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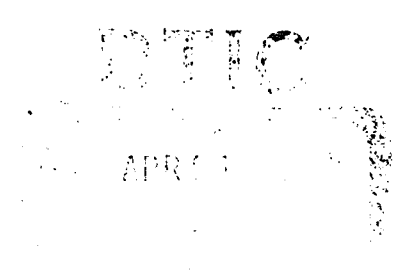
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**COLDEX-86: FLUID AND ELECTROLYTE CHANGES DURING  
PROLONGED COLD WATER IMMERSION**

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## **TECHNICAL REVIEW AND APPROVAL**

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The experiments reported herein were conducted according to the principles set forth in the current edition of the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

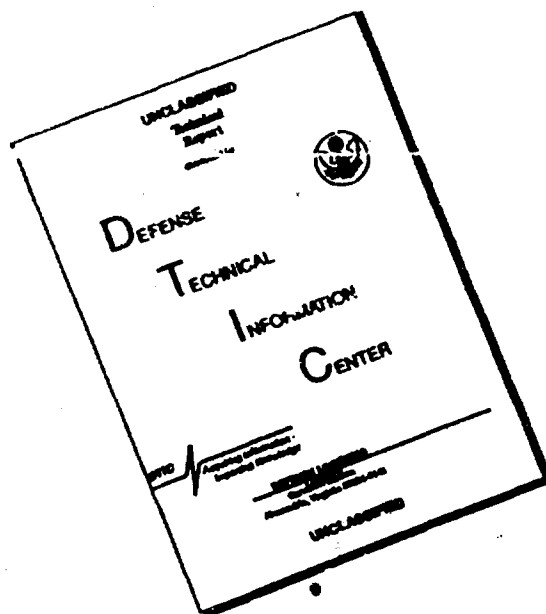
This technical report has been reviewed by the NMRI scientific and public affairs staff and is approved for publication. It is releasable to the National Technical Information Service where it will be available to the general public, including foreign nations.

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<p>Dehydration and hypothermia are major inhibitors of diver performance in cold water. To characterize the dehydration that accompanies cold water immersion, 16 U.S. Navy divers participated in two 5-day air saturation dives (ASD) at a depth of 6.1 meters sea water (msw). During each ASD, divers completed two immersions in 5 °C water: one began at 1000 h (AM) and the other at 2200 h (PM); a period of 54 h separated the immersions. Divers wore dry suits for thermal protection, and full face masks during immersions that lasted 3-6 h. All divers consumed identical diets. Blood samples were collected before and after each immersion, and urine was collected for 24 h in three separate periods on immersion days: for 12 h prior to immersion, during immersion, and after immersion until the end of the 24 h period. Plasma volume decreased significantly by approximately 17% during both AM and PM immersions. During the daytime, pre-immersion urine flow rate was 2.35 ± 0.27 ml/min; during immersion flow rate was 4.14 ± 0.45 ml/min. The nighttime pre-immersion urine flow rate was 1.14 ± 0.10 ml/min, while the nighttime immersion urine</p>			
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## INTRODUCTION

Body fluid and electrolyte shifts are consistent findings during head-out immersion (4,5,10-15,24), cold exposure (11,17,28), and under hyperbaric conditions (6,21,22,25). The most prominent manifestation of these shifts is an increase in urine flow, or diuresis. The diuresis induced by such conditions results in a reduction of total body water, primarily through a decrease in plasma volume (13). A state of dehydration ensues that precipitates a decrement in work performance and disturbances in thermoregulation (2,3,7). The inability to maintain core body temperature causes hypothermia, which continues to be a major limitation for divers in cold water (26). The triad of dehydration, hypothermia and loss of work capacity clearly serve to compromise the ability of U.S. Navy divers to successfully complete assigned tasks. Therefore, characterizing fluid and electrolyte shifts under conditions which simulate cold water diving missions are critical concerns of the U.S. Navy. Documenting the degree of dehydration under simulated diving conditions would permit future development of recommendations to counteract and/or prevent the detrimental consequences of mission-related physiologic changes during diving.

Although there is a body of data describing fluid/electrolyte shifts during headout immersion (4,5,10-15,24), saturation dives (6,21,22,25) and cold air exposure (11,17,28), there is a paucity of data describing the extent of such shifts during prolonged immersion in cold water for thermally protected subjects. Furthermore, there are several factors which affect an individual's responses to cold water immersion, and these factors require consideration for interpretation of results. For example, state of hydration prior to testing (14), physical conditioning level (4), and body position

during experiments (10) are all factors which can modify physiologic responses to immersion.

In addition to the factors mentioned above, there is also evidence that the ability to tolerate a cold environment is related to prior dietary intake (16,19,20). Cold studies have been conducted in the fasted state, and it has been clearly demonstrated that such conditions favor cold intolerance (19,20). It has been suggested that normal carbohydrate metabolism is necessary for maintenance of body temperature (20). In particular, the availability of glycogen reserves has been shown to improve cold tolerance, and levels of glycogen reserves may be essential determinants of the intensity of the hypothermic response to cold challenges (20). Hence, prior dietary intake of carbohydrates (CHO) may effect the extent of cold- and immersion-induced diuresis. Hydration status, dietary intake, and the aforementioned factors were not always controlled for or characterized during earlier experiments, and differences in methodologies render generalization of previous results to mission scenarios difficult.

The present study was conducted to determine the extent of fluid and electrolyte losses during prolonged cold water exposure at a depth of 6.1 msw under conditions in which hydration and conditioning level were similar in all subjects. Dietary intakes of all subjects were controlled to minimize dietary-induced differences. U.S. Navy divers were studied to answer the following questions: 1) Is diving in passive thermal protection suits associated with fluid and electrolyte shifts equivalent to shifts noted for thermally unprotected subjects? 2) Does the time of day (AM versus PM) affect fluid/electrolyte changes? 3) Does intake of a high CHO diet prior to exposure alter diving-induced changes as compared to a standard American mixed diet?

## MATERIALS AND METHODS

The present study was conducted in the Summer of 1986 at the Diving Medicine Department, Naval Medical Research Institute. Technical details of the project have been previously described (9). A brief summary follows. After giving informed consent 16 U.S. Navy divers each participated in two 5-day air saturation dives (ASD) in a hyperbaric chamber maintained at 6.1 msw (20 fsw). A team of 4 divers participated in each ASD. During one ASD series a high CHO diet was consumed, and during the other ASD series a standard mixed American diet was provided. A period of 9 days separated the two ASD series.

During each ASD series the 4 divers participated in two whole body immersion studies in water maintained at a temperature of  $5.0 \pm 0.1$  °C; 2 divers were immersed at the same time. The immersions, which were scheduled to last 6 h, were conducted while the divers were equipped with full face masks and dry suits for passive thermal protection (mean suit insulation = 1.4 clo). Immersions began at either 1000 hours (AM) or at 2200 hours (PM) with a period of 54 h separating the two immersions. The order of the AM and PM immersions was reversed for each of the two ASD series to minimize order effects. The ASD began at 0700 when the AM immersions were scheduled first, and at 1300 when the PM immersions were scheduled first. While in the chamber physical activity was restricted, and sleep hours of 2200 - 0600 were enforced except during the PM immersion. No sleep periods were instituted prior to the PM immersions.

During each immersion the divers pedalled on a bicycle ergometer at 50 revolutions per minute for 3 min at each of three workloads (50, 70, and 90 watts). One group (n=8) performed the exercise every hour during the immersion, and another group of 8 divers exercised only every 3 h, at the third and sixth hours of immersion. The immersions were scheduled to last

6 h, but immersions were terminated earlier if equipment malfunctioned, medical problems arose, core temperature dropped below 35 °C, or if the diver chose voluntary termination. Upon completion or termination of the immersion, divers were rewarmed passively until their core temperature returned to within 0.5 °C of baseline.

#### Dietary regimens and assessment.

Two different diets were served to the divers. For the first ASD series, a high carbohydrate diet (HCD) was provided, and during the second series a standard mixed American diet (MD) was consumed. Both diets provided approximately 3,000 calories per day, but the percent of calories derived from CHO and fat differed (Table 1). Despite attempts to construct diets providing similar amounts of essential nutrients, some differences between the two diets were noted (Table 2). Compared to the Recommended Dietary Allowances (RDA), the MD was low in zinc, copper, and vitamin B6, while the HCD was only slightly low in zinc. The MD was also higher in cholesterol as compared to the HCD.

Meals were provided 3 h before the start of immersions. On AM immersion days subjects undergoing the immersion were not served lunch, but dinners were augmented to provide 3,000 kcal. On PM immersion days three regularly spaced meals were provided. Subjects were allowed free access to water, non-caffeinated diet sodas, and decaffeinated tea and coffee. Ingestion of fluids was encouraged. After completing the immersion, 16 oz of warm apple or cranberry juice was provided during the rewarming period.

For 3 days prior to the ASD series, divers were instructed to follow a dietary regimen similar to the one they would be served in the chamber. Thorough instructions were given, and detailed 3-day dietary records were completed by each diver prior to entering the chamber. The dietary records

were used to document compliance with dietary recommendations. However, as indicated in Table 1, evaluations of dietary intakes over the weekend before the HCD diet indicated that their diet was not high in CHO. Because intakes over each weekend before the ASD were not significantly different, nutrient intakes from the diet records were pooled; they are referred to as control diets (CD).

#### Urine and blood collections.

Blood samples were collected on 2 different days prior to participating in the ASD. One sample was obtained at 0700 in the fasting state, and the second at 1600 on another day (4 h after lunch). These samples were considered control values and were also used to delineate differences due to diurnal rhythms. One 24 h control urine collection was also completed prior to the ASD series under free-living conditions.

Blood samples were obtained on several occasions during each ASD series: 1) in the fasting state at 0700 on day one of each ASD series prior to entering the hyperbaric chamber, 2) 30 min before each AM and PM immersion, 3) 15 min upon completion of each AM and PM immersion. Twenty-four hour urine collections were obtained for each immersion day in three separate collection periods. For the AM immersion, the first collection was begun at 2200 the evening before the immersion and continued until the immersion began at 1000 the next day. The second period was during the immersion. Details of this collection procedure have been previously described (9), but in brief, the divers wore condom catheters connected to urine collection bags. The third period began immediately at the end of immersion until 2200. In contrast, the first PM immersion collection period began at 1000 on the day of the immersion and continued until the exposure began at 2200. The second period was during

the immersion; the third period began at the end of the immersion and continued until 1000 the next day.

#### Sample processing and biochemical analyses.

Blood samples (25 ml) were drawn from an antecubital vein with minimum stasis. Each sample was divided into three tubes: 1) chilled tube containing lithium-heparin, 2) EDTA tube for hematocrit (Hct), hemoglobin (Hb), and complete blood counts, and 3) plain tube for serum osmolality (Osm), sodium (Na), potassium (K), chloride (Cl), inorganic phosphate (Pi), creatinine (Cr), total protein (TP) and albumin (ALB). The total urine volume (V) for each collection period was carefully recorded, and urine samples were analyzed for Cr, osmolality, Na, and K.

Serum and urine Osm was measured by the freezing point method. Urinary Na and K were measured with a Radiometer Na/K analyzer. Urinary Cr, and serum Na, K, Cl, Pi, Cr, TP and ALB were determined with a Technicon AutoAnalyzer by a clinical laboratory. Blood Hb was determined by the cyanomethemoglobin method (Coulter Hemoglobinometer) and Hct was determined by centrifugation. All samples were measured in triplicate, with coefficients of variation for all methods less than 5%.

#### Calculations.

Blood volume before the immersion (BV1) was estimated from height (in meters) and weight (in kg) as described by Allen et al. (1) according to the following equation:

$$BV1 \text{ (liters)} = 0.417 \cdot HT^3 + 0.045 \cdot WT - 0.03$$

Blood volume after the immersion (BV2) was estimated from pre- and post-immersion Hb (Hb1 and Hb2, respectively) and BV1 as described by Dill and Costill (8) using the formula below:

$$BV2 = BV1 \cdot Hb1/Hb2$$

Pre- and post-immersion plasma volumes (PV1 and PV2) were calculated from BV and Hct values as follows:

$$PV1 = BV1 \cdot (100 - Hct1)/100 \text{ and } PV2 = BV2 \cdot (100 - Hct2)/100$$

The percent change in plasma volume (%CPV) from pre- to post-immersion was calculated according to the following equation:

$$\%CPV = 100 \cdot (PV2 - PV1)/PV1$$

Total content of blood constituents was calculated from plasma volumes and serum concentrations of the specific constituents pre- and post-immersions.

The rate of urine flow (VU) in ml/min was calculated by dividing the total urine V collected for the designated period by the total time of the collection period. Glomerular filtration rate (GFR) was estimated by endogenous Cr clearance (CCr) according to the following equation:

$$CCr \text{ (ml/min)} = (\text{Urine Cr} \cdot \text{Urine V})/\text{Serum Cr}$$

Clearance (C) rates for other solutes in ml/min were calculated similarly for both pre- and post-immersion time periods. Free water clearance ( $CH_2O$ ) was calculated as:

$$CH_2O = CO_{sm} - VU$$

Total 24 h urinary excretion of electrolytes was calculated by summing the total amount excreted during each of the three collection periods.

#### Statistical analyses.

The computer package SAS was used for all statistical analyses (23). Data were analyzed as a 2 x 2 x 2 factorial with repeated measures by multivariate ANOVA. When significant main effects were detected, Duncan's comparison procedure was used to determine differences between treatment means. Data are expressed as mean  $\pm$  standard error (SEM) or  $\pm$  standard deviation (SD). Standard correlation and regression techniques were used to

identify associations among variables. The level of significance was set at 0.05.

## RESULTS

As presented in Table 3, the divers were lean and in above average physical condition. Of the 64 immersions, 27 lasted 6 h. Mean exposure times for all exposures were  $299 \pm 79$  minutes for AM and  $275 \pm 86$  for PM immersions. Seven immersions lasted less than 3 h and provided insufficient information for comparisons to prolonged immersions. Data from these shorter immersions were therefore excluded from the analyses presented herein. Thus, the data presented are only for the 57 immersions which lasted over 3 h. Mean exposure times for these immersions were  $317 \pm 12$  min for AM (n=29) and  $304 \pm 12$  min (n=28) for PM immersions. Rectal temperature decreased  $1.03 \pm 0.1$  °C and  $1.3 \pm 0.1$  °C during the AM and PM immersions, respectively, with a significantly greater drop observed during the PM ( $P < 0.01$ ). There were no significant differences in the decline in core temperature between the two exercise groups.

### Dietary effects.

Although on the weekend prior to the ASD series in which the HCD was provided the subjects did not consume a high carbohydrate diet, there were no differences between the first and second immersions. Thus, their failure to comply with dietary instructions did not alter the results. In general, analysis of the data for dietary effects yielded primarily negative results. The only statistically significant differences detected were in total urinary excretion of K. Urinary excretion of K over the entire 24 h collection periods was significantly higher on immersion days when the HCD was consumed ( $119.4 \pm 10.3$  mEq/day) as compared when the MD ( $92.5 \pm 6.2$  mEq/day) or CD ( $66.3 \pm 3.8$  mEq/day) was consumed. These differences could be attributed to

differences in dietary intake rather than immersion since there were significant differences in K intake among the diets (HCD: 180 mEq/day; MD: 136.4 mEq/day; BMD: 70.4 mEq/day and BHCD: 84.8 mEq/day). Because there were no differences in the response to immersion on the experimental diets, the results for the two diets were pooled.

#### Blood volume changes.

Post-immersion Hct and Hb increased significantly from pre-immersion values (Table 4) indicating a decrease in blood volume. Decreases in blood volume of  $11.7 \pm 0.9\%$  and  $10.3 \pm 0.9\%$  were noted for the AM and PM immersions, respectively. The change in blood volume was due almost entirely to a decrease in plasma volume, with decreases of  $17.3 \pm 1.1\%$  and  $16.9 \pm 1.3\%$  calculated for the AM and PM immersions, respectively. Estimated losses of plasma volume were  $564.1 \pm 38.6$  and  $567.2 \pm 47.9$  ml, for the AM and PM immersions, respectively. These losses were associated with immersion induced urine volume losses of  $478.4 \pm 197.2$  (AM) and  $521.8 \pm 113.1$  ml (PM). The correlation between loss of plasma volume and exposure time for all subjects was significant (Figure 1,  $r = 0.417$ ,  $p \leq 0.001$ ). Changes in plasma volume were not related to the drop in core temperature ( $r = -0.08$ ;  $P = 0.58$ ).

Although post-immersion red blood cell counts (RBC) were significantly higher than pre-immersion counts, this change was due to the decrease in plasma volume rather than a true increase in cell number. Post-immersion cell volumes were  $98.1 \pm 8.1\%$  and  $100.5 \pm 6.1\%$  of pre-immersion cell volumes for AM and PM immersions, respectively. No significant differences were detected for any of the hematologic data as a function of time of day.

#### Serum constituents.

Concentrations of constituents in serum pre- and post-immersion are presented in Table 5. There were no significant changes in the concentrations

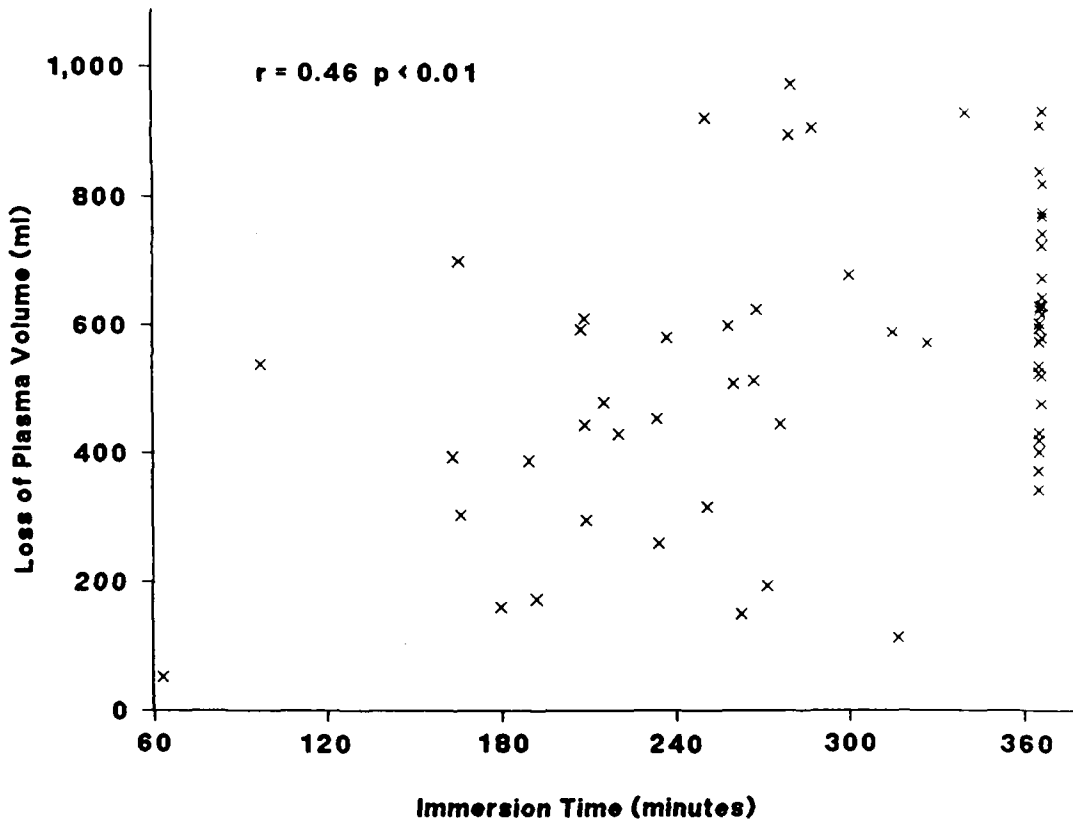


Figure 1. Relation between estimated loss of plasma volume and duration of immersion in cold water.

of serum Na, Cl or Osm, but a significant increase in Pi and decrease in K were noted. Also included in Table 5 are the concentrations one would expect to observe as a result of the decrease in plasma volume alone (expected concentration). The expected value is what the concentration in serum would have been if there had been no net gain or loss in total serum content of the specific constituent. Differences between the post-immersion concentration and the expected concentration indicate the net change in total serum content. While there was no significant net loss of Pi or protein (data not shown), a net loss of Na, K, and Cl was observed, the magnitudes of which were similar for AM and PM immersions. The average losses during each immersion were  $74.5 \pm 4.4$ ,  $60.6 \pm 3.5$  and  $3.0 \pm 0.2$  mEq, for Na, K, and Cl, respectively. Individual correlation coefficients for loss of serum Na, K and Cl content with loss of plasma volume were 0.95, 0.65, and 0.96, respectively (all  $P < 0.001$ ).

#### Urine volume and constituents.

Significant diurnal differences in urine V and VU were noted, with pre-immersion V and VU significantly greater during the PM collection period (1000 - 2200 hrs: V:  $1.63 \pm 0.2$  L; VU:  $2.35 \pm 0.28$  ml/min; mean  $\pm$  SEM) as compared to the AM collection period (2200 - 1000 hrs: V:  $0.820 \pm 0.07$  L; VU:  $1.14 \pm 0.10$  ml/min). Thus, pre-immersion urine flow was greater during the day. Similarly, V and VU during immersion were significantly higher during AM (V:  $1.26 \pm 0.13$  L; VU:  $4.12 \pm 0.43$  ml/min) as compared to during PM (V:  $0.928 \pm 0.135$  L; VU:  $2.96 \pm 0.36$  ml/min) immersions. Figure 2A compares VU over the 24 h collection period around AM and PM immersion. Flow rates during AM immersions were significantly greater than pre-immersion values for AM immersions only. AM immersion increased flow by  $3.21 \pm 0.47$  ml/min whereas PM immersion increased flow only  $0.55 \pm 0.48$  ml/min.

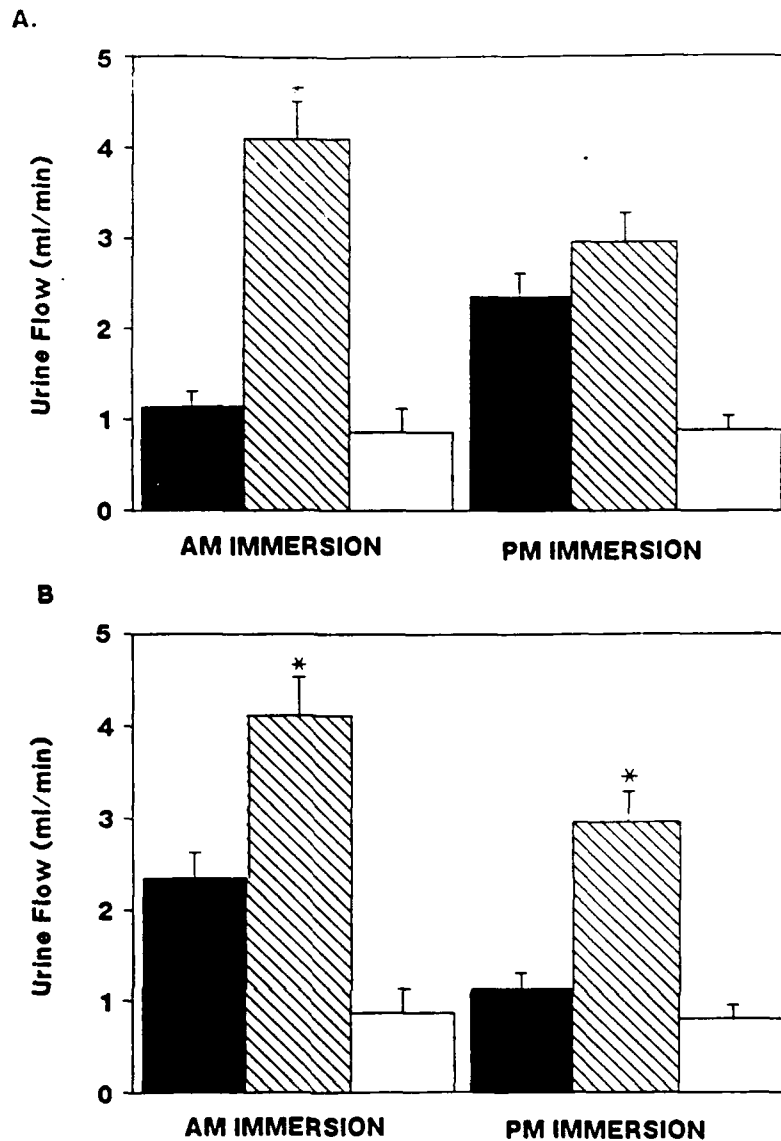


Figure 2. Rate of urine flow for AM and PM immersions days. Panel A: Before immersion collection periods (solid bars) were 2200 to 1000 hours for AM and from 1000 to 2200 hours for PM immersions. During immersion collection periods (hatched bars) were from start of immersion until the end of immersion, and after immersion collection periods (open bars) were from the end of immersion until 2200 for AM and until 1000 for PM immersions. Panel B: Before immersion collection periods (solid bars) were from 1000 to 2200 hours for AM and from 2200 to 1000 hours for PM immersions. During immersion (hatched bars) and after immersion (open bars) collection periods were as described in Panel A.

In contrast, if pre-immersion urine flow rates obtained during the daytime are compared to flow rates during daytime (AM) immersions ( $2.35 \pm 0.28$  vs  $4.12 \pm 0.43$  ml/min) and pre-immersion flow rates during the nighttime are compared to flow rates during nighttime (PM) immersions ( $1.14 \pm 0.10$  vs  $2.96 \pm 0.36$  ml/min), a different pattern emerged. Figure 2B illustrates that the magnitude of the increase from pre- to during immersion was similar for AM and PM, with increases of  $1.75 \pm 0.58$  and  $1.83 \pm 0.40$  ml/min, respectively. Urine flow decreased significantly after all immersions regardless of time of day, and in seven divers no urine was produced. Because VU during the day were higher than during the night, rates averaged over the entire 24 h period were significantly higher on PM immersion days as compared to AM immersion and non-diving control days. Flow rates over the 24 h periods averaged  $1.36 \pm 0.25$ ,  $1.64 \pm 0.10$ , and  $2.08 \pm 0.24$  ml/min for control, non-dive days and AM and PM immersion days, respectively. Similarly, mean total urine volumes over the 24 h periods were significantly higher around PM immersion days as compared to AM immersions and control, non-dive days (Figure 3). The correlation between loss of plasma volume and urine volume produced during the immersions was 0.408 ( $P < 0.01$ ).

Total urinary excretion of Na and K over the 24 h collection periods is presented in Figure 4. No significant differences in Na excretion were noted for control, non-dive days and immersion days. In contrast, total urinary K excretion was significantly greater on immersion days as compared to control, non-dive days. Urinary excretion rates for Na and K before, during and after the immersions are presented in Figure 5A and B. Excretion of Na increased significantly during both AM and PM immersions, and no significant diurnal differences were noted. Similar rates of excretion were noted during both AM and PM immersions. Diurnal differences in pre-immersion excretion were noted

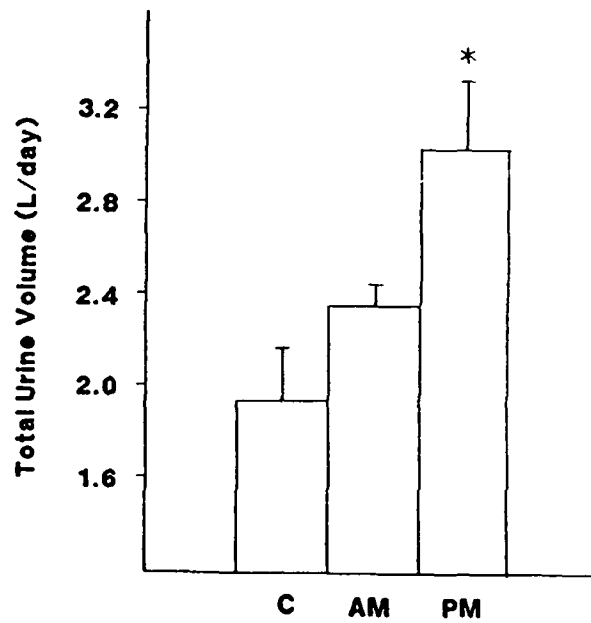


Figure 3. Total volume of urine collected over a 24 h period for control non-dive days (C), and for AM and PM immersion days.

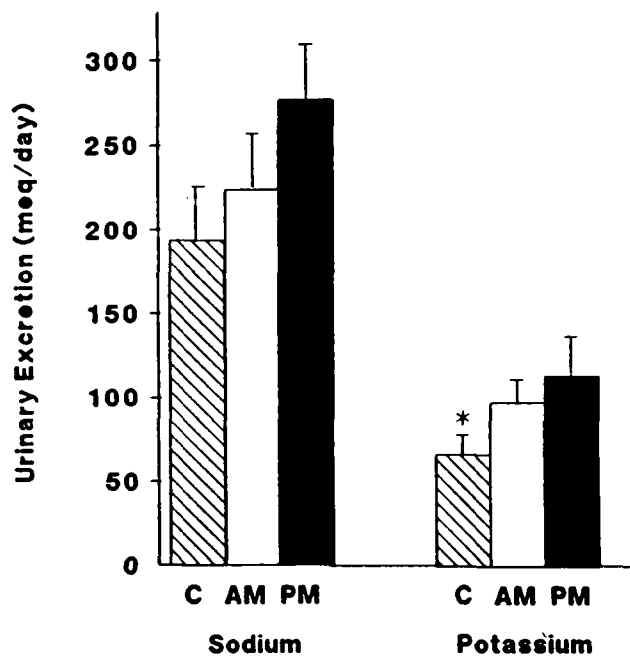


Figure 4. Total 24 h excretion of sodium and potassium on control, non-dive days (C - hatched bars), and on AM (open bars) and PM (solid bars) immersion days.

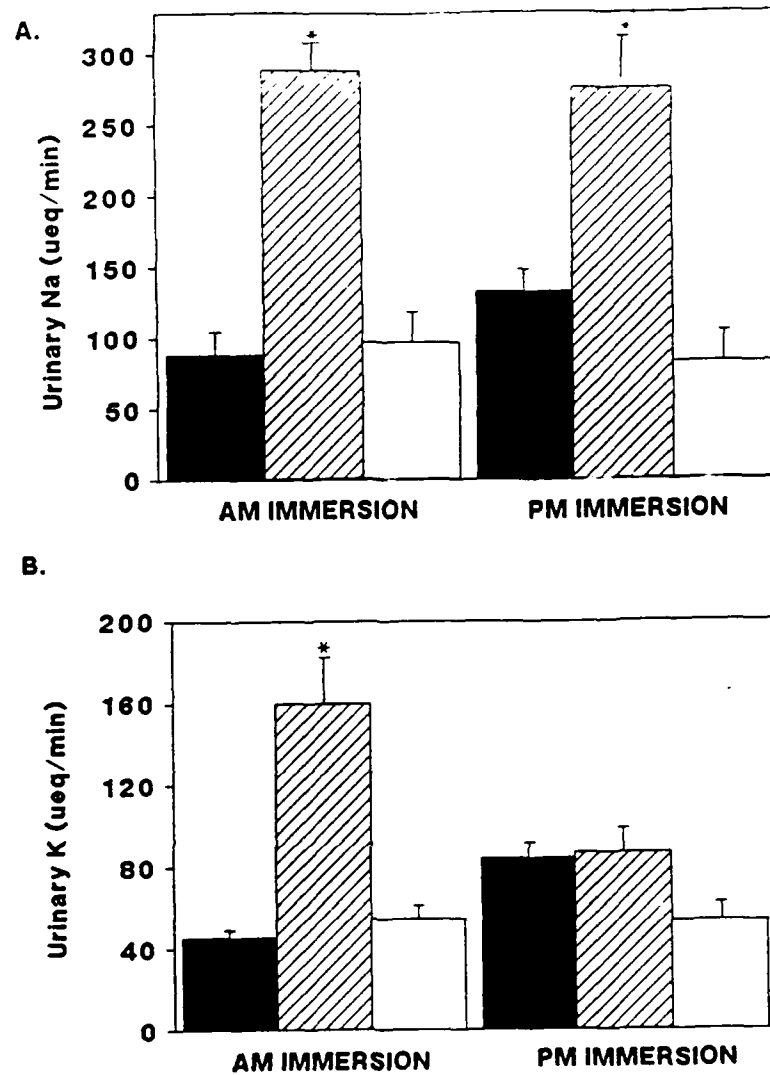


Figure 5. Urinary excretion rates for sodium (Panel A) and potassium (Panel B) before, during, and after AM and PM immersions. See legend for Figure 1 Panel A for details describing periods of collection for urinary flow rates.

for K, with excretion greater during the day than during the night. In addition, excretion of K was significantly higher during AM than PM immersions.

Selected clearance rates and fractional excretion of Na and K are presented in Table 6. While both  $C_{Cr}$  and  $C_{H_2O}$  increased during immersion, these values were not significantly different from pre-immersion values due to the large variability. Thus, there was no significant immersion-induced increase in GFR or free water excretion. During immersion  $C_{osm}$  increased significantly for AM, but not PM exposures. For Na and K, greater increases occurred during AM immersions as compared to PM immersions. However, differences in AM and PM immersions were partially due to differences between pre-immersion clearance rates. When pre-immersion clearance rates were compared to during immersion clearance rates obtained during the same time periods, the magnitude of change from pre-immersion to during immersion was similar for both AM and PM immersions. Diurnal differences were also noted for clearance of K, with pre-immersion clearance rates significantly lower during the night ( $9.71 \pm 0.96$  ml/min) as compared to during the day ( $18.95 \pm 1.73$  ml/min). Similarly, immersion clearance rates were significantly attenuated during PM immersions. Finally, when clearance rates for Na and K were expressed as ratios to creatinine clearance to estimate fractional excretion of the ions, differences between pre- and during immersion were not statistically significant (Table 6).

#### DISCUSSION

As early as 1945 it was suggested that dehydration may accompany exposure of man to cold (11). When man is exposed to cold air, a diuresis commences within the first 30 min; this diuresis is associated with an increase in solute excretion and loss of plasma volume (11). In particular, there are

reported increases in the urinary excretion of Na, K, Cl and Pi (11). A similar diuresis accompanies head-out immersion in thermoneutral water (10). The results of the present study provide the evidence that when men in thermal protective gear undergo whole body immersion in cold water for extended periods, there is a diuresis, natriuresis, kaliuresis, and significant decrease in plasma volume. Further, there are diurnal variations in the responses.

Fluid exchange and fluid balance during head-out immersion have been studied in detail; a profound diuresis, natriuresis, and kaliuresis are common observations (4,10,13). Urine flow rates have been shown to increase from pre-immersion values of approximately 0.5 ml/min up to 6 ml/min during head-out immersions lasting 4-8 h in thermoneutral water (13). An increase in the urinary excretion of Na and K accompanies the increase in urine flow, with changes from pre-immersion values of around 30 and 80 mEq/min for K and Na, respectively, to values of over 60 and 300 mEq/min during immersions lasting 4-8 h (13).

Despite differences in the experimental designs and treatments, these literature values are in line with those from the present study where pre-immersion excretion of K and Na was  $85.1 \pm 7.6$  and  $132.9 \pm 11.8$  mEq/min, respectively. During immersions lasting 3-6 h, excretion rose to  $159.8 \pm 19.6$  and  $288.4 \pm 6.1$  mEq/min. The similarity between reported literature values and the results of this study suggest that the immersion rather than the cold may be the primary stimulus for the observed fluid/electrolyte shifts during immersion in cold water. However, further studies are required to confirm this possibility.

Differences between responses to head-out immersion conducted during the day and the night have been compared previously. Krishna and Danovitch (15), and Shiraki et al. (24) noted that the rate of urine flow during nighttime

immersion was significantly lower than flow during daytime immersion, and urinary excretion of K and Na was blunted at night. The results of the present study with whole body immersion in cold water are similar to their results, and support their findings of diurnal differences in urine flow and K excretion. However, no attenuation in Na excretion during the PM immersions was noted in the present study. Despite an attenuation in the excretion of K during PM immersions, total urinary K excretion over the entire 24 h collection period was increased on immersion days as compared to control days.

Data on total urinary excretion of solutes are limited, but Neuman et al. (22) reported that daily excretion of Na was unchanged while K excretion was significantly elevated during open-sea saturation diving at 850 fsw. This finding is consistent with the present study. Thus, there may be a kaliuresis associated with living at increased depths. Alternatively, it is possible that the greater excretion on immersion days was due to the higher intake of K while in the chamber. Neuman et al. did not report dietary K intake, but intake of K during the present study supports this possibility. However, because the control, non-dive day 24 h urine collections were not conducted on the same day they recorded their dietary intakes, this possibility cannot be confirmed. The results of the present study indicate that K excretion should be monitored on several non-immersion and immersion days under constant dietary intake of K to determine whether immersion has a significant impact on the magnitude of the total urinary K loss.

Changes in plasma volume have also been documented during head-out immersion, but the extent of the change is controversial (4,10,12-14,27). In this study, there was a marked decline in plasma volume regardless of time of day, with losses of around 17% noted. Some investigators (13) have reported declines of up to 15% for head-out immersion, whereas others have reported no

change (14). Differences between studies may be due to variables such as body position, Na intake and hydration status. For example, when subjects are dehydrated prior to immersion, the diuresis and loss of plasma volume appear to be attenuated, or even abolished, while the natriuresis is maintained (14,27).

In the present study, the loss of plasma volume was related to the magnitude of the immersion-induced diuresis, but urinary loss could not account for all of the volume lost. Other potential routes of fluid loss include respiratory water loss and sweat loss. There may have been sweat losses when the divers put on their thermal protection attire, which took place after the blood had been drawn. Alternatively, there may have been an increase in the intracellular fluid during immersion. Such a phenomenon has been reported during exercise (18) and cold air exposure (17). Finally, the assumptions on which the formulas for estimating change in plasma volume were based may not be valid for immersion in cold water. Further study would be required to make this determination.

Although water and electrolyte exchange during exposure to cold air has been extensively studied, limited attention has focused on fluid/electrolyte shifts during cold water immersion for prolonged periods. Young et al. (28) recently compared 90 minutes of exposure to cold air ( $T_{\text{ambient}} = 5^{\circ}\text{C}$ ,  $\text{rh} = 30\%$ ) with head-out immersion in cold water ( $T_{\text{water}} = 18^{\circ}\text{C}$ ), with a greater diuresis noted during cold water immersion. The increase in urinary flow rate above pre-immersion values during their cold water immersion study was similar to values found in the present study. These investigators also monitored Na and K excretion during cold water immersion, and found that urinary Na increased from 90 to 280 mEq/min, and urinary K from 40 to 130 mEq/min. These values are also similar to those noted for AM immersion values in the present

study. The rates of increase for Na and K clearance on AM immersion days for the present study were also comparable to their values. Furthermore, Young et al. (28) noted a 16.6% loss in plasma volume, similar to our value of 17.1%.

Although the findings of Young et al. (28) were similar to those of this study were differences in the experimental conditions. The water temperature in their study was only 18 °C and immersion time was only 90 min as compared to 5 °C water and immersions over 3 h in duration for the present study. Further, their subjects were wearing only bathing suits, whereas subjects in this study were wearing thermal protection garments. Under the experimental conditions of their study, a 0.79 °C decline in rectal temperature was noted in the subjects over a 90 min period (0.53 °C/hr), as compared to a mean drop of  $1.17 \pm 0.07$  °C for our subjects over a 310 min period ( $0.23 \pm 0.02$  °C/hr). Thus, while immersion per se caused the same degree of fluid and solute loss, the rate of change in core temperature may be important in regulating the time over which such changes occur. This concept is supported by earlier work wherein the diuresis induced by exposure to cold air occurred more rapidly as the temperature was lowered (17). However, the drop in core temperature observed in the present study was not related to any of the observed changes in Na, K, or loss of plasma volume. Further investigation is needed to precisely document interactions between the magnitude of fluid and electrolyte changes with water temperature, thermal protection, and the rate of change in body heat content.

Although no differences were noted between the HCD and MD, these findings do not negate potential advantages for feeding carbohydrate diets. Previous research strongly suggests that the availability of glycogen stores is requisite to the maintenance of core temperature via shivering thermogenesis

(16,19,20). Theoretically, glycogen stores should have been greater during the HCD than during the MD. However, explanations can be offered as to why the two diets did not produce different results in our study. First, the divers were wearing thermal protective attire and rapid drops in core temperature were not seen. Second, there was infrequent shivering, which is known to depend on glycogen. Third, the thermogenic effect of periodic, light exercise might have lessened the need to shiver. Finally, it is unlikely that the intensity of the exercise performed during the immersions would have depleted glycogen stores with either diet. This is supported by the finding that peak  $O_2$  consumption during the exercise was, at most, 70% of maximal capacity. Further, post-immersion lactates were only  $2.13 \pm 0.21$  and  $1.80 \pm 0.18$  mM as compared to pre-immersion values of  $1.39 \pm 0.05$  and  $1.28 \pm 0.07$  mM, for AM and PM immersions, respectively. Thus, the conditions of the experiment were such that glycogen stores were probably adequate, and no specific dietary effects would be observed. Whether an HCD would be protective in the absence of thermal protection attire, in the presence of more rapid cooling, or with more vigorous exercise will require further investigation.

The results of this study indicate that plasma volume decreases and electrolyte excretion increases during prolonged underwater immersion in cold water despite thermal protection. The apparent extent of dehydration and loss of Na is similar during AM and PM immersions, whereas there are AM and PM differences in urine flow and excretion of K. Total urinary volume losses over the 24 h periods were significantly higher when immersions were conducted during the night. Although this particular aspect might be controlled if sleep patterns were modified, it is unlikely that such schedules would be feasible during missions. Thus, it appears that emphasis should focus on

electrolyte and hydration status both before and after immersions to protect divers from the potentially detrimental consequences associated with dehydration and hypothermia. In addition, although a nocturnal diuresis has been reported at great depths (25), which might impose additional complications during extended cold water immersion, this phenomenon was not observed at 6.1 msw. Finally, fluid and electrolyte replacement during immersion needs to be examined to determine whether the detrimental effects of dehydration can be avoided.

## REFERENCES

1. Allen, TH, MT Peno, KP, Chen, TF Huang, C Chang, and HS Fang. Prediction of blood volume and adiposity in man from body weight and cube of height. *Metab Clin Exper* 1956; 5:328-343.
2. Bergh, U and B Ekblom. Influence of muscle temperature on maximal muscle strength and power output in human skeletal muscles. *Acta Physiol Scand* 1979; 107:33-37.
3. Biersner, RJ. Motor and cognitive effects of cold water immersion under hyperbaric conditions. *Human Factors* 1976; 18:299-304.
4. Boening, D, HV Ulmer, U Meier, W Skipka, and J. Stegemann. Effects of a multi-hour immersion on trained and untrained subjects: I. Renal function and plasma volume. *Aerospace Med* 1972; 43:300-305.
5. Boening, D, HV Ulmer, U Meier, and J. Stegemann. Effects of a multi-hour immersion on trained and untrained subjects: II. Blood protein and electrolyte concentrations. *Aerospace Med* 1972; 43:415-518.
6. Claybaugh, JR, SK Hong, N Matsui, H Nakayama, YS Park, M Matsuda. Responses of salt and water regulating hormones during a saturation dive to 31 ATA (SEADRAGON IV). *Undersea Biomed Res* 1984; 11:65-80.
7. Coppin, EG, SD Livingstone, and LA Kuehn. Effects on handgrip strength due to arm immersion in a 10 °C water bath. *Avait Space Environ Med* 1978; 49:1322-1326.
8. Dill, DB and DL Costill. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol* 1974; 37:247-248.
9. Doubt, TJ, PA Duester, AJ Dutka, DJ Smith, RP Weinberg, and ET Flynn. Technical aspects of 6 hour cold water dives. *NMRI Technical Report Protocol 86-02*.

10. Epstein, M. Renal effects of head-out water immersion in man: Implications for an understanding of volume homeostasis. *Physiol Rev* 1978; 58:529-581.
11. Fregly, MJ. Water and electrolyte exchange during exposure to cold. *Pharmac Ther* 1982; 18:199-231.
12. Greenleaf, JE, E Shvartz, S Kravik, and LC Keil. Fluid shifts and endocrine responses during chair rest and water immersion in man. *J Appl Physiol* 1980; 48:79-88.
13. Greenleaf, JE, E Shvartz, and C Keil. Hemodilution, vasopressin suppression, and diuresis during water immersion in man. *Aviat Space Environ Med* 1981; 52:329-336.
14. Harrison, MH, LC Keil, CA Wade, JE Silver, G Geelen, and JE Greenleaf. Effect of hydration on plasma volume and endocrine responses to water immersion. *J Appl Physiol* 1986; 61:1410-1417.
15. Krishna, GG and GM Danovitch. Renal response to central volume expansion in humans is attenuated at night. *Am J Physiol* 1983; 244:R481-R486.
16. Jacobs, I. Muscle glycogen depletion during exercise at 9 °C and 21 °C. *Eur J Appl Physiol* 1985; 54:35-39.
17. Lennquist, S. Cold-induced diuresis. *Scand J Urol Nephrol Suppl* 1972; 9:1-46.
18. Lundvall, JS, H Mallander, H Westling, and T White. Fluid transfer between blood and tissues during exercise. *Acta Physiol Scand* 1972; 85:258-269.
19. MacDonald, IA, T Bennett, R Sainsbury. The effect of a 48 h fast on the thermoregulatory responses to graded cooling in man. *Clinical Science* 1984; 67:445-452.

20. Mager, M and R Francesconi. The relationship of glucose metabolism to hypothermia. In: The nature and treatment of hypothermia, Pozos RS and LE Wittmers Jr (eds). Minneapolis: University of Minnesota Pres, 1983; pp 100-120.
21. Nakayama, H, SK Hong, JR Claybaugh, N Matsui, YS Park, Y Ohta, K Shiraki, and M Matsuda. Energy and body fluid balance during a 14-day dry saturation dive at 31 ATA (SEADRAGON IV). In: Underwater physiology VII, Proceedings of the seventh symposium on underater physiology, Bachrach AJ and MM Matzen (eds). Bethesda: Undersea Medical Society, 1981; pp 541-554.
22. Neuman, TS, RF Gpad, D Hall, RM Smith JR Claybaugh, and SK Hong. Urinary excretion of water and electrolytes during open-sea saturation diving to 850 fsw. Undersea Biomed Res 1979; 6:291-302.
23. SAS Institute Inc. SAS user's guide: Statistics, 1982 Edition, Carey, NC SAS Institute Inc.
24. Shiraki, K, N Konda, S Sagawa, JR Claybaugh, and SK Hong. Cardiorenal-endocrine responses to head-out immersion at night. J Appl Physiol 1986; 60:176-183.
25. Shiraki, K, S Sagawa, N Konda, H Nakayama, and M Matsuda. Hyperbaric diuresis at a thermoneutral 31 ATA HeO<sub>2</sub> environment. Undersea Biomedical Research 1984; 11:341-353.
26. Taylor, RF and DW Yesair. Diver hypothermia: Current status and future control. Sea Technology 1982; 23:26-31.
27. Von Amelin, H, M Lanioado, L Rocker, and KA Kirsch. Effects of dehydration on the vasopressin response to immersion. J Appl Physiol 1985; 58:114-120.

28. Young, AJ, SR Muza, MN Sawka, and KB Pandolf. Human vascular fluid responses to cold stress are not altered by cold acclimation. Undersea Biomed Res 1987; 14:55-62.

Table 1. Comparison of calories and percent of total calories derived from protein, fat and carbohydrate for divers during the ASD for the high carbohydrate diet (HCD) and the standard American mixed diet (MD), and under free-living conditions before the MD (BMD) and the HCD (BHCD).

	MD	HCD	BMD	BHCD
Calories	3006.4*	2921.0	2637.8	3067.7
Protein	16.3	15.7	18.2	16.0
Fat	46.4	10.7	32.3	
Carbohydrate	37.3	73.6	39.0	45.0

\* Means for the 5-day diets.

**Table 2.** Essential nutrient content of the two diets consumed by divers during the ASD compared with recommended dietary allowances (RDA).

	MD	HCD	RDA
Sodium (meq)	151.0*	137.9	43 - 144
Potassium (meq)	136.4	180.4	48 - 144
Calcium (mg)	1,438	1,657	800
Magnesium (mg)	341.0	593.0	350
Zinc (mg)	12.5	14.8	15
Iron (mg)	17.1	23.1	10
Copper (mg)	1.2	2.4	2 - 3
Vitamin B6 (mg)	2.1	3.7	2.2
Cholesterol (mg)	814.5	213.3	< 300

\* Means for the 5-day diets.

Table 3. General characteristics of the 16 U.S. Navy divers.

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AGE (years)	27 ± 3*	23 - 33**
HEIGHT (cm)	180 ± 7	170 - 193
WEIGHT (kg)	79.6 ± 5.5	71.5 - 90.1
BODY FAT (%) <sup>1</sup>	12.7 ± 3.7	8 - 22
MAX O <sub>2</sub> UPTAKE <sup>2</sup> (ml/min/kg)	45.4 ± 4.1	38 - 52

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\* Mean ± SD; \*\* Range of values;

1 Determined by hydrostatic weight.

2 Determined by incremental bicycle workloads.

Table 4. Hematologic changes before and after cold water immersions lasting for at least three hours during the AM and PM.

	AM IMMERSION		PM IMMERSION	
	BEFORE	AFTER	BEFORE	AFTER
	0930	1630	2130	0430
Hct (%)	45.5 ± 0.4	49.8 ± 0.4*	44.9 ± 0.4	49.8 ± 0.4*
Hb (g/dl)	15.8 ± 0.2	17.6 ± 0.2*	15.7 ± 0.1	17.2 ± 0.2*
RBC (10 <sup>6</sup> /mm <sup>3</sup> )	5.0 ± 0.2	5.7 ± 0.1*	5.0 ± 0.1	5.6 ± 0.1*
WBC (10 <sup>3</sup> /mm <sup>3</sup> )	5.8 ± 0.2	9.8 ± 0.6*	6.8 ± 0.3	10.3 ± 0.5*

Mean ± SEM; \* P < 0.01 for comparisons between before and after immersion values.

**Table 5.** Serum concentrations of selected ions before (B) and after (A) cold water immersions lasting from three to six hours. Expected (E) values were calculated based on changes in plasma volume.

		AM IMMERSION	PM IMMERSION
Serum K (meq/l)	B	4.50 ± 0.05	4.43 ± 0.06
	A	4.21 ± 0.06*	4.29 ± 0.05*
	E	5.47 ± 0.10	5.34 ± 0.10
Serum Na (meq/l)	B	143.4 ± 0.5	143.8 ± 0.7
	A	145.4 ± 0.5*	145.3 ± 0.7*
	E	174.0 ± 2.4	173.3 ± 2.7
Serum Cl (meq/l)	B	143.4 ± 0.5	143.8 ±
	A	104.3 ± 0.4*	104.9 ± 0.5*
	E	128.3 ± 1.8	126.9 ± 2.1
Serum Pi (mg/dl)	B	3.41 ± 0.09	4.05 ± 0.17
	A	4.25 ± 0.12	4.29 ± 0.11
	E	4.15 ± 0.14	4.87 ± 0.23
Serum Osm (mosm/l)	B	288.4 ± 1.5	294.4 ± 2.1
	A	296.4 ± 5.4*	296.1 ± 2.4*
	E	356.4 ± 4.9	366.4 ± 5.3

Mean ± SEM;

\* Means for A values bearing superscripts within AM and PM immersion are significantly different from E values (P < 0.01).

**Table 6.** Clearance and fractional excretion of serum electrolytes and serum constituents before and during cold water AM and PM immersions lasting from three to six hours.

	AM IMMERSIONS		PM IMMERSIONS	
	BEFORE 2200 - 1000	DURING 1000 - EOI1	BEFORE 1000 - 2200	DURING 2200 - EOI
C <sub>Cr</sub> (ml/min)	127.7 ± 15.9	276.5 ± 81.7	149.9 ± 17.9	236.5 ± 70.5
C <sub>osm</sub> (ml/min)	1.33 ± 0.21	4.65 ± 0.92	1.89 ± 0.14	2.55 ± 0.55
C <sub>H<sub>2</sub>O</sub> (ml/min)	-0.34 ± 0.13	0.10 ± 0.07	0.03 ± 0.30	0.23 ± 0.38
C <sub>Na</sub> (ml/min)	0.62 ± 0.07	1.98 ± 0.42*	0.92 ± 0.08	1.89 ± 0.31*
C <sub>K</sub> (ml/min)	9.71 ± 0.96	36.73 ± 4.58*	18.95 ± 1.73	20.39 ± 2.82
Na/Cr (x102)	0.7 ± 0.1	1.0 ± 0.2	1.2 ± 0.3	1.7 ± 0.3
K/Cr (x102)	10.5 ± 1.2	23.4 ± 4.2	21.5 ± 4.5	17.1 ± 2.9

Mean ± SEM; 1 EOI = end of immersion.

\* P < 0.01 for comparisons between before and during immersion values.

APPENDIX 1. INDIVIDUAL SERUM BIOCHEMISTRIES

SUBJ#	TIME	DIET = GLYCOGEN		AM IMMERSION		
		ALBUMIN	PROTEIN	SODIUM	CHLORIDE	PI
1	before	4.40	6.70	145	105	3.2
1	after	5.30	8.20	144	106	4.6
10	before	5.40	8.10	144	106	2.8
10	after	5.10	9.20	147	103	4.1
11	before	4.70	8.50	143	103	2.8
11	after	5.40	9.60	146	102	4.0
12	before	5.40	7.70	146	108	3.2
12	after	5.60	8.50	NS	NS	4.2
13	before	4.70	7.70	143	107	3.2
13	after	5.40	8.90	144	105	4.1
14	before	6.00	10.10	144	108	4.8
14	after	6.30	9.80	144	104	4.8
15	before	4.80	7.80	144	106	3.1
15	after	5.40	9.00	148	105	4.4
16	before	4.90	7.60	144	106	3.4
16	after	5.40	8.50	145	105	3.8
17	before	5.10	7.80	145	106	3.0
17	after	NS	NS	NS	NS	NS
3	before	4.30	8.10	141	106	3.1
3	after	4.30	8.30	147	105	4.3
5	before	4.90	6.80	133	100	3.0
5	after	5.20	7.30	136	101	3.0
6	before	4.80	7.10	146	107	4.0
6	after	5.60	8.30	148	104	4.3
7	before	4.90	7.60	144	106	3.4
7	after	8.30	12.70	141	100	5.7
8	before	5.60	7.90	145	103	2.6
8	after	5.70	9.10	147	103	3.9
9	before	4.00	7.70	144	107	3.2
9	after	4.60	8.80	148	106	5.4

NS = No Sample

APPENDIX 1. INDIVIDUAL SERUM BIOCHEMISTRIES

SUBJ#	TIME	DIET = GLYCOGEN		PM IMMERSION		
		ALBUMIN	PROTEIN	SODIUM	CHLORIDE	PI
1	before	5.50	8.40	147	105	4.6
1	after	5.00	7.50	143	104	3.7
10	before	5.10	9.10	139	101	4.2
10	after	5.00	9.20	146	103	4.1
12	before	5.00	7.50	144	107	3.8
12	after	5.50	8.80	148	109	5.1
13	before	4.50	7.40	144	106	4.7
13	after	5.30	8.90	144	104	4.6
14	before	5.30	10.10	143	105	3.9
14	after	6.40	10.80	143	102	4.6
15	before	4.50	7.30	145	103	4.6
15	after	5.40	8.90	147	103	4.7
17	before	3.90	6.00	151	108	2.9
17	after	4.40	6.70	149	105	3.3
3	before	3.50	6.80	141	104	3.5
3	after	4.10	8.10	148	106	4.0
4	before	5.00	7.20	145	105	3.0
4	after	5.70	8.40	145	107	4.2
5	before	5.00	7.00	143	107	4.1
5	after	5.20	7.40	144	107	3.5
6	before	4.60	6.70	145	105	4.0
6	after	5.20	7.60	145	103	4.0
7	before	6.60	9.70	146	106	5.0
7	after	6.00	9.00	145	104	4.3
8	before	6.40	8.40	145	104	3.7
8	after	6.10	9.00	147	103	3.8
9	before	3.30	6.60	140	109	3.2
9	after	4.40	8.50	146	106	4.8

NS = No Sample

APPENDIX 1. INDIVIDUAL SERUM BIOCHEMISTRIES

SUBJ#	TIME	DIET = MIXED		AM IMMERSION		
		ALBUMIN	PROTEIN	SODIUM	CHLORIDE	PI
1	before	4.80	7.20	147	106	2.9
1	after	5.20	7.90	147	107	3.9
10	before	4.60	8.40	143	105	3.8
10	after	5.10	9.30	146	103	4.1
11	before	4.60	8.00	143	106	3.2
11	after	5.20	9.20	146	104	3.8
12	before	4.90	7.80	144	107	2.8
12	after	6.00	8.80	146	109	4.2
13	before	NS	NS	NS	NS	NS
13	after	6.50	10.80	143	102	4.6
14	before	5.80	9.80	140	103	3.9
14	after	6.70	11.30	145	103	4.2
15	before	4.80	7.80	143	103	3.8
15	after	5.70	9.40	146	104	4.5
16	before	4.50	7.00	143	109	4.3
16	after	5.60	8.60	147	105	4.0
3	before	3.70	7.10	141	108	4.0
3	after	4.40	8.50	147	105	4.4
5	before	5.10	7.10	141	104	3.4
5	after	5.40	7.70	145	105	3.3
6	before	4.70	7.00	144	105	3.3
6	after	4.60	6.80	143	105	3.5
7	before	5.70	8.50	145	105	4.1
7	after	6.20	9.50	146	103	4.6
8	before	5.70	8.10	144	104	3.7
8	after	6.40	9.10	147	102	3.8
9	before	3.90	7.90	146	111	3.4
9	after	4.40	8.80	148	109	5.6

NS = No Sample

APPENDIX 1. INDIVIDUAL SERUM BIOCHEMISTRIES

SUBJ#	TIME	DIET = MIXED		PM IMMERSION		
		ALBUMIN	PROTEIN	SODIUM	CHLORIDE	PI
1	before	4.70	7.20	143	106	4.7
1	after	5.40	8.00	140	106	4.2
10	before	4.50	7.90	142	104	3.6
10	after	4.90	8.80	147	104	3.9
11	before	5.00	8.90	146	106	3.6
11	after	5.30	9.20	147	103	4.6
12	before	4.70	7.40	151	109	3.5
12	after	4.90	7.80	147	109	4.1
13	before	NS	NS	142	104	NS
13	after	5.10	8.40	144	104	4.3
14	before	6.40	10.70	133	96	4.9
14	after	6.30	10.90	143	104	5.1
15	before	6.10	10.10	139	102	6.8
15	after	6.10	10.20	148	106	5.7
16	before	4.70	7.20	144	107	3.8
16	after	5.40	8.40	145	106	3.9
17	before	3.80	5.60	147	106	2.3
17	after	4.10	6.10	152	106	3.8
3	before	3.60	6.90	143	106	3.5
3	after	4.10	7.90	146	107	5.2
4	before	4.80	7.10	140	105	3.7
4	after	5.30	8.00	133	99	4.4
7	before	5.40	8.00	145	106	5.1
7	after	5.80	8.80	145	106	4.3
8	before	5.80	8.00	144	104	4.4
8	after	7.30	10.30	140	100	4.4
9	before	3.60	6.90	146	109	3.6
9	after	3.70	5.20	152	110	3.2

NS = No Sample

APPENDIX 2. INDIVIDUAL HEMATOLOGICAL DATA

SUBJ#	TIME	DIET = GLYCOGEN			AM IMMERSION	
		HCT	HB	RBC	WBC	CREATININE
1	before	45.3	15.4	5.20	6.0	1.04
1	after	45.3	15.4	5.96	7.6	1.05
10	before	43.0	16.1	4.45	6.2	1.10
10	after	43.0	16.1	5.20	7.5	1.00
11	before	48.3	16.3	4.85	4.8	0.90
11	after	48.3	16.3	7.54	9.0	0.90
12	before	45.0	15.7	5.19	6.5	1.20
12	after	45.0	15.7	5.84	13.3	1.00
13	before	45.0	15.0	4.99	5.2	1.10
13	after	45.0	15.0	5.61	9.9	1.00
14	before	48.0	16.9	5.53	5.0	1.40
14	after	48.0	16.9	5.96	6.7	1.30
15	before	47.0	16.0	5.35	5.9	1.20
15	after	47.0	16.0	6.19	8.3	1.10
16	before	48.0	16.3	5.10	4.2	1.10
16	after	48.0	16.3	5.48	6.1	0.90
17	before	45.0	15.3	4.98	6.4	1.00
17	after	45.0	15.3	5.35	9.9	NS
3	before	46.0	16.1	5.19	5.3	1.20
3	after	46.0	16.1	5.79	10.6	1.00
5	before	42.5	15.1	5.31	4.2	1.11
5	after	42.5	15.1	5.31	4.7	0.97
6	before	48.0	16.8	4.94	8.6	1.11
6	after	48.0	16.8	5.96	15.7	1.04
7	before	45.0	15.4	5.09	6.0	1.04
7	after	45.0	15.4	5.93	13.6	1.03
8	before	47.2	17.2	4.36	5.1	1.00
8	after	47.2	17.2	5.13	8.7	0.90
9	before	47.0	16.6	5.20	6.3	1.10
9	after	47.0	16.6	6.00	14.4	1.30

NS = No Sample

APPENDIX 2. INDIVIDUAL HEMATOLOGICAL DATA

SUBJ#	TIME	DIET = GLYCOGEN			PM IMMERSION	
		HCT	HB	RBC	WBC	CREATININE
1	before	45.0	15.1	5.05	6.4	1.24
1	after	45.0	15.1	5.13	7.3	1.09
10	before	48.0	16.3	5.41	7.8	1.00
10	after	48.0	16.3	5.75	11.6	1.00
12	before	44.0	15.3	5.21	8.0	1.20
12	after	44.0	15.3	6.01	13.1	1.20
13	before	43.0	14.9	4.82	5.6	1.10
13	after	43.0	14.9	5.43	9.5	1.00
14	before	46.0	16.4	5.26	5.8	1.30
14	after	46.0	16.4	5.80	10.6	1.30
15	before	43.0	15.5	5.19	6.6	1.10
15	after	43.0	15.5	6.09	NS	1.10
17	before	45.0	16.0	5.20	5.9	0.80
17	after	45.0	16.0	5.84	12.3	0.90
3	before	45.0	15.5	5.07	6.3	1.10
3	after	45.0	15.5	5.81	15.6	1.00
4	before	47.0	16.0	5.01	6.7	0.92
4	after	47.0	16.0	5.30	10.7	0.92
5	before	46.0	15.8	5.42	6.2	1.00
5	after	46.0	15.8	5.52	8.3	1.07
6	before	47.0	16.2	4.77	9.3	0.90
6	after	47.0	16.2	5.61	9.3	0.92
7	before	43.0	14.9	4.93	5.0	1.20
7	after	43.0	14.9	5.77	6.9	1.00
8	before	49.0	17.4	5.53	6.6	1.00
8	after	49.0	17.4	5.88	8.4	0.90
9	before	45.0	15.2	4.75	3.8	1.00
9	after	45.0	15.2	5.94	12.9	1.20

NS = No Sample

APPENDIX 2. INDIVIDUAL HEMATOLOGICAL DATA

SUBJ#	TIME	DIET = MIXED			AM IMMERSION	
		HCT	HB	RBC	WBC	CREATININE
1	before	45.0	15.3	5.19	6.4	1.05
1	after	45.0	15.3	5.61	8.0	1.00
10	before	48.0	16.5	5.43	7.4	1.00
10	after	48.0	16.5	5.72	12.9	1.10
11	before	43.0	13.6	4.48	7.6	1.00
11	after	43.0	13.6	5.08	14.6	1.00
12	before	43.0	15.3	4.96	5.8	1.30
12	after	43.0	15.3	5.59	11.5	1.10
13	before	43.0	14.5	4.57	4.4	1.10
13	after	43.0	14.5	5.42	8.0	1.30
14	before	47.0	15.9	5.26	4.7	1.40
14	after	47.0	15.9	4.56	8.0	1.20
15	before	44.0	15.1	4.49	5.5	1.20
15	after	44.0	15.1	5.95	8.2	1.20
16	before	42.0	14.7	4.49	4.8	1.20
16	after	42.0	14.7	5.19	5.8	1.20
3	before	47.0	16.0	5.04	6.0	1.00
3	after	47.0	16.0	6.44	12.2	1.10
5	before	44.0	15.0	5.20	5.8	1.12
5	after	44.0	15.0	5.55	8.5	0.99
6	before	48.2	16.8	5.02	7.6	1.04
6	after	48.2	16.8	4.92	7.6	0.96
7	before	46.0	16.0	5.24	5.2	1.10
7	after	46.0	16.0	5.88	7.8	1.10
8	before	43.0	16.4	5.16	5.2	1.00
8	after	43.0	16.4	5.66	11.8	1.00
9	before	47.0	16.4	5.29	6.5	1.10
9	after	47.0	16.4	5.76	13.2	1.30

NS = No Sample

APPENDIX 2. INDIVIDUAL HEMATOLOGICAL DATA

SUBJ#	TIME	DIET = MIXED			PM IMMERSION	
		HCT	HB	RBC	WBC	CREATININE
1	before	43.0	15.1	4.94	6.4	1.18
1	after	43.0	15.1	5.50	14.7	1.05
10	before	44.0	15.4	5.20	7.7	1.20
10	after	44.0	15.4	5.64	13.6	1.00
11	before	47.0	14.9	4.94	10.5	1.10
11	after	47.0	14.9	4.97	11.4	1.00
12	before	45.0	15.7	5.26	8.6	1.40
12	after	45.0	15.7	5.41	10.6	1.40
13	before	44.0	14.4	4.54	6.5	1.20
13	after	44.0	14.4	5.18	8.8	1.00
14	before	48.0	16.7	5.41	6.0	1.60
14	after	48.0	16.7	5.71	8.9	1.50
15	before	45.0	15.3	4.91	7.4	1.70
15	after	45.0	15.3	5.32	9.1	1.40
16	before	44.0	15.3	4.72	5.8	1.10
16	after	44.0	15.3	5.24	7.7	1.30
17	before	42.0	14.5	4.69	7.5	0.90
17	after	42.0	14.5	5.43	12.2	0.80
3	before	44.0	15.7	4.89	7.0	1.20
3	after	44.0	15.7	5.55	8.5	1.10
4	before	46.0	16.1	5.19	7.0	1.12
4	after	46.0	16.1	5.60	7.5	1.03
7	before	40.0	14.5	4.78	6.0	1.10
7	after	40.0	14.5	5.64	9.0	1.00
8	before	48.0	16.6	5.10	7.2	1.10
8	after	48.0	16.6	5.34	7.9	1.10
9	before	43.0	15.1	4.89	7.1	1.30
9	after	43.0	15.1	5.36	NS	0.90

NS = No Sample

APPENDIX 3. INDIVIDUAL URINE VALUES

SUBJ#	TIME	DIET = GLYCOGEN		AM IMMERSION		OSMOLALITY	VOLUME	CREATININE
		DIVE TIME	SODIUM	POTASSIUM				
1	before	369	NS	NS	NS	NS	NS	NS
1	during	369	23.0	37.0	128	0.560	201.6	201.6
1	after	369	NS	NS	293	0.840	1024.8	1024.8
10	before	369	32.9	18.6	370	0.620	1345.4	1345.4
10	during	369	323.7	130.8	286	3.270	3629.7	3629.7
10	after	369	45.6	26.4	289	0.300	639.0	639.0
11	before	369	16.5	22.0	352	0.500	605.0	605.0
11	during	369	32.5	32.5	272	1.160	986.0	986.0
11	after	369	NS	NS	576	1.140	5027.4	5027.4
12	before	268	33.6	2.9	372	0.210	296.1	296.1
12	during	268	NS	NS	NS	1.760	NS	NS
12	after	268	18.3	13.1	492	0.130	224.9	224.9
13	before	276	193.9	44.2	302	1.920	2304.0	2304.0
13	during	276	NS	NS	NS	NS	NS	NS
13	after	276	27.7	12.4	238	0.180	367.2	367.2
14	before	220	37.8	23.8	680	0.440	1641.2	1641.2
14	during	220	NS	NS	NS	NS	NS	NS
14	after	220	0.0	0.0	0	0.000	0.0	0.0
15	before	208	73.7	43.5	773	0.630	1701.0	1701.0
15	during	208	246.6	77.3	327	2.090	815.1	815.1
15	after	208	28.5	27.4	698	0.230	506.0	506.0
16	before	233	111.6	42.8	525	0.930	1190.4	1190.4
16	during	233	37.6	21.8	190	0.990	148.5	148.5
16	after	233	56.0	24.6	365	0.780	343.2	343.2
17	before	287	35.5	24.5	400	0.500	285.0	285.0
17	during	287	77.4	65.1	461	0.880	624.8	624.8
17	after	287	NS	NS	NS	NS	NS	NS
3	before	370	83.0	35.0	320	1.000	790.0	790.0
3	during	370	123.2	68.7	191	2.020	444.4	444.4
3	after	370	NS	NS	NS	NS	NS	NS
5	before	208	56.7	24.3	500	0.810	1449.9	1449.9
5	during	208	50.3	34.0	211	1.360	516.8	516.8
5	after	208	64.8	32.6	530	0.480	936.0	936.0
6	before	369	41.3	20.6	680	0.375	2167.5	2167.5

APPENDIX 3. INDIVIDUAL URINE VALUES (continued)

SUBJ#	TIME	DIET = GLYCOGEN		AM IMMERSION		OSMOLALITY	VOLUME	CREATININE
		DIVE TIME	SODIUM	POTASSIUM				
6	during	369	122.4	58.5	493	0.900	1017.0	
6	after	369	NS	NS	689	0.225	891.0	
7	before	369	NS	NS	NS	NS	NS	
7	during	369	83.0	37.0	274	1.000	630.0	
7	after	369	29.9	27.0	516	0.575	1167.3	
8	before	315	36.3	22.0	300	1.100	1628.0	
8	during	315	21.9	23.8	242	0.950	494.0	
8	after	315	13.3	7.3	386	0.170	232.9	
9	before	370	56.4	41.6	448	0.570	456.0	
9	during	370	62.7	38.3	154	1.530	765.0	
9	after	370	38.1	37.7	460	0.340	370.6	

NS = No Sample; 0 = No Urine Excreted

APPENDIX 3. INDIVIDUAL URINE VALUES

SUBJ#	TIME	DIET = GLYCOGEN		PM IMMERSION		OSMOLALITY	VOLUME	CREATININE
		DIVE TIME	SODIUM	POTASSIUM				
1	before	317	77.1	122.2	256	1654.4		
1	during	317	13.3	54.6	566	676.0		
1	after	317	46.9	11.9	517	636.9		
10	before	251	NS	NS	202	1751.6		
10	during	251	NS	NS	NS	NS		
10	after	251	61.6	56.1	368	902.0		
12	before	370	7.8	5.1	218	952.8		
12	during	370	NS	NS	NS	NS		
12	after	370	23.0	23.0	196	209.3		
13	before	370	125.6	75.5	193	1483.3		
13	during	370	27.3	9.7	410	1020.0		
13	after	370	NS	NS	NS	NS		
14	before	258	NS	NS	NS	NS		
14	during	258	NS	NS	NS	NS		
14	after	258	25.8	12.6	368	235.2		
15	before	340	65.7	53.5	368	838.3		
15	during	340	117.0	32.8	248	530.4		
15	after	340	NS	NS	NS	NS		
17	before	327	141.8	105.5	250	2479.5		
17	during	327	33.5	10.5	271	1070.0		
17	after	327	8.8	6.8	688	116.1		
3	before	250	103.7	96.3	501	1852.5		
3	during	250	11.3	4.1	134	35.1		
3	after	250	0.0	0.0	0	0.0		
4	before	369	95.5	46.5	415	207.5		
4	during	369	260.2	26.6	368	548.7		
4	after	369	52.6	37.6	571	958.8		
5	before	192	75.4	80.6	376	1976.0		
5	during	192	78.1	36.3	327	753.3		
5	after	192	51.5	26.5	487	1038.8		
6	before	237	64.2	33.6	608	1020.0		
6	during	237	177.0	37.0	466	851.2		
6	after	237	51.6	17.7	575	408.9		
7	before	207	128.5	81.9	454	1612.8		

APPENDIX 3. INDIVIDUAL URINE VALUES (continued)

SUBJ#	TIME	DIET = GLYCOGEN		PM IMMERSION		OSMOLALITY	VOLUME	CREATININE
		DIVE TIME	SODIUM	POTASSIUM	OSMOLALITY			
7	during	207	59.8	11.6	190	0.430	124.7	
7	after	207	64.1	18.1	370	0.475	432.3	
8	before	369	70.1	39.0	63	3.895	584.3	
8	during	369	93.8	62.0	241	1.675	1139.0	
8	after	369	8.7	10.8	251	0.300	381.0	
9	before	279	88.7	52.7	501	0.620	465.0	
9	during	279	NS	NS	NS	NS	NS	
9	after	279	25.2	16.8	385	0.210	266.7	

NS = No Sample; 0 = No Urine Excreted

## APPENDIX 3. INDIVIDUAL URINE VALUES

SUBJ#	TIME	DIVE TIME	DIET = MIXED		AM IMMERSION		OSMOLALITY	VOLUME	CREATININE
			SODIUM	POTASSIUM	SODIUM	POTASSIUM			
1	before	234	80.6	46.6	382	1.260	756.0		
1	during	234	96.4	51.2	301	1.220	NS		
1	after	234	NS	NS	NS	NS	NS		
10	before	369	63.8	39.2	288	1.120	1220.8		
10	during	369	40.8	54.1	251	1.020	612.0		
10	after	369	27.5	12.0	400	0.500	610.0		
11	before	369	30.4	23.4	235	0.780	413.4		
11	during	369	NS	NS	NS	1.240	706.8		
11	after	369	130.2	38.4	792	0.320	1603.2		
12	before	300	68.9	24.1	192	0.560	324.8		
12	during	300	7.8	4.6	698	0.900	144.0		
12	after	300	17.9	13.6	272	0.160	129.6		
13	before	370	53.3	59.0	480	0.820	697.0		
13	during	370	35.5	38.9	398	1.110	1221.0		
13	after	370	0.0	0.0	0	0.000	0.0		
14	before	260	59.9	25.9	360	0.540	237.6		
14	during	260	74.7	45.4	316	0.770	277.2		
14	after	260	28.5	17.9	284	0.380	273.6		
15	before	370	34.8	20.7	305	0.940	470.0		
15	during	370	132.7	87.7	235	2.370	1944.1		
15	after	370	0.0	0.0	0	0.000	0.0		
16	before	280	NS	NS	NS	0.680	NS		
16	during	280	176.3	79.6	416	1.170	620.1		
16	after	280	0.0	0.0	0	0.000	0.0		
3	before	370	37.1	16.9	215	0.545	278.0		
3	during	370	57.4	40.8	74	1.510	151.0		
3	after	370	9.5	17.2	322	0.170	190.4		
5	before	369	60.2	33.8	449	0.940	1513.4		
5	during	369	24.5	20.0	280	0.500	250.0		
5	after	369	45.2	20.1	553	0.330	986.7		
6	before	195	62.5	68.6	539	0.880	1557.6		
6	during	195	36.6	29.0	285	0.690	448.5		
6	after	195	5.4	10.7	805	0.120	45.6		
7	before	369	80.5	67.1	433	1.220	1573.8		

APPENDIX 3. INDIVIDUAL URINE VALUES (continued)

SUBJ#	TIME	DIET = MIXED		AM IMMERSION		OSMOLALITY	VOLUME	CREATININE
		DIVE TIME	SODIUM	POTASSIUM				
7	during	369	NS	NS	NS	NS	NS	NS
7	after	369	NS	NS	NS	NS	NS	NS
8	before	369	134.4	40.3	421	1.680	554.4	NS
8	during	369	NS	NS	NS	0.530	NS	NS
8	after	369	39.8	14.8	644	0.300	732.0	NS
9	before	370	67.2	13.4	339	0.560	604.8	NS
9	during	370	NS	NS	NS	NS	NS	NS
9	after	370	52.0	33.3	216	0.520	442.0	NS

NS = No Sample; 0 = No Urine Excreted

APPENDIX 3. INDIVIDUAL URINE VALUES

SUBJ#	TIME	DIVE TIME	DIET = MIXED			PM IMMERSION			OSMOLALITY	VOLUME	CREATININE
			SODIUM	POTASSIUM	POTASSIUM	POTASSIUM	OSMOLALITY				
1	before	369	128.4	98.0	199	1.690	828.1				
	during	369	NS	NS	NS	NS	NS				
1	after	369	92.2	52.4	452	0.970	1154.3				
10	before	369	NS	NS	380	1.370	1685.1				
10	during	369	NS	NS	NS	NS	NS				
10	after	369	NS	NS	NS	NS	NS				
11	before	263	104	74.6	285	2.260	1559.4				
11	during	263	15.5	15.8	387	0.360	NS				
11	after	263	13	8.9	207	0.370	395.9				
12	before	209	70.1	52.4	581	0.935	897.6				
12	during	209	24.8	3.6	202	0.330	158.4				
12	after	209	9.6	24.0	161	0.200	328.0				
13	before	370	183	73.2	248	1.830	933.3				
13	during	370	150.8	46.8	336	1.300	234.0				
13	after	370	13.7	11.8	180	0.380	64.6				
14	before	189	84.3	58.2	605	0.685	1774.2				
14	during	189	53.9	18.9	503	0.350	266.0				
14	after	189	0	0.0	0	0.000	0.0				
15	before	267	67.7	57.4	318	1.025	594.5				
15	during	267	32.4	11.3	343	0.390	97.5				
15	after	267	21.2	15.9	632	0.270	245.7				
16	before	370	45.6	16.3	608	0.800	952.0				
16	during	370	132.2	44.5	421	1.560	1138.8				
16	after	370	0	0.0	0	0.000	0.0				
17	before	370	116.6	56.4	302	1.880	620.4				
17	during	370	203.8	35.6	191	2.370	4123.8				
17	after	370	15.7	21.3	647	0.280	596.4				
3	before	370	170.1	50.4	126	3.150	882.0				
3	during	370	75.7	19.4	132	0.880	193.6				
3	after	370	12	26.8	223	0.250	172.5				
4	before	215	69.3	61.6	NS	0.770	NS				
4	during	215	NS	NS	NS	NS	NS				
4	after	215	64.5	11.4	426	0.520	639.6				
7	before	369	162.4	77.1	364	1.640	1623.6				

APPENDIX 3. INDIVIDUAL URINE VALUES (continued)

SUBJ#	TIME	DIET = MIXED		PM IMMERSION		OSMOLALITY	VOLUME	CREATININE
		DIVE TIME	SODIUM	POTASSIUM	POTASSIUM			
7	during	369	110.9	39.6	252	0.990	613.8	
7	after	369	NS	NS	NS	NS	NS	
8	before	272	74.8	52.8	120	4.400	1452.0	
8	during	272	67.9	23.8	425	0.700	903.0	
8	after	272	45.9	26.1	288	0.900	711.0	
9	before	370	144.9	64.4	182	0.800	184.0	
9	during	370	45.1	10.4	158	0.740	244.2	
9	after	370	NS	NS	NS	NS	NS	

NS = No Sample; 0 = No Urine Excreted