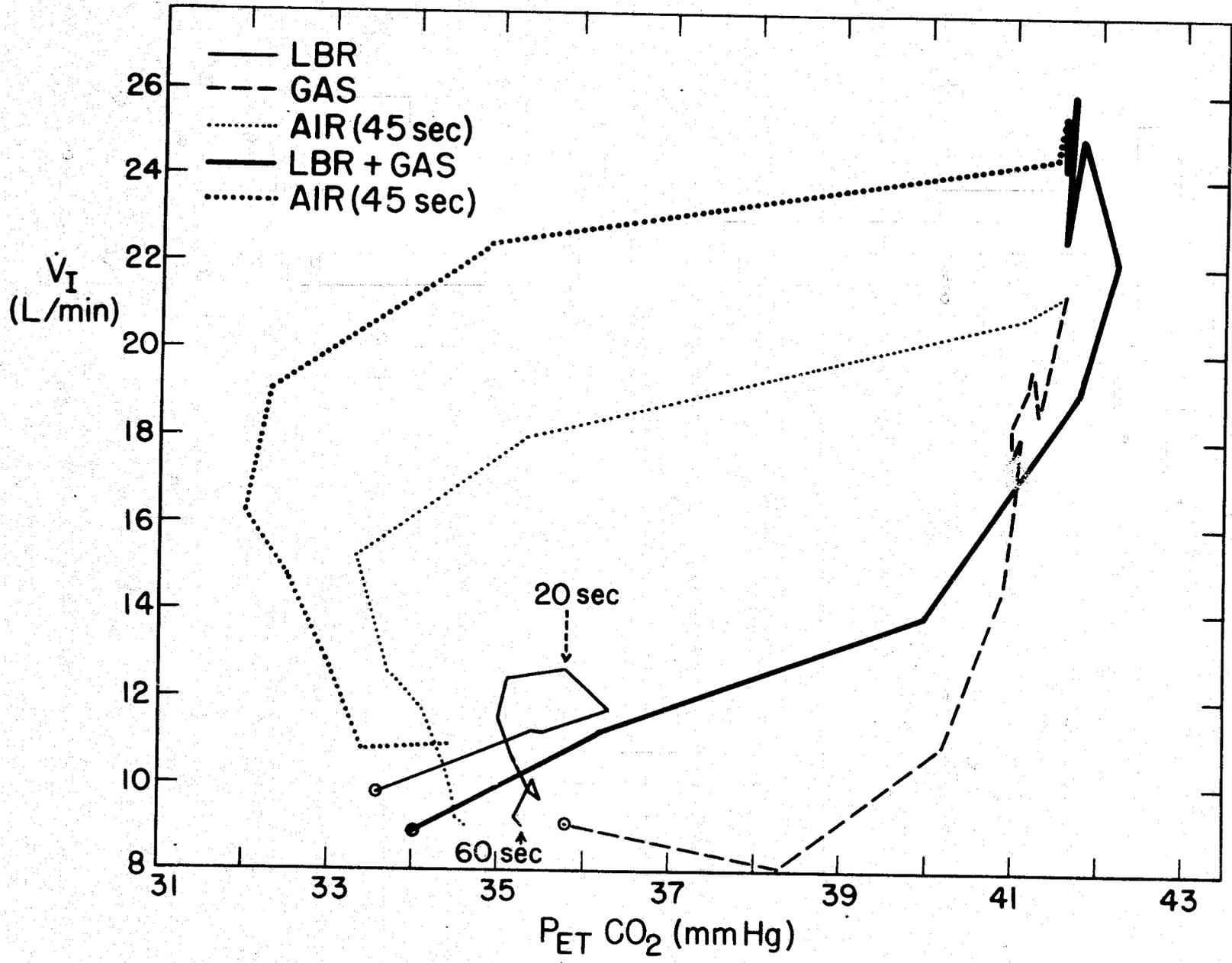
FIGURE 6

Mean response of tidal volume (V_T) and inspiratory drive ($\dot{V}_I$) to changes in end-tidal gases for 3 breaths after the onset of each procedure. V_T and $\dot{V}_I$ response corresponds to the end-tidal gas value preceeding that inspiration. The baseline value was the average of the previous 4 breaths.
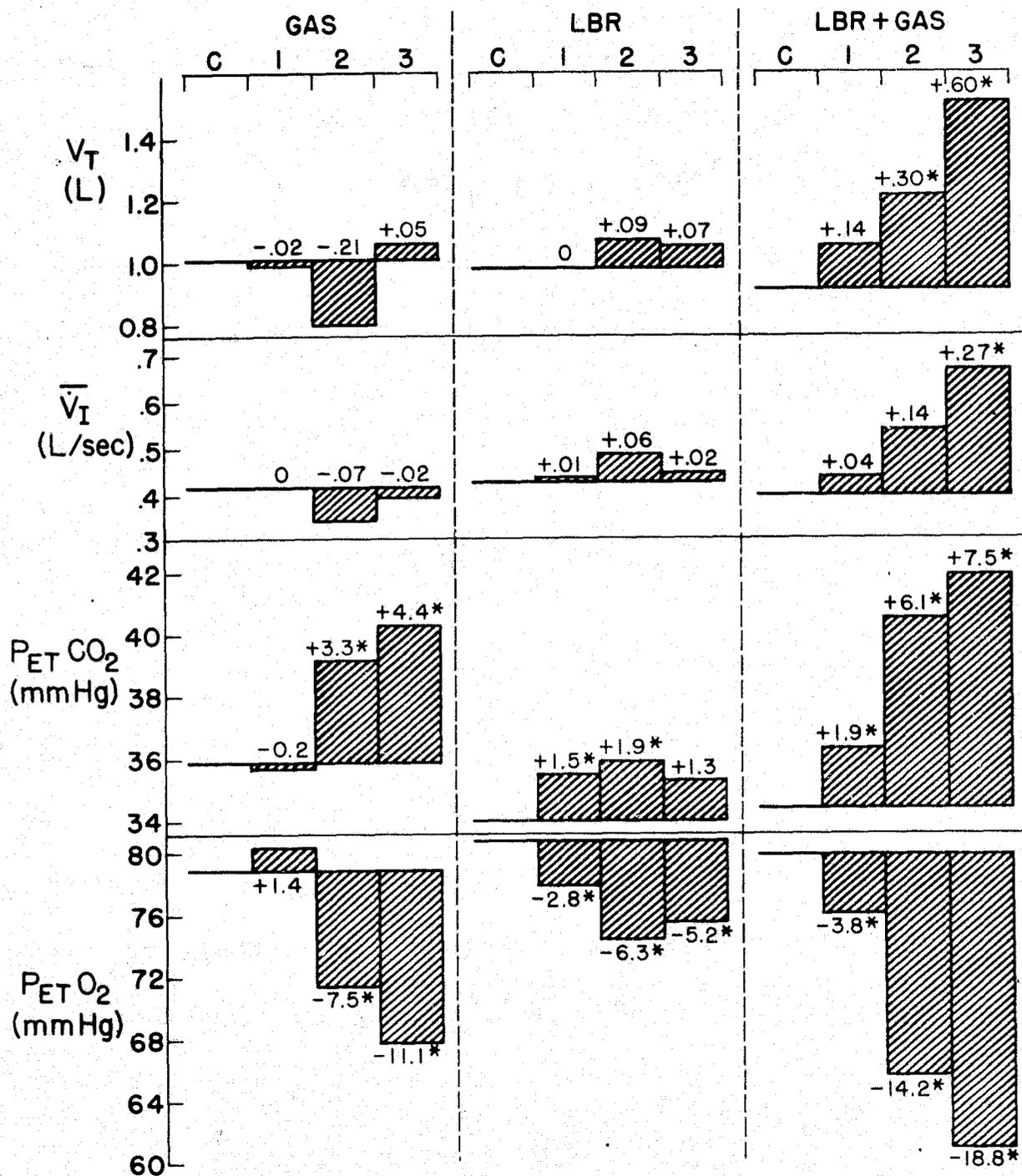
IV

CARDIORESPIRATORY RESPONSES TO ARM EXERCISE

WITH AND WITHOUT LOWER BODY NEGATIVE PRESSURE

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ABSTRACT

The reciprocal cardiorespiratory effects of arm exercise and lower body negative pressure (LBNP) were studied in 6 subjects by exposing them to LBNP for 10 min at -40 Torr (L), arm cranking for 8 min at a work load of 225 kpm/min (E) and the two combined (L+E) with E beginning 2 min after L. The early responses of ventilation ($\dot{V}_I$) and $\dot{V}_{O_2}$ were curtailed and heart rate after the first min was significantly higher for L+E than E, reflecting the less accessible venous reservoir and reduced stroke volume due to L. Leg volume was significantly reduced after 30 sec of E then continued to decline and remained below baseline during 6 min of recovery. With L+E leg volume remained constant after E began, probably indicating both a shift of blood from legs to arms in spite of L and reduced extravasation with LBNP. End-tidal P_{O_2}, $\dot{V}_I$ and $\dot{V}_I/\dot{V}_{O_2}$ tended to be higher and P_{CO_2} lower during the latter stages of L+E than during E, indicating less effective lung perfusion and greater alveolar deadspace when L was superimposed on E. During recovery from L+E the release of pooled blood from the lower body caused a greater $\dot{V}_I$, $\dot{V}_{O_2}$ and lower R_E than after E and produced a marked rise in P_{CO_2} and fall in P_{O_2}, thereby slowing the recovery of gas exchange.

INTRODUCTION

Previous investigations have shown marked differences in the response

of man to exericse when gravitational stress was altered by postural

changes (2) or acceleration (14). Studies of this type have documented

the apparent reduction in blood perfusing the lungs and muscles with

increased stress which impinges on the normal efficiency of ventilation

and tissue oxygenation. The shift of blood away from the central volume

results in a smaller venous return and lower stroke volume while heart

rate increases in an attempt to maintain a sufficient cardiac output.

At least two studies have been undertaken to study the influence

of gravitational stress induced by changing posture on the response to

arm exercise. In these studies the passive shift of blood to the legs

while upright, away from the exercising muscles, altered the cardio-

respiratory responses to the exercise. Stenberg et al. (15) noted

a 22% fall in cardiac output and stroke volume with little change in

heart rate in 10 subjects exercising in the upright sitting posture

compared to supine at a work load requiring an oxygen consumption of

1.1 liters/min. Ventilation and mean arterial blood pressure were

slightly, but not significantly lower while upright. Bevegård et al.

(1) noted the same fall in cardiac output in 6 subjects, but documented

a larger reduction in stroke volume (31%) and a 17% increase in heart

rate when 6 subjects exercised during orthostasis (sitting upright)

at the same workload of 1.1 liters/min. They also reported a 20%

increase in ventilation and lactic acid production while arterial

pressure remained about the same as in the supine posture. The more marked effects of orthostasis in the latter study can be attributed to the fact that the subjects had their legs freely suspended while sitting, whereas in the former (15) the subjects' legs were partially supported. This support would reduce the amount of blood removed from the central circulation by orthostasis.

Apparently no studies to date have investigated the effects of lower body negative pressure (LBNP) on the exercise response to upper body exercise. The combination of LBNP and exercise offers a unique situation in which the effect of one on the response to the other can be studied with the subject remaining in the same posture so as not to produce measurement artifacts. The general purpose of this study was to compare the extent and time course of some commonly measured cardio-respiratory responses to arm exercise in the same subjects with and without the superimposition of the additional circulatory stress of LBNP. By measuring the same variables during LBNP alone it was also possible to determine the influence of arm exercise on the LBNP responses.

METHODS

Six male subjects took part in the study. Their mean age, height, weight and $\dot{V}_{O2max}$ (with standard deviations) was 30 yr (± 7), 180 cm (± 6), 70.3 kg (± 6.4) and 46 ml/kg·min (± 11), respectively. The $\dot{V}_{O2max}$ was determined with a progressive test in the sitting position with leg exercise performed on a bicycle ergometer (10). Each of the subjects performed the three parts of the experiment in one session with 20 min of recovery allowed between each procedure. The sequence of the procedures was varied for each subject and the subjects were assigned to each sequence in random fashion.

The experiments were performed in Albuquerque at an elevation of 5,400 ft (P_B = 630 mmHg) and an ambient temperature of 24°C.

Ventilation ($\dot{V}_I$), oxygen consumption at the mouth ($\dot{V}_{O2}$) and the respiratory exchange ratio (R_E) were obtained from calculations based on mixed-expired gas collections with Douglas bag and analyses by mass spectrometer (MEDSPECT-MS8) which was calibrated with gases analyzed by the Scholander technique. The ventilation equivalent for O_2 ($\dot{V}_I/\dot{V}_{O2}$) could thus be calculated. End-tidal O_2 and CO_2 ($P_{ET}O_2$ and $P_{ET}CO_2$) were obtained by averaging the end-tidal gas deflections for each breath during the bag collection intervals. These respiratory gases were sampled continuously by mass spectrometer from a capillary inserted into the Rudolph (Model 2600) respiratory valve. Respiratory frequency (f) was calculated from the end-tidal record. With these values it was possible to calculate tidal volume (V_T), alveolar ventilation ($\dot{V}_A$) and the ventilatory efficiency ($\dot{V}_A/\dot{V}_I$). Heart rate (HR) was

obtained from forehead and two chest leads. Changes in leg volume (LV) were estimated from a mercury-in-rubber strain gauge attached around the left calf (5, 9). The end-tidal gases, HR and LV output signals were recorded on a Honeywell (Model 1508A) Visicorder. The LBNP apparatus and procedure has been previously described (9). The work load for the arm cranking exercise was 225 kpm/min for each subject and was chosen as a result of preliminary trials in order to allow the subjects to work for 8 min without undue discomfort.

During LBNP only (L) baseline values were averaged over 3 min during the Douglas bag collection. The negative pressure was then applied to the lower body below the iliac crest and stabilized at -40 Torr in a few sec. The negative pressure was held at this level for 10 min with a 2-min gas collection during the first 2 min and the last 2 min. LBNP was then instantaneously released and 3 bags collected during 6 min of recovery. The successive bags were of 1-min, 2-min and 3-min duration. With LBNP and exercise (L+E) the same protocol was followed, except that the subject began arm cranking at 225 kpm/min on the Monarch ergometer at 50 cpm to a metronome after 2 min of LBNP. The ergometer was mounted above the subject so that the pedal sprocket was vertically above the shoulders with the arms nearly extended during the long stroke. Two 1-min gas collections were added to the L procedure, one during the first and one for the second min of exercise. After 8 min of exercise, LBNP and exercise were terminated simultaneously and recovery measurements made as during L. With exercise alone (E) the resting baseline measurements (3 min) were followed by another 2 min of inactivity. Exercise was then begun and continued for 8 min, bags being collected during each of the first two and for the last two min. Recovery bag collections were similar to those for L and L+E.

The O_2 debt for E and L+E was obtained by subtracting the baseline $\dot{V}_{O_2}$ from the total $\dot{V}_{O_2}$ measured during the 6-min recovery period. For L the $\dot{V}_{O_2}$ for the last 2-min collection during LBNP was subtracted from the total $\dot{V}_{O_2}$ measured during recovery. The O_2 deficit for L was obtained by assuming that the true metabolic rate remained at the baseline $\dot{V}_{O_2}$ value throughout the 10 min LBNP exposure. The $\dot{V}_{O_2}$ during the first and last 2-min bag collections and the mean of these 2 values (for 6 min) were each subtracted from the baseline $\dot{V}_{O_2}$ and the resulting sum was the O_2 deifict. In ml/min values it was computed as:

$$O_2 \text{ deficit in ml, (L)} = 10(\text{control}) - 5(\text{1st bag}) - 5(\text{last bag}) \quad (1)$$

During E and L+E it was assumed that the $\dot{V}_{O_2}$ measured during the last 2 min of exercise represented the true O_2 cost of work. The values for the first two 1-min gas collections were subtracted from this value as was the mean of the second and last bag (for 4 min). The simplified equation is shown below:

$$O_2 \text{ deficit in ml, (E and L+E)} = 4(\text{last bag}) - 3(\text{2nd bag}) - (\text{1st bag}) \quad (2)$$

Paired t-tests were used to analyze the data to determine statistically significant differences ($p < .05$) in the data between the various procedures or at different times during the same procedure.

RESULTS

All mean values for the ventilatory and HR responses have been summarized in Table 1.

The negative pressure resulted in a reduction in apparent $\dot{V}_{O2}$ during the first 2 min of 62 ml, seen in Table 1, for L and L+E. This alteration in $\dot{V}_{O2}$ was statistically significant ($p < .05$). During L the 15% depression of $\dot{V}_{O2}$ continued throughout the 10 min of exposure. After L there was a 112% increase in $\dot{V}_{O2}$ during the first min of recovery, followed by a return to the pre-LBNP baseline during the next 2 min. For E the $\dot{V}_{O2}$ during the last 2 min was 800 ml/min and the measurements during the first and second min with linear interpolation indicated that the ½ time for $\dot{V}_{O2}$ was approximately 76 sec. During recovery, $\dot{V}_{O2}$ had not completely returned to the control value within 6 min. With L+E the O_2 cost was about 7% higher (not statistically significant), however a time lag in the $\dot{V}_{O2}$ response to the work was evidenced by a 17% lower $\dot{V}_{O2}$ during the first min of exercise when LBNP was superimposed on the exercise with an estimated ½ time of 89 sec. For the first 3 min during recovery $\dot{V}_{O2}$ was significantly higher after L+E than after E.

The O_2 deficits for the 3 procedures calculated from the mean data in Table 1 with equation 1 and 2 were 375, 768 and 1010 ml for L, E and L+E respectively. The corresponding O_2 debts were 368, 644 and 1011 ml. For each procedure the O_2 debt was the same or smaller

than the estimated O_2 deficit, with no significant differences noted between the two. This indicates that there was no lactacid debt since for it to have existed the O_2 debt would have had to exceed the O_2 deficit. Furthermore, it is clear that the larger O_2 fluctuations during L+E than E were the result of imposing the O_2 deficit and O_2 debt due to L on the responses to E since the sum for the latter two procedures, 1143 ml for O_2 deficit and 1012 ml for O_2 debt, were nearly equal to the values for L+E.

The response of $\dot{V}_I$ to L was not notable except during the first min of recovery where 79% increase was noted, corresponding to the higher $\dot{V}_{O_2}$ (Table 1). The differences in $\dot{V}_I$ between L+E and E were again similar to those for $\dot{V}_{O_2}$, with it being lower during the first min and higher during the last 2 min with the combined stress, although neither difference was significant. During recovery $\dot{V}_I$ was significantly greater following the combined stress for the first 3 min.

Since the responses of $\dot{V}_{O_2}$ and $\dot{V}_I$ paralleled one another, there was no significant difference in $\dot{V}_I/\dot{V}_{O_2}$ between E and L+E. During the first min of recovery from L there was a 16% reduction in $\dot{V}_I/\dot{V}_{O_2}$ which was about the same as that noted after L+E, whereas after E there was virtually no change.

The differences in $\overline{R}_E$ between E and L+E paralleled those for $\dot{V}_I/\dot{V}_{O_2}$. No notable differences were seen during exercise, but in recovery $\overline{R}_E$ fell from 1.07 to 0.97 during the first min and was significantly lower than after E where there was no change upon terminating the exercise. $\overline{R}_E$ was also transiently reduced when LBNP was terminated.

The breathing patterns in terms of f and V_T did not show any remarkable change with L although both values peaked during the first

min of recovery. In the two exercise procedures both values were higher (not significantly) during the last 2 min of exercise with LBNP. During the first min of recovery, both f and V_T were higher after L+E but only the latter difference was significant.

The end-tidal gases did show some significant alterations with L as $P_{ET}CO_2$ was about 2 mmHg lower and $P_{ET}O_2$ about 6 mmHg higher compared with the control values. During recovery both gases overshot the baseline value during the first min. During the last two min of L+E the $P_{ET}CO_2$ was significantly lower and $P_{ET}O_2$ significantly higher than during E. During the first min of recovery after L+E, $P_{ET}CO_2$ increased but remained below baseline by 1 to 2 mmHg for 6 min, the opposite pattern from that of $P_{ET}O_2$.

The changes in $\dot{V}_A$ were similar to those noted earlier for $\dot{V}_I$, showing a nearly 100% increase during the first min after L. $\dot{V}_A$ tended to be lower during the first min and higher during the last 2 min of L+E than with E and was significantly greater during the first three min of recovery after L+E. The ventilatory efficiency $(\dot{V}_A/\dot{V}_I)$ did not show any dramatic alterations with L, remaining at a value of about 0.67. In comparison, the values during and after both exercise sessions were considerably higher (0.75). The only difference noted between E and L+E was during the first min of exericse where $\dot{V}_A/\dot{V}_I$ was significantly lower for L+E, but this is probably not a difference due to L since the corresponding baseline value was also significantly lower than it was prior to E.

The response of HR to the 3 procedures is shown in Table 1 as well as Fig 1. The mean HR increased 11 bpm after one min with L and remained at this higher level during the 10 min exposure. It

then returned promptly to baseline during the first min of recovery and fell below baseline during the next 5 min. During exercise, HR was higher throughout with L, the differences being significant after the first min (p<.01). The absolute and percentage difference in HR became greater as exercise progressed, being 8% during the first min and 23% during the last min. The effect on HR of L+E was more than the summed response to L and E applied individually. The potentiating effect amounted to 4 bpm during the second min and 10 bpm during the last min. The recovery values for HR were similar after both exercises, however the reudction during the first min was of greater magnitude after L+E (38%) compared to E (27%).

The LV responses to LBNP and the two exercises are shown in Fig 2. With the onset of L the LV rose asymptotically during the first min to a value of 1.6%. After this it continued upward at a lesser but near linear rate throughout LBNP, reaching a value of 2.6% at the end. With the termination of LBNP, LV declined rapidly in asymptotic fashion to 0.3% after 60 sec and remained near this value for the following 5 min. With the onset of exercise with no LBNP, LV was reduced below baseline within the first 30 sec (p<.01) and continued to decline during the duration of the exercise, showing a significant reduction of 0.6% at the end of 8 min. After exercise LV was reduced an additional 0.1% after 30 sec and then returned to and remained at the level recorded at the end of exercise. During the first 2 min of L+E when only LBNP was applied the rise in LV was identical to that for L. However with the onset of exercise any further rise in LV due to LBNP was prevented and there was no appreciable change in LV during exercise between the first and last min. During the last min of exercise LV was 0.7% less for L+E than E (p<.01). In the first min

of recovery the course of LV was very similar for L and L+E with the latter remaining below L by about the same amount as was seen during the last min of exercise. The LV after L+E fell below the baseline 25 sec after exercise, dropping to its lowest value after 1 min and 15 sec of recovery (-0.4%) and then began an upward trend back towards baseline. In summarizing the changes in LV in Fig 2 it is clear that arm exercise alone reduced LV and attenuated the rise in LV during LBNP.

DISCUSSION

The fluctuations in apparent $\dot{V}_{O2}$ resulting from alterations in gravitational stress such as postural changes or LBNP have been previously reported from this laboratory (6, 7, 9). The increased blood pooling in the lower extremities produces an apparent O_2 deficit in $\dot{V}_{O2}$ measured at the mouth since the O_2 demands of the lower body tissues are being met by the extraction of O_2 from the pooled capillary blood so that the venous O_2 content is reduced and CO_2 content is increased during the course of the exposure (13). With the release of LBNP, this hypercapnic and hypoxic blood is returned to the central circulation and the rise in $\dot{V}_{O2}$ (Table 1) is indicative of the increased demands on gas exchange to resaturate this blood as it courses through the lungs. This also results in a transient hyperpnea probably due to the stimulation of chemoreceptors.

It has been estimated previously (9) that LBNP for 10 min at -40 Torr results in the shift of 630 ml of blood from the intravascular compartment of the central circulation to the lower body, with an additional 270 ml loss of plasma to the tissues of the lower body by extravasation. When arm exercise was superimposed on LBNP it was noted in Fig 2 that any further increase in LV was arrested during the 8 min of exercise. Whether the constant LV during L+E was the result of a withdrawal of blood from the legs to the working muscle with a counterbalancing of plasma loss to the tissues as seen during L is

not discernible from the data during LBNP. However, the course of

LV after release of LBNP provides more information on this point.

With L alone, after the fast phase of recovery was over, a residual

LV of 0.3% above the baseline was seen due to edema from the previous

extravasation. In contrast, after L+E the LV dropped well below the

baseline within 60 sec of LBNP release, approaching the values after

exercise alone. This may mean either that there was less edema after

L+E or that there was less blood left in the legs due to the preceding

arm exercise, or both. However, the fact that LV after L+E remained

higher than after E alone suggests that some extravasation was present.

Certainly the major part of the intravascular blood shift had been

completed during the 2 min of LBNP preceding the exercise (4). Based

on the observations that the recovery curves for LV after E and L+E

were quite similar for the first 60 sec it would seem that the intra-

vascular blood volume shifted after exercise was also similar since

the two are closely related (9). Also speaking in favor of the

reduced intravascular plasma loss during L+E compared to L, was the

observation that the O_2 deficit and debt of L+E were very close to

the sums of those for L and E when performed individually. Both the

O_2 debt and deficit during LBNP have been related to the intravascular

volume shifts (9) and should be relatively free of plasma volume differences.

On the other hand LV was reduced during E and it is difficult to en-

vision a process that can draw fluid from the lower body tissues into

the vascular compartment 30 sec after arm exercise begins to result

in a net loss in LV. With these considerations it would appear that

both events may be of significance, i.e. arm exercise induces a mobilization

of blood from the vascular compartment of the legs to the working

muscles while some plasma loss due to LBNP continues. This seems reasonable since a comparable observation has been made previously wherein LV showed a striking increase when leg exercise was begun in the supine posture (8). This showed the rapid redistribution of blood to working muscles inspite of hydrostatic forces in the opposite direction.

Although none of the subjects felt that L+E was subjectively more difficulty than E, it is apparent from the difference in HR (Fig 1) that the pooling of blood prior to exercise and the conflicting demands for the blood volume (working tissue and pooling tendency) placed on the central circulation during exercise altered the cardiodynamic response. If one assumes that the change in cardiac output during arm exercise due to upright posture (-22%), reported in the two studies mentioned previously (1, 15) for subjects working at a slightly greater intensity than in the present study, apply to these subjects then the HR differences noted in Table 1 would translate to a 37% reduction in exercise stroke volume during LBNP. This would reflect the reduced venous return from the relatively inaccessible venous reservoir and results in the potentiation of the HR response. Since these earlier studies noted no significant change in mean arterial pressure during the two postures it would seem that the total peripheral vascular resistance was some 20 to 30% higher during L+E than during E only. We had earlier noted only a 5% reduction in mean pressure during LBNP of this intensity without exercise (9). The greater vascular resistance during L+E than E along with increased venous tone (3) probably prevented blood pooling and hence reduced the loss of plasma as proposed previously.

The influence of LBNP on the respiratory response to exercise
was notable (Table 1), but in most cases of borderline statistical
significance during exercise. The response of $\dot{V}_I$ to exercise was
slower with LBNP during the first min of work, although not significantly.
This is probably related to the attenuated rise in venous return
with superimposed LBNP because the blood volume in the lower body
was not as readily mobilized. The venous return has previously been
related to $\dot{V}_I$ at exercise onset (8, 16). Towards the end of exercise
both $\dot{V}_I$ and $\dot{V}_I/\dot{V}_{O2}$ tended to be higher with LBNP, probably reflecting
an increase in alveolar deadspace which can be inferred from the rela-
tively lower $P_{ET}CO_2$ (Table 1) during L+E than E. Two studies with leg
exercise during acceleration reported an increase in alveolar deadspace
at rest with an additional enlargement (14) and no further increase
(12) noted at 3G. However, for their calculations they assumed P_aCO_2
was equal to the alveolar P_{CO_2}, an assumption that has recently been
shown to be invalid and misleading (11). Since these acceleration studies
showed little change in P_aCO_2 during exercise with or without the addi-
tional stress, we may speculate that the smaller $P_{ET}CO_2$ during L+E
(Table 1) in indeed an indication of a 4 mmHg increase in the end-tidal
to arterial CO_2 gradient which in turn is proportional to the alveolar
deadspace. The fact that $\dot{V}_A/\dot{V}_I$ was nearly identical during exercise
with or without LBNP does not preclude a difference in alveolar dead-
space, it only indicates a relatively constant anatomical deadspace.
Larger alveolar deadspace values and greater $\dot{V}/\dot{Q}$ mismatching in the
lungs is usually reflected by a greater $\dot{V}_I$ and $\dot{V}_I/\dot{V}_{O2}$, especially during
LBNP (9) and in pulmonary patients with unequal ventilation and perfusion
(11).

During recovery from L+E the three variables pertaining to ventilation ($\dot{V}_I$, V_T, and $\dot{V}_A$) all showed a slower decline than after E. This demonstrates the overriding influence of the ventilatory stimulus arising from the influx of previously pooled blood, also evidenced by the greater fall in R_E and $P_{ET}O_2$ and larger increment in $P_{ET}CO_2$ after exercise with LBNP. The HR fell more rapidly after L+E, which may be indicative of a relatively greater sympathetic response during exercise with LBNP and, when this higher sympathetic tone was withdrawn by the vagal reaction initiated by the baroreceptors, the drop was more precipitous.

REFERENCES

1. Bevegård, S., U. Freyschuss and T. Strandell. Circulatory adaptation to arm and leg exercise in supine and sitting position. J. Appl. Physiol. 21:37-46, 1966.

2. Bevegård, S., A. Holmgren and B. Jonsson. The effect of body position on the circulation at rest and during exercise, with special reference to the influence on the stroke volume. Acta Physiol. Scand. 49:279-298, 1960.

3. Bevegård, B.S. and J.T. Shepherd. Changes in tone of limb veins during supine exercise. J. Appl. Physiol. 20:1-8, 1965.

4. Foux, A., R. Seliktar and A. Valero. Effects of lower body negative pressure (LBNP) on the distribution of body fluids. J. Appl. Physiol. 41:719-726, 1976.

5. Holling, H.E., H.C. Boland and E. Russ. Investigation of arterial obstruction using a mercury-in-rubber strain gauge. Am. Heart J. 62:194-205, 1961.

6. Loeppky, J.A. Cardiorespiratory responses to orthostasis and the effects of propranolol. Aviat. Space Environ. Med. 46:1164-1169, 1975.

7. Loeppky, J.A. and U.C. Luft. Fluctuations in O_2 stores and gas exchange with passive changes in posture. J. Appl. Physiol. 39:47-53, 1975.

8. Loeppky, J.A., M.D. Venters and U.C. Luft. Gravitational effects on blood distribution, ventilation, and gas exchange at the onset and termination of exercise. In: <u>Environmental Stress: Individual Human Adaptations</u>, edited by L.J. Folinsbee et al. New York: Academic Press, 1978, p. 225-245.

9. Loeppky, J.A., M.D. Venters and U.C. Luft. Blood volume and cardiorespiratory responses to lower body negative pressure. <u>Aviat. Space Environ. Med.</u> 49:1297-1307, 1978.

10. Luft, U.C., D. Cardus, T.P.K. Lim, E.C. Anderson and J.L. Howarth. Physical performance in relation to body size and composition. <u>Annals of New York Academy of Sciences.</u> 110:795-808, 1963.

11. Luft, U.C., J.A. Loeppky and E.M. Mosytn. Mean alveolar gases and alveolar-arterial gradients in pulmonary patients. Accepted for publication in <u>J. Appl. Physiol.</u>

12. Nunneley, S.A. Gas exchange in man during combined +Gz acceleration and exercise. <u>J. Appl. Physiol.</u> 40:491-495, 1976.

13. Reeves, J.T., R.F. Grover, S.G. Blount, Jr. and G.F. Filley. Cardiac output response to standing and treadmill walking. <u>J. Appl. Physiol.</u> 16:283-288, 1961.

14. Rosenhamer, G. Influence of increased gravitational stress on the adaptation of cardiovascular and pulmonary function to exercise. <u>Acta Physiol. Scand.</u> 68(Suppl. 276):1-61, 1967.

15. Stenberg, J., P. Åstrand, B. Ekblom, J. Royce and B. Saltin. Hemodynamic response to work with different muscle groups, sitting and supine. <u>J. Appl. Physiol.</u> 22:61-70, 1967.

16. Wasserman, K., B.J. Whipp and J. Castagna. Cardiodynamic hyperpnea: hyperpnea secondary to cardiac output increase. _J. Appl. Physiol._ 36:457-464, 1974.

TABLE 1

MEAN RESPIRATORY AND HEART RATE RESPONSES OF 6 SUBJECTS DURING AND AFTER LBNP (L), ARM EXERCISE (E) AND A COMBINATION OF THE TWO (L+E).

		REST	← LBNP →	← EXERCISE →			RECOVERY		
Time(min):		(0-3)	(4-5)	(6)	(7)	(12-13)	(1)	(2-3)	(4-6)
$\dot{V}_{O_2}$	L	229	194	--	--	189	400	212	226
(ml/min)	E	217	--	452	660	800	580	275	272
	L+E	215	188	373	683	858	788**	335**	281
$\dot{V}_I$	L	6.64	6.32	--	--	5.77	10.32	6.26	7.01
(L/min)	E	6.96	--	16.15	19.86	25.99	19.15	10.57	9.60
	L+E	6.16	6.58	11.99	19.63	31.68	24.99*	12.57*	10.31
$\dot{V}_I/\dot{V}_{O_2}$	L	29.0	32.3	--	--	30.8	25.9	29.7	30.9
	E	32.1	--	34.0	30.2	32.2	33.0	38.6	35.5
	L+E	28.7	34.9	32.3	28.9	36.9	31.6	38.0	36.8
R_E	L	0.78	0.87	--	--	0.80	0.74	0.78	0.81
	E	0.88	--	0.95	0.95	1.06	1.06	1.16	1.00
	L+E	0.76	0.89	0.87	0.91	1.07	0.97*	1.11	1.00
f/min	L	12.0	10.1	--	--	12.3	13.8	12.8	12.6
	E	11.3	--	12.9	15.5	20.9	16.7	13.1	12.0
	L+E	12.4	11.3	13.0	15.5	26.2	20.9	15.5	14.9
V_T	L	0.75	0.68	--	--	0.60	0.88	0.62	0.65
(L)	E	0.71	--	1.33	1.35	1.27	1.32	0.97	0.93
	L+E	0.66	0.66	1.03	1.33	1.31	1.49*	0.92	0.84
HR/min	L	65	76	--	--	79	65	62	60
	E	65	--	90	90	100	73	68	66
	L+E	65	73	97	108**	123**	76	70	66
$P_{ET}CO_2$	L	35.7	33.8	--	--	34.0	36.1	35.7	35.7
(mmHg)	E	35.7	--	34.4	35.8	35.2	35.7	35.0	34.7
	L+E	36.5	33.3	33.8	35.3	31.3*	35.2	34.5	34.3
$P_{ET}O_2$	L	77.7	84.3	--	--	81.0	74.8	78.0	78.3
(mmHg)	E	80.8	--	83.9	83.2	87.2	87.5	89.2	86.2
	L+E	77.5	86.2	84.1	83.3	92.1*	85.6	89.2	87.2
$\dot{V}_A$	L	4.36	4.36	--	--	3.88	7.11	4.07	4.46
(L/min)	E	4.67	--	11.96	15.43	20.98	14.65	7.88	6.91
	L+E	3.90	4.35	8.26	15.16	25.35	18.90*	9.25*	7.13
$\dot{V}_A/\dot{V}_I$	L	.66	.70	--	--	.67	.69	.65	.65
	E	.68	--	.73	.77	.81	.77	.74	.71
	L+E	.63**	.67	.69**	.77	.80	.76	.74	.69

Significance of difference between values for E and L+E noted by: *, $p < .05$ and **, $p < .01$.

FIGURE 1

Mean heart rate response of 6 subjects to LBNP
beginning at 3 min and arm exercise beginning
at 5 min and a combination of the two (LBNP +
EX). Values for LBNP + EX were significantly
higher than E (p<.01) after the first min.
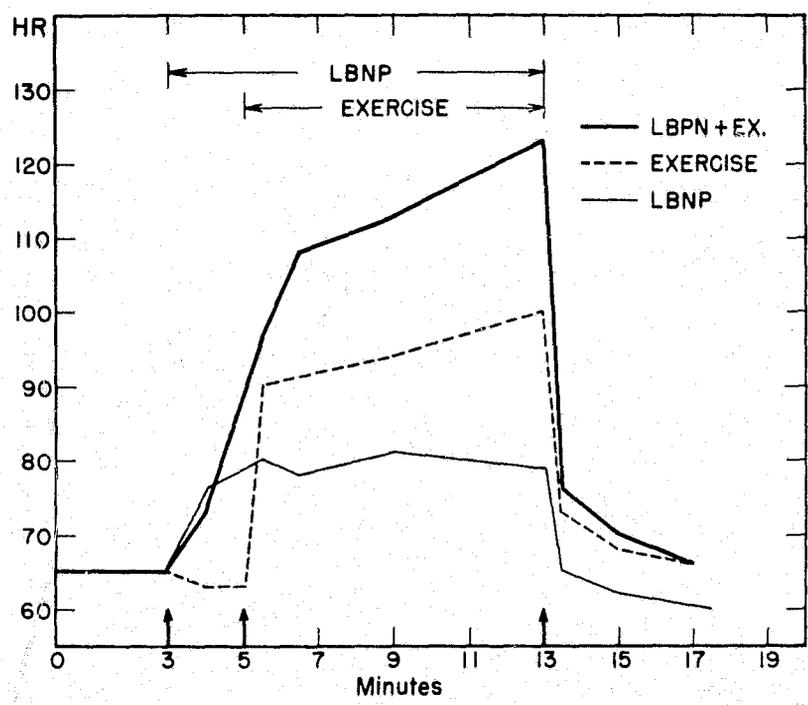
FIGURE 1

FIGURE 2

Mean leg volume response of 6 subjects to LBNP
beginning at 3 min and arm exercise beginning
at 5 min and a combination of the two (LBNP + EX).

FIGURE 2