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The Measurement of Human Body-fluid Volumes: Resting Fluid Volumes Before and After Heat Acclimation

Mark J. Patterson, Jodie M. Stocks, Nigel A.S. Taylor and Denys Amos

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ABSTRACT

The study evaluated changes in human body-fluid volumes accompanying a 21-day heat-acclimation regime. Eight unacclimatised males, were studied for 22 consecutive days, including 19 days of heat exposure. Heat stress tests and acclimation trials were undertaken at 40°C and relative humidity 60%. Total body water increased from Day 1 to 8 but a further 13 days acclimation did not elicit a further increase. This trend was reflected within the extracellular space, with the extracellular fluid volume significantly elevated after 8 days, without further expansion beyond Day 8. A plasma volume expansion of 18% was evident by Day 8, and, by Day 22, had increased to 23.6% of pre-acclimation levels. The interstitial fluid volume increase from Day 1 to Day 8 was not significant. Proportionately more of the extracellular fluid volume expansion occurred within the plasma volume and was ascribed to increases in the total plasma protein which accompanied acclimation. The intracellular volume remained stable and erythrocyte volume was similarly unaltered. Body-fluid enlargement was restricted therefore to the extracellular compartment. It was concluded that the plasma volume was expanded rapidly in response to heat acclimation, with the majority of this expansion taking place within the first 8 days. A unique observation was the tendency for the plasma volume to remain elevated from Day 8 to Day 22, indicating that further heat acclimation would maintain this expanded volume.

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Executive Summary

During military operations and exercises in northern Australia soldiers frequently have been transferred from the cooler southern states and have been required to operate at peak efficiency shortly after arrival in the tropics. Such soldiers have not been heat acclimatised and are prone to varying degrees of heat strain during operations. Heat illness occurs most frequently during the first five days of heat exposure with an inability to continue work being the most common disorder. Humans have evolved to produce mechanisms that enable adaptation to environmental conditions. These physiological changes can be brought about in response to natural climatic changes and to artificial heat exposure. The latter is most important for the acclimation of ADF personnel prior to relocation to the tropics.

This report examines the changes in human body fluid volumes during a 21-day heat acclimation regime. Eight healthy, physically active but unacclimatised males were studied for 22 consecutive days, including 19 days of heat exposure in a climate-controlled chamber. Heat stress tests and acclimation trials were undertaken at 40°C and 60% relative humidity. Total body water increased from Day 1 to Day 8 but further acclimation did not produce a further increase. This trend was reflected within the extracellular space, with the extracellular fluid significantly elevated after 8 days but with little expansion after Day 8. A plasma volume expansion of 18% was evident by Day 8 and this increased to 23% of pre-acclimation levels by Day 22. There was an insignificant increase in interstitial fluid volume from Day 1 to Day 8. Proportionally more of the extracellular volume expansion occurred within the plasma volume and was ascribed to increases in the total plasma protein accompanying acclimation. The intracellular volume remained stable and erythrocyte volume was unaltered. Body-fluid enlargement was restricted therefore to the extracellular compartment.

It may be concluded that the plasma volume was expanded rapidly in response to heat acclimation and that the majority of the expansion took place within the first 8 days. This volume increase will serve a protective function during subsequent heat exposure. For military purposes, an 8-day heat acclimation regime would be of considerable benefit from the perspective of body-fluid changes. However, it would not be correct to assume that only 8 days of combined exercise and heat acclimation will provide the optimum heat acclimation; changes in other physiological functions can take up to 14 days to evolve.

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1. Introduction

The incidence of heat illness is greatest during the first five days of an unaccustomed heat exposure (Armstrong and Maresh, 1991), with exertional heat exhaustion the most commonly reported heat disorder. Humans have evolved mechanisms so that heat dissipation and conservation pathways are able to adapt to a wide range of environmental conditions, as well as to the combined thermal stresses of exercise and external heat. Accordingly, the reduced physiological strain following acclimatisation is mediated by the following adaptations:

(i) an elevation of the skin blood flow response to heat stress (Fox *et al.*, 1963),

(ii) an expansion of the plasma volume (PV; Senay, 1979);

(iii) cardiac stroke volume increases and cardiac frequency is reduced (Mitchell *et al.*, 1976; Shapiro *et al.*, 1981; Cadarette *et al.*, 1984);

(iv) there is an enhancement of the sweat (sudomotor) response:

* increased sweat rate ($\underline{m}_{sw}$: Libert *et al.*, 1983; Sato *et al.*, 1990);
* increased sweat gland sensitivity relative to core temperature (T_c);
* decreased T_c threshold for sweating (Nadel *et al.*, 1974);
* sweat glands also reabsorb a greater amount of sodium and chloride from the sweat (Allan and Wilson, 1971).

Such changes permit a reduction in both mean skin temperature (T_{sk}) and T_c for a fixed level of exercise and heat stress (Mitchell *et al.*, 1976; Pandolf *et al.*, 1977; Houmard *et al.*, 1990).

These general physiological modifications may be induced through exposure to natural climatic changes (acclimatisation: Hellon *et al.*, 1956), artificial heat exposure (acclimation: Nadel *et al.*, 1974; Roberts *et al.*, 1977, Wells *et al.*, 1980) and to endurance exercise producing significant elevations in body core temperature (Gisolfi and Robinson, 1969; Henane *et al.*, 1977; Pandolf *et al.*, 1977). This report primarily focuses upon human body-fluid changes that accompany a 21 day, combined heat and exercise acclimation regime.

Human fluid volumes may be subdivided into several compartments: total body water (TBW), extracellular fluid (ECFV), plasma volume (PV), interstitial fluid (IFV), intracellular water (ICW), red cell volume (RCV) and blood volume (BV). Such fluid volumes are not static but are in continual motion between the various compartments, with water passing through the cell membranes and vascular walls under the influence of osmotic, oncotic and hydrostatic forces. Various stresses have been shown to induce body-fluid shifts. Of these, exercise and heat exposure are of direct relevance to military personnel.

It is well established that the PV is expanded in endurance-trained individuals (Kjellberg *et al.*, 1949; Dill *et al.*, 1974; Brotherhood *et al.*, 1975; Maw *et al.*, 1996). Recently, we have observed a whole-body hyperhydration in such subjects, where all body-fluid compartments were expanded in comparison with a standard population (Maw *et al.*, 1996). Observations of an elevation in PV have also been reported following short-term (Green *et al.*, 1990; Gillen *et al.*, 1991; Luetkemeier *et al.*, 1994) and long-term endurance training regimes (Fortney and Senay, 1979; Convertino *et al.*, 1991). However, the combination of a hot environment with endurance training (heat acclimation) will elicit an even greater PV expansion than that seen with exercise training alone (Harrison *et al.*, 1981; Kirby and Convertino, 1986; Nielsen *et al.*, 1993). Nevertheless, the resultant effect of heat acclimation on the other body-fluid compartments is neither well documented nor well understood. This report summarises the effects of a 21 day heat acclimation regime upon resting, thermoneutral body-fluid volumes.

Heat acclimatisation data indicate that PV, total body water, extracellular fluid and red cell volumes tend to be larger in summer than during the winter months (Bazett *et al.*, 1940; Yoshimura, 1958). The PV expansion with acclimation, appears to be a result of protein entering the vascular space. Shapiro *et al.* (1981) found that when acclimation was undertaken at the end of both the summer and winter months, some level of summer acclimatisation had already been induced. However, the subjects were not fully acclimated since this required exercise in the heat and appeared dependent upon BV changes, before cardiovascular stability could be achieved during a combined exercise and heat exposure. They found that the blood volume change, measured during exercise, was due to red cell contraction and a PV expansion. Since these changes were also found to occur on the control days (*i.e.* they were acclimatisation affects), these volumes shifts were ascribed to the effects of exercise. The most pronounced fluid-volume changes (haemodilution) were observed between Days 6-8 of the heat-acclimation regime.

It has been almost universally demonstrated that heat acclimation elevates the PV (Greenleaf and Greenleaf, 1970; Wyndham, 1973; Sciaraffa *et al.*, 1980; Harrison 1986; Mack and Nadel, 1996). Wyndham (1973) suggested this expansion could be due to retention of sodium, following an elevation of aldosterone secretion[1]. It is also likely that antidiuretic hormone[2] is released in response to a progressive dehydration and increased extracellular osmolarity. Such dehydration leads to circulatory instability during the course of acclimation. This will not only trigger volume and blood pressure

[1] Aldosterone is the primary mineralocorticoid. Its site of action is the distal tubule of the kidney, where it promotes Na^+ reabsorption and K^+ excretion during urine formation. As a direct result of Na^+ reabsorption, water is also retained.

[2] Antidiuretic hormone is produced by the pituitary gland and stored in the posterior pituitary. Its release is triggered by a water deficit, which is detected by a hypertonicity of the extracellular fluid. Antidiuretic hormone increases water reabsorption through the tubular cells of the distal tubules of the nephron and collecting ducts.

receptors but will also activate hypothalamic osmoreceptors, leading to the retention of sodium and water and an isotonic expansion of the extracellular space. If water alone was retained, the ECFV would become hypotonic, resulting in a fluid flux into the cells and expanding the ICW. However, if electrolytes were retained without water, the intracellular fluid space would decline due to a cellular efflux. Therefore, the combination of both water and electrolyte retention is critical to the establishment and maintenance of an expanded PV. However, Senay (1979) found that heat exposure after acclimation, resulted in a haemodilution, which was mediated by proteins moving from the interstitium into the vascular space. While proteins are not the most powerful osmotic constituents of either the intracellular or extracellular spaces, they are osmotically active. Since proteins are generally not excreted, do not freely pass between ECFV compartments (PV and IFV) and attract a large volume of water per unit mass (*e.g.* albumin attracts 18 $mL \cdot g^{-1}$), they can play a pivotal role in inter-compartment fluid movements.

While PV variations are almost unequivocally supported, relatively little is known concerning the other body-fluid compartments during exercise and heat acclimation. Bass *et al.* (1955) used a chemical marker antipyrine to measure TBW. They found that TBW increased slightly (<1 L) over the course of a 14-day acclimation schedule. Their data indicate that the ECFV was expanded at the expense of ICW after 5 days, with a small decline in PV between Days 5 and 14, while the ECFV remained constant. However, no statistical analyses were employed and it is impossible to determine whether such changes were mere chance events. Wyndham *et al.* (1968) measured TBW during a 16 day acclimation trial, using the ^3H marker. They found an elevation in TBW at Day 5, while TBW for Days 1 and 17 were equivalent. It was also observed that the ECFV compartment was unchanged (Day 1 versus 5, p>0.05). This would imply that the intracellular compartment had expanded. However, the PV was also augmented on Day 5 but approached the control volume again on Day 17 (p>0.05). It appears that, since the PV is part of the extracellular space, fluid had moved from extravascular portion of the ECFV into the vascular compartment, without altering the total volume of the ECFV. Since we consider such fluid movements to be passive, then for these changes to have occurred, there must have been a shift in one or more osmotically active components between the fluid spaces (electrolytes or proteins). Due to the relatively free mobility of electrolytes, it is most likely that such changes are attributable to the movement of proteins between these compartments.

Senay *et al.* (1976) reported that both TBW and ECFV remained unchanged following heat acclimation over 10 days (n=4), while the PV was expanded. This group concluded that the interstitial space provided protein and water to the intravascular volume to facilitate such fluid expansion. However, TBW was approximated only from changes in body mass. It was assumed that ECFV and ICW were unchanged and, therefore, IFV was reduced in face of the PV expansion. In the most recent review of body-fluid changes during acclimation, Mack and Nadel (1996) accept the position of Senay *et al.* (1976), and conclude that the acclimation-induced increase in PV is at the expense of IFV. Mack and Nadel (1996) cite the observations of both Bass *et al.* (1955) and Wyndham *et al.* (1968) to reinforce their conclusion that neither TBW nor ECFV are altered following acclimation. Therefore, the elevation in PV was assumed to be at the expense of the IFV. However, Bass *et al.* (1955) observed an increase in the ECFV, without a change in TBW, whereas Wyndham *et al.* (1968) observed an elevation in TBW, with no change in the ECFV. Therefore, the interpretation of Mack and Nadel (1996) is more a mixture of the results from both studies rather than explicit observations of both groups.

Due to the lack of a clear consensus concerning both the intracellular and extracellular fluid compartments following heat acclimation, the current study sought to address the following questions.

(i) Does heat acclimation result in a whole-body hyperhydration, or is the volume expansion restricted only to the vascular space?
(ii) What are the dynamics of this fluid-volume expansion?
(iii) Is the PV expansion an iso-osmotic change and what are the driving forces behind this expansion?

2. Experimental

2.1 Subjects

Eight healthy, physically-active males (Table 1), without a previous history of heat acclimation, were acclimated during the months August-October to minimise seasonal acclimatisation effects. During this time, the average daily maximum and minimum temperatures were 20.5°C (S.D. 4.4)[3] and 10.3°C (S.D. 3.3). Each person was in good health and asymptomatic for cardiovascular dysfunction. The body-fluid volumes of the subjects were studied over 22 days, including 19 days of heat exposure in a climate-controlled chamber. Data were obtained during thermoneutral rest, prior to heat exposure, on Days 1, 8 and 22. All procedures were approved by the Human Research Ethics Committee, University of Wollongong, and all subjects provided informed consent. All procedures were conducted under sterile conditions, and in accordance with the requirements of the International Commission on Radiological Protection (1975) and the International Committee for Standardization in Haematology (1980). The total radiation dose imposed by these measurements amounted to 1.5 millisieverts, which is approximately one-thirteenth of the annual dose permitted for an industrial worker (International Commission on Radiological Protection, 1975).

[3] Data are presented as means with either standard deviations (S.D.) or standard errors of the means (±).

Table 1. Characteristics of subjects.

Subject	Age (y)	Mass (kg) Day 1	Mass (kg) Day 8	Mass (kg) Day 22	Height (cm)	V_{O2peak} ($L.min^{-1}$) Day 0
S1	20	66.3	66.6	65.2	179.0	4.179
S2	20	85.1	84.9	85.8	196.8	6.694
S3	18	85.8	83.6	83.6	182.4	3.817
S4	24	76.1	77.3	76.9	180.5	4.549
S5	20	75.5	74.8	75.0	183.8	4.254
S6	19	59.1	59.2	59.5	169.9	3.332
S7	28	84.2	83.8	81.4	180.1	3.815
S8	24	87.9	89.2	88.1	189.3	-
Mean	21.63	77.5	77.4	77.0	182.7	4.377
S.D.	3.38	10.35	10.21	10.07	7.86	1.093

Abbreviations: V_{O2peak} = peak oxygen uptake (aerobic power) measured during an incremental, semi-recumbent cycle protocol.

2.2 Procedural overview

The heat-acclimation protocol consisted of cycle exercise in a climate chamber at an air temperature (T_a) of 39.8°C with relative humidity (RH) controlled at 60%. Wind speed was less than 0.5 $m.s^{-1}$, and black globe temperature was within 0.5°C of T_a. Heat stress tests (HST) were conducted on Days 1, 8 and 22 (Table 2). Days 7, 14 and 21 were non-exposure, rest days to ensure subjects were both rested and adequately hydrated before each HST. On all other days, except for Days 6 and 19, subjects cycled in the heat for 90 min.

Two days before heat exposure, and on Days 6 and 19, subjects performed peak aerobic power tests in an air-conditioned laboratory (20°C). On Days 6 and 19, the subsequent heat-acclimation exposure was shortened to 60 min. Each peak power test involved semi-recumbent cycling to volitional exhaustion (Lode Excalibur ergometer, The Netherlands), with expired gases collected and analysed to derive peak oxygen consumption (V_{O2peak})[4], using an automated respiratory system (2900 Sensormedics, U.S.A.). Data from these tests were used to determine work rates to be used during the both the HSTs, and the combined exercise and heat-acclimation exposures.

2.2.1 Experimental standardisation

Subjects were strictly and thoroughly informed concerning their daily and pre-experimental requirements. Accordingly, subjects refrained from strenuous exercise and consumption of alcohol or caffeine 24 hr prior to each stress test. Fluid intake (15 mL.kg^{-1} before bed, and 15 mL.kg^{-1} on day of HST) and food intakes (high carbohydrate diet) for 24 hours prior to each HST was closely prescribed, to ensure uniform carbohydrate intake and euhydration. Subjects wore only cycle shorts or swimming costumes, and open-toed sandals throughout both the HSTs and the acclimation regime. Clothing was controlled within subjects.

2.3 Heat acclimation protocol

The heat-acclimation regimen commenced the day after the first HST, and incorporated two distinct phases.

Phase I: Heat stress tests (Days 1, 8 and 22): Each HST involved both rest and exercise in the heat (Table 2). Subjects started by resting (semi-recumbent) in the climate chamber at 28°C (60% RH). During this phase, subject preparation was completed. The chamber temperature was then elevated, over 20 minutes, to its target temperature (~40°C) and relative humidity (~60%). At this time, subjects started a 30 min resting heat exposure, followed by three 30 min stages of semi-recumbent cycling (Quinton Excalibur ergometer, Quinton Instrument Company, U.S.A.), with 2 min rest between each exercise bout. These work rates were designed to elicit a controlled elevation in body core temperature to ~39°C.

(i) *Stage 1:* Day 1: 30% peak work rate (110 watts); Day 8: 30% peak work rate (110 watts); Day 22: 30% peak work rate (115 watts).
(ii) *Stage 2:* Day 1: 28.9% peak work rate (104 watts); Day 8: 28.9% peak work rate (104 watts); Day 22: 28.9% peak work rate (108 watts).
(iii) *Stage 3:* Day 1: 27.5% peak work rate (98 watts); Day 8: 27.5% peak work rate (98 watts); Day 22: 28.4% peak work rate (101 watts).

After 90 min, the work rate was increased in a ramp function (4% peak power per min: 14.5 watts·min^{-1}) until the subjects reached volitional exhaustion, T_c reached the pre-

[4] Peak aerobic power is reported in absolute units (L.min^{-1}) since the exercise performed was semi-recumbent cycling, and body mass was fully supported throughout exercise.

determined upper cut-off point (≥39.5°C), or cardiac frequency (f_c) increased to 95% of the f_c reserve. Fluid replacement was not permitted until the HST was completed, when an iso-osmotic fluid, approximately equal in mass to the mass change of the subject during the HST, was consumed under supervision.

Phase II: 16 heat-acclimation days: During these exposures (Table 2) subjects cycled upright (Monark 868 ergometer, Sweden) for 90 minutes. Each session started with 30 min cycling to elevate T_c to ~38.5°C. On the first heat-acclimation day (Day 2), the mean work rate was 163 watts (S.D. 72), which represented 44% peak aerobic power. During the remaining 60 min of each exposure, subjects continued cycling but external work rate was adjusted to maintain, or slightly elevate T_c. Subjects were provided with 200 mL of water at 30 and 60 min within the acclimation exposure, at which time they rested for 2 min and then consumed an iso-osmotic drink at the conclusion of each session to replace their fluid loss.

Table 2. Details of daily heat acclimation protocol.

Heat-acclimation day	Protocol summary
1	heat stress day
2	acclimation day
3	acclimation day
4	acclimation day
5	acclimation day
6	peak aerobic power + acclimation
7	rest day: no heat exposure
8	heat stress day
9	acclimation day
10	acclimation day
11	acclimation day
12	acclimation day
13	acclimation day
14	rest day: no heat exposure
15	acclimation day
16	acclimation day
17	acclimation day
18	acclimation day
19	peak aerobic power + acclimation
20	acclimation day
21	rest day: no heat exposure
22	heat stress day

2.4 Determination of compartmental volumes and plasma constituents

On each HST day, subjects arrived at the laboratory at 0700 hours, and were transferred from their car to the laboratory by wheelchair, to negate postural influences upon subsequent fluid-volume measurements (Maw *et al.*, 1995). Once in the laboratory, a resting, seated blood sample was drawn from the right antecubital vein, without stasis. This sample was used as a background for beta (β) and gamma (γ) counting, and for labelling red blood cells. Subjects then ingested an aliquot of tritiated water (150 μCi, ^3H_2O) and an energy-controlled and fluid-regulated breakfast was consumed (38 $kJ \cdot kg^{-1}$ and 15 $mL \cdot kg^{-1}$ of plain water).

Packed erythrocytes from the first 10-mL blood sample were incubated, for 30 min at room temperature, with 8 μCi of sodium-radiochromate ($Na^{51}Cr$), washed three times and resuspended to an approximate volume of 10 mL in isotonic saline. At 0800 hours, and after breakfast was completed, a 21-gauge catheter was inserted into the left antecubital vein, and an aliquot of sodium-radiobromide (20 μCi, $Na^{82}Br$) was infused. Between 0930 and 1000 hours, a background blood sample was drawn from the catheter for Evan's blue dye (T-1824). A 21-gauge butterfly needle was placed in the right antecubital vein, through which the T-1824 was infused. At 10 min intervals, a venous blood sample was drawn from this catheter to determine T-1824 concentration over a 30 min basal period. The quantity of each injection was determined gravimetrically. The labelled red cells were infused at the same time as the T-1824. However, due to problems in constituting the count standards, this technique provided unrealistic red cell volumes, resulting in f-cell ratios greater than 1.00. Therefore, these fluid volumes were not analysed further.

Body mass was determined after completing plasma volume assessment, before entering the climate chamber. A urine void was collected and measured. Subject preparation was then conducted in the climate chamber, which was equilibrated to an air temperature of 27.3°C and 60% RH. Subjects assumed a semi-recumbent seated position for at least 60 min before data was collected. At approximately 1200 hours, two resting blood samples were drawn:

(i) Sample One, treated with ethylenediamine tetra-acetic acid (EDTA), was used to determine TBW, ECFV, haematocrit (Hct), and haemoglobin concentration (Hb).

(ii) Sample Two, treated with heparin, was used to quantify plasma concentrations of sodium, potassium, plasma osomolality, total plasma protein, and plasma albumin concentration.

Blood samples, for volume determinations, were centrifuged at 1700 g for 40 min, to minimise trapped plasma, separated into 1 mL aliquots and stored at 4°C for subsequent counting. Samples for plasma constituents were spun at 1700 g for 15 min, the plasma removed and stored at -20°C.

2.4.1 Extracellular fluid volume

Erythrocyte, plasma and urine aliquots were counted, for 15 min each, in the ^{82}Br energy band using a γ counter (Wallac 1480 Wizard™ 3", Finland), on the day of each HST assessment. This was necessary due to the 36.5 hour half life of the nuclide. Activity was expressed in counts per minute. Erythrocyte aliquots and urine samples were counted, since ^{82}Br penetrates into the red blood cells, and is lost from the vascular space during the 3 h equilibration period. Erythrocytes aliquots were haemolysed before counting with saponin. Both counts were used to correct the derived ECFV, which was calculated as:

$$ECFV = \frac{d}{r}\left(\frac{(S_B \times S_d \times S_V) - U_V(U_B - cU_C) - RCV(E_B - cE_C)}{P_B} - PV \right) + PV$$

where: d = protein displacement factor

r = Gibbs-Donnan ratio

S_B = [^{82}Br] of the ^{82}Br standard

S_d = dilution of the ^{82}Br standard

S_V = volume of the $Na^{82}Br$ injection

U_V = volume of urine collected

U_B = [^{82}Br] in the urine collected

c = the ratio of ^{51}Cr detected in the ^{82}Br and ^{51}Cr energy ranges

U_C = [^{51}Cr] in the urine collected

RCV = erythrocyte volume

E_B = [^{82}Br] in the erythrocyte aliquot

E_C = [^{51}Cr] in the erythrocyte aliquot

P_B = [^{82}Br] in the plasma aliquot

PV = plasma volume.

The protein displacement factor (d) was calculated as:

$$d = \frac{100 - (0.073 \times [PP])}{100}$$

w

here

[PP] = plasma protein concentration.

On Day 8, the ^{82}Br nuclide was not available for subjects 1 to 4, due to a reactor shut down at ANSTO. Therefore, ECFV was not measured and ICW and IFV could not be derived.

2.4.2 Total body water

Plasma and urine samples were assessed for ^{3}H activity (12 year half-life) at the end of the complete test battery, as the ^{82}Br activity band would influence counting in the ^{3}H band. A β liquid-scintillation counter was used to count for ^{3}H activity. From the original aliquots, 500 μL was solubilised with 50 μL of 1 M hydrochloric acid, with 10 mL of liquid-scintillation cocktail (Starscint, Packard) added 15 min before samples were counted. TBW was calculated as:

$$TBW = d\left(\frac{(S_H \times S_d \times S_V) - (U_V \times U_H)}{P_H}\right)$$

where d = protein displacement factor;

S_H = [^{3}H] of the ^{3}H standard

S_d = dilution of the ^{3}H standard (see Section 3.1.3.2)

S_V = volume of the $^{3}H_2O$ injection

U_V = volume of urine collected

U_H = [^{3}H] in the urine collected

P_H = [^{3}H] in the plasma aliquot

2.4.3 Plasma volume

Five 1 mL plasma aliquots were placed in cuvettes for the measurement of PV: two background and 10, 20 and 30 min. The second background plasma was combined with diluted T-1824 dye (1:100 distilled water) and placed in a cuvette. The technique for using the Evan's blue dye to measure PV was largely in accordance with the procedures of Greenleaf *et al.* (1980). This technique used an extraction procedure in which the dye was first unbound from the protein albumin, using a detergent and cellulose columns. Due to the stringent dietary requirements, both on the morning of each HST and on the night before each HST, subjects possessed low levels of plasma lipids, with no visual evidence of haemolysis, due to the method of blood collection. Therefore, the above extraction procedure was not used. A major limitation of using a cellulose column is the possibility of not collecting all the dye caught in the cellulose. This was frequently observed in the current investigation, and is indicated by the presence of a bluish tinge of the cellulose. This further supported the use of raw plasma, in which all the dye within the 1 mL sample was present. Similar problems of extraction have been reported by Farjanel *et al.* (1997). The spectrophotometer (Shimadzu UV-1601, Japan) was set to zero using distilled water, and the samples were analysed at a wavelength of 640 nm. PV was calculated as:

$$PV = \frac{S_{Ab} \, x \, S_d \, x \, S_V}{P_{Ab} \, x \, 1.03}$$

w here S_{Ab} = absorbency of T-1824 standard;

S_d = dilution of the T-1824 standard;

S_V = volume of the T-1824 injection;

P_{Ab} = absorbency of T-1824 plasma aliquot;

1.03 = correction.

2.4.4 Other fluid volumes

Additional body-fluid volumes were derived from the measured fluid volumes:

ICW = TBW - ECW. where ECW = extracellular water and is derived from ECFV corrected for total volume of plasma solutes (Chien and Gregersen, 1962).

IFV = ECFV - PV.

BV and RCV were derived using PV and Hct.

2.4.5 Analysis of plasma and urine constituents

Haemoglobin concentrations were measured spectrophotometrically using the cyanmethemoglobin method (Sigma Proc. 525, USA). Haematocrit was measured in triplicate using microcapillary tubes and corrected for trapped plasma (0.98; Chaplin and Mollison, 1952) and whole-body haematocrit (0.94; Maw *et al.*, 1993). Plasma concentrations of total protein and albumin were determined spectrophotometrically using Lowry's method (Lowry *et al.*, 1951), and bromcresol green (Sigma Proc. 631, USA) respectively. Plasma sodium and potassium concentrations were determined using flame photometry (Ciba-Corning 410, England). Plasma osmolality was determined using a vapour pressure osmometer (Wescor 5100C, USA).

2.5 Statistical Analysis

Statistical analyses were performed using data collected on Days 1, 8 and 22 using one-way analysis of variance, with repeated measures. Sources of significant difference were isolated using *Tukey's HSD* statistic. Paired *t*-tests were used for comparisons between Days 1 and 22 for ECFV, ICW and IFV. *Alpha* was set at the 5% level for all analyses. Data are presented as means with standard errors of the means, unless otherwise stated.

3. Results and Discussion

3.1 Compartmental fluid volumes - pre-acclimation volumes

The control (pre-exposure, pre-acclimation) body-fluid volumes are summarised in Table 3. The data are within the expected range for healthy, physically active subjects who typically display a high PV (Oscai *et al.*, 1968; Schmidt *et al.*, 1988). Well trained male runners typically have a BV of up to 107 $mL \cdot kg^{-1}$ (PV of 66 $mL \cdot kg^{-1}$), compared with the BV of 75-85 $mL \cdot kg^{-1}$ in untrained males (Kjellberg *et al.*, 1949; Dill *et al.*, 1974). Accordingly, the control body-fluid volumes of the present sample were slightly greater than the standard reference volumes (International Commission on Radiological Protection, 1975; International Committee for Standardization in Haematology, 1980) but were considered normal for physically active subjects (Pace *et al.*, 1945; Sjöstrand, 1962; Maw *et al.*, 1996). Since muscle is about 75% water, while adipose is only about 10% water, differences in TBW and other fluid volumes, between trained and sedentary subjects may simply be attributable to differences between their respective muscle and fat content. Thus, well-trained subjects tend to have a greater TBW.

Table 3. Pre-exposure and pre-acclimation body-fluid volumes.

Subject	TBW ($mL \cdot kg^{-1}$)	ECFV ($mL \cdot kg^{-1}$)	PV ($mL \cdot kg^{-1}$)	IFV ($mL \cdot kg^{-1}$)	ICW ($mL \cdot kg^{-1}$)	RCV ($mL \cdot kg^{-1}$)
1	723.17	331.56	38.32	293.02	391.83	22.70
2	800.01	285.44	56.45	228.64	514.92	35.04
3	642.34	274.14	34.48	239.47	368.39	18.40
4	686.22	299.74	45.85	251.57	386.81	29.67
5	691.11	370.70	39.41	331.06	320.64	25.88
6	681.21	278.85	48.05	230.54	402.62	29.50
7	569.02	264.33	37.60	226.50	304.92	18.51
8	591.21	263.00	43.75	218.99	328.47	27.39
Mean	673.04	294.22	43.24	251.77	339.16	25.89
S.D.	73.35	51.49	7.24	53.08	43.42	5.78

Abbreviations: TBW = total body water; ECFV = extracellular fluid volume; PV = plasma volume; IFV = interstitial fluid volume; ICW = intracellular fluid volume; RCV = red cell volume.

3.2 Compartmental fluid volumes - post-acclimation volumes

Total body water increased 3.6% from Day 1 to Day 8 (Figure 1; $p<0.05$). However, an additional 13 days of heat acclimation did not elicit a further TBW elevation, resulting in equivalent TBW for Day 8 and Day 22, but still a significantly elevated TBW for Day 22 compared with Day 1 (Figure 1; $p<0.05$). These TBW changes, either for the first week or for the entire acclimation regime, were consistent with some previous observations (Bass *et al.*, 1955; Wyndham *et al.*, 1968), but not others (Senay *et al.*, 1976). However, this hyperhydration was not reflected in total body mass changes: 77.5 kg (±10.35), 77.4 kg (±10.21) and 77.0 kg (±10.07), respectively (Table 1; $p>0.05$). While it may be expected that both indices would show similar trends, it is possible both were influenced by altered body composition. Over a 21-day programme, there is adequate time for such changes to occur, even in well-trained subjects. Thus, a loss of adipose, in combination with an increased lean body mass, could result in negligible mass change but an increased capacity to hold water. It must be highlighted that Senay *et al.* (1976) approximated TBW solely from changes in body mass. Accordingly, the discrepancy between the current data, showing an increased TBW, and the observation of Senay *et al.* (1976) may be ascribed to methodological differences, with the latter open to considerable uncertainty.

This trend was reflected within the extracellular space, and ECFV was significantly elevated after 8 days (Figure 1; $p<0.05$). A further expansion was not seen by Day 22 ($p>0.05$). However, a reactor shut-down prevented ECFV from being measured on Day 8 in four subjects and Figure 1 only includes data for four of the eight subjects. Separate analyses were therefore performed for all eight subjects comparing Days 1 and 22. These data are shown in the inset panels of Figure 1, and revealed that, while the ECFV had reached a plateau by Day 8, it still remained significantly greater on Day 22 than on Day 1 ($p<0.05$). The combination of these analyses demonstrates that the ECFV is expanded within the first eight days of heat acclimation, with this elevation being maintained through the full days of acclimation. This observation contradicts earlier results of Bass *et al.* (1955) and Wyndham *et al.* (1968).

In accordance with evidence from the literature, PV expansion was evident by Day 8 of heat acclimation (Figure 1; $p<0.05$). This volume increase represented 18% of the pre-exposure PV. By Day 22, the volume expansion had increased to 23.6% of the pre-acclimation level. However, while being significantly greater than the PV measured on Day 1, the difference between values for Days 8 and 22 were not significant ($p>0.05$). Although showing a 15.2% IFV elevation from Day 1 to Day 8, this change was not significant when either four (Days 1, 8 and 22) or eight subjects (Days 1 and 22) were used within the analyses (Figure 1; $p>0.05$). However, it is evident that the IFV showed a similar trend to that observed for ECFV (expansion on Day 8, with a volume

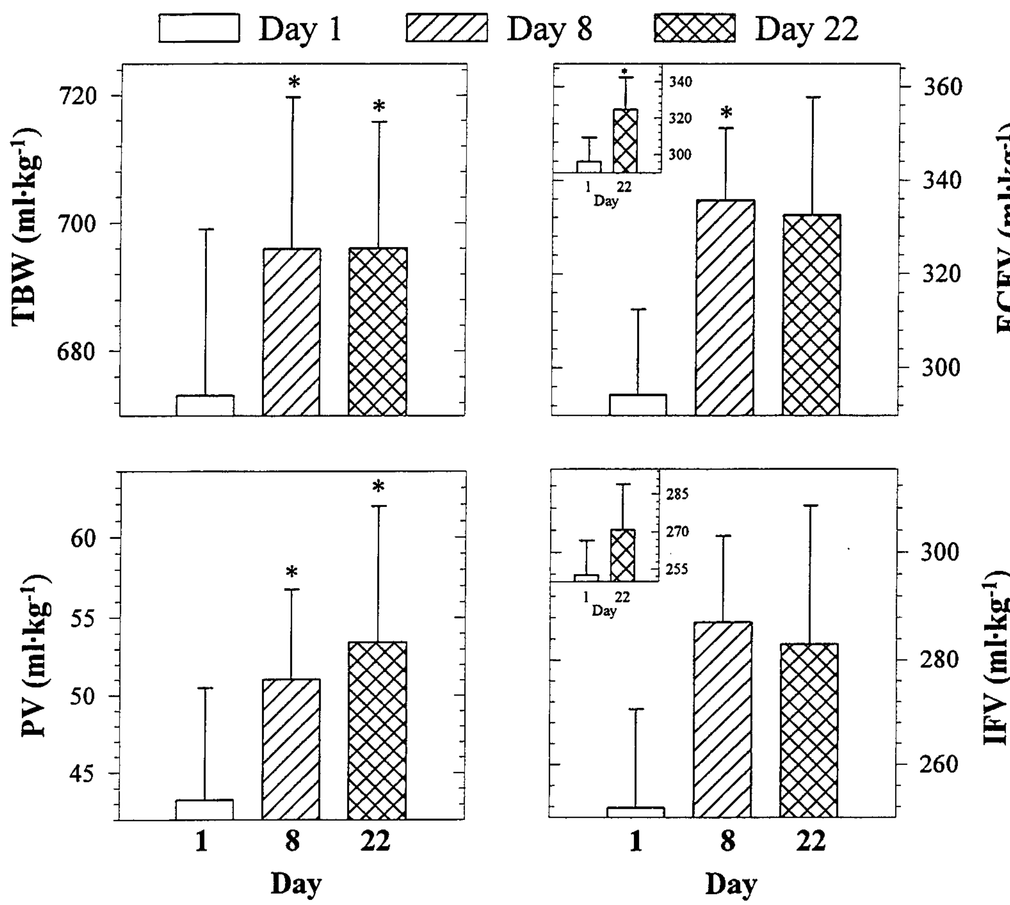

Figure 1. *Resting total body water (TBW), plasma volume (PV), and extracellular fluid volume (ECFV) and interstitial fluid volume (IFV) before, during and after a 21-day heat-acclimation protocol, where subjects were exposed to an air temperature of 39.8°C, with relative humidity controlled at 59.2%. Data are means with standard errors of the means (n=8 for TBW, PV and insert figures; n=4 fpr ECFV and IFV). Significant differences (p<0.05) between Day 1 and Days 8 and 22 are indicated with an asterisk.*

maintenance through to Day 22) but proportionately more of the retained fluid was distributed to the PV.

These results indicate that the retention of fluid within the vascular compartment is a rapid response occurring within 8 days. Several groups have found an even earlier expansion of the PV. For instance, Gillen *et al.* (1991) reported an 8% elevation in PV, 24 hr after a single exercise-training session. Similarly, Senay *et al.* (1976) observed a 9% elevation in PV after 2 days of heat acclimation, plateauing at 23% after 6 to 10 days. The current data imply that the majority of the PV expansion, accompanying exercise and heat acclimation, occurs within the first eight days of the protocol, since the PV for Day 8 did not differ from that observed on Day 22, and this supports the observation of Senay *et al.* (1976).

A unique observation from this investigation is the tendency for the PV to remain elevated from Day 8 to Day 22. Previously, both Senay *et al.* (1976) and Mack and Nadel (1996) have suggested that after longer periods of heat acclimation (up to 20 days), the PV tends to return towards pre-acclimation levels. Early work by Bass *et al.* (1955) and Wyndham *et al.* (1968) found that the PV declined after the fifth day of acclimation. While the present investigation suffered technical limitations with respect to measuring ECFV on four subjects, such difficulties were not experienced for the PV measurements and all eight subjects provided data. However, the total number of subjects used by Bass *et al.* (1955), Wyndham *et al.* (1968) and Senay *et al.* (1976) was only 12. The last two investigations required their subjects to undergo physical training (three and two months respectively) prior to experiencing the heat-acclimation protocol in order to isolate the effects of heat alone. Furthermore, the exercise intensity used in each investigation was kept constant. Thus, unlike the current project, where the workload was adjusted as the subjects became accustomed to the exercise and heat, the physiological stress placed on their subjects would have been reduced as the heat-acclimation protocol progressed. In addition, their subjects drank water *ad libitum,* while the current subjects experienced progressive dehydration throughout each heat exposure. Finally, with the small subject numbers in these earlier investigations, and with the usual non-uniform responses seen within some subjects to heat acclimation, it is perhaps not surprising that the current observations differ from those in the literature.

It may be concluded that, from the present data, the PV is rapidly expanded in response to heat acclimation, with the majority of this expansion occurring within the first 8 days. Further heat acclimation will serve to maintain this expanded volume. However, the conclusion that only eight days of heat acclimation are needed to gain the optimal effects on PV, while appropriate for practical purposes, may be too simplistic from a purely physiological perspective. That is, heat acclimation, from a PV viewpoint, should more correctly be viewed as a continuum, rather like physical fitness, so that while it is possible to induce PV changes very rapidly, perhaps even within less than eight days (Bass *et al.*, 1955), further gains may be elicited with a more protracted protocol. Nevertheless, from a practical perspective, the current data show clear evidence of diminishing returns to scale, similar to that seen with the benefits of endurance training on physical fitness, so that an additional 14 days of combined exercise and heat acclimation did not elicit a significant PV elevation, relative to the expansion observed on Day 8. It is therefore concluded that, for military purposes, an extended heat-acclimation regimen will be of limited physiological benefit.

The intracellular compartment was unchanged during the heat-acclimation protocol. The ICW remained stable (Figure 2; $p>0.05$), and the RCV was similarly unaltered, although it tended towards an expansion during acclimation (Figure 2; $p>0.05$). Thus, with an expansion of the TBW, and an elevation in the ECFV without a concomitant reduction in either the ICW or the RCV, these data indicate that the body-fluid enlargement was restricted to the extracellular compartment. We have previously demonstrated that endurance-trained athletes showed a whole-body hyperhydration (Maw *et al.*, 1996), however, the current data strongly suggest that a 21 day combined exercise and heat acclimation results in an enlargement of only the extracellular space. Bass *et al.* (1955) found that the ECFV was expanded at the expense of ICW. This is refuted by the present observations, which clearly show that the ECFV is preferentially enlarged following heat acclimation.

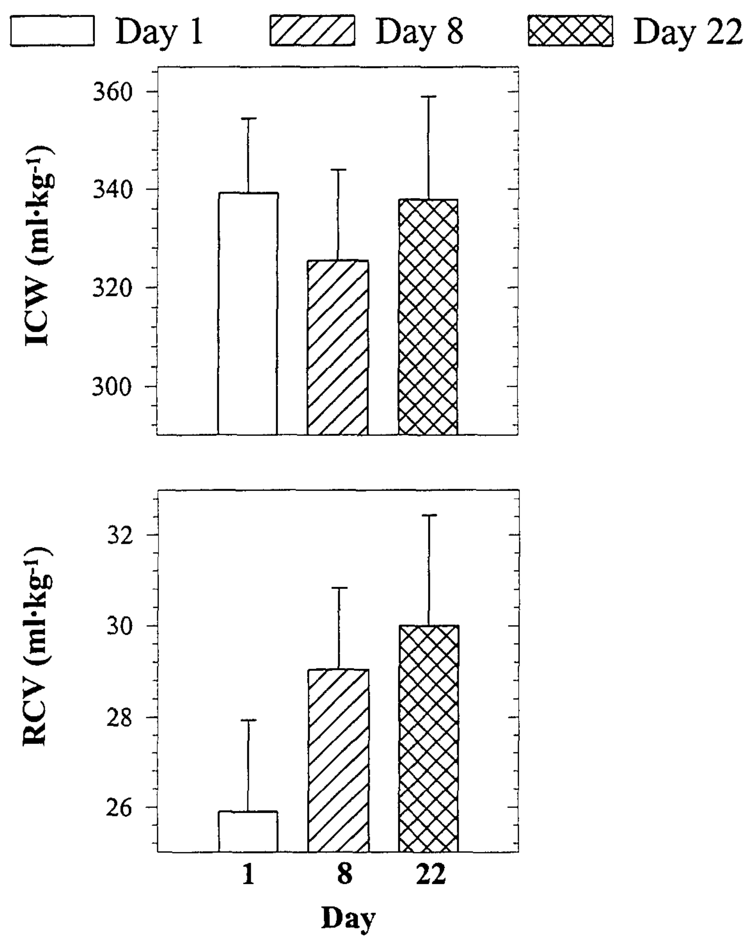

Figure 2. *Resting intercellular fluid volume (ICW) and red cell volume (RCV) before, during and after a 21 day heat-acclimation protocol, where subjects were exposed to an air temperature of 39.8°C with relative humidity controlled at 59.2%. Data are means with standard errors of the means (n=8 for RCV; n=4 for ICW).*

3.3 Plasma constituents

The total plasma sodium, potassium, albumin and consolidated proteins showed step-wise increments from Days 1 to 22, with plasma osmolality mirroring these changes (Figure 3, $p<0.05$). However, when expressed as concentrations, each plasma constituent was unchanged across the heat-acclimation regime (Figure 3, insets; $p>0.05$). Since the PV was significantly expanded on Days 8 and 22 (Figure 1), these changes in the plasma concentrations are consistent with those that are observed during an iso-osmotic volume expansion.

The greater expansion of the PV when compared to the IFV may be attributed to differences in protein content, particularly albumin which accounts for 75% of the oncotic pressure of plasma, since water and electrolytes can freely pass between extracellular compartments. Albumin slowly leaks from the plasma into the interstitium from the capillary. However, it cannot pass directly from the interstitium to the plasma, but must make that journey via the lymphatic system. Since the total protein concentration in the IFV is far less than that observed in the plasma, it would seem that with the iso-osmotic expansion of PV, the whole ECFV is expanded. The selectively greater expansion of PV is most probably due to its increased total protein and albumin content. However, it is uncertain whether this protein is derived from the interstitium, as suggested by Senay *et al.* (1976), or is due to an increased synthesis of new protein (Carraro *et al.*, 1990).

Both components of the extracellular compartment (PV and IFV) experience a volume increase if electrolytes are retained. However, since the vascular walls are freely permeable to both water and electrolytes, then a PV expansion may be primarily attributed to an influx of proteins into the vascular space. Luetkemeier *et al.* (1994) suggested that about 60% of such a PV expansion could be attributed to total protein, while the other 40% may be electrolyte driven. In the current investigation, about 83% of the observed PV expansion on Day 8 could be explained by total electrolyte retention which occurs within both the interstitium and the plasma. After 22 days, such retention could explain only 53% of the PV change.

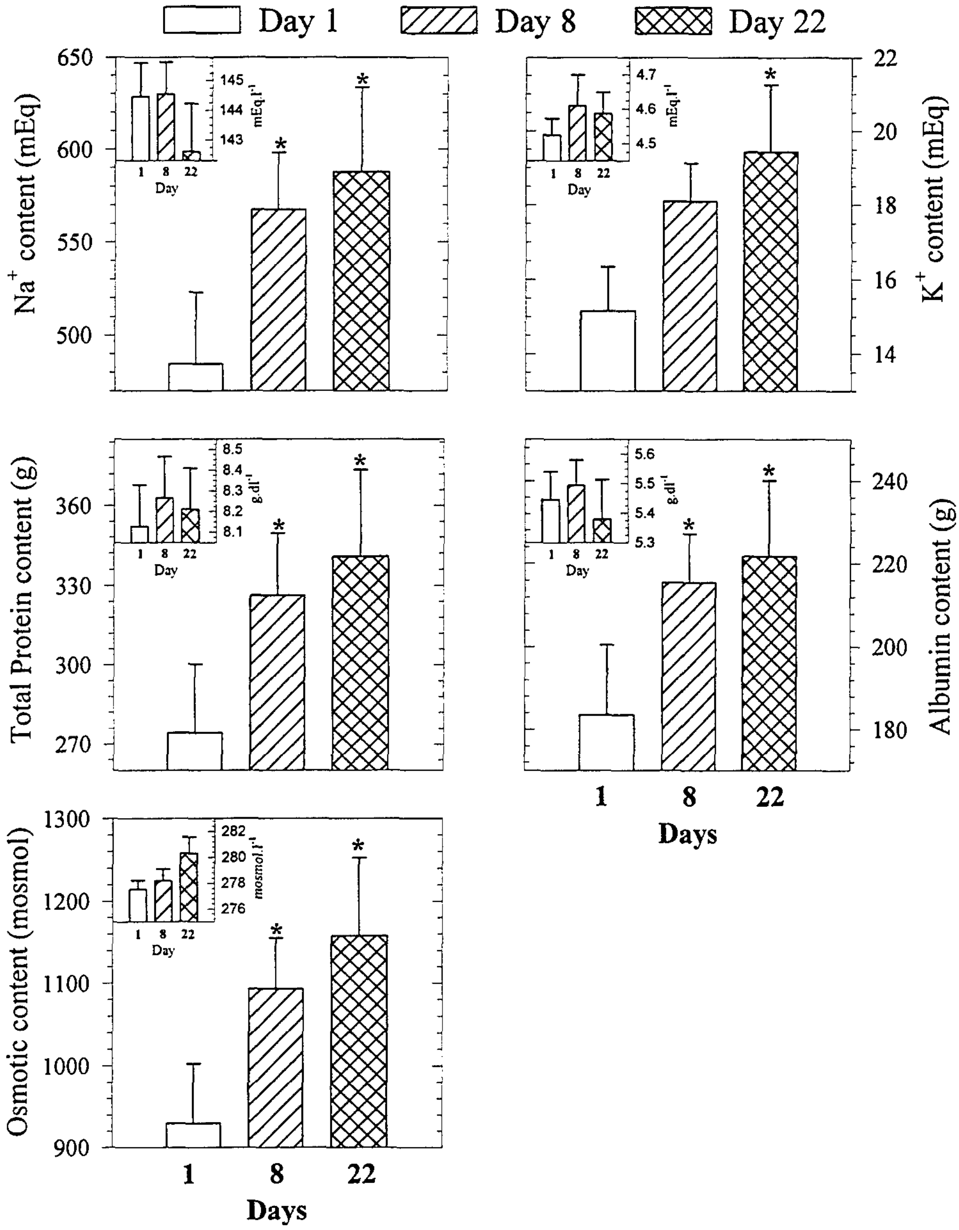

Figure 3. *Plasma electrolytes, proteins and osmolality in absolute (main graphs) and relative terms (inset graphs). Data were obtained from resting blood samples taken before, during and after a 21 day heat-acclimation protocol, where subjects were exposed to an air temperature of 39.8°C with relative humidity controlled at 59.2%. Data are means with standard errors of the means (n=8). Significant differences (p<0.05) between Day 1 and Days 8 and 22 are indicated with an asterisk.*

After 8 days of heat acclimation, plasma albumin increased by 31.9 g (17.4%). Given that one gram of albumin can attract 18 mL of water, the increase in plasma albumin alone could account for a PV change of about 575 mL, which was 99% of the total PV change observed on Day 8 (580.9 mL). After 22 days, the expansion of the PV which may be attributed to an increased albumin content, was approximately 88% of the expansion in PV. Therefore, while both electrolyte and plasma protein changes in the interstitium and the plasma play significant water-retention roles, it is believed that, since the PV increased to a greater extent than did the IFV, the differential changes in these two extracellular compartments are due to an influx of protein into the vascular space.

4. Conclusions

The current data indicate that a 21 day, combined exercise and heat acclimation results in an enlargement of the total body water content, with this fluid retention being primarily within the extracellular space. Furthermore, the plasma component of this fluid space increased more than the interstitial fraction. A unique observation was the tendency for the PV to remain elevated from Days 8 to 22, indicating that further heat acclimation will maintain this expanded volume.

On the basis of plasma protein and electrolyte assays, it is concluded that, while a retention of electrolytes may account for a significant part of the extracellular expansion, the differential changes in the fluid volumes of the two extracellular compartments were due to an influx of protein into the vascular space.

It may be concluded that the PV is rapidly expanded in response to heat acclimation, with the majority of the expansion taking place within the first 8 days. This volume increase will serve a protective function during subsequent heat exposure. For military purposes, an extended heat-acclimation regime will be of limited benefit from the perspective of body fluid changes induced by acclimation.

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The Measurement of Human Body-Fluid Volumes: Resting Fluid Volumes Before and After Heat Acclimation.

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19. ABSTRACT

The study evaluated changes in human body-fluid volumes accompanying a 21-day heat-acclimation regime. Eight unacclimatised males, were studied for 22 consecutive days, including 19 days of heat exposure. Heat stress tests and acclimation trials were undertaken at 40°C and relative humidity 60%. Total body water increased from Day 1 to 8 but a further 13 days acclimation did not elicit a further increase. This trend was reflected within the extracellular space, with the extracellular fluid volume significantly elevated after 8 days, without further expansion beyond Day 8. A plasma volume expansion of 18% was evident by Day 8, and, by Day 22, had increased to 23.6% of pre-acclimation levels. The interstitial fluid volume increase from Day 1 to Day 8 was not significant. Proportionately more of the extracellular fluid volume expansion occurred within the plasma volume and was ascribed to increases in the total plasma protein which accompanied acclimation. The intracellular volume remained stable and erythrocyte volume was similarly unaltered. Body-fluid enlargement was restricted therefore to the extracellular compartment. It was concluded that the plasma volume was expanded rapidly in response to heat acclimation, with the majority of this expansion taking place within the first 8 days. A unique observation was the tendency for the plasma volume to remain elevated from Day 8 to Day 22, indicating that further heat acclimation would maintain this expanded volume.

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