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Human body-fluid distribution: postural, thermal, and exercise stress

Graeme J. Maw

University of Wollongong

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HUMAN BODY-FLUID DISTRIBUTION:
POSTURAL, THERMAL, AND EXERCISE STRESS

A thesis submitted in fulfilment of the requirements for the award of the degree

DOCTOR OF PHILOSOPHY

from

UNIVERSITY OF WOLLONGONG

by

GRAEME J. MAW, MSc, BSc (Hons)

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BIOMEDICAL SCIENCE
1994
Abstract

Human body-Fluid Distribution:
Postural, Thermal, and Exercise Stress

Although it is known that body-fluid distribution is dependent on posture, environment, and exercise, previous research has concentrated on intravascular fluid shifts, describing redistributions through changes in venous haematocrit and haemoglobin concentration. It was the purpose of this investigation to examine the distribution of fluid throughout the body during postural, thermal, and exercise stress, using the dilution of radionuclides.

Body-fluid distribution was measured in eight physically active males, using the simultaneous dilution of 450 µCi of tritiated water, 20 µCi of radiobromine, 2 µCi of radioiodinated fibrinogen, and 8 µCi of radiochromated erythrocytes, to measure total body water, extracellular fluid, plasma (PV), and erythrocyte (RCV) volumes, respectively.

During 30 min of upright rest following sitting, intravascular volume (BV) decreased by 406 ml (± 89; mean ± SEM), causing a concurrent expansion of interstitial volume (655 ± 165 ml); BV tended to increase (89 ± 82 ml) during supine rest, this time depleting the interstitium (-86 ± 145 ml). Changes in BV were largely accounted for by changes in PV (-233 ± 64 and +52 ± 70 ml, respectively), probably mediated by changes in capillary hydrostatic pressure.

BV decreased during 30 min of seated cool exposure (-302 ± 76 ml; 14°C), and increased during heat exposure (124 ± 150 ml; 36°C), although considerable intersubject variation occurred in the heat. Fluid shifts were again accounted for by changes in PV (-205 ± 60 and +108 ± 123 ml, respectively), attributed to changes in venomotor tone.

BV decreased during 10 min of cycle exercise, by 287 (± 60), 114 (± 86), and 470 (± 192) ml in cool, control, and hot environments, respectively. BV subsequently recovered in cool and control as exercise progressed, but remained depleted throughout 50 min in the heat, reflecting increased peripheral blood flow and general dehydration; 861 (± 100) ml of fluid were lost from the body, drawn proportionately from the intra- and extracellular compartments. In all three environments changes in BV were largely accounted for by changes in PV, although RCV also decreased in each case, by a mean of 88 (± 13) ml at minute 20.

Under all circumstances, changes in BV were considered important in the regulation of cardiovascular strain, while changes in the dynamics of blood flow apparently distorted the relationship between whole-body and venous haematocrits. Thus, it was considered that changes in venous haematocrit did not accurately reflect intravascular fluid shifts during cycle exercise.
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and the eight young, healthy males, who willingly gave their time, blood, and friendship for the pursuit of science and the opportunity to sit in an air-conditioned room.

Finally, I wish to thank Mary and Fiona, for their love and support, their voices on the phone, and the debt which one day might even be repaid.

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Clay lies still, but blood's a rover;
Breath's a ware that will not keep;
Up, lad: when the journey's over
Then there'll be 'nough time to sleep.

A.E. Hourman: "Reveillé"

With love to Mary,
Across the miles.
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Chapter One
Introduction and Hypotheses

Water is the most abundant substance in the human body, accounting for between 50% and 70% of adult body mass (see Sheng & Huggins, 1979). As such, it is spread throughout the body, encompassing both intra- and extracellular compartments, within and outside the vascular space. Approximately 55% of body water is contained within cells, with the remaining 45% being extracellular, divided between both the interstitial (38%) and the plasma (7%) phases; erythrocytes account for approximately 5% of total body water, making a total of 12% contained within the vascular space (International Commission on Radiological Protection, 1975; Figure 1.1).

Throughout the body, the water is critically involved in a variety of functions, including electrolyte equilibration, thermoregulation, and nutrient and gas transportation. Hence, its distribution is carefully regulated, as a disturbance can result in impairment of physiological function. For example, a disturbance in the distribution of fluid between the intra- and extracellular compartments can change membrane potential, and therefore impair the function of the affected cells (Sjøgaard et al., 1985); a loss of fluid from the intravascular compartment can decrease the drives for sweating and vasodilation, and hence impair thermoregulation (Fortney et al., 1981; see Morimoto, 1990, for review); and a general loss of body fluid can impair physical performance, to the point of exhaustion or ultimately to the point of death (Shibolet et al., 1967; see Sawka, 1992, for review).

The regulation of body-fluid distribution is effected by a combination of hydrostatic, osmotic, and oncotic forces, which balance across the cellular and vascular membranes to maintain equilibria between the various fluid compartments (Starling, 1896; see Sjöstrand, 1962, and Aukland & Nicolaysen, 1981, for reviews). However, the magnitude of these forces is dependent on such circumstances as posture, environmental temperature, and exercise state, and hence a change in circumstances can also cause a change in the distribution of body fluid. For example, moving from a supine to an upright posture causes an increase in extravascular fluid volume (Waterfield, 1931; Stick
Total body water
\( \sigma = 600 \text{ ml kg}^{-1} \)
\( \varphi = 500 \text{ ml kg}^{-1} \)

Intracellular fluid volume
\( \sigma = 340 \text{ ml kg}^{-1} \)
\( \varphi = 300 \text{ ml kg}^{-1} \)

Extracellular fluid volume
\( \sigma = 260 \text{ ml kg}^{-1} \)
\( \varphi = 200 \text{ ml kg}^{-1} \)

Extravascular cells
\( \sigma = 310 \text{ ml kg}^{-1} \)
\( \varphi = 275 \text{ ml kg}^{-1} \)

Erythrocyte volume
\( \sigma = 30 \text{ ml kg}^{-1} \)
\( \varphi = 25 \text{ ml kg}^{-1} \)

Plasma volume
\( \sigma = 40 \text{ ml kg}^{-1} \)
\( \varphi = 40 \text{ ml kg}^{-1} \)

Interstitial fluid volume
\( \sigma = 220 \text{ ml kg}^{-1} \)
\( \varphi = 160 \text{ ml kg}^{-1} \)

Intravascular (blood) volume
\( \sigma = 70 \text{ ml kg}^{-1} \)
\( \varphi = 65 \text{ ml kg}^{-1} \)

**Figure 1.1** The normal volume and distribution of fluid within adult male (\( \sigma \)) and adult female (\( \varphi \)) bodies (after the International Commission on Radiological Protection, 1975, and the International Committee for Standardization in Haematology, 1980).
et al., 1993), due to an extravasation of plasma following an increase in capillary hydrostatic pressure (Pollack & Wood, 1949). Moving from a cold to a warm environment can expand plasma volume (Bazett et al., 1940; Harrison et al., 1981), due to a reduction in capillary hydrostatic pressure following rapid venodilation (Harrison, 1985). Exercise can reduce the total volume of intravascular fluid (Kaltreider & Meneely, 1940; Åstrand & Saltin, 1964), while simultaneously increasing the volume of intramuscular fluid, due to the accumulation of intramuscular metabolites and the resultant osmotic gradient (Jacobsson & Kjellmer, 1964; Sjøgaard & Saltin, 1982).

The effects of posture, environmental temperature, and exercise on body-fluid distribution are apparently interactive, such that, the accumulation of extravascular fluid during upright rest is reversed when upright exercise is allowed (Stick et al., 1993). Similarly, the expansion of intravascular volume during heat exposure is reversed during seated exercise in the heat (Adolph et al., 1969; see Harrison, 1985, for review). The decrease in intravascular volume during exercise is further dependent on posture, with the decrease in blood volume being greater during seated exercise than during an equivalent upright task (Diaz et al., 1979; Gore et al., 1992; see Senay & Pivarnik, 1985, for review).

To date, little is known about the effect of posture on the extravascular distribution of body fluid, or about the effect of exercise in the cold on the distribution of fluid throughout the body. Investigation of extravascular fluid volume during upright rest, for example, has been confined to measurements of leg volume rather than actual measurements of internal fluid volumes (Waterfield, 1931; Stick et al., 1993), and has therefore been unable to distinguish between changes in the intra- and extracellular fluid compartments. Investigation of exercise in the cold has largely been confined to measurements of circulatory, metabolic, and thermoregulatory functions (Claremont et al., 1975; Suzuki et al., 1980; Kruk et al., 1990), generally ignoring the response of body-fluid distribution (Dann et al., 1990); certainly, no attempt has been made to directly compare fluid distribution during exercise in the cold with that during exercise in the heat.
In addition, information concerning body-fluid distribution during exercise in the heat is largely limited to descriptions of intravascular volumes, with only occasional concern for the distribution of the remaining body-fluid compartments. Both Costill et al. (1976) and Nose et al. (1988) examined the effects of exercise in the heat on the volume of fluid in the intra- and extracellular compartments, but took measurements only before and after prolonged exercise, and therefore ignored the interim changes associated with the onset of exercise. Furthermore, in both studies, extracellular fluid volume was calculated from changes in plasma chloride concentration, which may itself have been susceptible to changes in membrane potential during the exercise period (Sjøgaard & Saltin, 1982). Hence, the effect of exercise on the distribution of extravascular fluid is currently unclear, regardless of the environmental temperature.

The exact effect of exercise, and of posture and environmental temperature, on the distribution of intravascular fluid may also still be undetermined, due to possible flaws in the method currently favoured for its measurement. Changes in intravascular fluid volumes are currently calculated from changes in venous haematocrit (Hctv) and haemoglobin concentration ([Hb]; after Elkinton et al., 1946, Strauss et al., 1951, Van Beaumont, 1972, or Dill & Costill, 1974), rather than from volumes measured using various dilution methods. Thus, resultant calculations may be erroneous if the volumes of plasma and erythrocytes change simultaneously (Dill & Costill, 1974; Greenleaf et al., 1979), or if there is a change in the relationship between Hctv and the whole-body haematocrit (Hctw; Harrison et al., 1982).

It is known that Hctw is typically greater than Hctv, possibly due to the presence of erythrocyte deficient blood in the microcirculation (Hahn et al., 1942; Sawka et al., 1992); however, it is presently not known whether this relationship is constant during postural, thermal, or exercise stress. Apparently only Fricke (1965) and Costill and Saltin (1974) have examined the relationship during short-term stress, and while the former found an increase in the ratio between Hctw and Hctv (f-ratio) during exercise, the latter found no change during combined exercise and heat stress. Thus, it cannot presently be postulated whether changes in Hctv reflect changes in the distribution of fluid throughout the circulation, and therefore whether Hctv can be used to determine
changes in intravascular fluid volumes.

It appeared, therefore, that current knowledge concerning the effects of posture, environmental temperature, and exercise on the distribution of intravascular fluid needed verifying using more direct measurements than those afforded through Hct, and [Hb]. Furthermore, it appeared necessary to investigate the effects of postural, thermal, and exercise stress on the distribution of fluid outside the vascular space, including a comparison of the effects of heat and cool on the distribution of body fluid during both rest and exercise.

1.1 Aims and Hypotheses

It was the purpose of this investigation to examine the distribution of body fluid during postural, thermal, and exercise stress, using the dilution of radionuclides to simultaneously measure total body water, extracellular fluid volume, and plasma and erythrocyte volumes. Body-fluid distribution was to be measured during supine, seated, and upright rest in a control environment, during short-term seated exposure to hot, control, and cool environments, and during moderate, seated exercise in hot, control, and cool environments.

It was therefore necessary to develop a radionuclide dilution method that would enable serial measurements of body-fluid distribution during short-term stress, and that could be used to repeatedly assess subjects within a relatively short calendar period. It was proposed to develop a method using the simultaneous dilution of tritiated water, radiobromine, radioiodinated serum fibrinogen, and radiochromated erythrocytes to measure total body water, extracellular fluid volume, and plasma and erythrocyte volumes, respectively, and hence to calculate the volumes of intracellular, interstitial, and intravascular fluid.
It was hypothesised that:

1.1.1 Postural manipulation would cause intravascular fluid volume to increase during supine compared to seated rest, and to decrease during upright compared to seated rest, while interstitial fluid volume would decrease during supine rest, and would increase during upright rest;

1.1.2 Thermal stress during seated rest would cause intravascular fluid volume to increase during heat exposure compared to a control environment, and to decrease during cool exposure compared to a control environment, while interstitial fluid volume would decrease during heat exposure, and would increase during cool exposure;

1.1.3 Exercise would cause total body water, intracellular water, extracellular fluid, intravascular, plasma and erythrocyte volumes to decrease, with the decreases being progressively greater during exposure to a hot compared to control and cool environments;

1.1.4 Body-fluid losses, consisting of sweat and respiratory water, would increase during exercise in a hot compared to a control environment, and would decrease during exercise in a cool compared to a control environment.
1.2 References


Clinical Investigation, 30, 862-868.


Chapter Two
Measurement of Body-Fluid Distribution:
A History of Methods

The measurement of body-fluid distribution has interested medical, environmental, and exercise scientists, resulting in the proposal and refinement of a multitude of measurement methods. For example, nutritionists currently measure body-fluid distribution using an application of bioelectrical impedance (Kushner & Schoeller, 1986), while exercise scientists examine changes in intravascular fluid distribution by monitoring changes in venous haematocrit (Hct,) and haemoglobin concentration ([Hb]; after Strauss et al., 1951, and Dill & Costill, 1974). In contrast, the original determinations of body-fluid distribution were made using relatively direct methods, either through dissection and dehydration, or using the dilution of various exogenous or innate tracers (see Lawson, 1962, for review).

2.1 Early Measurements of Blood Volume

According to Keith et al. (1915), direct measurements of body-fluid distribution date from the mid-nineteenth century, when Haller (1854) and Bischoff (1856) bled several criminals to death to determine their blood volume. The blood was collected, and its volume was assumed to equal that in the intact circulation. However, the method underestimated circulating blood volume, as it had previously been shown that further blood could be removed by flushing the circulation with water after bleeding had ceased (Lehman & Weber, 1850, Welcker, 1854, see Erlanger, 1921). The method was also obviously unsuitable for general use, and for the measurement of blood volume in vivo.

In vivo measurement of blood volume reputedly began some years earlier, when Valentin (1847) developed a dilution method using the infusion into the circulation of a measured volume of distilled water (see Keith et al., 1915). The method required that the water equilibrated throughout the circulation, with its concentration subsequently measurable in a small blood sample. It was essential that the water remained entirely within the circulation between infusion and sampling, and that its effect on blood
concentration was precisely measured. Blood volume was then calculated by comparing the known dose of water infused with the resultant change in blood concentration, assuming that the water had accessed the entire vascular space. Thus, the method highlighted the general principle of dilution, whereby an unknown volume was determined by comparing the volume and concentration of a known tracer with its subsequent concentration in the unknown space. The unknown volume \( V_U \) was then calculated from:

\[
V_T \times c_T = V_U \times c_U
\]

\[
\Rightarrow V_U = \frac{V_T \times c_T}{c_U}
\]

where: 
- \( V_T \) = volume of the infusion;
- \( c_T \) = concentration of the tracer in the infusion;
- \( c_U \) = concentration of the tracer in the unknown sample.

Unfortunately, with distilled water, the infusion was not wholly contained within the circulation prior to blood sampling, and hence its dilution volume overestimated the volume of blood. Investigation therefore turned to the use of alternative dilution tracers to gain a more accurate measurement of circulating blood volume.

Various attempts were made to measure blood volume using the dilution of innate substances, such as sodium chloride, glucose, and serum, but in each case it proved difficult to determine the effect of the infusion on the existing concentration of the substance in the body (see Erlanger, 1921, for review). Hence, in 1899, Haldane and Smith (1899) introduced a method of measuring blood volume, using the dilution of carbon monoxide, as ordinarily the gas was largely absent from the body. The method required the rebreathing of carbon monoxide until it reached equilibrium throughout the circulation; the volume of inhaled gas was carefully controlled, and its subsequent concentration in the blood was precisely measured. However, a small fraction of the inhaled gas also escaped from the circulation, to become associated with tissue myoglobin, and hence this method also tended to overestimate blood volume.

The development of dye-dilution methods supposedly obviated the extravasation of
dilution tracers, as the dyes were thought to remain within the circulation sufficiently long to reach a stable equilibrium (Keith et al., 1915; Dawson et al., 1920). However, regardless of the chosen dye, the measurement of its concentration proved problematic, as its resultant colour was affected by the composition as well as the volume of the circulating blood (Gregersen et al., 1935; Senn & Karlson, 1958). This problem was not overcome until the introduction of radionuclide dilution tracers, the concentration of which could be measured precisely using radiation scintillation counters.

2.2 Measurement of Body-Fluid Volumes Using Radionuclide Dilution
Radionuclide dilution tracers also proved suitable for the measurement of body-fluid compartments other than the vascular space, as they could be attached to different sized molecules which effectively discriminated between the various fluid compartments. For example, by attaching tritium (³H) to the inulin molecule it proved possible to estimate extracellular fluid volume, as the inulin freely crossed the vascular membrane but could not penetrate into cells (Sjøgaard & Saltin, 1982). Conversely, by attaching a radionuclide to erythrocytes it was possible measure erythrocyte volume, as the combination was confined wholly within the vascular space (Ebaugh et al., 1953). Thus, it proved possible to measure not only blood volume, but also the volumes of circulating erythrocytes and plasma, and the volumes of extracellular fluid and total body water.

2.2.1 Measurement of Erythrocyte Volume
Hahn and Hevesy (1940) were apparently the first to use radionuclides to measure the distribution of human body fluid, labelling erythrocytes in vivo with radiophosphorous (³²P) to measure the volumes of circulating blood and erythrocytes. However, the labelling proved unstable during the course of volume measurement, making it difficult to determine the resultant dilution volume; hence, ³²P was soon superseded by the use of radioiron (⁵⁹Fe; Hahn et al., 1941, 1942). The labelling of erythrocytes with ⁵⁹Fe was itself problematic, requiring prolonged incubation and therefore the use of donor cells. In contrast, the later use of radiochromate (⁵¹Cr) enabled the labelling of autologous cells, as the radionuclide attached to erythrocytes within 60 min of incubation at room temperature (Gray & Sterling, 1950; Sterling & Gray, 1950). Specifically, the gamma-
emitting ($\gamma$)\footnote{Gamma emissions ($\gamma$) are those generated in the form of rays, when an unstable atomic nucleus transforms a neutron into a proton and an electron but maintains both elements within the nucleus, resulting in expendable radioactive energy (see Wang, 1969).} nuclide attached to the beta-polypeptide chain of the erythrocyte’s globin molecule, and remained there throughout the cell’s natural life (Ebaugh \textit{et al.}, 1953; Pearson 1963). Thus, it was trapped within the cell, and therefore within the circulation, making circulatory [$^{51}\text{Cr}$] essentially constant for up to 24 h following infusion (Sterling & Gray, 1950; Dyrbye & Kragelund, 1970). It later proved possible to label erythrocytes with $^{51}\text{Cr}$ during just 15 min of incubation at 37°C, and thus the method was adopted by the International Committee for Standardization in Haematology (ICSH, 1973, 1980).

The currently recommended method involves the incubation of freshly collected erythrocytes with $^{51}\text{Cr}$ for 15 min at 37°C, followed by an equilibration period in the circulation of at least 20 min following injection (ICSH, 1980). Erythrocyte volume is then calculated by comparing the $^{51}\text{Cr}$ concentration of the injection with that in a sample of erythrocytes, which can be collected at anytime within the subsequent 24 h.

\textbf{2.2.2 Measurement of Plasma Volume}

A similar radionuclide dilution method, also using a $\gamma$-emitting nuclide, was developed for the measurement of plasma volume, using the dilution of radioiodinated serum albumin (RISA; Fine & Seligman, 1943). However, the method was limited by the constant loss of tracer albumin across the vascular membrane (Wasserman & Mayerson, 1951; see Landis & Pappenheimer, 1963, for review), such that its dilution volume extended beyond the vascular space. Several methods were proposed to compensate for this extravasation, including the multiplication of [RISA] by a standard correction factor (Andersen & Gabuzda, 1964; Ekelund & Holmgren, 1964); however, it was the extrapolative technique of Erlanger (1921) that was finally adopted by the ICSH (1980).

Briefly, the technique involved the measurement of [RISA] in several postinfusion blood samples, and subsequent extrapolation to estimate the theoretical [RISA] at the time of infusion (Figure 2.1). This concentration was taken to represent that which would have occurred had tracer mixing been instantaneous, or had the tracer been confined solely
Figure 2.1 The loss of radioiodinated serum albumin (RISA) from the circulation, prior to and during cycle exercise (manipulation), extrapolated to calculate initial and subsequent plasma volumes (from Maw, 1992). Initial plasma volume is calculated using the estimated theoretical [RISA] prior to its extravasation ($P_{10}$), while subsequent volumes are calculated by comparing [RISA] predicted from extrapolation of the curve ($P_{lp}$), with that measured in the plasma at the corresponding time ($P_{lm}$).
within the circulation. The theoretical concentration was then compared with the concentration of the RISA infusion to calculate plasma volume.

Despite the use of this extrapolation technique, plasma volume measured using RISA dilution still exceeded those measured using the dilution of larger tracers, such as radiiodinated serum fibrinogen (RISF) and macroglobulin (Andersen, 1962; Andersen & Gabuzda, 1964; Larsen, 1968; Bent-Hansen, 1989). Thus, it appeared that the dilution of RISA still exaggerated plasma volume, possibly due to a rapid extravasation of the tracer during its initial mixing period (Jaenike et al., 1957; Baker, 1963; Larsen, 1968; Swan & Nelson, 1971). Bent-Hansen (1991; Bent-Hansen & Svendsen, 1991) recently confirmed that RISA escaped from the circulation faster during its first few minutes of mixing than it did during the remaining period of equilibration, and also that this initial rate was faster than the corresponding loss of RISF. Hence, the ICSH (1980) suggested that a more accurate measurement of plasma volume might be achieved using the dilution of a larger tracer, such as radiolabelled serum fibrinogen.

The movement of RISA across the vascular membrane also hindered its use for the measurement of acute changes in plasma volume, as it was not thought possible to distinguish between simultaneous movements of the tracer and of plasma fluid. However, Gibson and Evans (1937), and later Harrison and his colleagues (1975, 1976), reported a method of calculating acute changes in plasma volume by comparing a predicted [RISA] with that measured in the blood at the corresponding time. The method required that the rate of RISA loss from the circulation was established prior to experimental manipulation, and that it was then extrapolated through the experimental period, to predict the concentrations that would occur if plasma volume remained constant (Figure 2.1). It was assumed that the rate of extravasation remained constant throughout the experiment, so that the difference between the predicted and measured [RISA] was indicative of a change in plasma volume. Harrison and Edwards (1976) showed that the rate of RISA extravasation remained constant during both thermal and exercise stress, and hence that the method could be used to measure resultant changes in plasma volume; however, the rate apparently changed during postural manipulation, such that RISA was lost at a faster rate when subjects were supine than when they sat
upright. The method was therefore thought fallible during postural manipulation, when resultant changes in plasma volume may have been exaggerated. In contrast, the rate of extravasation of fibrinogen-sized molecules is minimal at all times, and is apparently unaffected by such stresses as venous congestion and exercise (Grotte, 1956; Arturson & Kjellmer, 1964; Renkin, 1979). Indeed, the volume of circulating fibrinogen is essentially unchanged during a variety of environmental and exercise stresses (De Lanne et al., 1958), and it is therefore considered that the dilution of RISF could be used to measure acute changes in plasma volume during postural, as well as thermal, and exercise stress.

The process would involve the infusion of a known amount of RISF, and the collection of subsequent plasma samples, from which to determine the rate of RISF extravasation and, through extrapolation, the initial volume of plasma (after the ICSH, 1980). The extravasation of RISF would then be extrapolated through the experimental period, to predict subsequent [RISF] that would occur if plasma volume remained constant. The actual plasma volumes would be estimated by comparing the predicted [RISF] with those actually measured in the corresponding plasma samples (after Gibson & Evans, 1937, and Harrison & Edwards, 1976).

2.2.3 Measurement of Extracellular Fluid Volume

Similar to the measurement of plasma volume, the measurement of extracellular fluid volume was hampered by the unavailability of a tracer that was both distinct from innate body substances and wholly confined to the extracellular compartment. Indeed, many of the early measurements of the extracellular compartment were frustrated by the loss of the infused tracer, either excreted from the body or subject to cellular penetration. For example, thiocyanate, iodide, bromide, and sulfate, which all mimicked the body’s chloride (Cl) space, rapidly appeared in urine, gastrointestinal fluid, and erythrocytes, and hence exaggerated the extracellular space (Gamble et al., 1953; Walser et al., 1953).

In contrast, larger molecules, such as sucrose, mannitol, and inulin, were largely confined within the extracellular space, but were slow to diffuse across the vascular membrane (Lavietes et al., 1936); hence, they required prolonged equilibration, frequently through constant tracer infusion, making them impractical for general use (see
Gradually, the use of radiobromine ($^{82}\text{Br}$) became popular, as, despite its rapid loss from the extracellular compartment, it equilibrated within 3 h, and could be measured easily using a $\gamma$ scintillation counter (Berson & Yallow, 1955; Staffurth & Birchall, 1960; Spears et al., 1974). In addition, its deficiencies could be corrected for, to produce a reliable estimation of extracellular fluid volume. Leth and Binder (1970), for example, showed that with corrections for urinary loss, intracellular penetration, and imbalance across the vascular membrane*, the $^{82}\text{Br}$ dilution volume varied little during the course of a day, or between repeat assessments of the same subject.

Recently the use of $^{82}\text{Br}$ to measure extracellular fluid volume has been superseded by the use of radiochromated ethylenediamine tetra-acetic acid ($^{51}\text{Cr-EDTA}$), as the latter tracer is slow to enter gastrointestinal fluids and does not penetrate into cells (Ljungqvist et al., 1990; G. Heigenhauser, personal communication, January 28, 1993). However, $^{51}\text{Cr-EDTA}$ cannot be used in conjunction with the measurement of erythrocyte volume, as its $^{51}\text{Cr}$ label would prevent detection of the radiochromated erythrocytes. On such occasions, it is necessary to measure extracellular fluid volume using the dilution of $^{82}\text{Br}$, by comparing $[^{82}\text{Br}]$ in the infusion with that present in the plasma after 3 h, and correcting for urine and erythrocyte losses, and for imbalance across the vascular membrane.

2.2.4 Measurement of Total Body Water

The measurement of total body water traditionally required little correction, as the dilution volume of hydrogen isotopes was thought to accurately reflect the distribution of the body's innate water-bound hydrogen (Hevesy & Hofer, 1934). Thus the measurement of total body water was initially achieved using the dilution of deuterium oxide ($^2\text{HO}$), which was found to distribute to a volume similar to the volume of water

*As an anion, radiobromine equilibrates at a higher concentration in interstitial fluid than that achieved in plasma, due to the relative hyperconcentration of anionic protein in plasma. The relationship between the two concentrations is described by the Gibbs-Donnan ratio, which, according to Manery (1954), averages 1.02 for monovalent anions such as radiobromine.
determined using dissection and dehydration (Moore, 1946). However, measurement of [2HO] in plasma required the use of either mass spectrometry or the falling-drop method (Moore, 1946; see Pinson, 1952, for review), both of which were cumbersome and lacked the sensitivity of radionuclide scintillation counting. For example, mass spectrometry of 2HO required the conversion of the nuclide to a gas prior to its measurement, while the falling-drop method required extensive distillation of the plasma, to compare the density of 2H2O with that of pure water. Hence, with the advancement of liquid scintillation techniques (Langham et al., 1956; Kragelund & Dyrbye, 1966), the use of 2HO was largely replaced by the use of the beta-emitting (β) tritium (3H) nuclide.

The 3H dilution volume was similarly found to approximate the volume of body water determined using dissection and dehydration (Pace et al., 1947), and also to be stable within 2 h of infusion (Schloerb et al., 1950). However, it was later suggested that 3H dilution actually exaggerated total body water by up to 5%, due to the exchange of infused radioactive hydrogen with the body’s innate hydrogen stores (Forbes, 1987; Heymsfield & Waki, 1991); hence, it is current practise to reduce the 3H dilution volume by 5% in order to approximate the true volume of body water. However, this correction factor has not been consistently established against dissection measures, and indeed the dissection method itself may underestimate total body water by several percent (Schoeller & Jones, 1987). In addition, the body’s stores of exchangeable hydrogen are thought to account for only approximately 2% of body mass (Schloerb et al., 1950; Pinson, 1952), placing the true difference between the dilution and dissection volumes within the error of the respective measurements. It is therefore considered at this time that the dilution volume of 3H is representative of total body water, without the need for further correction.

Total body water can therefore be determined using the dilution of 3H, in the form of tritiated water, simply by comparing [3H] in the infusion with that achieved in plasma

1Beta emissions (β) are those generated as radioactive electrons, when an unstable atomic nucleus transforms a neutron into a proton and an electron and emits the electron in an attempt to stabilise its own electromagnetic balance (see Wang, 1969).
at any time beyond 2 h after infusion.

2.2.5 Simultaneous Measurement of Body-Fluid Volumes

The accuracy and reliability of radionuclide dilution methods led to their combination, to enable the simultaneous measurement of several compartments of body fluid. Critically, in such combined methods, the radioemissions of the various nuclides could be distinguished during counting, and hence the respective fluid volumes could be simultaneously determined with minimal correction for internuclide interference.

2.2.5.1 Combined Measurement of Plasma and Erythrocyte Volumes

Initially, the labelled-albumin and $^{51}$Cr dilution methods were combined, to simultaneously measure the volumes of plasma and erythrocytes, and hence to directly determine the total volume of blood (Hahn et al., 1942; Gray & Frank, 1953; Gurney & Bolt, 1956). This was in contrast to previous calculations of blood volume, which had relied on the measurement of a single intravascular phase with appropriate adjustment for the venous haematocrit ($Hct_v$). It was immediately apparent that the blood volume indicated by combining simultaneous measurements of plasma and erythrocyte volume was significantly larger than that calculated from measuring a single phase and adjusting for $Hct_v$, and also that the ratio between erythrocyte volume and total blood volume ($Hct_r$) was lower than $Hct_v$ (Gibson et al., 1946). The respective discrepancies were attributed to the presence of relatively cell-deficient blood in the microcirculation, which would not have been detected by $Hct_v$ (Gibson et al., 1946; Tuckman et al., 1959); however, it was later suggested that the differences were due to the exaggeration of plasma volume by the use of RISA. The difference between $Hct_r$ and $Hct_v$ still existed when plasma volume was measured using RISF, and hence it was recommended that blood volume should only be calculated from simultaneous independent measurements of plasma and erythrocyte volumes (Wright et al., 1975). The ICSH (1980) currently recommends measuring blood volume using the simultaneous dilution of RISA and $^{51}$Cr-labelled erythrocytes.

2.2.5.2 Measurement of Whole-Body Fluid Distribution

The addition of several further radionuclide tracers enabled the simultaneous
measurement of the extravascular, as well as the intravascular, fluid volumes. For example, Moore et al. (1956) simultaneously measured the volumes of total body water, extracellular fluid, plasma, and erythrocytes, using the dilution of $^2$H, $^{82}$Br, Evans' Blue dye, and $^{51}$Cr-labelled erythrocytes, respectively. However, the method was cumbersome, requiring the passage of 36 h and the withdrawal of 200 ml of blood. Similar methods followed (McMurrey et al., 1958; Shires et al., 1960; Dyrbye & Kragelund, 1970), but it was not until 1974 that a method was reported enabling the truly simultaneous measurement of several body-fluid volumes. Then, Spears et al. (1974) measured total body water, extracellular fluid, plasma, and erythrocyte volumes in just 3 h, using the dilution of $^3$H, $^{82}$Br, RISA, and $^{51}$Cr-labelled erythrocytes, respectively. The method required the withdrawal of just 50 ml of blood, and, during repeated assessments, was reliable to within 4% for each of the individual fluid volumes.

Despite the simultaneous use of four radionuclides, the method was safe for human study, imposing less than half the radiation dose of typical bone, brain, or testicular scans (Carey et al., 1983). It would therefore have been classed as a "moderate" x-ray dose by the International Commission on Radiological Protection (ICRP, 1970), and was equivalent to between just 1% and 4% of the dose likely to be genetically effective (ICRP, 1970). Furthermore, the subject could be protected from thyroid-uptake of radioiodine through the appropriate administration of potassium iodide prior to and following assessment (ICSH, 1980)*. Following assessment, the radionuclides were known to leave the body in just a few days. For example, $^{51}$Cr, the most biologically stable of the radionuclides, had a reported half-time in the body of approximately 12 d (ICRP, 1968), making it possible to perform repeated assessments within a relatively short period of time. Indeed, Fortney et al. (1991) performed weekly assessments on a group of subjects during a 56-d period, without any adverse incidents. The total radiation dose imposed by three such assessments was approximately one-fifteenth of the maximum annual dose permitted for an industrial worker (ICRP, 1968).

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*The thyroid gland is the organ likely to receive the largest radiation dose following the infusion of radioiodine. However, thyroid-uptake of radioiodine can be prevented by administering 60 mg d$^{-1}$ of potassium iodide, commencing 2 d prior to radioiodine infusion and continuing for a period of between 14 and 28 d (International Committee for Standardization in Haematology, 1980).
2.3 Indirect Measurements of Body-Fluid Volumes

Despite their reliability and safety, radionuclide dilution methods did not prove universally applicable for the measurement of body-fluid distribution. For example, it was difficult to measure acute changes in plasma volume using the dilution of RISA, due to the tracer’s constant escape from the circulation (Costill, 1977). Hence, several less direct methods were developed for the measurement of body-fluid distribution, based on changes in the concentration of innate substances, such as erythrocytes (Hct), haemoglobin ([Hb]), and chloride ([Cl]), to determine respective compartmental fluid volumes.

2.3.1 Application of the Venous Haematocrit

The measurement of acute changes in intravascular fluid volumes has typically been achieved using various combinations of Hct and [Hb], to determine relative changes in blood, plasma, and erythrocyte volumes. For example, Van Beaumont (1972) used Hct to indicate changes in plasma volume, while assuming a constant volume of erythrocytes. In contrast, Elkinton et al. (1946), Strauss et al. (1951), and Dill and Costill (1974) used both Hct and [Hb] to determine simultaneous changes in plasma and erythrocyte volumes. The inclusion of [Hb] in the calculations was thought to account for any changes in the volume of individual erythrocytes (Dill & Costill, 1974). However, regardless of the particular method, the calculations only equated to changes in blood, plasma, and erythrocyte volumes if the ratio between Hct and Hct (f-ratio) remained constant throughout the period of study; otherwise the magnitude and even the direction of the calculated volume changes were potentially erroneous (Harrison et al., 1982).

There is currently a paucity of information regarding the response of the f-ratio to acute stress, largely due to the difficulties of measuring acute changes in plasma volume. Costill and Saltin (1974) examined the f-ratio following thermal- and exercise-induced dehydration, but allowed their subjects to rest for 30 min prior to the postdehydration measurement. Fricke (1965), on the other hand, measured the f-ratio before and immediately after exercise, but used arterial rather than venous blood to determine the respective haematocrits. Hence, the effect of acute stress on the f-ratio remains unclear,
and it is therefore considered inadvisable to indirectly determine changes in intravascular fluid volumes until the concurrent behaviour of the $f$-ratio has been elucidated.

### 2.3.2 The Chloride Method

Changes in extracellular fluid volume have largely been determined through changes in tissue [Cl] (Costill et al., 1976; Nose et al., 1988), using a method developed by Bergström (1962). The method is based on an assumed quantity of Cl being present in the body, and being contained almost entirely within the extracellular compartment. However, to determine extracellular fluid volume, it is necessary to correct for the slight intracellular Cl presence and for its imbalance across the vascular membrane (see Section 2.2.3). It is also necessary to assume that both the tissue membrane potential and the ratio between intra- and extravascular [Cl] remain constant during the period of study (Costill et al., 1976; Sjøgaard & Saltin, 1982); otherwise it is difficult to distinguish between shifts of fluid and concurrent movements of Cl. It is therefore possible that the chloride method is erroneous during acute thermal and exercise stress (Sjøgaard & Saltin, 1982).

### 2.4 Conclusion

In conclusion, it would appear that the most accurate and reliable measurements of body-fluid distribution can presently be achieved using the dilution of radionuclides. Radionuclide dilution can be used to measure the static volume of body-fluid compartments, and, due to the stability or correction of their dilution volumes, to measure acute changes in body-fluid compartments. Individual methods can also be combined, to enable the simultaneous measurement of several body-fluid compartments, and hence to determine whole-body fluid distribution. For example, the dilutions of radiochromated erythrocytes, radioiodinated fibrinogen, radiobromine, and tritiated water could be combined to simultaneously measure the volumes of erythrocytes, plasma, extracellular fluid, and total body water, respectively. Crucially, their radioemissions could be distinguished in the counting process, and hence it is considered that such a method could be applied to the study of acute changes in body-fluid distribution during postural, thermal, and exercise stress.
2.5 References


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Chapter Three
Measurement of Body-Fluid Distribution Using
Simultaneous Radionuclide Dilution

Of the methods previously proposed for the measurement of whole-body fluid distribution, the most convenient and the most recent was that of Spears et al. (1974), who measured the volumes of total body water, extracellular fluid, plasma, and erythrocytes using the simultaneous dilution of tritiated water ($^3$H$_2$O), radiobromine ($^{82}$Br), radioiodinated serum albumin (RISA), and radiochromated erythrocytes ($^{51}$Cr). The procedure was complete within 3 h, at which time, or any time thereafter, all the respective volumes could be determined from a single 10-ml blood sample. The repeated-measures variability for each of the volumes was less than 4%, and the whole procedure could be repeated within a matter of days without adverse effects to the subject (Spears et al., 1974; Fortney et al., 1991). Hence, the method has since been used by both medical and nutritional scientists interested in the overall distribution of human body fluid (Elwyn et al., 1975; Szeluga et al., 1984; Fortney et al., 1991).

However, despite its convenience, reliability, and safety the method suffered from the limitations of the component dilution methods, such that $^3$H$_2$O, $^{82}$Br, and RISA possibly exaggerated the volume of their respective compartments. Tritium dilution, for example, is thought to exaggerate the total volume of body water, due to the exchange of some of the infused hydrogen with the body’s organic hydrogen stores (Forbes, 1987; Heymsfield & Waki, 1991). However, the amount of exchangeable hydrogen in the human body accounts for less than 2% of body mass (Schloerb et al., 1950; Pinson, 1952), placing the exaggeration of total body water within the error of the dilution method. Hence, the $^3$H$_2$O dilution volume can probably be considered to be representative of total body water without the need for further correction.

The $^{82}$Br dilution volume, on the other hand, requires considerable correction to represent extracellular fluid volume, due to the diffusion of $^{82}$Br into gastrointestinal and even intracellular fluids (Staffurth & Birchall, 1960; Dyrbye & Kragelund, 1970). Hence, the use of $^{82}$Br to measure extracellular fluid volume has recently been superseded by
the use of a larger molecule, radiochromated ethylenediamine tetra-acetic acid ($^{51}$Cr-EDTA), which does not penetrate into either gastrointestinal or intracellular fluids (Ljungqvist et al., 1990). Unfortunately, it has not been possible to incorporate the latter tracer into the simultaneous-dilution method, as its radiolabel would interfere with the determination of erythrocyte volume, which also uses the dilution of $^{51}$Cr. Therefore, it remains necessary to use $^{82}$Br to measure extracellular fluid volume during simultaneous dilutions, and to appropriately correct its dilution volume to obtain a reliable measurement of the extracellular space (Leth & Binder, 1970).

The measurement of plasma volume similarly requires a correction for the loss of RISA from the vascular space. Spears et al. (1974) applied the currently recommended correction technique, using the extrapolation of the RISA elution curve to estimate its theoretical concentration prior to extravasation (International Committee for Standardization in Haematology [ICSH], 1980b; see Figure 2.1). However, despite this correction process, RISA dilution may still overestimate plasma volume, due to rapid loss of the tracer during its initial mixing period (Jaenike et al., 1957; Baker, 1963; Swan & Nelson, 1971). Hence, in 1980, the ICSH (1980b) suggested that a more accurate measurement of plasma volume might be achieved using a larger dilution tracer, such as radioiodinated serum fibrinogen, which measures a volume approximately 6% smaller than RISA dilution (Larsen, 1968; Bent-Hansen, 1989). This advice has gone largely unheeded and has yet to be incorporated into the simultaneous-dilution method.

It was the purpose of this study to develop a method for measuring the distribution of body fluid using the simultaneous dilution of several radionuclides, including radioiodinated serum fibrinogen in combination with tritium, radiobromine, and radiochromated erythrocytes.

### 3.1 Methods

#### 3.1.1 Subjects

The body-fluid distribution of eight adult males (Table 3.1) was measured on three occasions in autumn, over a minimum period of 56 d. The subjects were all healthy and
### Table 3.1

**Physical Characteristics of Subjects**

<table>
<thead>
<tr>
<th></th>
<th>Age (y)</th>
<th>Height (cm)</th>
<th>Mass (kg)</th>
<th>Σ8SkF (mm)</th>
<th>V_{O2peak} (ml·kg⁻¹·min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
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<td>165.7</td>
<td>72.62</td>
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<td>53.6</td>
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<tr>
<td>S2</td>
<td>24.5</td>
<td>180.2</td>
<td>64.55</td>
<td>60.0</td>
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<tr>
<td>S3</td>
<td>23.2</td>
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<td>85.81</td>
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<td>69.1</td>
</tr>
<tr>
<td>S4</td>
<td>24.3</td>
<td>184.3</td>
<td>88.67</td>
<td>83.0</td>
<td>68.7</td>
</tr>
<tr>
<td>S5</td>
<td>21.0</td>
<td>176.4</td>
<td>78.64</td>
<td>89.3</td>
<td>59.4</td>
</tr>
<tr>
<td>S6</td>
<td>27.8</td>
<td>183.9</td>
<td>90.07</td>
<td>95.5</td>
<td>53.6</td>
</tr>
<tr>
<td>S7</td>
<td>33.8</td>
<td>174.5</td>
<td>73.60</td>
<td>64.3</td>
<td>74.6</td>
</tr>
<tr>
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<td>185.6</td>
<td>80.85</td>
<td>44.5</td>
<td>64.0</td>
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<td>179.3</td>
<td>79.35</td>
<td>73.0</td>
<td>66.0</td>
</tr>
<tr>
<td>σ</td>
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<td>6.4</td>
<td>8.25</td>
<td>15.9</td>
<td>10.7</td>
</tr>
</tbody>
</table>

Abbreviations: Σ8SkF = the sum of biceps, triceps, subscapular, midaxillary, suprailliac, abdominal, thigh, and calf skinfold thicknesses; 
V_{O2peak} = maximal aerobic power (estimated after Jones, 1988); 
σ = standard deviation.
regularly participated in physical exercise. They were fully informed of the experimental procedures, which were approved by the University’s Human Experimentation Ethics Committee, and subsequently provided informed consent. Their three assessments were each separated by a minimum of 28 d, to permit maximal decay of residual radioactivity between times.

3.1.2 Procedures

Subjects arrived at the laboratory 1 h prior to assessment, in a rested state, following a 12-h overnight fast*. After 10 min of seated rest, 20 ml of blood were collected without stasis, from an antecubital vein; 10 ml were treated with EDTA (1.8 mg ml⁻¹ of blood) and stored as a radiation-background reference, and 10 ml were treated with 10% citrate phosphate dextrose adenine solution (CPD-A), for use in the preparation of the chromate injection (see Section 3.1.3.1). A urine void was also collected, from which a sample was stored as a background reference. A standard breakfast was then provided, containing approximately 38 kJ·kg⁻¹ of body mass, supplemented by 5 ml·kg⁻¹ of water, to ensure euhydration. Euhydration was apparent from the consistency of subjects’ body mass between repeat assessments, which varied on average by 1.01% (mean coefficient of variation {v}, p=0.989, power {φ}=0.812; Table 3.2). At least 30 min prior to assessment, with breakfast completed, the subject was seated at rest in a control environment (22.0°C ± 1.0°C, 52% ± 6% relative humidity), to stabilise circulation. This posture was then maintained throughout the remainder of the assessment.

Following local anaesthesia (0.25 ml of 2% xylocaine solution), a 16-gauge teflon cannula, attached to a length of teflon tubing, was inserted into a second antecubital vein and secured with a covering of adhesive plaster; venous placement was confirmed by the ease of blood flow through the tubing. The dead-space of the combined cannula and tubing was less than 1 ml.

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*On each of the 2 d prior to assessment, and for the subsequent 14 d, subjects consumed 60 mg of potassium iodide, to protect their thyroid gland from irradiation (after the International Committee for Standardization in Haematology, 1980b; see Section 2.2.5.2).
<table>
<thead>
<tr>
<th>Subject</th>
<th>Mass$_1$ (kg)</th>
<th>Mass$_2$ (kg)</th>
<th>Mass$_3$ (kg)</th>
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<td>77.64</td>
</tr>
<tr>
<td>σ</td>
<td>8.22</td>
<td>8.60</td>
<td>9.12</td>
</tr>
</tbody>
</table>

Abbreviations: Mass$_1$, Mass$_2$, & Mass$_3$ = body mass prior to first, second, and third assessments, respectively; σ = standard deviation.
Radionuclide injections were then administered, via the cannula, within a 30-s period. Two microcuries (μCi) of radioiodinated human serum fibrinogen (RISF; Amersham Australia, 125I Human Fibrinogen), containing less than 1.6% of the dose as free iodine⁴, 8 μCi of sodium radiochromated autologous erythrocytes (Amersham Australia, Na⁵¹Cr; see Section 3.1.3.1), 20 μCi of sodium radiobromine (Australian Radioisotopes, Na⁸²Br), and 500 pCi of tritiated water (Amersham Australia, ³H₂O) were injected sequentially, to respectively measure the volumes of plasma (PV), erythrocytes (RCV), extracellular fluid (ECFV), and total body water (TBW). The midtime of the fibrinogen injection was considered as the commencement of assessment, time-zero (t₀). The cannula was immediately flushed with sufficient saline to remove visual traces of erythrocytes, a minimum of 15 ml, followed by 5 ml of heparinised saline (50 i.u. ml⁻¹). The total flush was considered sufficient to clear the cannula of radionuclides, rendering it suitable for subsequent blood sampling (Ladegaard-Pedersen & Engell, 1969).

Ten-millilitre blood samples were collected after 15, 30, 60, and 180 min, without stasis and with the forearm at approximately heart level. Samples were immediately treated with EDTA. Before each sample, 5 ml of fluid were removed from the cannula and discarded, and, following each sample, the cannula was flushed with 10 ml of heparinised saline. A urine void was collected after 180 min, and measured volumetrically to a precision of 5 ml.

3.1.3 Radionuclide Preparations

The radionuclide injections were prepared under sterile conditions, in accord with the New South Wales Department of Health’s Code of Safe Practice (1987), using procedures developed with and approved by the Illawarra Regional Hospital’s Haematology Service (Maw & Comer, 1993 [Appendix A]).

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⁴J. Critchlow, Technical Specialist Amersham Australia, personal communication, January 12, 1993.
3.1.3.1 Erythrocyte Labelling

The 10 ml of CPD-A treated blood were centrifuged for 10 min at 1500 \( g \)\(^*\). The supernatant and buffy coat were then removed, and 8 \( \mu \text{Ci} \) of Na\(^{51}\)Cr were gently mixed with the remaining erythrocytes. The mixture was incubated for 20 min at 37°C, to facilitate chromium labelling of the cells. The erythrocytes were then washed three times to remove free \(^{51}\)Cr. Each time, the cells were gently mixed with approximately 10 ml of isotonic saline and centrifuged for 10 min at 1500 \( g \); the supernatant was removed and the procedure was repeated. On several occasions the final wash supernatant was assessed for the presence of \(^{51}\)Cr, and was always found to contain less than 1.0% of the \(^{51}\)Cr remaining in the erythrocytes. After washing, the packed erythrocytes were resuspended in isotonic saline, to a combined volume of approximately 10 ml, which was drawn into a syringe ready for injection. Quantities of \(^{3}\text{H}_2\text{O}\), Na\(^{82}\)Br, and RISF, containing 500, 20, and 2 \( \mu \text{Ci} \) of the respective nuclides, were drawn into similar syringes, also ready for injection.

3.1.3.2 Radionuclide Standards

Approximately 0.5 ml of the radiochromated erythrocyte preparation was diluted with distilled water, to a combined volume of 250 ml. This dilution was chosen to approximate that expected for the remaining radiochromated erythrocytes after their equilibration in the body. The exact dilution was determined gravimetrically, by weighing the syringe before and after expulsion, to a precision of 0.001 g (Denver Instruments, AL-300). A 3-ml aliquot, determined volumetrically to an accuracy of \( \pm 0.28\% \)\(^*\) (Labsystems, Finnpipette), was then removed from the dilution and stored in a syringe ready for injection.

\(^*\)The centrifugal force was calculated from the speed and diameter of the centrifuge (after the International Committee for Standardization in Haematology, 1980a), such that the current centrifuge (Clements Electronics, GS200), with an effective radius of 14.75 cm, was operated at 3000 revs-min\(^{-1}\) in order to achieve a force of 1500 \( g \).

\(^*\)Volumetric accuracy was determined as the mean volume-error of twenty 3 ml samples of distilled water. The error of each sample was considered as the absolute difference between the intended and exact volumes, measured gravimetrically to a precision of 0.001 g (Denver Instruments, AL-300), and expressed as a percentage of the 3-ml intention. Independent determinations were similarly made for plasma and erythrocytes, with gravimetric erythrocyte volumes adjusted for red cell density (after the International Committee for Standardization in Haematology, 1973).
glass vial as the $^{51}$Cr-dose standard. Tritium, $^{82}$Br, and $^{125}$I standards were similarly prepared by diluting approximately 0.1, 0.1, and 0.2 ml of the $^3$H$_2$O, Na$^{82}$Br, and RISF preparations with distilled water, to volumes of 500, 500, and 250 ml, respectively. A 3-ml aliquot of distilled water was also stored as a background reference. The standards were later used to determine the exact injected doses of the four radionuclides, in the calculation of respective compartmental fluid volumes (see Section 3.1.6).

3.1.4 Blood and Urine Analyses

Blood samples, including the background reference, were assessed for haemoglobin concentration ([Hb]) and haematocrit (Hct), to respective accuracies of ± 0.78% and ± 0.83%, using the cyanmethaemoglobin and electronic cell-counting methods, respectively (Coulter Electronics, S-Plus IV). The cyanmethaemoglobin method involved the conversion, by a lysing agent, of haemoglobin to a stable cyanide-containing pigment, the concentration of which was then determined photoelectrically; the cell-counting method measured changes in the electrical conductivity of the blood, produced by variations in the concentration of cells. Blood samples were then centrifuged for 40 min at 1500 $g$, to separate plasma from erythrocytes. The resultant plasma was considered to be free of platelets**, while trapped plasma was taken as 2% of the packed erythrocytes (after Chaplin & Mollison, 1952). In seven assessments, a 3-ml aliquot of whole blood was removed prior to blood separation, to assist in the calculation of extracellular fluid volume (see Section 3.1.6.2).

Three-millilitre aliquots of plasma and erythrocytes were dispensed from each blood sample into glass vials, and refrigerated at 4°C pending gamma-radiation ($\gamma$) counting; a 3-ml aliquot from each urine sample was similarly stored. Erythrocyte aliquots were

---

*The accuracy of the haemoglobin ([Hb]) and haematocrit (Hct) measurements was determined from daily calibrations using commercial standards of known [Hb] and Hct, (Coulter Electronics), by the Department of Haematology at the Illawarra Regional Hospital.

**The removal of platelets did not affect the plasma concentration of radioiodinated fibrinogen ($^{125}$I). The $^{125}$I in 10 platelet-free samples, centrifuged for 10 min at 1500 g, did not differ from that in 10 samples which retained platelets, centrifuged for 10 min at 300 g ($p=0.952$, power($\phi$)=0.17).
haemolysed prior to storage, using a trace of powdered saponin (Sigma Chemical Company, S-1252). Further 0.5-ml aliquots of plasma and urine, and 0.5-ml aliquots of the $^3$H and $^{125}$I standards, and of distilled water, were dispensed into glass vials for preparation for beta-radiation ($\beta$) counting. This preparation involved vigorously mixing each aliquot with 0.05 ml of 1 molar hydrochloric acid, to solubilise all solid tissues, followed by 9 ml of a commercially prepared, aqueous liquid scintillation cocktail** (Packard Instruments, Emulsifier-Safe). The combination of the acid and the cocktail effected a clear solution, regardless of whether it was added to plasma, urine, or water, and hence the extent of intrinsic radiation absorption (quench) was similar for all prepared samples.

After radiation counting, plasma aliquots were assessed in triplicate for protein concentration, to a precision of 0.1 g$^{-1}$, using a refractometer (Otago, 93032). Plasma protein concentration ([PP]) was considered as the mean of the three refractometer readings**.

3.1.5 Radiation Counting
Tritium activity was counted using a liquid scintillation counter (LKB Wallac, 1219 Rackbeta), while $^{82}$Br, $^{125}$I, and $^{51}$Cr were counted using a well-type $\gamma$ scintillation counter (Abbott Laboratories, Auto-LOGIC). The liquid scintillation counter was calibrated to measure $\beta$ energy between 0.005 and 0.132 MeV**, to an efficiency of between 33% and 37%. The $\gamma$ counter was calibrated to measure the optimum energy ranges of $^{82}$Br, $^{125}$I, and $^{51}$Cr (Figure 3.1), to efficiencies of between 25% and 29%. Calibrations were achieved by counting the respective radionuclide standards in various

**The liquid scintillation cocktail (Packard Instruments, Emulsifier Safe) acted as a deposit for the radioactive energy in the plasma, resulting in fluorescent excitation and the release of light, which was subsequently detected during liquid scintillation counting.

**In 20 samples, refractometer readings (Otago, 93032) did not differ between three repeated measurements ($p=0.998$, power$\{\phi\}=0.11$). In addition, the means of the three measures were similar to plasma protein concentrations ([PP]) determined by spectrophotometry (Kodak, Ektachem 700XR; $p=0.704$, $\phi=0.17$; $r^2=0.967$). The difference between the methods averaged 0.32% of [PP].

**$1$ MeV (million electron volt) = $1.6 \times 10^{-13}$ joules.
Figure 3.1 The gamma-radiation (γ) spectra and optimum energy ranges of radiobromine (\(^{82}\)Br), radioiodine (\(^{125}\)I), and radiochromate (\(^{51}\)Cr), determined during calibration of the well-type γ scintillation counter (Abbott Laboratories, Auto-LOGIC).
energy ranges, to determine the optimum range for detecting each nuclide. Counting efficiencies were determined during calibration, by comparing the measured count rate with that expected for the corresponding standard. Each standard was expected to emit $2.22 \times 10^6$ disintegrations per minute per microcurie (Wang, 1969), with some disintegrations going undetected due to intrinsic energy absorption or through failing to excite the respective counter. The ratio between the detected and the expected number of disintegrations was considered as counting efficiency.

Radiobromine was counted on the day of assessment, with all other counting delayed for 14 d, pending the decay of $^{82}$Br. Attempts were made to count $^{125}$I and $^{51}$Cr after 10 d, but then it was still possible to detect $^{82}$Br in both plasma and erythrocytes, and so impossible to differentiate between respective radioemissions. After 14 d, due to its half-life of 35.6 h, $^{82}$Br had decayed to approximately 0.14% of its original activity, and was therefore undetectable in the presence of other radionuclides.

All aliquots were counted twice for $^{82}$Br, each time for 12 min, and three times for $^3$H, $^{125}$I, and $^{51}$Cr, for 2, 6, and 10 min, respectively. For each radionuclide, the sequence of vials was reversed between counts. The duration of the counts was chosen to ensure that a minimum of 10000 counts were aggregated for each radiated aliquot in each counting range. Radionuclide concentrations were considered as the mean of the respective counts, minus the mean of the relevant distilled water, plasma, erythrocyte, or urine background reference, and were expressed as counts per minute per millilitre.

3.1.6 Determination of Compartmental Fluid Volumes

In determining TBW and ECFV, corrections were made for the fluid displaced from the plasma aliquots by the presence of protein. The protein displacement factor ($d$) was calculated as (after Chien & Gregersen, 1962):

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

where: $[PP]$ = plasma protein concentration.

$^{a}$Radiobromine ($^{82}$Br) was counted twice, rather than three times, to negate the effect of radiation decay during the counting process (Moore et al., 1963).
3.1.6.1. Total Body Water

TBW was calculated from \[^{3}H\]\ in the plasma collected at \(t_{180}\), corrected for the presence of plasma protein and \(^{125}\text{I}\), and for the loss of \(^{3}H\) in urine. The presence of \(^{125}\text{I}\) was accounted for by subtracting a portion of the radioactivity detected in the \(^{125}\text{I}\) energy range from that detected in the \(^{3}H\) range. This portion was determined as the ratio \(i\) between the radioactivity of the \(^{125}\text{I}\) standard detected in the \(^{3}H\) energy range and that detected in the \(^{125}\text{I}\) range. TBW was thus calculated as:

\[
\text{TBW} = d \left( \frac{(S_{H} \times S_{d} \times S_{v}) - U_{v}(U_{H} - iU_{i})}{P_{H} - iP_{I}} \right)
\]

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 \(^{3}H_{2}O\) injection;
- \(U_{v}\) = volume of urine collected at \(t_{180}\);
- \(U_{H}\) = \[^{3}H\] in the urine collected at \(t_{180}\);
- \(i\) = ratio of \(^{125}\text{I}\) detected in the \(^{3}H\) and \(^{125}\text{I}\) energy ranges;
- \(U_{i}\) = \(^{125}\text{I}\) in the urine collected at \(t_{180}\);
- \(P_{H}\) = \[^{3}H\] in the \(t_{180}\) plasma aliquot;
- \(P_{I}\) = \(^{125}\text{I}\) in the \(t_{180}\) plasma aliquot.

3.1.6.2 Extracellular Fluid Volume

ECFV was calculated from \(^{82}\text{Br}\] in the plasma collected at \(t_{180}\), corrected for the presence of plasma protein, for the loss of \(^{82}\text{Br}\) in erythrocytes and urine, and for the Gibbs-Donnan electrolyte balance (see Section 2.2.3). Erythrocyte and urine \(^{82}\text{Br}\] were themselves corrected for the presence of \(^{51}\text{Cr}\], using the ratio \(c\) between the radioactivity of the \(^{51}\text{Cr}\) standard detected in the \(^{82}\text{Br}\) energy range and that detected in the \(^{51}\text{Cr}\) range. The Gibbs-Donnan ratio \(r\) was taken as 1.02 (Manery, 1954). ECFV was thus calculated as:

\[
\text{ECFV} = d \left( \frac{(S_{B} \times S_{d} \times S_{v}) - U_{v}(U_{B} - cU_{C}) - RCV(E_{B} - cE_{C})}{P_{B}} - PV \right) + PV
\]

where:
- \(d\) = protein displacement factor;
- \(r\) = Gibbs-Donnan ratio;
- \(S_{B}\) = \(^{82}\text{Br}\] of the \(^{82}\text{Br}\) standard;
- \(S_{d}\) = dilution of the \(^{82}\text{Br}\) standard (see Section 3.1.3.2);
- \(S_{v}\) = volume of the \(\text{Na}^{82}\text{Br}\) injection;
In seven assessments $^{82}\text{Br}$ erythrocyte loss was also calculated from the difference between $^{82}\text{Br}$ in plasma and that measured in whole blood. The whole-blood concentration was corrected for the presence of $^{51}\text{Cr}$, as described above. Erythrocyte $^{82}\text{Br}$ was thus calculated as:

$$E_B = P_B + \frac{(W_B - cW_C) - P_B}{Hct_v}$$

where:
- $P_B = [^{82}\text{Br}]$ in the $t_{180}$ plasma aliquot;
- $W_B = [^{82}\text{Br}]$ in the $t_{180}$ whole-blood aliquot;
- $c = \text{ratio of } ^{51}\text{Cr} \text{ detected in the } ^{51}\text{Cr} \text{ and } ^{82}\text{Br} \text{ energy ranges};$
- $W_C = [^{51}\text{Cr}]$ in the $t_{180}$ whole-blood aliquot
- $Hct_v = \text{haematocrit of the } t_{180} \text{ blood sample.}$

### 3.1.6.3 Plasma Volume

PV was calculated using extrapolation of $[^{125}\text{I}]$ in the plasma collected at $t_{15}$, $t_{30}$, and $t_{60}$, to estimate the theoretical plasma $[^{125}\text{I}]$ at $t_0$. In seven assessments, however, no blood sample was collected at $t_{60}$, making it necessary to estimate the corresponding $[^{125}\text{I}]$. Several methods of estimation were explored, using the 15 assessments in which complete sets of samples were collected as a measure of estimation accuracy. Ratios were calculated comparing the measured $[^{125}\text{I}]$ at $t_{60}$ to the concentrations at surrounding times, $t_{30}$ and $t_{180}$; a ratio was calculated expressing $[^{125}\text{I}]$ at $t_{60}$ as a percentage of $^{125}\text{I}$ dose; and curves were fitted through concentrations from $t_{15}$ to $t_{180}$, to predict individual concentrations at $t_{60}$.

None of the ratios appeared sufficiently consistent, either within or between subjects, to satisfactorily estimate $[^{125}\text{I}]$ at $t_{60}$; but, in contrast, it was considered that the $^{125}\text{I}$ mixing/elution curve was adequately described using the function $y=a+(b/x)$ (Function
A; where \( y \) is \([^{125}\text{I}]\) and \( x \) is time). The mean coefficient of determination \((R^2)\) for Function A, considering the 15 assessments with complete sets of samples, was 0.961 (± 0.012; mean ± SEM), compared to 0.879 (± 0.059) and 0.623 (± 0.044) for exponential and semilogarithmic functions (Functions B and C, respectively); the mean standard error of the curve fit (\(\sqrt{\text{MSE}}\); root mean square of the error) for Function A was 19.4 (± 4.2), compared to 49.0 (± 19.5) and 69.7 (± 11.1), for Functions B and C, respectively.

Function A continued to adequately describe the mixing curve when the \( t^\) concentration was omitted. In 22 such cases \( R^2 \) was 0.935 (± 0.018), compared to 0.856 (± 0.069) and 0.699 (± 0.041) for Function B and C, and \(\sqrt{\text{MSE}} \) was 32.0 (± 5.9), compared to 33.4 (± 15.1) and 82.9 (± 12.7), respectively.

In the 15 assessments with complete sets of samples, the \([^{125}\text{I}]\) at \( t^\) estimated by Function A did not differ from that actually measured in the plasma \((p=0.453, \text{power}(\phi)=0.17)\). The mean absolute difference between estimated and measured concentrations was 3.8% (± 0.8%) of measured \([^{125}\text{I}]\), which was probably within the methodological variability involved in measuring the radionuclide concentration. The ratios of estimated \([^{125}\text{I}]\) at \( t^\) to the concentrations measured at surrounding times, and as a percentage of \(^{125}\text{I}\) dose, were in agreement with assessments on the same subject during which complete sets of samples were collected \((p=0.871, \phi=0.10)\). The \([^{125}\text{I}]\) at \( t^\) was therefore considered, when not measured, as that estimated by Function A.

Radioiodine concentrations at \( t_{15}, t_{30}, \) and \( t^\) were then used to estimate the theoretical plasma \([^{125}\text{I}]\) at \( t_0\), using a semilogarithmic function \((\ln y = a + bx\), where \( y \) is \([^{125}\text{I}]\) and \( x \) is time) to account for the extravasation of tracer fibrinogen (see Figure 2.1). The concentration at \( t_0 \) was used to calculate PV as:

\[
PV = \frac{S_i \times S_d \times S_v}{P_{t0}}
\]

where: \( S_i = \([^{125}\text{I}]\) of the \(^{125}\text{I}\) standard; 
\( S_d = \) dilution of the \(^{125}\text{I}\) standard (see Section 3.1.3.2); 
\( S_v = \) volume of the RISF injection; 
\( P_{t0} = \) theoretical \([^{125}\text{I}]\) in plasma at \( t_0\).
To examine the mixing dynamics of RISF, PV was also calculated using the extrapolation of $[^{125}\text{I}]$ in just the $t_{30}$ and $t_{60}$ plasma aliquots. The same semilogarithmic function and process were used as described above.

### 3.1.6.4 Erythrocyte Volume

RCV was calculated from the mean of $[^{51}\text{Cr}]$ in the erythrocytes collected at $t_{30}$ and $t_{60}$, corrected for $^{51}\text{Cr}$ urine loss. The $t_{30}$ and $t_{60}$ aliquots were used to ensure that complete circulatory mixing of Na$^{51}\text{Cr}$ had occurred prior to blood sampling, and to reduce the error involved in the use of a single 3-ml erythrocyte aliquot. RCV was thus calculated as:

$$ RCV = \frac{(S_c \times S_d \times S_v) - 0.25(U_c \times U_v)}{E_c} $$

where:
- $S_c = [^{51}\text{Cr}]$ of the $^{51}\text{Cr}$ standard;
- $S_d =$ dilution of the $^{51}\text{Cr}$ standard (see Section 3.1.3.2);
- $S_v =$ volume of the Na$^{51}\text{Cr}$ injection;
- 0.25 accounts for the first 45 min of the $t_{180}$ urine collection;
- $U_c = [^{51}\text{Cr}]$ in the urine collected at $t_{180}$;
- $U_v =$ volume of urine collected at $t_{180}$;
- $E_c =$ mean of $[^{51}\text{Cr}]$ in the $t_{30}$ and $t_{60}$ erythrocyte aliquots.

To examine the mixing dynamics and stability of the Na$^{51}\text{Cr}$, the concentration of $^{51}\text{Cr}$ at $t_{15}$ and $t_{180}$ was also examined, correcting the $^{51}\text{Cr}$ dose for the corresponding proportions of the $^{51}\text{Cr}$ present in the $t_{180}$ urine collection.

### 3.1.6.5 Intracellular, Interstitial, and Total Blood Volumes

Intracellular water volume (ICW) was calculated as the difference between TBW and extracellular water volume (ECW), where ECW was first calculated by adjusting ECFV for the presence of all plasma solutes (after Chien and Gregersen (1962):

$$ ECW = ECFV - PV(1 - d) $$

where:
- ECFV = extracellular fluid volume;
- PV = plasma volume;
- $d =$ protein displacement factor.

Interstitial fluid volume (IFV) was calculated as the difference between ECFV and PV,
and total blood volume (BV) was calculated as the sum of PV and RCV. Whole-body haematocrit (Hct\textsubscript{w}) was then calculated as the ratio of RCV to BV, and the $f$-ratio was considered as the ratio between Hct\textsubscript{w} and the haematocrit in the corresponding venous blood samples (Hct\textsubscript{v}).

### 3.1.7 Analysis
One subject was unable to complete three assessments due to injuries unrelated to the study, but provided satisfactory data on one occasion. Retest reliability was therefore determined from the fluid volumes of the remaining seven subjects, as the mean coefficient of variation for each volume. Variation coefficients were calculated for each compartmental volume of each subject, based on three repeat assessments, and were then averaged across subjects to give a mean coefficient for each compartment. Subsequent analyses were conducted on the fluid volumes of all eight subjects, with the volumes of seven subjects considered as the mean of their three assessments and data for the eighth subject included from a single assessment.

One-way analysis of variance was used to determine differences in the respective fluid volumes between assessments, while paired $t$-tests were used to determine differences in individual volumes within an assessment (Statistical Package for Social Scientists [SPSS], 1988). In the event of a significant result, specific differences were determined using Tukey's test of Wholly Significant Difference (Tukey\textsubscript{WSD}, Howell, 1992).

Differences were considered significant if the probability that the difference was due to chance was less than 5% ($\alpha=0.05$). When differences were not significant, the power ($\phi$) of the analysis was computed (SPSS, 1988). Analysis of variance summary tables are included in Appendix E, while data in the text are means with standard errors of the means (SEM), unless otherwise stated as standard deviations ($\sigma$).

### 3.2 Results
The process and considerations involved in the calculation of body-fluid volumes are illustrated by the results of one subject, N2. The subject was 24.5 y old, 180.2 cm tall,
and weighed 64.92 kg. Following his radionuclide injections, 10-ml blood samples were collected after 15, 30, 60, and 180 min, and a urine void, of 350 ml, was collected after 180 min. The injected and sampled radionuclide concentrations are shown in Table 3.3, with the ensuing calculations given in Figure 3.2. His TBW was calculated as 44010 ml, and his PV, calculated using the extrapolation of $[^{125}\text{I}]$ in the $t_{15}$, $t_{30}$, and $t_{60}$ plasma aliquots, was 2405 ml; however, when the $t_{15}$ aliquot was omitted, his PV was 3560 ml. His RCV, calculated using the mean $[^{51}\text{Cr}]$ in the $t_{30}$ and $t_{60}$ erythrocyte aliquots, was 2590 ml; calculated from $[^{51}\text{Cr}]$ in the $t_{15}$ and $t_{180}$ aliquots, his RCV was 2410 and 2530 ml, respectively.

From examination of these data, and of the accompanying $^{125}\text{I}$ and $^{51}\text{Cr}$ mixing curves, it was considered that PV calculated using the $t_{15}$ plasma aliquot was unrepresentative of the subject's fluid volume, possibly due to incomplete tracer mixing at that time. Radionuclide concentrations in the $t_{15}$ blood sample were therefore disregarded, and PV and RCV were calculated using the respective $t_{30}$ and $t_{60}$ plasma and erythrocyte aliquots. His PV and RCV were thus taken as 3560 and 2590 ml, respectively, making his ECFV, ICW, IFV, and BV 18645, 25560, 15090, and 6150 ml, respectively (Figure 3.2). His Hct, equated to 0.421, while the mean of his Hct at $t_{30}$ and $t_{60}$ was 0.438; hence, his $f$-ratio was 0.961.

The discrepancy observed for Subject N2, between the PV calculated using the first three plasma aliquots and that calculated omitting the earliest sample, was similarly evident for many of the assessments on the remaining subjects. On average, PV calculated using the extrapolation of $[^{125}\text{I}]$ in the $t_{15}$, $t_{30}$, and $t_{60}$ plasma aliquots was 2727 (± 808) ml, compared to 3673 (± 341) ml when the first sample was omitted (means ± σ; $p=0.001$). Similarly, RCV calculated using the $t_{15}$ erythrocyte aliquot was smaller (2324 ± 586 ml) than those calculated using the mean of $[^{51}\text{Cr}]$ in the $t_{30}$ and $t_{60}$ aliquots, or using $[^{51}\text{Cr}]$ at $t_{180}$ (2627 ± 227 and 2711 ± 419 ml, respectively; means ± σ; $p=0.016$).

It was subsequently considered that PV calculated using $[^{125}\text{I}]$ in the $t_{15}$ plasma aliquot was generally unrepresentative of subjects' fluid volumes. It was below the range
Table 3.3
Radionuclide Doses (upper box) and Blood Sample Concentrations (lower box) for Subject N2

<table>
<thead>
<tr>
<th></th>
<th>[Nuclide]</th>
<th>Dilution</th>
<th>Volume (ml)</th>
<th>Dose (x10³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>³H₂O</td>
<td>158003</td>
<td>500:0.136</td>
<td>2.087</td>
<td>1212324</td>
</tr>
<tr>
<td>Na⁸²Br</td>
<td>1208</td>
<td>500:0.212</td>
<td>3.302</td>
<td>9409</td>
</tr>
<tr>
<td>RISA</td>
<td>842</td>
<td>250:0.333</td>
<td>2.583</td>
<td>1632</td>
</tr>
<tr>
<td>Na⁵¹Cr</td>
<td>40</td>
<td>250:0.603</td>
<td>7.623</td>
<td>128</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>t₁₅</th>
<th>t₃₀</th>
<th>t₆₀</th>
<th>t₁₈₀</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pₜ</td>
<td>51933</td>
<td>30207</td>
<td>27036</td>
<td>26946</td>
<td>17043</td>
</tr>
<tr>
<td>P_b</td>
<td>834</td>
<td>469</td>
<td>453</td>
<td>433</td>
<td>183</td>
</tr>
<tr>
<td>E_b</td>
<td>638</td>
<td>397</td>
<td>372</td>
<td>383</td>
<td></td>
</tr>
<tr>
<td>P_t</td>
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<td>397</td>
<td>354</td>
<td>313</td>
<td>310</td>
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<tr>
<td>E_c</td>
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<td>46</td>
<td>54</td>
<td>51</td>
<td>0</td>
</tr>
<tr>
<td>[PP] (g.l⁻¹)</td>
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<td>72.3</td>
<td>70.3</td>
<td>75.0</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>0.941</td>
<td>0.951</td>
<td>0.949</td>
<td>0.945</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: [Nuclide] = radionuclide concentration of the standard; Dilution = dilution of the standard; Volume = volume of the injection; Urine = radionuclide concentration in the urine collected at t₁₈₀; Pₜ = [³H] in the plasma aliquots; P_b = [⁸²Br] in the plasma aliquots; E_b = [⁸²Br] in erythrocyte aliquots; P_t = [¹²⁵I] in plasma aliquots; E_c = [⁵¹Cr] in erythrocyte aliquots; [PP] = plasma protein concentration; d = protein displacement factor.
Figure 3.2 Calculation of total body water (TBW), extracellular fluid (ECFV), plasma (PV), erythrocyte (RCV), extracellular water (ECW), intracellular water (ICW), interstitial fluid (IFV), and total blood (BV) volumes for subject N2.
considered normal (ICSH, 1980b), and was also only marginally greater than RCV. Hence, Hct calculated using this PV, in conjunction with the mean RCV, was 0.496 (± 0.004), while omission of the t15 [^{125}I] produced an Hct of 0.418 (± 0.006). Hct averaged 0.446 (± 0.003) throughout the first 60 min of assessment, such that corresponding f-ratios were 1.171 (± 0.008) and 0.939 (± 0.015), respectively. Examination of the {^{125}I} and {^{51}Cr} mixing curves (Figure 3.3) suggested that [^{125}I] and [^{51}Cr] were disproportionately elevated at t15 compared to their later equilibria, possibly due to incomplete tracer mixing, and it was thus decided to disregard the t15 radionuclide concentrations during the calculation of compartmental fluid volumes. PV, RCV, and BV were therefore subsequently determined using the t^ and t^ blood samples, while TBW, ECFV, ICW, and IFV were determined using blood collected at t180.

TBW, ECFV, PV, and RCV thus averaged 51822 (± 4698), 20420 (± 1770), 3673 (± 341), and 2627 (± 227) ml, respectively (means ± σ; Table 3.4). Hence, ICW, IFV, and BV were 31659 (± 3237), 16747 (± 1518), and 6348 (± 521) ml, respectively (means ± σ; Table 3.4). These body-fluid volumes did not differ within subjects between repeat assessments, with the mean intrasubject coefficients of variation generally being less than 6% (Table 3.4). The exception was for PV, with a coefficient of variation of 8.44%, for which considerable physiological variation was possible during the 56-d course of the study. The coefficient of variation embraced both physiological and methodological variation, and hence, for all volumes, variation due to methodological error could be considered to be less than the reported coefficient. Measurement variability was probably unaffected by the presence of residual radiation, as, at the time of reassessment, less than 0.01% of the previous {^3}H, {^{125}I}, and {^{51}Cr} doses remained in the blood; no residual {^{82}Br} was detected, reflecting the 35.6-h half-life of the {^{82}Br} nuclide.

The measurement of ECFV was, however, dependent on the method of calculating intracellular losses of {^{82}Br}. In the seven assessments when intracellular [^{82}Br] was both measured directly from erythrocytes and calculated from the plasma and whole-blood [^{82}Br], the apparent intracellular [^{82}Br] was higher in the latter measure (p=0.001). The corresponding ratio between erythrocyte [^{82}Br] and that in plasma was 0.804 (± 0.019), compared to 0.703 (± 0.011) when erythrocyte and plasma concentrations were both
Figure 3.3 Radioiodine and radiochromium mixing curves for subjects seated at rest for 3 h following simultaneous radionuclide injections (means ± SEM; expressed as a percentage of the respective concentration 15 min after injection).
Table 3.4

Body-Fluid Volumes of Eight Physically Active Males

<table>
<thead>
<tr>
<th></th>
<th>TBW</th>
<th>ICW</th>
<th>ECFV</th>
<th>IFV</th>
<th>BV</th>
<th>PV</th>
<th>RCV</th>
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</thead>
<tbody>
<tr>
<td>Absolute volume (ml):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>51822</td>
<td>31659</td>
<td>20420</td>
<td>16747</td>
<td>6348</td>
<td>3673</td>
<td>2627</td>
</tr>
<tr>
<td>σ</td>
<td>4698</td>
<td>3237</td>
<td>1770</td>
<td>1518</td>
<td>521</td>
<td>341</td>
<td>227</td>
</tr>
<tr>
<td>Relative volume (ml·kg⁻¹):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>654.2</td>
<td>399.4</td>
<td>258.1</td>
<td>211.5</td>
<td>80.4</td>
<td>46.6</td>
<td>33.3</td>
</tr>
<tr>
<td>σ</td>
<td>13.4</td>
<td>14.6</td>
<td>12.1</td>
<td>7.6</td>
<td>6.9</td>
<td>4.9</td>
<td>2.9</td>
</tr>
<tr>
<td>Reliability</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.327</td>
<td>0.234</td>
<td>0.118</td>
<td>0.499</td>
<td>0.799</td>
<td>0.803</td>
<td>0.633</td>
</tr>
<tr>
<td>φ</td>
<td>0.25</td>
<td>0.66</td>
<td>0.45</td>
<td>0.10</td>
<td>0.58</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>υ (%)</td>
<td>3.24</td>
<td>5.82</td>
<td>4.93</td>
<td>5.46</td>
<td>6.00</td>
<td>8.44</td>
<td>3.89</td>
</tr>
</tbody>
</table>

Abbreviations: TBW = total body water; ICW = intracellular water volume; ECFV = extracellular fluid volume; IFV = interstitial fluid volume; BV = blood volume; PV = plasma volume; RCV = erythrocyte volume; σ = standard deviation; Reliability = analysis of variance comparing three repeat assessments on each subject; p = statistical significance; φ = statistical power; υ = mean intrasubject coefficient of variation.
measured directly. Consequently, ECFV was 532 (± 54) ml smaller when erythrocyte [$^{82}$Br] was derived from plasma and whole-blood concentrations, compared to the volume determined directly from plasma and erythrocytes (p=0.001). The discrepancy was difficult to explain, as, had the direct measure of erythrocyte [$^{82}$Br] been influenced by its presence in trapped plasma, it would probably have exceeded the indirect [$^{82}$Br]; in practice the reverse was true. In addition, the ratio between directly measured erythrocyte [$^{82}$Br] and plasma [$^{82}$Br] was similar to that previously reported during the calculation of extracellular fluid volume (Staffurth & Birchall, 1960; Moore et al., 1963). It was therefore determined that erythrocyte [$^{82}$Br] should be measured directly from packed cells, rather than calculated as a function of whole-blood concentration, during the measurement of the extracellular space. This procedure is further recommended to others adopting similar measurement techniques.

Analysis of urine voids showed that 1.25% (± 0.08%) of the $^{82}$Br dose was excreted during the 3-h assessments. Similarly, an average of 0.85% (± 0.10%), 15.60% (± 0.97%), and 0.44% (± 0.07%) of respective $^3$H, $^{125}$I, and $^{51}$Cr doses was also excreted during the course of assessment.

3.3 Discussion

3.3.1 Interpretation of Body-Fluid Volumes

The distribution of body fluid was measured in eight physically conditioned males, using the simultaneous dilution of four radionuclides. Similar studies have examined the whole-body fluid distribution of normal or bed-ridden populations (Dyrbye & Kragelund, 1970; Spears et al., 1974; Fortney et al., 1991), or the intravascular fluid volumes of athletes (Kjellberg et al., 1949; Dill et al., 1974; Magnusson et al., 1984a). However, apparently none have previously examined the distribution of both intra- and extravascular fluids in physically conditioned subjects.

The resultant compartmental body-fluid volumes were all slightly enlarged compared to previously reported normal values (International Commission on Radiological Protection [ICRP], 1975; ICSH, 1980b), but were considered normal in light of the subjects'
athletic history and body composition (Pace & Rathbun, 1945; Sjöstrand, 1962; see Section 3.3.2). The subjects were all habitually active, generally participating in endurance-type exercise every day, and, on the basis of their estimated maximal aerobic power, were inside the top 10% of Australian males (Gore & Edwards, 1992). Similarly, based on their skinfold measurements, subjects were among the leanest 5% of Australian males (Gore & Edwards, 1992).

Low adiposity is known to be accompanied by a large volume of total body water relative to body mass, due to the higher concentration of water in lean tissue than that present in fat (Pace & Rathbun, 1945). Hence, in comparison to more adipose populations, the present lean subjects possessed enlarged TBW. As a result, ICW and ECFV were also expanded compared to normal populations. TBW, ICW, and ECFV were 654, 399, and 258 ml·kg⁻¹, respectively, compared to values of 600, 340, and 260 ml·kg⁻¹ considered normal by the ICRP (1975). Values ranging from 605 to 610, 352 to 385, and 220 to 265 ml·kg⁻¹, respectively, have previously been measured in normal males, using radionuclide dilutions similar to the present method (Dyrbye & Kragelund, 1970; Spears et al., 1974; Fortney et al., 1991).

The relative contributions of ICW and ECFV to TBW, however, remained similar to those considered normal in sedentary populations. ICW and ECFV accounted for 61% and 39% of TBW, respectively, compared to values ranging from 57% to 63% and from 37% to 43%, respectively, in sedentary males (Dyrbye & Kragelund, 1970; Spears et al., 1974; ICRP, 1975). Similarly, the relationships between TBW and intravascular fluid volumes were essentially maintained. BV, PV, and RCV accounted for 12.3%, 7.1%, and 5.1% of TBW, respectively, compared to previous values ranging from 11.2% to 12.4%, 6.9% to 7.7%, and 4.3% to 5.2%, respectively (Dyrbye & Kragelund, 1970; Spears et al., 1974; ICRP, 1975).

In terms of absolute volume, the intravascular fluids were also larger than those considered normal for more sedentary populations. BV averaged 80 ml·kg⁻¹, consisting of 47 ml·kg⁻¹ of plasma and 33 ml·kg⁻¹ of erythrocytes. In comparison, the ICSH (1980b) suggested that normal blood volume, measured using the simultaneous dilution
of radioiodinated serum albumin (RISA) and radiochromated erythrocytes (\(^{51}\)Cr), was between 65 and 75 ml·kg\(^{-1}\). Of this, plasma accounted for 40 ml·kg\(^{-1}\) and erythrocytes accounted for between 25 and 35 ml·kg\(^{-1}\). Similarly, Sawka et al. (1992) found that the blood volume of 22 healthy young males was 69 ml·kg\(^{-1}\), including 43 ml·kg\(^{-1}\) of plasma and 26 ml·kg\(^{-1}\) of erythrocytes. Normal blood volume may actually be slightly smaller than these values, as plasma volume measured using RISA may be an overestimate of the true circulating plasma. For example, it has been shown that plasma volume is enlarged when measured using RISA dilution compared to that measured using the dilution of RISF, as was the case in the present study (Larsen, 1968; Bent-Hansen, 1989).

The present intravascular fluid volumes were, however, comparable to those previously measured in similarly conditioned populations. For example, blood volumes of between 79 and 85 ml·kg\(^{-1}\) have been measured in trained cyclists and runners (Sjöstrand, 1962; Glass et al., 1969; Magnusson et al., 1984a), and larger volumes, of between 93 and 104 ml·kg\(^{-1}\), have been reported for superior athletes, undergoing systematic sports training and with maximal aerobic power of approximately 73 ml·kg\(^{-1}\)·min\(^{-1}\) (Dill et al., 1974; Kjellberg et al., 1949; Brotherhood et al., 1975). Thus, it was considered that the present mild hypervolaemia was the result of physical conditioning rather than an error in the measurement of intravascular volumes, and that the method used provided a valid means of determining the distribution of intravascular and extravascular fluids.

### 3.3.2 Mechanisms of Exercise-Induced Hypervolaemia

It is presently unclear whether exercise-induced hypervolaemia is directly related to physical conditioning *per se*, or whether it is a consequence of other accompanying physical adaptations, such as reduced adiposity or increased capillarisation. Holmgren and Åstrand (1966) reported a correlation coefficient of 0.88 between blood volume and maximal aerobic power for 20 trained cyclists, suggesting that blood volume expanded with improved physical condition. However, Young et al. (1993) produced no expansion of blood volume during 8 weeks of cycle training, despite an increase of 13% in aerobic power. It appears therefore that prolonged habitual training may be necessary to produce supportive physical adaptations prior to the chronic expansion of blood volume.
Exercise-induced hypervolaemia might then reflect increased hydrostatic filling of chronically expanded heart and blood vessels (Brotherhood et al., 1975), or an osmotically-induced plasma expansion, in response to an increase in the total mass of circulating protein (Harrison et al., 1975, 1976; Convertino et al., 1980a; 1980b). Indeed, when total circulating protein does not increase, blood volume also remains stable, despite other indications of improved physical condition (Young et al., 1993).

However, an osmotic expansion of plasma volume would tend to deplete the volume of interstitial fluid (Senay et al., 1976), and would also reduce the circulating haematocrit. In the present study, IFV was not decreased compared to previous normal values (ICRP, 1975), and Hct, was not low compared to that considered normal by Magnusson et al. (1984b) in a study of sports anaemia. It is therefore more likely that the hypervolaemia was a reflection of whole-body hyperhydration, including the proportionate expansion of all compartments of body fluid. Indeed, while haematocrit has been found to decrease during short periods of exercise training (Convertino et al., 1980a; Oscai et al., 1968), it has been shown to recover as training progresses, due to a proportionate expansion of plasma and erythrocyte volumes (Holmgren et al., 1960; Kjellberg et al., 1949; Schmidt et al., 1988). The increase in erythrocyte volume has been attributed to the stimulation of erythropoietin production following renal hypoxia during exercise (Brotherhood et al., 1975; Schmidt et al., 1988; Schwandt et al., 1991), although this increase does not always occur (Klausen et al., 1993). Hence, it is considered possible that the chronic expansion of erythrocyte volume occurs as a homeostatic response to balance the compartmental distribution of body fluid throughout the body.

The increased volume of total body water necessary to support such a whole-body hyperhydration may be accounted for by increased water retention due to increased plasma renin, aldosterone, and vasopressin activity following exercise (Convertino et al., 1981, 1983; see Wade, 1984, for review). In particular, increased sodium retention, due to increased renal sensitivity to aldosterone, may be an important factor in exercise-induced water retention (Convertino, 1991).

In addition, part of the exercise-induced hyperhydration may be accounted for by
accompanying reductions in adiposity. Dill et al. (1974) suggested that at least one-third of exercise-induced hypervolaemia could be explained by reduced adiposity, and examination of the present data suggested that differences in adiposity between trained and untrained individuals may actually account for considerably more of the apparent hyperhydration. For example, PV adjusted for adiposity (estimated from TBW after Pace & Rathbun, 1945) equated to approximately 52 ml·kg\(^{-1}\) of fat free mass, compared to a volume of 50 ml·kg\(^{-1}\) for a normal male (ICSH, 1980b) with a body-fat content of 20%. It is therefore suggested that, when considering the chronic status of body-fluid volumes, consideration must be given to the relative adiposity of the subject, while recognising the inaccuracies of estimating body-fat content.

3.3.3 Methodological Considerations

3.3.3.1 Reliability

The present method for measuring body-fluid volumes, using the simultaneous dilution of \(^3\)H\(_2\)O, Na\(^{82}\)Br, RISF, and \(^{51}\)Cr-labelled erythrocytes, produced results that were not only considered normal for the appropriate population, but were also reliable between repeated assessments. Seven subjects underwent three assessments, spaced at 28-d intervals, and the resultant coefficient of variation between assessments was less than or equal to 6% for all measured and derived volumes (Table 3.4). The sole exception was for PV, which produced a coefficient of variation of 8.4%, but this may be attributed to physiological changes in PV between repeat assessments, as well as to methodological error. Considerable physiological variation is possible in PV, and indeed in all other body-fluid volumes, due to changes in such circumstances as environmental temperature and health (Bazett et al., 1940; Fortney et al., 1991), and such variations may well have occurred during the 56-d course of the present study. Spears et al. (1974) reported variation in plasma volume of just 1.2% during a 7-d period, and variations of 0.5%, 1.3%, and 3.9% in total body water, extracellular fluid, and erythrocyte volumes, respectively. It was therefore considered that, in the present study, the variation in volume measurements due to methodological error was probably less than the calculated
coefficients of variation, and may have approached 5% for all respective volumes***.

3.3.3.2 Radiobromine Dilution

The determination of PV and RCV was somewhat hampered by the use of $^{82}$Br to measure ECFV, as the activity of $^{82}$Br interfered with the detection of $^{125}$I and $^{51}$Cr. Spears et al. (1974) previously suggested allowing between 5 and 7 d to elapse prior to the measurement of $^{125}$I and $^{51}$Cr, to permit the decay of $^{82}$Br; however, in the present study, considerable $^{82}$Br was still detectable in both plasma and erythrocytes 10 d after their collection, making it impossible to differentiate the respective activities. It was therefore necessary to allow $^{82}$Br to decay for 14 d before measuring $^{125}$I and $^{51}$Cr, at which time only 0.14% of the original $^{82}$Br remained, due to its half-life of 35.6 h.

3.3.3.3 Whole-Body Haematocrit and the f-ratio

The subsequent measurement of $^{125}$I and $^{51}$Cr, and the calculation of PV and RCV enabled the calculation of whole-body haematocrit ($H_{ct_{w}}$), and hence allowed further investigation of its relationship with venous haematocrit ($H_{ct}$). During the study, $H_{ct_{w}}$ averaged 0.418, while $H_{ct}$ averaged 0.446, with the mean ratio between them ($f$-ratio) being 0.939. Larsen (1968) produced apparently the only other determination of the $f$-ratio using RISF and radiochromated erythrocyte dilutions, and reported a somewhat lower value of 0.882. However, he calculated plasma volume from [RISF] in plasma collected 15 min after injection, without correction for its extravasation; hence, plasma volume was probably exaggerated, lowering both the whole-body haematocrit and the resultant $f$-ratio.

Previous studies using the simultaneous dilution of radioiodinated albumin (RISA) and radiochromated erythrocytes have reported $f$-ratios ranging from 0.89 to 0.94 (Tuckman et al., 1959; Shires et al., 1960; Albert et al., 1965; Sawka et al., 1992), similar to that

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***The accuracies of the radioiodinated serum fibrinogen (RISF) and radiochromated erythrocyte ($^{51}$Cr) dilution methods were determined in vitro, using volumes of distilled water ranging between 2 and 7 l, as being ± 2.50% and ± 2.82%, respectively. In vitro accuracies were not determined for the tritiated water or sodium radiobromine dilution methods due to the overall expense of the investigation.
determined in the present study. Thus, it would appear that the \( f \)-ratio was unaffected by the use of RISF instead of RISA, and therefore that the difference between \( \text{Hct}_w \) and \( \text{Hct}_v \) cannot be attributed to the exaggeration of plasma volume by RISA, as had previously been suggested (Swan & Nelson, 1971; Baker & Wycoff, 1961).

Initially, the difference between \( \text{Hct}_w \) and \( \text{Hct}_v \) was attributed to the presence of relatively anaemic blood in the microcirculation (Gibson \textit{et al.}, 1946; Tuckman \textit{et al.}, 1959). However, it is unlikely that a true cell deficiency exists in the microcirculation, as logically the erythrocytes that enter the capillaries must progress through to the veins without leaving the vasculature. In contrast, some of the plasma entering the capillaries escapes to the interstitium, potentially increasing the haematocrit of the microcirculation, and therefore negating the \( f \)-ratio (Swan & Nelson, 1971).

A more likely explanation for the \( f \)-ratio may therefore lie with the differential flow rates of plasma and erythrocytes. It is known that erythrocytes, travelling in the centre of blood vessels, flow faster than plasma, which lines the walls of the vessels (Larsen, 1968). Hence, a sample of free-flowing blood will collect an amount of erythrocytes proportionate to their flow-rate rather than to their volume, making the composition of the blood sample (\( \text{Hct}_v \)) similarly representative of the ratio between erythrocyte and plasma flow-rates (Fahraeus, 1929; Fahraeus & Lindqvist, 1931; Meier & Zierler, 1954). Thus, when erythrocytes flow faster than plasma, \( \text{Hct}_v \) will be more concentrated than the composition of the whole circulation (\( \text{Hct}_w \)), and the ratio between \( \text{Hct}_w \) and \( \text{Hct}_v \) will be less than one. Furthermore, the ratio will be susceptible to changes in the dynamics of blood flow.

In the present study, the \( f \)-ratio ranged between 0.836 and 1.102, suggesting that the dynamics of blood flow were not consistent between individuals (Table 3.5). Indeed, the \( f \)-ratio also varied within individuals between assessments, measuring, for example, 0.925, 0.961, and 0.918 during three assessments of Subject 2. Previously, the \( f \)-ratio has been found to vary within individuals depending on their health status, consciousness, or surrounding environment (Gurney & Bolt, 1956; Albert \textit{et al.}, 1965). Thus, it appears that the application of a nominal \( f \)-ratio for the correction of \( \text{Hct}_v \) is
Table 3.5
Whole-Body and Venous Haematocrits, and the Corresponding f-Ratio

<table>
<thead>
<tr>
<th>Subject</th>
<th>Hct_w</th>
<th>Hct_v</th>
<th>f-ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0.430</td>
<td>0.463</td>
<td>0.929</td>
</tr>
<tr>
<td>N1</td>
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<td>0.453</td>
<td>0.976</td>
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<td>H1</td>
<td>0.382</td>
<td>0.443</td>
<td>0.861</td>
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<td>C2</td>
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<tr>
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<td>0.438</td>
<td>0.961</td>
</tr>
<tr>
<td>H2</td>
<td>0.423</td>
<td>0.461</td>
<td>0.918</td>
</tr>
<tr>
<td>C3</td>
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<td>1.102</td>
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<td>0.459</td>
<td>0.929</td>
</tr>
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</tr>
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</tr>
<tr>
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<td>0.438</td>
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</tr>
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</tr>
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<td>0.459</td>
<td>0.977</td>
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<td>0.457</td>
<td>0.890</td>
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<td>N7</td>
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<td>0.949</td>
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<td>0.421</td>
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<td>C8</td>
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<td>Mean</td>
<td>0.418</td>
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<tr>
<td>SEM</td>
<td>0.006</td>
<td>0.003</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Abbreviations: Hct_w = whole-body haematocrit, from blood collected at t_30 and t_60;
Hct_v = mean venous haematocrit of blood collected at t_30 and t_60;

f-ratio = Hct_w / Hct_v;
SEM = standard error of the mean.
probably inadvisable, and that the use of Hct, to indicate changes in the composition of blood may be erroneous during circumstances that affect the dynamics of blood flow.

3.3.4 Conclusion

In conclusion, it was shown that the simultaneous dilution of $^3$H$_2$O, Na$^{82}$Br, RISF, and $^{51}$Cr-labelled erythrocytes provided a valid and reliable method for measuring body-fluid distribution. Each of the respective dilution volumes required several corrections, but, having done so, retest variance was generally less than 6%. The resultant compartmental fluid volumes were generally larger than those considered normal for adult males, but were accepted as normal in light of the subjects’ athletic history and body composition. Hence, physically conditioned males showed a whole-body hyperhydration, but retained a normal distribution of fluid between body compartments. In addition, the relationship between their whole-body and venous haematocrits was similar to that previously reported for adult males, but may have been susceptible to changes in the dynamics of blood flow. It was considered inadvisable to apply a nominal correction to venous haematocrit to equate it to whole-body haematocrit, or to use venous haematocrit to indicate changes in whole-body blood composition during circumstances that might affect the dynamics of blood flow.
3.4 References


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Chapter Four
Body-Fluid Distribution During Supine, Seated, and Upright Rest

It is well documented that the distribution of body fluid is dependent on the posture of the subject (Thompson et al., 1928; Maxfield et al., 1941; Hinghofer-Szalkay & Moser, 1986). Thus, body-fluid distribution determined during seated rest (see Chapter Three) would probably change if the subject moved to either a supine or an upright posture. For example, central blood volume would probably increase during supine rest, stimulating the release of atrial natriuretic peptide, increasing urine flow, and consequently decreasing total body water (Norsk et al., 1993). In contrast, total blood volume would decrease during upright rest (Thompson et al., 1928; Fawcett & Wynn, 1960; Hagan et al., 1978), increasing the volume of extravascular fluid and potentially leading to the development of peripheral oedema (Youmans et al., 1934).

Thompson et al. (1928) and Waterfield (1931a) were apparently the first to show that blood volume decreased during upright rest, reporting decreases of 320 and 512 ml, respectively, when standing followed prolonged recumbency. In each case, the majority of blood loss was from the plasma phase, probably filtered into the interstitium in response to an increase in intravascular hydrostatic pressure (Pollack & Wood, 1949; Stick et al., 1993b). Several reports have since confirmed that plasma volume decreases during upright rest following prolonged recumbency (Fawcett & Wynn, 1960; Hagan et al., 1978; Hinghofer-Szalkay & Moser, 1986), and have suggested that extravascular volume consequently in the legs (Waterfield, 1931b; Stick et al., 1993a).

The contribution of erythrocytes to intravascular fluid loss during upright rest is, however, less clear. Erythrocyte volume is generally thought to remain constant during upright rest, but this conclusion has frequently been drawn from calculations based on changes in venous haematocrit (Hct,) and plasma volume, rather than from actual measurements of erythrocyte volume (Thompson et al., 1928; Hinghofer-Szalkay, 1986). Such calculations may be inaccurate during postural manipulation, as the value of Hct, is then dependent on the chosen sample site (Youmans et al., 1934; Eisenberg, 1963).
For example, Thompson et al. (1928) showed that Hct, in the foot was affected considerably more by standing up than was Hct, in the arms. Hence, the calculated change in erythrocyte volume was also dependent on the site from which blood was collected.

Despite these limitations associated with the use of Hct,, few attempts have been made to actually measure erythrocyte volume during upright rest. Waterfield (1931a) measured erythrocyte volume using the carbon monoxide rebreathing technique, and found a decrease of 85 ml during 60 min of standing still, following 65 min of recumbency. In contrast, Hagan et al. (1978), also using carbon monoxide rebreathing, found no change in erythrocyte volume during 35 min of standing, following 35 min supine. Further measurements are therefore needed to clarify the response of erythrocyte volume to upright rest.

Similarly, there is a paucity of information comparing erythrocyte volume, and indeed plasma volume, during periods of seated and supine rest. Apparently only Maxfield et al. (1941) have previously compared erythrocyte volume during seated rest with that during a supine period, and their study involved less than 12 min of sitting and examined only 3 subjects. Diaz et al. (1979) and Hagan et al. (1980) calculated the plasma response to seated rest, in comparison to both standing and lying, and found that the magnitude of plasma loss during sitting was approximately half that associated with standing following supine rest. However, both studies calculated fluid shifts from Hct,, and therefore need confirming with more direct measurements of plasma and erythrocyte volume. No attempt has yet been made to measure both plasma and erythrocyte volumes simultaneously during supine, seated, and upright rest.

Despite the acknowledged effects of postural change on the volume of intravascular fluids, no attempt has been made to measure the corresponding effect on the volume and distribution of extravascular fluids. The volume of the legs has been found to increase during upright rest (Waterfield, 1931b; Stick et al., 1993a), and pitting oedema has similarly been found to develop during prolonged standing (Youmans et al., 1934); however, the magnitude of extravascular fluid accumulation, and its division between
intra- and extracellular compartments has not yet been quantified.

It was the purpose of this study to determine the distribution of both intra- and extravascular fluids during supine, seated, and upright rest, using simultaneous measurements of total body water, extracellular fluid volume, and plasma and erythrocyte volumes.

4.1 Methods

4.1.1 Subjects
The body-fluid distribution of eight healthy, physically active males (see Table 3.1) was measured using the simultaneous radionuclide dilution method described in Chapter Three. The subjects were fully informed of the experimental procedures, which were approved by the University’s Human Experimentation Ethics Committee, and subsequently provided informed consent. All assessments were conducted in autumn, over a period of 56 d.

4.1.2 Procedures
Preliminary procedures and radionuclide preparations were those described in Sections 3.1.2 and 3.1.3. Subjects arrived at the laboratory in a rested, fasted state, consumed breakfast (38 kJ·kg\(^{-1}\) of body mass, plus 5 ml·kg\(^{-1}\) of water), and were seated at rest, at least 30 min prior to assessment. Before breakfast, a urine void and 20 ml of venous blood were collected, for use as reference blanks and for radiochromate labelling. Approximately 5 ml of erythrocytes were labelled with radiochromate, using 20 min of incubation at 37°C; the labelled cells were then washed three times and resuspended in saline, and drawn into a syringe ready for injection. Quantities of tritiated water, radiobromine, and radioiodinated serum fibrinogen were drawn into similar syringes, also ready for injection. The volumes of these injections, and of the radionuclide standards, were determined gravimetrically, to a precision of 0.001 g (Denver Instruments, AL-300).

A 16-gauge teflon cannula was inserted into an antecubital vein, through which to
administer the radionuclide injections. Two microcuries (µCi) of radioiodinated human serum fibrinogen (RISF; Amersham Australia, 125I Human Fibrinogen), 8 µCi of sodium radiochromated autologous erythrocytes (Amersham Australia, Na51Cr), 20 µCi of sodium radiobromine (Australian Radioisotopes, Na82Br), and 500 µCi of tritiated water (Amersham Australia, 3H2O) were injected sequentially, within a 30-s period. The radionuclides were used to measure plasma (PV) and erythrocyte (RCV) volumes, extracellular fluid volume (ECFV), and total body water (TBW), respectively. The midtime of the fibrinogen injection was considered as the commencement of assessment, time-zero (t₀). Immediately following the injections, the cannula was flushed with a minimum of 15 ml of saline followed by 5 ml of heparinised saline, rendering it suitable for subsequent blood sampling (Ladegaard-Pedersen & Engell, 1969).

Ten-millilitre blood samples were collected after 30, 60, and 180 min, from which to determine initial fluid volumes (see Sections 3.1.4 to 3.1.6). Samples were collected without stasis with the forearm at approximately heart level, and were immediately treated with ethylenediamine tetra-acetic acid (EDTA; 1.8 mg·ml⁻¹ of blood). Before each sample, 5 ml of fluid were removed from the cannula, and, following each one, the cannula was flushed with 10 ml of heparinised saline. A urine void was collected after 180 min, and measured to a precision of 5 ml.

The 180 min following the radionuclide injections were spent seated at rest, with at least the last 30 min being in a control environment of 22.0°C (± 1.0°; standard deviation), 52% (± 6%) relative humidity (rh). Air movement was less than 0.5 m·s⁻¹, and black-globe temperature was within ± 0.5°C of ambient temperature. The environment was maintained, in the University's Environmental Physiology Research Laboratory, using a combination of heaters, coolers, and dehumidifiers, controlled by microcomputer (see Maw & Williamson, 1992 [Appendix B]).

The subsequent 150 min were also spent at rest in the control environment, during which time several changes in posture were effected. Thirty minutes were spent in each of supine, seated, and upright postures, separated by 30 min of seated rest. The postures were effected in an order counterbalanced between subjects. In each posture,
environmental exposure was maximised, by respectively utilising a string hammock, a
string-backed chair, and a support frame with hand rail. A blood sample was collected
immediately prior to assuming each posture, while the subject remained seated, and then
after 15 and 30 min in each posture. A urine void was collected every 30 min, whenever
posture was changed. Cardiac frequency ($f_c$) was recorded, from ventricular
depolarisations, at 5-s intervals throughout assessment, using a telemetry system (Polar
Electro, PE3000; Figure 4.1)*.

4.1.3 Blood and Urine Analyses

Blood and urine collections were analysed as described in Section 3.1.4. All blood
samples were assessed for venous haematocrit ($Hct_v$) and haemoglobin concentration
([Hb]), to accuracies of ± 0.83% and ± 0.78%, respectively (Coulter Electronics, S-Plus
IV; see Section 3.1.4), and then centrifuged for 40 min at 1500 g, to separate plasma
from erythrocytes. Trapped plasma was taken as 2% of the resultant packed erythrocytes
(after Chaplin & Mollison, 1952).

Three-millilitre aliquots of plasma and erythrocytes were dispensed from each blood
sample into glass vials, and refrigerated at 4°C, pending gamma radiation ($\gamma$) counting.
A 3-ml aliquot from each urine void and from each of the $^{125}$I, $^{51}$Cr, and $^{82}$Br standards
was similarly stored. Erythrocyte aliquots were haemolysed prior to storage, using a
trace of powdered saponin (Sigma Chemical Company, S-1252). Further 0.5-ml aliquots
of plasma and urine, and 0.5-ml aliquots of the $^3$H and $^{125}$I standards, and of distilled
water, were dispensed into glass vials, vigorously mixed with 0.05 ml of 1 molar
hydrochloric acid and 9 ml of liquid scintillation cocktail (Packard Instruments,
Emulsifier-Safe), and refrigerated pending beta radiation ($\beta$) counting. All aliquots were
measured volumetrically, to an accuracy of ± 0.2% (Labsystems, Finnpipette; see
Section 3.1.3.2).

*The telemetry system (Polar Electro, PE3000) has been shown to provide a record of
cardiac frequency ($f_c$) similar to that measured using a 5-lead electrocardiograph (ECG;
Quinton Instruments, Q5000), during seated rest, cycle exercise at 150 W, and seated
recovery (Figure 4.1, from Osborne, 1994). Averaged across all three states, the
correlation coefficient ($r^2$) was 0.999, while the difference between the two measures
averaged ± 0.65% of the ECG frequency.
Figure 4.1 Validation of cardiac frequency measurement during seated rest, cycle exercise at 150 W, and seated recovery, comparing a 5-lead electrocardiograph (ECG; Quinton Instruments, Q5000) and a telemetry system (Polar Electro, PE3000; from Osborne, 1994).
After radiation counting, plasma aliquots were assessed, in triplicate, for protein concentration, to a precision of 0.1 g⁻¹, using a refractometer (Otago, 93032). The mean of the three refractometer readings was considered as plasma protein concentration ([PP]; see Section 3.1.4).

4.1.4 Radiation Counting
Radiation counting was conducted as described in Section 3.1.5. Radioiodine, ^{51}\text{Cr}, and ^{82}\text{Br} were counted using a well-type $\gamma$ scintillation counter, calibrated to measure the optimum range of radiant energy of the respective nuclides, to efficiencies of between 25% and 29% (Abbott Laboratories, Auto-LOGIC; see Figure 3.1); ^{3}\text{H} was counted using a liquid scintillation counter, calibrated to measure $\beta$ energy between 0.005 and 0.132 million electron volts (MeV)*, to an efficiency of between 33% and 37% (LKB Wallac, 1219 Rackbeta).

Radiobromine was counted on the day of assessment, with all other counting delayed for 14 days, pending the decay of ^{82}\text{Br}. Aliquots were counted twice for ^{82}\text{Br}, each time for 12 min, and three times for ^{125}\text{I}, ^{51}\text{Cr}, and ^{3}\text{H}, for 6, 10, and 2 min, respectively. For each radionuclide, the sequence of vials was reversed between counts. The duration of the counts ensured a minimum of 10000 counts were aggregated for each radiated aliquot in each of the calibrated ranges. The mean of the respective counts, minus the mean of the relevant distilled water, plasma, erythrocyte, or urine background reference, was considered as radionuclide concentration. Concentrations were expressed as counts per minute per millilitre.

4.1.5 Determination of Compartmental Fluid Volumes
Initial fluid volumes were calculated from radionuclide concentrations in the blood collected 30, 60, and 180 min after the radionuclide injections (see Section 3.1.6). Later volumes, during changes in posture, were similarly calculated, by comparing the radionuclide concentrations in the blood samples with the respective radionuclide doses. Volumes were thus calculated for minutes 0, 15, and 30 of each posture.

*1 \text{MeV} = 1.6 \times 10^{-13} \text{ joules.}
4.1.5.1 Total Body Water, Extracellular Fluid, and Erythrocyte Volumes

TBW, at any given time (t.), was calculated from [3H] in the appropriate plasma aliquot, corrected for the presence of plasma protein and 125I, and for 3H urine loss; ECFV was calculated from [82Br] in the appropriate plasma, corrected for the presence of protein, for 82Br erythrocyte and urine losses, and for the Gibbs-Donnan electrolyte ratio; RCV was calculated from [51Cr] in the appropriate erythrocytes, corrected for 51Cr urine loss.

The presence of protein was corrected for by calculating the protein displacement factor using [PP] (after Chien & Gregersen, 1962; see Section 3.1.6). Radionuclide urine losses were calculated from the respective radionuclide concentration and the volume of the urine voids collected up to t. The resultant radionuclide contents were summed up to t, or, in the case of the void following an experimental posture, were apportioned to the preceding 30 min on a linear basis. The Gibbs-Donnan ratio was taken as 1.02 for radiobromine (Manery, 1954).

4.1.5.2 Plasma Volume

PV was calculated from a comparison of [125I] measured in the plasma collected at t, with that predicted, using semilogarithmic extrapolation of the 125I elution curve, for the equivalent time (after Gibson & Evans, 1937, and Harrison & Edwards, 1976; see Section 2.2.2 and Figure 2.1). The elution curve, describing the loss of RISF from the circulation, was established using [125I] in plasma collected from t30 through t180 in all cases, plus any immediately succeeding samples collected during seated rest. For example, during three assessments, the seated posture occurred first, before any change in posture was effected, providing two further blood samples, up to t210, for the curve-fitting process. PV was then calculated as:

\[ PV = \frac{P_{te}}{P_{im}} \times PV_0 \]

where: 
- \(P_{te}\) = [125I] predicted for t;
- \(P_{im}\) = [125I] in the t plasma aliquot;
- \(PV_0\) = initial plasma volume.

4.1.5.3 Intracellular Water, Interstitial Fluid, and Total Blood Volumes

Intracellular water (ICW), interstitial fluid volume (IFV), and blood volume (BV) were
calculated as described in Section 3.1.6.5. ICW was calculated as the difference between TBW and extracellular water volume (ECW), where ECW was calculated from ECFV adjusted for the presence of all plasma solutes; IFV was calculated as the difference between ECFV and PV; BV was calculated as the sum of PV and RCV.

Whole-body haematocrit (Hctw) was calculated as the ratio of RCV to BV, and the f-ratio was considered as the ratio between Hctw and the haematocrit in the corresponding venous blood sample (Hctv).

**4.1.5.4 Relative Changes in Intravascular Fluid Volumes**

Relative changes in BV, PV, and RCV were calculated, with respect to values immediately preceding the assumption of each posture, from measured volumes, and also from changes in Hctv and [Hb] (after DiU & Costill, 1974):

\[
\Delta BV = \left( \frac{[Hb_b]}{[Hb_t]} - 1 \right) \times 100\%
\]

\[
\Delta PV = \left( \frac{[Hb_b] \times (1 - Hct_v)}{[Hb_t] \times (1 - Hct_b)} - 1 \right) \times 100\%
\]

\[
\Delta RCV = \left( \frac{[Hb_b] \times Hct_v}{[Hb_t] \times Hct_b} - 1 \right) \times 100\%
\]

where: [Hb]b = initial haemoglobin concentration;  
[Hb]v = haemoglobin concentration at tv;  
Hctb = initial venous haematocrit;  
Hctv = venous haematocrit at tv.

**4.1.6 Analysis**

Body-fluid volumes were normalised to their initial values, to negate lingering effect from the previous posture. Although the 30 min of seated rest between each posture were considered sufficient to allow fluids to return to their initial distribution (Hagan et al., 1978, 1980), it was considered prudent to normalise volumes to account for prolonged disturbances. Urine volumes displayed proportional variance, and were
therefore transformed to logarithms prior to further analysis (Montgomery & Peck, 1982).

Cardiac frequency was averaged for the minute surrounding each 5-min interval, such that values were derived for minutes 0, 5, 10, 15, 20, 25, and 30 of each posture. Data were also averaged to produce a mean $f_c$ across the duration of each posture.

Factorial analysis of variance was used to determine differences related to posture and time. One-way analysis of variance was used to determine differences when posture was the only independent variable (Statistical Package for Social Scientists [SPSS], 1988). In the event of a significant result, specific differences were determined using Tukey’s test of Wholly Significant Difference (TukeyWSD; Howell, 1992).

Differences were considered significant if the probability that the difference was due to chance was less than 5% ($\alpha=0.05$). When differences were not significant, the power ($\phi$) of the analysis was computed (SPSS, 1988). Analysis of variance summary tables are included in Appendix F; data in the text are means with standard errors of the means (SEM), unless otherwise stated as standard deviations ($\sigma$).

4.2 Results
Preexperimental TBW, ECFV, PV, and RCV were 52199 (± 5635), 20842 (± 1606), 3844 (± 645), and 2735 (± 322) ml, respectively (means ± $\sigma$). Hence, corresponding ICW, IFV, and BV were 31566 (± 4236), 16998 (± 1231), and 6579 (± 923) ml, respectively (means ± $\sigma$). Initial $f_c$ ranged between 41 and 75 b·min$^{-1}$, with a mean of 58 (± 8) b·min$^{-1}$ (mean ± $\sigma$).

Body-fluid volumes were subsequently disturbed by changes in posture, with the results of one subject, N3, illustrating the typical response to the supine, seated, and upright periods (Figure 4.2). The subject was 23.2 y old, 183.8 cm tall, and weighed 84.78 kg. His initial TBW, ECFV, PV, and RCV were 58010, 21905, 3870, and 3100 ml, respectively, with his initial ICW, IFV, and BV being 36370, 18040, and 6965 ml,
Figure 4.2 Intracellular water (ICW), extracellular fluid (ECF), interstitial fluid (IFV), blood (BV), plasma (PV), and erythrocyte (RCV) volumes for Subject N3 during 30 min of supine, seated, and upright rest in a control environment (22°C; normalised to preexperimental levels).
respectively. During the seated period, his BV remained essentially stable, gaining just 35 ml during the 30 min. In contrast, his BV gradually increased during the supine period and decreased while standing. The increase totalled 180 ml, due to the addition of 75 ml to PV and 105 ml to RCV; the decrease was 580 ml, including the loss of 190 ml from PV and 390 ml from RCV. Changes in BV were partially reflected by changes in IFV, such that IFV decreased by 320 ml while supine and increased by 400 ml when standing. Despite the fluid shifts across the vascular membrane, the distribution of fluid between intra- and extracellular compartments showed no consistent change regardless of posture, and with no urine being produced at all TBW was relatively stable.

The changes in BV were inversely related to changes in f_c. N3's f_c was initially 57 b·min\(^{-1}\), but fell during the supine period, when BV increased, and rose while standing, when BV decreased. His f_c averaged 45 (± 2), 56 (± 2), and 65 (± 2) b·min\(^{-1}\) during the 30 min of lying, sitting, and standing, respectively.

In general, the response of Subject N3 to postural manipulation was followed by the remaining subjects. BV increased by an average of 89 (± 82) ml during the supine period and decreased by 406 (± 89) ml during standing (p=0.003; Figure 4.3). The decrease in BV was rapid during standing, reaching 327 (± 78) ml by minute 15 of the period (p=0.003). Changes in BV were largely accounted for by changes in PV (Figure 4.3), such that PV tended to increase during supine rest (+52 ± 70 ml) and to decrease (-233 ± 64 ml) during standing (p=0.011). PV decreased by 197 (± 58) ml during the initial 15 min of standing upright (p=0.011). Fluctuations in RCV accounted for the remaining changes in BV, although RCV was not significantly affected by changes in posture (p=0.089, \(\phi=0.581\); Figure 4.3). RCV tended to decrease during the upright period (-174 ± 63) ml, but was relatively stable during the supine and seated periods (+38 ± 75 and +54 ± 70 ml, respectively).

The extravasation of fluid during the upright period was absorbed by the interstitial compartment, such that IFV increased during standing compared to the other two postures (p=0.014; Figure 4.3). IFV increased by 513 (± 104) and 655 (± 165) ml, respectively, during 15 and 30 min of standing, and tended to decrease while supine (-86
Figure 4.3 Intracellular water (ICW), extracellular fluid (ECFV), interstitial fluid (IFV), blood (BV), plasma (PV), and erythrocyte (RCV) volumes during 30 min of supine, seated, and upright rest in a control environment (22°C; means ± SEM; normalised to preexperimental levels).
± 145 ml). IFV also tended to increase during the seated period (+234 ± 158 ml).

Fluid shifts across the vascular membrane apparently did not disturb the distribution of fluid between the intra- and extracellular compartments, as no consistent changes were detected in either ECFV or ICW, regardless of posture ($p=0.271$, $\phi=0.368$; and $p=0.800$, $\phi=0.131$, respectively; Figure 4.3).

There were also no significant changes in TBW throughout the assessment ($p=0.842$, $\phi=0.118$), despite the loss of body fluid in all three postures. Fifty-six (± 20), 37 (± 12), and 62 (± 25) ml of urine were excreted following the supine, seated, and upright periods, respectively ($p=0.956$, $\phi=0.056$), and, an additional 15 to 20 ml of fluid were probably lost during each period, through evaporative channels of respiration and perspiration. However, it was considered that the total loss of fluid from the body, amounting to less than 0.2% of TBW, was probably within the sensitivity of the TBW measurement, and hence was undetectable using the tritium dilution method.

The similarity of urine production between postures was also attributed to the relatively small volumes involved, which was itself a consequence of the duration of postural manipulation. Generally, urine flow is increased during supine rest, due to increased central venous pressure suppressing the release of renin and aldosterone and stimulating the release of atrial natriuretic peptide (Solomon et al., 1986; Norsk et al., 1993). However, it was considered unlikely that such hormonal changes would be effective within 30 min, and hence, in the present study, urine production remained relatively constant between postures.

As with Subject N3, it appeared that changes in BV affected the rate of $f_c$. Cardiac frequency was consistently faster while standing, when BV was depleted, compared to $f_c$ during both the seated and supine periods ($p=0.001$). In contrast, $f_c$ was slowed during supine rest, when BV tended to increase, compared to the other two postures ($p=0.001$).

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*Seven of the same subjects lost an average of 15 (± 7) ml of fluid through evaporation during 30 min of seated rest in the same control environment (22.0°C, 52% relative humidity; see Section 5.2).*
Cardiac frequency averaged 61, 56, and 49 b·min$^{-1}$ during the 30 min of standing, sitting, and lying, respectively.

The response of BV to changes in posture was similar when determined using the indirect method of measurement, from changes in Hct, and [Hb] (after Dill & Costill, 1974), as it was when using radionuclide dilution. Relative changes in BV, measured using radionuclide dilution, equated to -6.0% (± 1.2%), 0.0% (± 1.6%), and +1.5% (± 1.3%) during the upright, seated, and supine periods, respectively, compared to values of -4.5% (± 1.0%), -1.0% (± 0.6%), and +2.0% (± 0.3%) indicated by the indirect method (p=0.782, φ=0.137). Similarly, relative changes in PV and RCV, measured using radionuclide dilution, did not differ from those indicated by the indirect method, regardless of posture or time. Changes in PV, measured using radionuclide dilution, equated to -6.0% (± 1.5%), -1.5% (± 1.1%), and +1.1% (± 1.8%) during standing, sitting, and lying, respectively, compared to values of -7.4% (± 1.8%), -0.9% (± 0.5%), and +3.7% (± 0.6%) indicated by the indirect method (p=0.357, φ=0.310). Corresponding changes in RCV were -5.7% (± 2.3%), +2.1% (± 2.8%), and +1.8% (± 2.8%), respectively, compared to -0.9% (± 0.3%), +0.1% (± 0.4%), and -0.1% (± 0.4%; p=0.184, φ=0.446).

The relationship between whole-body haematocrit (Hct$_w$) and Hct$_r$ (f-ratio), which initially averaged 0.931 (± 0.018), did not change throughout assessment, regardless of posture or time (p=0.091, φ=0.577). After 30 min of upright rest, the f-ratio was 0.915 (± 0.023), compared to 0.938 (± 0.025) and 0.941 (± 0.029) after the seated and supine periods, respectively.

### 4.3 Discussion

Initial fluid volumes, while larger than reference norms (International Commission on Radiological Protection, 1975; International Committee for Standardization in Haematology, 1980), were considered appropriate for endurance trained males; comparable volumes have previously been reported for similar athletic populations (Sjöstrand, 1962; see Section 3.3 for a complete discussion of resting fluid volumes).
With the exception of one subject, who experienced severe dizziness during the last 5 min of standing, the experimental procedure was completed without incident. Dizziness, and even fainting, have previously been reported during prolonged standing, and have been associated with marked decreases in circulating blood volume (Youmans et al., 1934; Hinghofer-Szalkay & Moser, 1986). Indeed, in the present study, the distressed subject lost 691 ml of blood during the 30 min, compared to a mean of 365 (± 79) ml for the remaining 7 subjects.

4.3.1 Body-Fluid Movements During Postural Manipulation

During the present study, BV decreased by 6.0% on standing from the seated posture, which was comparable to the intravascular expansion of between 2% and 7% suggested by the data of Tan et al. (1973), for subjects making the opposite movement. The majority of the present blood loss was accounted for by a decrease in PV, which occurred rapidly on assuming the upright posture. Plasma volume decreased by 197 ml (5.1%) during the initial 15 min of standing, progressing to 233 ml (6.0%) by the end of 30 min. It is not known whether PV, and hence BV, would have continued to decline after this time, although there was no significant difference between PV at minutes 15 and 30. Tan et al. (1973) and Hagan et al. (1978, 1980) suggested that posturally induced fluid shifts were essentially complete within 20 to 25 min of changing posture; however, others have shown that stability may not be achieved within 40 min or more (Waterfield, 1931b; Youmans et al., 1934). Hence, it is recommended that a posture be maintained for at least 30 min if it is desired to stabilise body-fluid distribution.

The decrease in PV during upright rest was apparently due to a filtration of plasma from the circulation into the interstitium, as a concurrent increase in IFV was also evident. The filtration was probably caused by an increase in capillary hydrostatic pressure in the dependent limbs, resulting directly from the body’s upright posture. Although standing upright is known to cause a widespread peripheral vasoconstriction that would tend to decrease capillary pressure (see Rowell, 1983), the weight of the upright venous columns would have tended to cause plasma to filter from the nonconstricted veno-capillaries (Stick et al., 1993a). Indeed, pressure in the saphenous vein has been shown to increase ten-fold on moving from supine to upright (Pollack & Wood, 1949; Stick et al., 1993b),
leading to the efflux of fluid from the microcirculation.

It appeared that the plasma filtrate was absorbed entirely by the interstitium, as there was no real change in the volume of the intracellular extravascular compartment. However, there remained a discrepancy between the magnitudes of BV contraction and IFV expansion during the upright period. BV decreased by 406 ml while IFV increased by 655 ml; however, the difference between the two equated to less than 1.5% of IFV, and was therefore probably within the error of the IFV measurement. Hence, it was considered that the discrepancy reflected the insensitivity of IFV measurement rather than the influx of fluid from another source, such that intravascular fluid was eluted only into the interstitium during upright rest.

The extent of IFV expansion was itself limited by mechanisms which automatically defend the volume of intravascular fluid. For example, the movement of fluid into the interstitium during upright rest was probably countered by increases in both interstitial hydrostatic pressure (Wells et al., 1938; Aratow et al., 1993) and plasma oncotic pressure, resulting from the hypotonic nature of the plasma filtrate (Youmans et al., 1934; Hinghofer-Szalkay & Moser, 1986; see Aukland & Nicolaysen, 1981, for review). Thus, the balance of homeostatic forces would have been restored, and the further movement of plasma into the interstitium would have been prevented.

Increased plasma oncotic pressure might also have been expected to dehydrate the erythrocytes, as mean erythrocyte volume is known to be dependent on plasma osmolarity (Costill et al., 1974). Indeed, RCV tended to decrease during the upright period, but this trend failed to reach statistical significance. Waterfield (1931a) reported a decrease of 85 ml in erythrocyte volume during 60 min of upright rest following prolonged recumbency, but this finding has since been opposed by both Hagan et al. (1978) and Hinghofer-Szalkay and Moser (1986). The response of erythrocyte volume to upright rest therefore remains unclear, although the present data suggest that changes in BV could not be accounted for solely by changes in PV.

Regardless of the origin of the eluted intravascular fluid, the decrease in BV during
upright rest was apparently sufficient to stimulate an increase in $f_c$. Cardiac frequency increased during standing, probably as a reflex response to decreased central blood volume and central blood pressure, which in turn invoke sympathetic vasoconstriction and a reduction in vagal tone. Central blood volume is known to fall in the upright posture, due to impaired venous return (Matzen et al., 1991), and consequently cardiac frequency has been found to be elevated during both rest and exercise in the upright posture, compared to equivalent tasks performed horizontally (Diaz et al., 1979; Matzen et al., 1991).

In contrast, both central and total blood volumes are thought to increase on assuming a supine posture (Fawcett & Wynn, 1960; Tan et al., 1973; Norsk et al., 1993). Hence, in the present study, BV tended to increase on moving from the seated to the supine posture. While this fluid shift did not reach statistical significance, it was accompanied by a slight decrease in IFV, and trends towards expansion of PV and RCV. It was therefore considered probable that, on lying horizontal, fluid was absorbed into the circulation from the interstitial compartment, in response to a decrease in mean circulatory pressure resulting from central blood pooling and reduced vasoconstriction (Norsk et al., 1993).

The intravascular influx amounted to 1.5% of BV during the 30 min of supine rest, which was similar to the fluid shift determined by Maxfield et al. (1941), on moving subjects from supine to seated. It was, however, less than that effected by both Diaz et al. (1979) and Hagan et al. (1980) on moving from supine to seated, although both of the latter studies determined intravascular fluid shifts from changes in Hct. It is therefore noteworthy that the apparent extent of intravascular fluid shifts determined from Hct, is dependent on the orientation of the blood sampling site to the rest of the body, such that a dependent blood sample exaggerates the intravascular response compared to blood collected at heart level (Wilkerson & Gaddis, 1984; Sullivan & Fuentes, 1993). In the present study, blood was collected from the forearm at approximately heart level regardless of posture, making it less susceptible to the effects of localised haemoconcentration and more representative of whole-body fluid distribution. In addition, both the present study and that of Maxfield et al. (1941)
measured intravascular volume using dilution methods, which are less susceptible to localised changes in blood composition than are methods using the application of Hct. It is therefore possible that the difference in fluid distribution between seated and supine postures is less than has been suggested previously, when intravascular volumes have been calculated using Hct.

4.3.2 Methodological Considerations
In the present study, relative changes in intravascular fluid volumes calculated using Hct, and [Hb] (after Dill & Costill, 1974) were similar in magnitude to those determined using radionuclide dilution, regardless of the posture or its duration. Ultimately, this was not surprising, as there was no change in the ratio between the whole-body and venous haematocrits (f-ratio) throughout the study, and care was taken to collect blood from a nondependent site. Thus, changes in Hct could be assumed to reflect changes in the composition of blood throughout the circulation. It is not known whether this would have been the case had the blood samples been collected from an alternative site, which may have been subject to localised haemoconcentration. Hence, it is recommended that, if Hct, and [Hb] are used to assess changes in intravascular volumes during postural manipulation, the necessary blood samples should be collected from a nondependent sample site.

4.3.3 Conclusion
In conclusion, it was confirmed that the distribution of body fluid is dependent on the posture of the subject. On assuming an upright posture following seated rest, intravascular volume rapidly decreased, due to the filtration of plasma into the interstitium. Conversely, during supine rest, intravascular volume tended to increase, reflecting the movement of fluid from the interstitium into the circulation. Thus, Hypothesis 1.1.1 (see Section 1.1) was accepted for both upright and supine postures. The extravasation of plasma during upright rest was accompanied by an increase in cardiac frequency, probably in response to decreases in central blood volume and central blood pressure. In addition, as plasma loss progressed, erythrocyte volume tended to diminish, probably reflecting an increase in plasma tonicity. During supine rest, the expansion of plasma and erythrocyte volumes was not as great as had previously been
suggested, possibly due to previous neglect towards the orientation of the blood sampling site. When blood was sampled from approximately heart level it was possible to accurately determine changes in intravascular volumes from changes in Hct, and [Hb], without the need for radionuclide dilution. It was not clear how long was required for intravascular volume changes to reach equilibrium; however, it is recommended that a posture is maintained for at least 30 min if it is desired to stabilise body-fluid distribution.
4.4 References


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Body fluid is critically involved in the regulation of body temperature, providing cooling through evaporation, and both cooling and warming through heat transfer from the circulation (see Stolwijk & Hardy, 1977). Thus, through evaporation and changes in the circulation, both the volume and the distribution of body fluid are susceptible to thermal influence. For example, in hot environments, where the need for evaporative cooling is increased, body-fluid losses exceed those incurred during equivalent tasks in cooler conditions (Adolph & Molnar, 1946; Morimoto et al., 1967). At the same time, changes in vasomotor tone redistribute blood from the central to the peripheral circulation, to facilitate the conduction and convection of heat to the environment (see Rowell, 1983). It follows, therefore, that not only the volume of total body fluid, but also the volume of specific fluid compartments, will be dependent on the prevailing environmental temperature.

The volume of the intravascular compartment appears to be particularly susceptible to environmental influence, decreasing in cold conditions (Bass & Henschel, 1956; Vogelaere et al., 1992), and potentially increasing in the heat, depending on the severity of the stress (Adolph et al., 1969; see Harrison, 1985, for review). In the cold, the decrease manifests as a reduction in plasma volume (Adolph & Molnar, 1946; Vogelaere et al., 1992), which has been attributed both to an increase in capillary hydrostatic pressure, caused by thermally induced venoconstriction (Harrison, 1985), and to an increase in urine production, due to the stimulation of antidiuretic hormones (Eliot et al., 1949). Hence, plasma volume has been found to contract during both long- and short-term cold stress (Lennquist et al., 1974; Harrison et al., 1983; see Fregly, 1982, for review).

Conversely, during heat exposure, the veno-capillaries relax to increase cutaneous perfusion and assist with thermoregulation, causing a reduction in capillary hydrostatic pressure, and potentially an increase in plasma volume. Indeed, plasma volume has been found to expand during various periods of heat exposure, imposed both naturally and
artificially (Bazett et al., 1940; Adolph et al., 1969; Harrison et al., 1981). However, plasma volume has also been shown to contract during heat stress (Diaz et al., 1979; Harrison et al., 1983), or to initially expand and then contract, as the stress is prolonged (Senay & Christensen, 1965; see Harrison, 1985, for review). According to Harrison (1985, 1986), the distinction between plasma expansion and contraction during heat exposure is related to the severity of the stress, such that warm environments produce a plasma expansion in response to venodilation, while extreme heat induces plasma loss due to vasodilation and body-fluid loss.

Extreme heat has also been shown to decrease erythrocyte volume (Costill & Fink, 1974; Harrison et al., 1981), presumably in response to an increase in plasma tonicity accompanying the development of dehydration (Costill & Saltin, 1974). Erythrocytes are known to be responsive to the tonicity of their surrounding medium (Costill et al., 1974), and plasma tonicity tends to increase during thermal dehydration (Senay & Christensen, 1965). Hence, the cells themselves may become dehydrated, reducing both the mean cell and total erythrocyte volumes. This finding has not, however, been universally corroborated (Myhre & Robinson, 1969), and may be dependent on the extent of dehydration and the accompanying rise in plasma tonicity.

Erythrocyte volume is therefore probably maintained during cold stress (Bass & Henschel 1956), when plasma tonicity remains essentially constant, despite the significant decrease in plasma volume (Young et al., 1987). However, there is presently a paucity of information regarding the response of erythrocyte volume to cold stress, and therefore the overall effect of cold on the distribution of intravascular fluid remains unclear.

The study of erythrocyte response to thermal stress has perhaps been hampered by the use of venous haematocrit (Hctv) to indicate changes in plasma volume, forcing the assumption that erythrocyte volume remains constant during the period of assessment (Van Beaumont, 1972; Greenleaf et al., 1979). Furthermore, while the use of both Hctv and haemoglobin concentration ([Hb]) accounts for simultaneous changes in both plasma and erythrocyte volumes (Dill & Costill, 1974), the method is still dependent on the
assumption that changes in Hct, accurately reflect changes in the composition of blood throughout the circulation. To date, no attempt has been made to validate this assumption using more direct measurements of intravascular fluid volumes during short-term thermal stress. Harrison (1974; Harrison & Edwards, 1976) used the dilution of radioiodinated serum albumin to show that plasma volume contracted during 2 h of heat stress, and Bazett et al. (1940) used the carbon monoxide rebreathing technique to show that erythrocyte volume expanded during 5 d in a hot environment. However, apparently only Costill and Saltin (1974) have combined dilution methods to measure both plasma and erythrocyte volumes simultaneously during thermal stress. They found that, while plasma volume significantly decreased during heat stress, erythrocyte volume was essentially constant. Unfortunately, the concurrent imposition of exercise makes the results inapplicable to resting subjects. Plasma and erythrocyte volumes have therefore not yet been measured simultaneously during short-term exposure to either heat or cold stress.

Similarly, the volume of extravascular fluids has not yet been measured during short-term thermal stress in resting humans. Both Costill et al. (1976) and Nose et al. (1988) examined the whole-body distribution of fluid during thermal dehydration, but used both heat and exercise to effect fluid changes. In addition, they used the chloride method, based on changes in plasma chloride concentration, to calculate changes in extracellular fluid volumes, making their results susceptible to changes in muscle membrane potential (Sjøgaard & Saltin, 1982). In contrast, Durkot et al. (1986) used a multiple radionuclide dilution method to simultaneously measure the volumes of total body water, extracellular fluid, and plasma during the passive dehydration of rats, finding that both the intra- and extracellular compartments contributed significantly to whole-body dehydration. A similar dilution method has been developed for the study of resting humans (see Chapter Three), and could therefore be used to examine the effects of thermal stress on human body-fluid distribution.

It was the purpose of this study to measure the distribution of body fluid in resting humans during short-term exposure to heat and cool, using simultaneous measurements of total body water, extracellular fluid volume, and plasma and erythrocyte volumes.
5.1 Methods

5.1.1 Subjects

The body-fluid distribution of seven adult males (Table 5.1) was measured using the simultaneous radionuclide dilution method described in Chapter Three. The subjects were all healthy and regularly participated in physical exercise. They were fully informed of experimental procedures, which were approved by the University's Human Experimentation Ethics Committee, and subsequently provided informed consent. Each subject was assessed on three occasions, including a hot, a control, and a cool condition, which were conducted in autumn at 28-d intervals, with the order counterbalanced between subjects. On each occasion, subjects wore only brief swimsuits and athletic shoes.

5.1.2 Procedures

Preliminary procedures and radionuclide preparations were those described in Sections 3.1.2 and 3.1.3. Subjects arrived at the laboratory in a rested, fasted state, consumed breakfast (38 kJ·kg⁻¹ of body mass, plus 5 ml·kg⁻¹ of water), and were seated at rest, at least 30 min prior to assessment. Before breakfast, a urine void and 20 ml of venous blood were collected, for use as reference blanks and for radiochromate labelling. Approximately 5 ml of erythrocytes were labelled with radiochromate, using 20 min of incubation at 37°C; the labelled cells were then washed three times and resuspended in saline, and drawn into a syringe ready for injection. Quantities of tritiated water, radiobromine, and radioiodinated serum fibrinogen were drawn into similar syringes, also ready for injection. The volumes of these injections, and of the radionuclide standards, were determined gravimetrically, to a precision of 0.001 g (Denver Instruments, AL-300).

A 16-gauge teflon cannula was inserted into an antecubital vein, through which to administer the radionuclide injections. Two microcuries (μCi) of radioiodinated human serum fibrinogen (RISF; Amersham Australia, ¹²⁵I Human Fibrinogen), 8 μCi of sodium radiochromated autologous erythrocytes (Amersham Australia, Na⁵¹Cr), 20 μCi of sodium radiobromine (Australian Radioisotopes, Na⁸²Br), and 500 μCi of tritiated water (Amersham Australia, ³H₂O) were injected sequentially, within a 30-s period. The
Table 5.1
Physical Characteristics of Subjects

<table>
<thead>
<tr>
<th></th>
<th>Age (y)</th>
<th>Height (cm)</th>
<th>Mass (kg)</th>
<th>Σ8SkF (mm)</th>
<th>V_{O_{peak}} (ml·kg^{-1}·min^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>24.6</td>
<td>165.7</td>
<td>72.62</td>
<td>80.7</td>
<td>53.6</td>
</tr>
<tr>
<td>S2</td>
<td>24.5</td>
<td>180.2</td>
<td>64.55</td>
<td>60.0</td>
<td>84.9</td>
</tr>
<tr>
<td>S3</td>
<td>23.2</td>
<td>183.8</td>
<td>85.81</td>
<td>66.8</td>
<td>69.1</td>
</tr>
<tr>
<td>S4</td>
<td>21.0</td>
<td>176.4</td>
<td>78.64</td>
<td>89.3</td>
<td>59.4</td>
</tr>
<tr>
<td>S5</td>
<td>27.8</td>
<td>183.9</td>
<td>90.07</td>
<td>95.5</td>
<td>53.6</td>
</tr>
<tr>
<td>S6</td>
<td>33.8</td>
<td>174.5</td>
<td>73.60</td>
<td>64.3</td>
<td>74.6</td>
</tr>
<tr>
<td>S7</td>
<td>28.8</td>
<td>185.6</td>
<td>80.85</td>
<td>44.5</td>
<td>64.0</td>
</tr>
<tr>
<td>Mean</td>
<td>26.2</td>
<td>178.5</td>
<td>78.02</td>
<td>71.6</td>
<td>65.6</td>
</tr>
<tr>
<td>σ</td>
<td>4.0</td>
<td>6.5</td>
<td>8.61</td>
<td>16.5</td>
<td>10.7</td>
</tr>
</tbody>
</table>

where: Σ8SkF = the sum of biceps, triceps, subscapular, midaxillary, suprailiac, abdominal, thigh, and calf skinfold thicknesses;
V_{O_{peak}} = maximal aerobic power (estimated after Jones, 1988);
σ = standard deviation.
radionuclides were used to measure plasma (PV) and erythrocyte (RCV) volumes, extracellular fluid volume (ECFV), and total body water (TBW), respectively. The midtime of the fibrinogen injection was considered as the commencement of assessment, time-zero \( (t_0) \). Immediately following the injections, the cannula was flushed with a minimum of 15 ml of saline followed by 5 ml of heparinised saline, rendering it suitable for subsequent blood sampling (Ladegaard-Pedersen & Engell, 1969).

Ten-millilitre blood samples were collected after 30, 60, and 180 min, from which to determine initial fluid volumes (see Sections 3.1.4 to 3.1.6). Samples were collected without stasis and with the forearm at approximately heart level, and were immediately treated with ethylenediamine tetra-acetic acid (EDTA; 1.8 mg ml\(^{-1}\) of blood). Before each sample, 5 ml of fluid were removed from the cannula, and, following each one, the cannula was flushed with 10 ml of heparinised saline. A urine void was collected after 180 min, and measured volumetrically to a precision of 5 ml.

The 180 min following the radionuclide injections were spent seated at rest, with at least the last 30 min being in a control environment of 22.0°C (± 1.0°; standard deviation), 52% (± 6%) relative humidity (rh). The subsequent 30 min were spent seated, in a string-backed chair, in either a hot (36.2°C ± 0.7°, 44% ± 3% rh), the control, or a cool (14.4°C ± 1.6°, 74% ± 9% rh) environment. In all the environments, air movement was less than 0.5 m s\(^{-1}\), and black-globe temperature was within ± 0.5°C of ambient temperature. The environments were maintained, in the University's Environmental Physiology Research Laboratory, using a combination of heaters, coolers, and dehumidifiers, controlled by microcomputer (see Maw & Williamson, 1992 [Appendix B]). Subjects were moved from the control environment into the Environmental Laboratory in a wheelchair, so that their seated posture was not disturbed. A change of posture from the seated control would have affected fluid distribution independently of the ensuing thermal stress (see Section 4.2).

At least 30 min prior to entering the Environmental Laboratory, a thermistor probe (London Hospital Medical College, zero-gradient aural thermometer) was inserted into the left aural canal, to a depth of approximately 10 mm. The probe, which was used to
measure deep-body temperature ($T_w$), was secured with a cotton wad, covered by a padded headset, and interfaced with an electrically-isolated microcomputer. The headset maintained the outer ear at the same temperature as the aural canal, using servo-heating, and hence eliminated the direct influence of environmental temperature from the measurement of $T_w$ (Keatinge & Sloan, 1975). Measured in this manner, $T_w$ closely corresponds to the temperature in the midesophagus, both quantitatively and dynamically, during both rest and exercise in a range of thermal environments (Maw & Taylor, 1992 [Appendix C]; Figure 5.1). $T_w$ was recorded at 5-s intervals throughout the 30-min environmental exposures.

Skin temperatures were also recorded at 5-s intervals throughout exposure, using surface thermistors (YSI, Mini-thermistor EU) positioned on the forehead, the left upper chest, the dorsal aspect of the left forearm and hand, and over the right scapula, right deltoid, right rectus femoris, and left gastrocnemius. The thermistors were secured with a single covering of waterproof adhesive tape, and interfaced with a data logger (Grant Instruments, 1200 series Squirrel). The skin thermistors and the aural probe were previously calibrated against a certified mercury-in-glass thermometer (Dobbie Instrument, Dobros total immersion)*.

Cardiac frequency ($f_c$) was recorded, from ventricular depolarisations, at 5-s intervals throughout exposure, using a telemetry system (Polar Electro, PE3000). The system provides a record of $f_c$ comparable to that measured using electrocardiography during rest, exercise, and recovery ($r^2=0.999$, from Osborne, 1994 [see Figure 4.1]).

Body mass was measured immediately before and immediately after environmental exposure, to an accuracy of ± 5 g, using a modified electronic scale (A&D, FW-150K). The scale, which was interfaced directly to a microcomputer, was calibrated prior to each assessment, in the prevailing environment, using calibrated standard masses. Output was found to be linear through a physiological range of mass, in each of the three

*The mercury-in-glass thermometer (Dobbie Instruments, Dobros total immersion) was certified to an accuracy of ± 0.05 °C by the National Association of Testing Authorities, Australia.
Figure 5.1 Oesophageal ($T_{oe}$) and aural ($T_{au}$) temperatures during 30 min of cycle exercise at 150 W in a neutral (24°C) environment, followed by 30 min of cycle exercise at 150 W in either a cool (13°C; upper graph) or a hot (36°C; lower graph) environment (means ± SEM, normalised to mean resting level; from Maw & Taylor, 1992 [Appendix C]).
environmental conditions.

Blood samples were collected, as described above, after 15 and 30 min of each environmental exposure, and a urine void was collected at the end of exposure, after the subject had been weighed.

5.1.3 Calculation of Thermoregulatory Responses

Mean skin temperature ($T_{sk}$) was calculated from the weighted mean of the eight skin temperatures, after the International Organization for Standardization (1992):

$$T_{sk} = 0.07(T_f + T_{ua} + T_{la}) + 0.175(T_{uc} + T_s) + 0.05T_h + 0.19T_t + 0.27T_c$$

where:
- $T_f$ = forehead temperature;
- $T_{uc}$ = upper chest temperature;
- $T_s$ = scapular temperature;
- $T_{ua}$ = deltoid temperature;
- $T_{la}$ = forearm temperature;
- $T_h$ = hand temperature;
- $T_t$ = rectus femoris temperature;
- $T_c$ = gastrocnemius temperature.

Evaporative water loss, including sweat and respiratory water, was calculated from the change in body mass during environmental exposure, corrected for the mass of interim blood collections.

5.1.4 Blood and Urine Analyses

Blood and urine collections were analysed as described in Section 3.1.4. All blood samples were assessed for venous haematocrit (Hct) and haemoglobin concentration ([Hb]), to accuracies of ± 0.83% and ± 0.78%, respectively (Coulter Electronics, S-Plus IV; see Section 3.1.4), and then centrifuged for 40 min at 1500 g, to separate plasma from erythrocytes. Trapped plasma was taken as 2% of the resultant packed erythrocytes (after Chaplin & Mollison, 1952).

Three-millilitre aliquots of plasma and erythrocytes were then dispensed from each blood sample into glass vials, and refrigerated at 4°C, pending gamma radiation ($\gamma$) counting. A 3-ml aliquot from each urine void and from each of the $^{125}$I, $^{51}$Cr, and $^{82}$Br standards
was similarly stored. Erythrocyte aliquots were haemolysed prior to storage, using a trace of powdered saponin (Sigma Chemical Company, S-1252). Further 0.5-ml aliquots of plasma and urine, and 0.5-ml aliquots of the $^3$H and $^{125}$I standards, and of distilled water, were dispensed into glass vials, vigorously mixed with 0.05 ml of 1 molar hydrochloric acid and 9 ml of liquid scintillation cocktail (Packard Instruments, Emulsifier-Safe), and refrigerated pending beta radiation ($\beta$) counting. All aliquots were measured volumetrically, to an accuracy of $\pm 0.2\%$ (Labsystems, Finnpipette; see Section 3.1.3.2).

After radiation counting, plasma aliquots were assessed, in triplicate, for protein concentration, to a precision of 0.1 g$^{-1}$, using a refractometer (Otago, 93032). The mean of the three refractometer readings was considered as plasma protein concentration ([PP]; see Section 3.1.4).

5.1.5 Radiation Counting

Radiation counting was conducted as described in Section 3.1.5. Radioiodine, $^{51}$Cr, and $^{82}$Br were counted using a well-type $\gamma$ scintillation counter, calibrated to measure the optimum range of radiant energy of the respective nuclides, to efficiencies of between 25% and 29% (Abbott Laboratories, Auto-LOGIC; see Figure 3.1); $^3$H was counted using a liquid scintillation counter, calibrated to measure $\beta$ energy between 0.005 and 0.132 million electron volts (MeV)*, to an efficiency of between 33% and 37% (LKB Wallac, 1219 Rackbeta).

Radiobromine was counted on the day of assessment, with all other counting delayed for 14 days, pending the decay of $^{82}$Br. Aliquots were counted twice for $^{82}$Br, each time for 12 min, and three times for $^{125}$I, $^{51}$Cr, and $^3$H, for 6, 10, and 2 min, respectively. For each radionuclide, the sequence of vials was reversed between counts. The duration of the counts ensured a minimum of 10000 counts were aggregated for each radiated aliquot in each of the calibrated ranges. The mean of the respective counts, minus the mean of the relevant distilled water, plasma, erythrocyte, or urine background reference,

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*1 MeV = 1.6 x $10^{-13}$ joules.
5.1.6 Determination of Compartmental Fluid Volumes

Initial fluid volumes were calculated from radionuclide concentrations in the blood collected 30, 60, and 180 min after the radionuclide injections (see Section 3.1.6). Later volumes, during environmental exposure, were similarly calculated, by comparing the radionuclide concentrations in the blood samples with the respective radionuclide doses. Volumes were thus calculated for minutes 0, 15, and 30 of each environmental exposure.

Fluid volumes during environmental exposure were calculated as described in Sections 4.1.5.1 and 4.1.5.2. TBW, at any given time \( t_i \), was calculated from \(^3\text{H}\) in the appropriate plasma aliquot, corrected for the presence of plasma protein and \(^{125}\text{I}\), and for \(^3\text{H}\) urine loss. ECFV was calculated from \(^{82}\text{Br}\) in the appropriate plasma, corrected for the presence of protein, for \(^{82}\text{Br}\) erythrocyte and urine losses, and for the Gibbs-Donnan electrolyte ratio. PV was calculated from a comparison of \(^{125}\text{I}\) measured in the appropriate plasma with that predicted, using semilogarithmic extrapolation of the \(^{125}\text{I}\) elution curve, for the equivalent time (after Gibson & Evans, 1937, and Harrison & Edwards, 1976). RCV was calculated from \(^{51}\text{Cr}\) in the appropriate erythrocytes, corrected for \(^{51}\text{Cr}\) urine loss.

The presence of protein was corrected for by calculating the protein displacement factor using [PP] (after Chien & Gregersen, 1962; see Section 3.1.6). Radionuclide urine losses were calculated from the respective radionuclide concentration and the volume of the voids collected up to \( t_i \). The resultant radionuclide contents were summed up to \( t_i \), or, in the case of a void following an environmental exposure, were apportioned to the preceding 30 min on a linear basis. The Gibbs-Donnan ratio was taken as 1.02 for radiobromine (Manery, 1954).

Intracellular water (ICW), interstitial fluid volume (IFV), and blood volume (BV) were calculated as described in Section 3.1.6.5. ICW was calculated as the difference between TBW and extracellular water volume (ECW), where ECW was calculated from ECFV.
adjusted for the presence of all plasma solutes; IFV was calculated as the difference between ECFV and PV; BV was calculated as the sum of PV and RCV.

Whole-body haematocrit (Hctₜ) was then calculated as the ratio of RCV to BV, and the $f$-ratio was considered as the ratio between Hctₜ and the haematocrit in the corresponding venous blood sample (Hctᵥ).

Relative changes in BV, PV, and RCV were calculated, with respect to values immediately preceding each environmental exposure, from measured volumes, and also from changes in Hctᵥ and [Hb] (after Dill & Costill, 1974; see Section 4.1.5.4).

5.1.7 Analysis

$T_{ac}$, $T_{sk}$, and $f_c$ were averaged for the minute surrounding each 5-min interval, such that values were derived for minutes 0, 5, 10, 15, 20, 25, and 30 of each environmental exposure. Data were also grouped to produce mean $T_{ac}$, $T_{sk}$, and $f_c$ across the duration of each exposure.

Body-fluid volumes were normalised to their initial values, to negate physiological variations occurring over the course of the study. Considerable physiological variations were possible over the minimum course of 56 d, which would have been unrelated to the experimental manipulations. It was therefore considered prudent to normalise the fluid volumes, and hence to consider absolute changes in volumes during subsequent analysis. Urine volumes displayed proportional variance, and were therefore transformed to logarithms prior to further analysis (Montgomery & Peck, 1982).

Factorial analysis of variance was used to determine differences related to environmental condition and time; one-way analysis of variance was used to determine differences when environmental condition was the only independent variable (Statistical Package for Social Scientists [SPSS], 1988). In the event of a significant result, specific differences were determined using Tukey's test of Wholly Significant Difference (Tukey WSD; Howell, 1992).
Differences were considered significant if the probability that the difference was due to chance was less than 5% (α=0.05). When differences were not significant, the power (ϕ) of the analysis was computed (SPSS, 1988). Analysis of variance summary tables are included in Appendix G; data in the text are means with standard errors of the means (SEM), unless otherwise stated as standard deviations (σ).

5.2 Results

Initially, while seated in the control environment, Tₐₑ ranged between 36.7° and 38.2°C, with a mean of 37.2°C (± 0.3°; mean ± σ). At the same time, Tₕₑ ranged between 29.2° and 34.5°C, with a mean of 31.4°C (± 0.7°), while fₑ averaged 61 (± 10) b·min⁻¹ (means ± σ).

5.2.1 A Case Study of Responses to Thermal Stress

Tₐₑ, Tₕₑ, and fₑ were subsequently influenced by changes in the environment, with the responses of one subject, N7, illustrating the effect of the hot and cool conditions (Figure 5.2). The subject was 28.8 y old, 185.6 cm tall, and weighed 78.74 kg. His initial Tₐₑ, Tₕₑ, and fₑ were 37.4°C, 31.4°C, and 54 b·min⁻¹, respectively. On entering the hot environment, Tₐₑ began to fall, dropping to 37.1°C by minute 15 of the exposure and remaining there for the remainder of the period. In the cool condition, Tₐₑ rose, reaching 37.6°C by minute 20, but subsiding to 37.5°C by the end of the period. Conversely, Tₕₑ rose during hot exposure and fell in the cool, reaching 35.8° and 26.5°C by the end of the respective periods. Cardiac frequency also rose in the heat and fell in the cool, averaging 58 and 47 b·min⁻¹ during the respective 30-min periods.

The response of Subject N7's fluid volumes was also fairly typical of the effects of the hot and cool exposures (Figure 5.3). His initial TBW, ECFV, PV, and RCV were 54065, 20485, 3750, and 2725 ml, respectively; hence, his ICW, IFV, and BV were 33840, 16735, and 6475 ml, respectively. BV subsequently contracted during heat exposure, losing 50 ml during the 30 min period. However, BV contracted considerably more during the cool period, losing 450 ml during the 30 min. Approximately forty-five percent of the blood losses were accounted for by changes in PV, with PV decreasing.
Figure 5.2 Aural ($T_{ac}$) and mean skin ($T_{sk}$) temperatures, and cardiac frequency ($f_c$) for Subject N7 during 30 min of hot (36°C), control (22°C), and cool (14°C) exposure.
Figure 5.3 Intracellular water (ICW), extracellular fluid (ECFV), interstitial fluid (IFV), blood (BV), plasma (PV), and erythrocyte (RCV) volumes for Subject N7 during 30 min of hot (36°C), control (22°C), and cool (14°C) exposure (normalised to mean preexposure levels).
by 35 and 170 ml during the hot and cool exposures, respectively; hence, RCV decreased by 15 and 275 ml, respectively.

Despite the movement of fluid across the vascular membrane, no consistent patterns were evident in N7's IFV, ECFV, and ICW, regardless of the environmental condition. Similarly, despite the noticeable loss of fluid from the body, TBW was relatively stable in all three environments; body-fluid losses totalled 60, 45, and 145 ml during the hot, control, and cool exposures, respectively, including 45, 30, and 85 ml of urine, and 15, 15, and 60 ml of evaporated water, respectively.

5.2.2 Thermoregulatory and Body-Fluid Responses to Thermal Stress

The response of Subject N7 was typical of the remaining subjects during the thermal stresses, for whom $T_{ac}$ decreased in the heat, and increased in the cool, compared to the control condition ($p=0.001$; Figure 5.4). On average, $T_{ac}$ fell by 0.4°C ($\pm 0.1°$) during the first 20 min in the heat, before stabilising, and rose by 0.2°C during the equivalent time in the cool, then remaining elevated for the remainder of the exposure ($p=0.001$). Conversely, $T_{sk}$ was higher in the heat than it was in the control condition, where in turn it was higher than in the cool ($p=0.001$; Figure 5.4). $T_{sk}$ rose throughout the hot exposure, reaching 35.8°C ($\pm 0.1°$) by the end of the period, and fell throughout the cool period, reaching a low of 28.1°C ($\pm 0.4°$) at minute 25. Changes in $T_{sk}$ were rapid, with deflections of $+3.2°$ ($\pm 0.1°$) and $-1.8°$ ($\pm 0.1°$) occurring during the first 5 min of the respective hot and cool periods ($p=0.001$).

Cardiac frequency was also increased in the heat, and in the cool, compared to the control environment (Figure 5.4). On average across the 30-min periods, $f_c$ was increased by 7 $b\cdot min^{-1}$ in the heat and 4 $b\cdot min^{-1}$ in the cool, compared to the control environment, with the difference between all conditions being significant ($p=0.001$).

Initially, prior to thermal manipulation, TBW, ECFV, PV, and RCV averaged 51172 ($\pm 5100$), 20061 ($\pm 1693$), 3555 ($\pm 370$), and 2719 ($\pm 368$) ml, respectively (means $\pm \sigma$). Hence, ICW, IFV, and BV were 31304 ($\pm 3739$), and 16506 ($\pm 1439$), and 6273 ($\pm 644$) ml, respectively (means $\pm \sigma$).
Figure 5.4  Aural ($T_a$) and mean skin ($T_{sk}$) temperatures, and cardiac frequency ($f_c$) during 30 min of hot (36°C), control (22°C), and cool (14°C) exposure (means ± SEM).
On exposure to the cool environment, BV tended to contract in comparison to a mild expansion of BV in the heat ($p=0.051, \phi=0.584$; Figure 5.5). BV decreased by $166 (\pm 63)$ ml and increased by $142 (\pm 125)$ ml, during the initial 15 min in the cool and hot environments, respectively, and then continued to contract in the cool, losing a total of $302 (\pm 76)$ ml during 30 min ($p=0.055, \phi=0.658$). In comparison, BV stabilised during the second 15 min of the heat exposure, being expanded by just $124 (\pm 150)$ ml by the end of the period.

The majority of the changes in BV were accounted for by changes in PV, such that PV also contracted in the cool compared to the hot environment ($p=0.040$; Figure 5.5). PV decreased by $144 (\pm 53)$ ml during the initial 15 min in the cool, progressing to $205 (\pm 60)$ ml by the end of the period, compared to respective expansions of $165 (\pm 108)$ and $108 (\pm 123)$ ml during heat exposure ($p=0.020$).

RCV therefore contributed little to the changes in BV in the respective environments. Indeed, RCV was relatively constant throughout assessment, regardless of the environmental condition ($p=0.447, \phi=0.257$; Figure 5.5). RCV was within $-97 (\pm 53)$ and $+16 (\pm 33)$ ml of its initial level at the end of the cool and hot exposures, respectively.

Despite the movement of fluid across the vascular membrane, IFV was apparently unaffected by changes in the environment, throughout the 30 min of each exposure ($p=0.641, \phi=0.179$; Figure 5.5). However, it should be noted that the largest change in intravascular volume during assessment, the decrease in BV of $302$ ml during cool exposure, could have changed IFV by less than 2%, and so was probably within the error of IFV measurement. Hence, it was considered that the apparent stability of IFV might have reflected the insensitivity of its measurement, instead of a truly constant volume.

Similarly, ECFV and ICW were apparently unchanged during the various exposures, regardless of the duration ($p=0.417, \phi=0.272$; and $p=0.589, \phi=0.198$, respectively; Figure 5.5). However, in this case, the stability might also have reflected the simple
Figure 5.5 Intracellular water (ICW), extracellular fluid (ECFV), interstitial fluid (IFV), blood (BV), plasma (PV), and erythrocyte (RCV) volumes during 30 min of hot (36°C), control (22°C), and cool (14°C) exposure (means ± SEM; normalised to mean preexposure levels).
transfer of fluid from plasma to the interstitium, within the extracellular compartment, as well as the insensitivity of ECFV and ICW measurements. Hence, the balance between the intra- and extracellular compartments may truly have been maintained, regardless of the environmental condition.

TBW remained constant throughout assessment \((p=0.810, \phi=0.126)\), despite the loss of fluid from the body. Fluid losses totalled 66 (± 13), 46 (± 12), and 118 (± 13) ml during the hot, control, and cool exposures, respectively, of which urine contributed 34 (± 14), 31 (± 12), and 59 (± 21) ml, respectively \((p=0.660, \phi=0.106)\). Hence, evaporative fluid losses, including sweat and respiratory water, accounted for 32 (± 12), 16 (± 6), and 59 (± 17) ml, respectively \((p=0.133, \phi=0.391)\). Again, the maximum fluid loss, of 118 ml in the cool, accounted for less than 0.3% of TBW, and so was probably within the error of TBW measurement.

The response of BV to the respective environmental conditions was similar when determined using the indirect method, from changes in Hct, and [Hb] (after Dill & Costill, 1974), as it was when using radionuclide dilution. Relative changes in BV, measured using radionuclide dilution, equated to +2.2% (± 2.7%), -0.6% (± 1.7%), and -5.0% (± 1.2%) at the end of the hot, control, and cool exposures, respectively, compared to values of +1.2% (± 1.3%), -1.2% (± 1.5%), and -4.3% (± 1.0%) calculated using the indirect method \((p=0.544, \phi=0.215)\). Similarly, relative changes in PV and RCV, measured using radionuclide dilution, did not differ from those calculated using the indirect method, regardless of the environmental condition or its duration. Changes in PV, measured using radionuclide dilution, equated to +3.4% (± 4.0%), -1.8% (± 1.2%), and -6.2% (± 2.0%) at the end of the hot, control, and cool exposures, respectively, compared to values of +2.5% (± 2.3%), -2.1% (± 1.0%), and -7.3% (± 1.7%) calculated using the indirect method \((p=0.562, \phi=0.208)\); corresponding changes in RCV were +0.7% (± 1.3%), +1.1% (± 3.0%), and -3.5% and (± 2.0%), respectively, compared to -0.1% (± 0.4%), 0.0% (± 0.4%) and -0.6% (± 0.2%) \((p=0.560, \phi=0.209)\).

The relationship between whole-body haematocrit (Hct,) and Hct, which initially averaged 0.967 (± 0.028), did not change throughout assessment, regardless of the
environmental condition or its duration \((p=0.551, \phi=0.212)\). After 30 min of heat exposure, the \(f\)-ratio was 0.982 (± 0.037), compared to 0.939 (± 0.028) and 0.939 (± 0.037) after the control and cool exposures, respectively.

5.3 Discussion

\(T_{\infty}, T_{sk},\) and \(f_c\) were within normal range (Gagge et al., 1967), and initial fluid volumes, although larger than reference norms (International Commission on Radiological Protection, 1975; International Committee for Standardization in Haematology, 1980), were appropriate for endurance trained males; comparable volumes have previously been reported for similar athletic populations (Sjöstrand, 1962; see Section 3.3 for a complete discussion of resting fluid volumes).

5.3.1 Thermoregulatory Responses to Thermal Stress

Thermoregulatory responses to the subsequent environmental stresses were also similar to those previously reported for resting subjects. For example, the respective depression and elevation of \(T_{\infty}\) in the hot and cool environments were similar to the decrease, of 0.5°C, and the increase, of 0.2°C, reported by Rowell et al. (1969) for right atrial temperature during skin heating and cooling, respectively. In addition, counter-movements have previously been reported for both rectal and aural temperatures during short-term thermal stress (Wyss et al., 1974; Harrison et al., 1983; Vogelaere et al., 1992), and have been attributed to rapid changes in vasomotor tone. Specifically, the decrease in deep-body temperature during heat stress has been attributed to peripheral vasodilation, which allows warm blood to leave the central circulation to be replaced by relatively cool blood from the periphery (Rowell et al., 1969). Conversely, the increase during cold stress is probably due to vasoconstriction, which inhibits normal heat loss, allowing deep-body temperature to temporarily increase (Adolph & Molnar, 1946; Rowell et al., 1969). Such changes in deep-body temperature are thought to prevail until the central and peripheral circulations have fully adjusted to their new environment, which may take up to 90 min in resting subjects (Rowell et al., 1969; Vogelaere et al., 1992). Hence, in the present study, when subjects were exposed for just 30 min, \(T_{\infty}\) remained depressed and elevated throughout the hot and cool periods, respectively.
Changes in $T^c$ were probably mediated through the effect of the environment on $T_{sk}$. In the heat, $T_{sk}$ rose, initially due to the change in ambient temperature, which led to vasodilation and an increase in peripheral blood flow. Conversely, in the cool, $T_{sk}$ fell, causing vasoconstriction and a decrease in peripheral flow (see Rowell, 1983). Changes in $T_{sk}$ were rapid, being seventy-five percent complete within 5 min of entering each environment; hence, the changes in vasomotor tone were almost equally rapid, mediating prompt responses from $T^c$.

5.3.2 Body-Fluid Responses to Thermal Stress

Changes in vasomotor tone, however, were probably not as rapid or extensive as changes in venomotor tone, which, according to Harrison (1985), is the primary determinant of blood volume during short-term thermal stress. For example, had vasoconstriction been dominant during cool exposure, cutaneous blood flow, and hence plasma filtration, would have decreased, whereas in reality plasma filtration must have increased to produce a depletion of intravascular fluid volume. Thus, it appears that rapid and extensive venoconstriction may have acted to increase capillary hydrostatic pressure, producing a net filtration of intravascular fluid into the interstitium. BV decreased by 166 ml during the initial 15 min of cool exposure, progressing to 302 ml by the end of the period. Similarly, blood volume has previously been found to decrease by 160 and 510 ml during 2 h exposure to environments of 15° and 1°C, respectively (Bass & Henschel, 1956; Vogelaere et al., 1992), suggesting that the extent of blood volume depletion is dependent on the severity and the duration of cold stress.

The depletion of BV during the present cool stress was largely accounted for by changes in PV, while RCV remained essentially constant. Bass and Henschel (1956) also found no change in erythrocyte volume during 2 h of cool exposure, during which plasma volume decreased by 200 ml. The loss of plasma was attributed to an increase in capillary hydrostatic pressure, while the stability of erythrocyte volume probably reflected the concurrent stability of plasma tonicity. Vogelaere et al. (1992) showed that plasma tonicity remained constant during prolonged cold stress, despite a significant reduction in plasma volume.
The destination of the lost plasma, however, is presently difficult to determine, as IFV, ECFV, and ICW were all apparently unchanged, regardless of the environmental condition. Such stability is actually unfeasible, considering the decrease in BV during cool exposure; however, it should be remembered that the decrease in BV, of 302 ml, equated to less than 2% of IFV and ECFV and less than 1% of ICW. It was therefore probably within the error of the respective volume measurements, and, as such reflected the insensitivity of the dilution methods rather than the disappearance of the plasma filtrate.

It was previously proposed that the plasma filtrate was actually lost from the body, removed from the circulation to supply the increased urine production incurred in cold environments (Eliot et al., 1949). The cold-induced diuresis was attributed to the inhibition of arginine vasopressin and the stimulation of atrial natriuretic peptide, in response to increased peripheral resistance and central blood pressure (Segar & Moore, 1968; see Fregly, 1982, for review). However, it was considered that the present 30 min of cool exposure was not of sufficient duration to effect such humoral responses, and indeed urine production was not increased in the cool compared to the two warmer conditions. Similarly, Young et al. (1987) found no increase in urine flow during 90 min of exposure to an environment of 5°C. In addition, it has previously been shown that the decrease in plasma volume during cold exposure greatly exceeds the accompanying diuresis (Adolph & Molnar, 1946; Vogelaere et al., 1992), and, in the present study, urine production averaged only 59 ml during the cool exposure, while PV decreased by 205 ml. Hence, it was unlikely that the plasma filtrate was actually excreted from the body.

Barbour et al. (1943) proposed instead that, during hypothermia, the plasma filtrate was absorbed by extravascular muscle cells, in response to increased intramuscular tonicity as a result of shivering. However, in the present study, subjects remained normothermic during cool exposure, and shivering was not particularly evident. Indeed, $T_{sc}$ was actually elevated compared to the control and hot exposures, and $f_c$ was only increased by 4 $b\text{min}^{-1}$ compared to its rate during the control period. It was therefore unlikely that much stimulus existed to draw the plasma filtrate, or any other fluid, from the
interstitium into the cells, and it was considered that the fluid therefore remained in the interstitium throughout the cool period. Both Adolph and Molnar (1946) and Vogelaere et al. (1992) previously suggested that the interstitium provided the reservoir for cold-induced plasma filtrate, and the latter further showed that the filtration was rapidly reversed on removing the cold stimulus. Plasma volume was essentially restored within 20 min of rewarming, after being depleted by 510 ml during 2 h at 1°C (Vogelaere et al., 1992). It was therefore concluded that, far from leaving the body, the plasma filtrate remained within the extravascular compartment and was simply shifted between the plasma and the interstitium in response to changes in capillary tone.

Similarly, during the present heat exposure, there appeared to be an exchange of fluid between the plasma and interstitium, although this time from the interstitium into the intravascular compartment, probably in response to a relaxation of venomotor tone. Rapid venodilation, or relaxation of constrictor tone, would have reduced capillary hydrostatic pressure, and hence induced an influx of interstitial fluid and a resultant expansion of blood volume (Harrison, 1985). BV increased by 142 ml during the initial 15 min of heat exposure, and then remained essentially constant for the remaining 15 min. Again there were apparently no changes in IFV, ECFV, and ICW, although again this was considered reflective of the insensitivity of their respective measurements, rather than being a true indication of fluid homeostasis.

The response of BV to the heat exposure showed considerable inter-subject variation, such that two subjects actually experienced a decrease in BV, while two others showed an initial increase followed by a decrease during the second 15 min of the period. Senay and Christensen (1965) previously showed that blood volume could initially expand and then contract during heat exposure, while others have found BV to either expand or contract, depending on the severity of the heat stress (Adolph et al., 1969; Harrison et al., 1983; see Harrison et al., 1985, for review). In moderate heat, blood volume is thought to expand, in response to widespread venodilation, while in severe heat, progressive vasodilation increases cutaneous blood flow to such an extent that venodilation is overwhelmed, and capillary filtration predominates (Harrison, 1985). Thus, it would appear that the present hot environment approached the boundary
between absorption and filtration, and hence the response of BV was probably dependent on the subjects' individual levels of heat tolerance.

Changes in BV were again borne largely by the plasma phase, while RCV again remained essentially constant. Previously, changes in erythrocyte volume during heat stress have been associated with increases in plasma tonicity and large decreases in total body water (Adolph et al., 1969; Costill & Fink, 1974; Costill & Saltin, 1974); however, during the present heat exposure, only 66 ml of fluid were lost from the body, and hence RCV was probably not challenged by large changes in plasma tonicity. Myhre and Robinson (1969) similarly reported no change in erythrocyte volume during prolonged heat stress, and cited an accompanying increase in plasma osmolality of less than 2%. It is therefore suggested that, in the absence of dehydration, the present heat stress caused a shift of isotonic fluid into the blood, which expanded PV, but barely affected both plasma tonicity and RCV.

The absence of dehydration in the heat was attributed to the short duration of the exposure, such that total evaporative water losses did not differ from those in the two cooler environments. Indeed, the similarity of evaporative losses between the three environments was testament to the role of both deep-body and skin temperatures in the regulation of sweating, as evaporative rate was maintained in the heat despite the depression of $T_w$. Both Nadel et al. (1971) and McCaffrey et al. (1979) have previously documented the interaction of deep-body and skin temperatures in the regulation of sweating, and therefore highlighted the thermoregulatory importance mean skin temperature.

5.3.3 Methodological Considerations

In the present study, relative changes in intravascular fluid volumes calculated using Hct, and [Hb] (after Dill & Costill, 1974) were similar in magnitude to those determined using radionuclide dilution, regardless of the environmental condition or its duration. Ultimately, this was not surprising, as there was no change in the ratio between the whole-body and venous haematocrits ($f$-ratio) throughout assessment; any such change, concurrent with a change in plasma volume, would potentially have led to erroneous
calculations of intravascular fluid shifts, based on \( \text{Hct} \) (Harrison et al., 1982). Changes in \( \text{Hct} \) were thus deemed to reflect changes in the composition of blood throughout the circulation, and hence to accurately indicate changes in the volume of intravascular fluids during the various thermal stresses.

5.3.4 Conclusion

In conclusion, it was confirmed that, when a constant posture is maintained, the distribution of body fluid is dependent on the prevailing environmental condition. On exposure to a cool environment, intravascular volume decreased rapidly, as plasma was filtered into the interstitium. Conversely, exposure to heat caused an expansion of intravascular volume, with fluid moving in the opposite direction, from the interstitium into the plasma. Thus, Hypothesis 1.1.2 (see Section 1.1) was accepted for both cool and hot environments. The extravasation of plasma in the cool was attributed to an increase in capillary hydrostatic pressure caused by rapid venoconstriction. The filtrate was probably isotonic to the remaining plasma, and hence erythrocyte volume was relatively unchallenged. In addition, the filtrate probably remained within the interstitium throughout the short period of cool exposure, and was not, as had previously been suggested, removed from the body to supply cold-induced diuresis. Similarly, fluid transfer in the heat was probably confined to the plasma and interstitium, although this time moving into the circulation in response to a decrease in capillary hydrostatic pressure, caused by rapid venodilation. The fluid was again probably isotonic to plasma, and hence erythrocyte volume remained unchallenged. The response to heat, however, showed considerable intersubject variation, such that some subjects actually experienced a decrease and others an oscillation of plasma volume during the 30-min period. The variations were attributed differences in vasomotor response, possibly related to subjects' individual levels of heat tolerance and state of acclimation. Even though intravascular fluid response varied between subjects, it was possible to accurately determine changes in volumes from changes in \( \text{Hct} \) and [Hb], without the need for radionuclide dilution.
5.4 References


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Chapter Six

Body-Fluid Distribution During Exercise in
Hot and Cool Environments

In addition to the influence of the environment, the distribution of body fluid is affected by metabolic heat production. For example, during exercise, when heat production is increased, fluid distribution is disturbed by an increase in evaporative fluid loss (Adolph et al., 1969) and by redistribution of blood to the skin to facilitate conductive and convective cooling (Savard et al., 1988). At the same time, fluid may be transferred between the body's compartments, with both plasma and erythrocyte volumes potentially decreasing (see Harrison, 1985, and Senay & Pivarnik, 1985, for reviews) and intramuscular fluid increasing (Jacobsson & Kjellmer, 1964) as the balance between hydrostatic and osmotic forces changes. Thus, both the total volume of body fluid, and its compartmental distribution, are dependent on exercise state and the prevailing environmental condition.

The volume of blood appears to be particularly susceptible to change during exercise, particularly in hot environments, when dehydration and increased plasma filtration tend to decrease plasma volume (Schneider & Havens, 1915; Kaltreider & Meneely, 1940; Sawka et al., 1984). Adolph et al. (1969), for example, showed that during exercise in the desert, plasma volume can decrease by as much as 28% when accompanied by a decrease in body mass of 11% (Adolph et al., 1969). Part of this decrease can be attributed to increased capillary hydrostatic pressure, which, during cycle exercise, causes an increase in capillary filtration to the interstitium (Lundvall et al., 1972). Thus, plasma volume has been found to decrease during cycle exercise in cold as well as hot environments (see Harrison, 1985, and Senay & Pivarnik, 1985, for reviews).

Erythrocyte volume has also been found to decrease during prolonged exercise in both hot and cold environments (Åstrand & Saltin, 1964; Costill et al., 1974; Diaz et al., 1979), probably reflecting an increase in plasma tonicity and a consequent cellular dehydration. However, decreased erythrocyte volume has not been universally corroborated, with several authors being unable to detect a change during exercise,
regardless of environmental condition (Nylin, 1947; Myhre & Robinson, 1969; Costill & Saltin, 1974). The discrepancy between these results may be due to differences in the respective measurement methods. For example, both Nylin (1947) and Costill and Saltin (1974) used radionuclide dilution techniques to study erythrocyte volume during exercise, and found no change in the circulating cell volume. In contrast, Van Beaumont (1972) proposed a method of measuring intravascular volume changes using venous haematocrit (Hct), which actually assumed that erythrocyte volume remained constant during the period of study. The additional use of haemoglobin concentration ([Hb]), as proposed by Dill and Costill (1974), enabled the simultaneous calculation of changes in both plasma and erythrocyte volumes, without the need for dilution techniques; however, the method was still susceptible to changes in the ratio between the whole-body haematocrit and Hct, (f-ratio). Thus, changes in intravascular fluid distribution coinciding with changes in the f-ratio might result in erroneous interpretations of Hct, (Harrison et al., 1982).

It is presently unclear whether the f-ratio changes during exercise, as only occasionally has Hct, been appropriately calculated using simultaneous dilution methods. For example, Fricke (1965) used the simultaneous dilution of blue dye and radiochromated erythrocytes, and found that the f-ratio increased following 30 min of cycle exercise. In contrast, Costill and Saltin (1974), found no change in the f-ratio following exercise-induced dehydration, using the simultaneous dilution of radioiodinated albumin and radiochromated erythrocytes. It therefore remains unclear whether changes in Hct, accurately reflect changes in intravascular fluid volumes during exercise, and therefore how the intravascular fluids respond to prolonged periods of exercise and thermal stress.

It is similarly undetermined how redistribution of intravascular fluid affect the volume and distribution of the extravascular fluid compartment during exercise. There is a paucity of information regarding the whole-body response of body fluid to exercise, and what little data does exist may be limited by the use of indirect measurement methods. For example, Nose et al. (1988) showed that plasma left the intravascular space during exercise, and suggested that it was taken up for evaporation and to defend the volume of extravascular cells. Similarly, Costill et al. (1976) found that the intracellular
compartment was defended, in comparison to the extracellular space, during the early stages of exercise-induced dehydration, but that the contribution of intracellular fluid increased as dehydration became more severe. Both studies, however, used the chloride (Cl) method, based on changes in the plasma [Cl], to determine changes in extracellular volume, and so were susceptible to changes in muscle membrane potential. For example, a change in muscle membrane potential during exercise may affect the balance of the Cl ion across the membrane, and therefore cause a change in [Cl] independent on any shift in fluid (Sjøgaard & Saltin, 1982). In contrast, Durkot et al. (1986) used a multiple radionuclide dilution method to determine the effects of dehydration on fluid distribution in rats, and found that the contribution of the intracellular phase increased as dehydration became more severe. A similar method could be used to determine the effects of exercise on the body-fluid distribution of humans, and therefore to describe the whole-body fluid response to exercise in both hot and cold conditions.

It was the purpose of this study to measure the whole-body distribution of body fluid during exercise in hot, control, and cool conditions, using the dilution of four radionuclides to simultaneously measure the volume of total body water, extracellular fluid, plasma, and erythrocytes.

6.1 Methods
6.1.1 Subjects
The body-fluid distribution of seven healthy, physically active males (see Table 5.1) was measured using the simultaneous radionuclide dilution method described in Chapter Three. The subjects were fully informed of the experimental procedures, which were approved by the University’s Human Experimentation Ethics Committee, and subsequently provided informed consent. Each subject was assessed on three occasions, including a hot, a control, and a cool condition, which were conducted in autumn at 28-d intervals with the order counterbalanced between subjects. On each occasion, subjects wore only brief swimsuits and athletic shoes.
6.1.2 Procedures

Preliminary procedures and radionuclide preparations were those described in Sections 3.1.2 and 3.1.3. Subjects arrived at the laboratory in a rested, fasted state, consumed breakfast (38 kJ·kg\(^{-1}\) of body mass, plus 5 ml·kg\(^{-1}\) of water), and were seated at rest, at least 30 min prior to assessment. Before breakfast, a urine void and 20 ml of venous blood were collected, for use as reference blanks and for radiochromate labelling. Approximately 5 ml of erythrocytes were labelled with radiochromate, using 20 min of incubation at 37°C; the labelled cells were then washed three times and resuspended in saline, and drawn into a syringe ready for injection. Quantities of tritiated water, radiobromine, and radioiodinated serum fibrinogen were drawn into similar syringes, also ready for injection. The volumes of these injections, and of the radionuclide standards, were determined gravimetrically, to a precision of 0.001 g (Denver Instruments, AL-300).

A 16-gauge teflon cannula was inserted into an antecubital vein, through which to administer the radionuclide injections. Two microcuries (\(\mu\)Ci) of radioiodinated human serum fibrinogen (RISF; Amersham Australia, \(^{125}\)I Human Fibrinogen), 8 \(\mu\)Ci of sodium radiochromated autologous erythrocytes (Amersham Australia, Na\(^{51}\)Cr), 20 \(\mu\)Ci of sodium radiobromine (Australian Radioisotopes, Na\(^{82}\)Br), and 500 \(\mu\)Ci of tritiated water (Amersham Australia, \(^3\)H\(_2\)O) were injected sequentially, within a 30-s period. The radionuclides were used to measure plasma (PV) and erythrocyte (RCV) volumes, extracellular fluid volume (ECFV), and total body water (TBW), respectively. The midtime of the fibrinogen injection was considered as the commencement of assessment, time-zero (\(t_0\)). Immediately following the injections, the cannula was flushed with a minimum of 15 ml of saline followed by 5 ml of heparinised saline, rendering it suitable for subsequent blood sampling (Ladegaard-Pedersen & Engell, 1969).

Ten-millilitre blood samples were collected after 30, 60, and 180 min, from which to determine initial fluid volumes (see Sections 3.1.4 to 3.1.6). Samples were collected without stasis with the forearm at approximately heart level, and were immediately treated with ethylenediamine tetra-acetic acid (EDTA; 1.8 mg·ml\(^{-1}\) of blood). Before each sample, 5 ml of fluid were removed from the cannula, and, following each one, the
cannula was flushed with 10 ml of heparinised saline. Urine voids were collected after 180 and 270 min, and measured volumetrically to a precision of 5 ml.

The 270 min following the radionuclide injections were spent seated at rest in a chair, with at least the last 60 min being in a control environment of 22.0°C (± 1.0°; standard deviation), 52% (± 6%) relative humidity (rh). The subsequent 60 min were spent exercising on a mechanically-braked cycle ergometer (Monark, 868) at 50% of maximal work-rate, in either a hot (36.2° ± 0.7°C, 44% ± 3% rh), the control (as above), or a cool (14.4° ± 1.6°C, 74% ± 9% rh) environment*. In all three environments, air movement was less than 0.5 m s⁻¹, and black-globe temperature was within ± 0.5°C of ambient temperature. The environments were maintained, in the University’s Environmental Physiology Research Laboratory, using a combination of heaters, coolers, and dehumidifiers, controlled by microcomputer (see Maw & Williamson, 1992 [Appendix B]). Subjects were moved from the control environment into the Environmental Laboratory in a wheelchair, so that their seated posture was not disturbed prior to exercise. A change of posture from the seated control would have affected fluid distribution independently of the ensuing exercise (see Section 4.2).

At least 30 min prior to entering the Environmental Laboratory, a thermistor probe (London Hospital Medical College, zero-gradient aural thermometer) was inserted into the left aural canal, to a depth of approximately 10 mm. The probe, which was used to measure deep-body temperature (Tₒ), was secured with a cotton wad, covered by a padded headset, and interfaced with an electrically-isolated microcomputer. The headset maintained the outer ear at the same temperature as the aural canal, using servo-heating, and hence eliminated the direct influence of environmental temperature from the measurement of Tₒ (Keatinge & Sloan, 1975). Measured in this manner, Tₒ closely corresponds to the temperature in the midoesophagus, both quantitatively and

*The same absolute work rate, calculated as 50% of maximal rate in a control environment (22.0°C, 52% rh), was used in each of the three conditions. Maximal work rate was determined, at least 3 days prior to the first assessment, using an incremental exercise test on a mechanically-braked cycle ergometer (Monark, 868). During the test, work rate was increased by 15 W every 30 s, with the rate at volitional exhaustion being considered maximal.
dynamically, during both rest and exercise in a range of thermal environments (Maw & Taylor, 1992 [Appendix C]; see Figure 5.1). $T_{sc}$ was recorded at 5-s intervals throughout exercise.

Skin temperatures were also recorded at 5-s intervals throughout exercise, using surface thermistors (YSI, Mini-thermistor EU) positioned on the forehead, the left upper chest, the dorsal aspect of the left forearm and hand, and over the right scapula, right deltoid, right rectus femoris, and left gastrocnemius. The thermistors were secured with a single covering of waterproof adhesive tape, and interfaced with a data logger (Grant Instruments, 1200 series Squirrel). The skin thermistors and the aural probe were previously calibrated against a certified mercury-in-glass thermometer (Dobbie Instrument, Dobros total immersion; see Section 5.1.2).

Cardiac frequency ($f_c$) was recorded, from ventricular depolarisations, at 5-s intervals throughout exercise, using a telemetry system (Polar Electro, PE3000). This system provides a record of $f_c$ comparable to that measured using electrocardiography during rest, exercise, and recovery ($r^2=0.999$, from Osborne, 1994 [see Figure 4.1]).

Body mass was measured immediately before, at 10-min intervals during, and then immediately after exercise, to an accuracy of ± 5 g, using a modified electronic scale (A&D, FW-150K). The scale was calibrated prior to each assessment, in the prevailing environment, and found to be linear through a physiological range of mass (see Section 5.1.2). The scale was positioned directly under the cycle, to minimise the duration and magnitude of the postural change required while standing to be weighed. Visible sweat was wiped from both the scale and the subject prior to weighing, and the subject's swimsuit and shoes were weighed before and after exercise, to determine absorption of sweat.

Expired gas was collected for 1 min, after 5, 15, and 45 min of exercise, using a respiratory face mask and non-diffusing collection bag (Hans Rudolph, Series 7910 face mask and gas collection bag). The mask was linked to the bag with 30 cm of 35 mm-diameter ethylene plastic tubing, which was prewarmed to minimise the condensation.
of expired water vapour. The temperature and humidity of the expired air were immediately measured, using a resistance hygrometer (Hygrodynamics, 15-3080E), previously calibrated against saturated salt solutions*. The volume of the air was measured, to an accuracy of ± 1.7%, using a rotary gas meter (Vacumetrics, Air Flow Meter 17150)†.

Blood samples were collected, as described above, at 10-min intervals throughout exercise, before corresponding measurement of body mass, and a urine void was collected after exercise, after the subject had been weighed.

6.1.3 Calculation of Thermoregulatory Responses
Mean skin temperature ($T_{sk}$) was calculated from the weighted mean of the eight skin temperatures, after the International Organization for Standardization (1992; see Section 5.1.3).

Sweat loss was calculated from the change in body mass during exercise, corrected for the metabolic exchange of oxygen and carbon dioxide, the absorption of sweat into clothing, the loss of respiratory water, and the mass of interim blood collections. Metabolic exchange was calculated as a function of the estimated oxygen consumption and respiratory exchange ratio prevalent during exercise, where oxygen consumption was estimated after Jones (1988), and the respiratory exchange ratio was approximated using the data of Solomon (1991), who measured the respiratory exchange of several of the present subjects during 60 min of cycle exercise at 50% of maximal aerobic power. Clothing absorption was determined from the difference between the mass of the

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*The hygrometer (Hygrodynamics, 15-3080E) was calibrated, in each of the three environments (cool: 14.4°C, 74% rh; control: 22.0°C, 52% rh; hot: 36.2°C, 44% rh), against water vapour collected over saturated solutions of dipotassium hydrogen phosphate ($K_2$HPO$_4$), diammonium sulphate ($\left(NH_4\right)_2$SO$_4$), sodium chloride (NaCl), and potassium dihydrogen phosphate (KH$_2$PO$_4$), providing a range of relative humidity from 44.5% to 98.0%. The coefficients of determination ($r^2$) of the calibration curves were 0.994, 0.998, and 0.991 in the cool, control, and hot environments, respectively.

†The gas meter was calibrated using a 3000 ml calibrated volume standard (Quinton Instruments, Model 1921; see Solomon, 1991), through a range of volumes from 0 to 150 l.
clothing before and after exercise, apportioned to the 10-min exercise intervals on a linear basis.

Respiratory water loss was calculated as a function of the relative humidity, temperature, and volume of the air expired during the 1-min collections. The relative humidity and temperature of the air were used to determine its water content (see Weast et al., 1989), with the volume then applied to calculate the rate of respiratory water loss ($M_{EH}$):

$$M_{EH} = M_{EH} \times V_{E_{exp}}$$

where: $M_{EH}$ = mass, in grams, of expired water per litre of air;
$V_{E_{exp}}$ = expiratory rate, in litres, as measured at ambient temperature, pressure, and saturation.

**6.1.4 Blood and Urine Analyses**

Blood and urine collections were analysed as described in Section 3.1.4. All blood samples were assessed for venous haematocrit ($Hct_s$) and haemoglobin concentration ($[Hb]$), to accuracies of ± 0.83% and ± 0.78%, respectively (Coulter Electronics, S-Plus IV; see Section 3.1.4), and then centrifuged for 40 min at 1500 g, to separate plasma from erythrocytes. Trapped plasma was taken as 2% of the resultant packed erythrocytes (after Chaplin & Mollison, 1952).

Three-millilitre aliquots of plasma and erythrocytes were then dispensed from each blood sample into glass vials and refrigerated at 4°C, pending gamma radiation ($\gamma$) counting; a 3-ml aliquot from each urine void and from each of the $^{125}$I, $^{51}$Cr, and $^{82}$Br standards was similarly stored. Erythrocyte aliquots were haemolysed prior to storage, using a trace of powdered saponin (Sigma Chemical Company, S-1252). Further 0.5-ml aliquots of plasma and urine, and 0.5-ml aliquots of the $^3$H and $^{125}$I standards, and of distilled water, were dispensed into glass vials, vigorously mixed with 0.05 ml of 1 molar hydrochloric acid and 9 ml of liquid scintillation cocktail (Packard Instruments, Emulsifier-Safe), and then refrigerated pending beta radiation ($\beta$) counting. All aliquots were measured volumetrically to an accuracy of ± 0.2% (Labsystems, Finnpipette; see Section 3.1.3.2).
After radiation counting, plasma aliquots were assessed, in triplicate, for protein concentration, to a precision of 0.1 g/1, using a refractometer (Otago, 93032). The mean of the three refractometer readings was considered as plasma protein concentration ([PP]; see Section 3.1.4).

6.1.5 Radiation Counting
Radiation counting was conducted as described in Section 3.1.5. Radioiodine, 51Cr, and 82Br were counted using a well-type γ scintillation counter, calibrated to measure the optimum range of radiant energy of the respective nuclides, to efficiencies of between 25% and 29% (Abbott Laboratories, Auto-LOGIC; see Figure 3.1); 3H was counted using a liquid scintillation counter, calibrated to measure β energy between 0.005 and 0.132 million electron volts (MeV)\(^1\), to an efficiency of between 33% and 37% (LKB Wallac, 1219 Rackbeta).

Radiobromine was counted on the day of assessment, with all other counting delayed for 14 days, pending the decay of 82Br. Aliquots were counted twice for 82Br, each time for 12 min, and three times for 125I, 51Cr, and 3H, for 6, 10, and 2 min, respectively. For each radionuclide, the sequence of vials was reversed between counts. The duration of the counts ensured a minimum of 10000 counts were aggregated for each radiated aliquot in each of the calibrated ranges. The mean of the respective counts, minus the mean of the relevant distilled water, plasma, erythrocyte, or urine background reference, was considered as radionuclide concentration. Concentrations were expressed as counts per minute per millilitre.

6.1.6 Determination of Compartmental Fluid Volumes
Initial fluid volumes were calculated from radionuclide concentrations in the blood collected 30, 60, and 180 min after the radionuclide injections (see Section 3.1.6). Later volumes, during exercise, were similarly calculated, by comparing the radionuclide concentrations in the blood samples with the respective radionuclide doses. Volumes were thus calculated for minutes 0, 10, 20, 30, 40, 50, and 60 of each exercise period.

\(^1\)1 MeV = 1.6 \times 10^{-13}\) joules.
The calculation of these volumes followed the method described in Sections 4.1.5.1 and 4.1.5.2. TBW, at any given time (t), was calculated from \( ^3H \) in the appropriate plasma, corrected for the presence of plasma protein and \( ^{125}I \), and for \( ^3H \) urine, sweat, and respiratory losses; ECFV was calculated from \( ^{82}Br \) in the appropriate plasma, corrected for the presence of protein, for \( ^{82}Br \) erythrocyte, urine, and sweat losses, and for the Gibbs-Donnan electrolyte ratio; PV was calculated from a comparison of \( ^{125}I \) measured in the appropriate plasma with that predicted, using semilogarithmic extrapolation of the \( ^{125}I \) elution curve, for the equivalent time (after Gibson & Evans, 1937, and Harrison & Edwards, 1976); RCV was calculated from \( ^{51}Cr \) in the appropriate erythrocytes, corrected for \( ^{51}Cr \) urine loss.

The presence of protein was corrected for by calculating the protein displacement factor using \([PP]\) (after Chien & Gregersen, 1962; see Section 3.1.6). Radionuclide urine losses were calculated from the respective radionuclide concentration and the volume of the urine voids collected up to \( t \). The resultant radionuclide contents were summed up to \( t \), or, in the case of the postexercise void, were apportioned to the 10-min exercise intervals on a linear basis. Tritium and \( ^{82}Br \) sweat and respiratory losses were calculated from respective sweat and respiratory water losses, and their estimated \( ^3H \) and \( ^{82}Br \). Sweat and respiratory water \( ^3H \) were assumed to be equal to plasma \( ^3H \) (Dyrbye & Kragelund, 1970), while sweat \( ^{82}Br \) was taken as a fraction of the plasma concentration, determined according to the relationship between sweat and plasma [chloride] reported by Costill and Miller (1980). The Gibbs-Donnan ratio was taken as 1.02 for radiobromine (Manery, 1954).

Intracellular water (ICW), interstitial fluid volume (IFV), and blood volume (BV) were calculated as described in Section 3.1.6.5. ICW was calculated as the difference between TBW and extracellular water volume (ECW), where ECW was calculated from ECFV adjusted for the presence of all plasma solutes. IFV was calculated as the difference between ECFV and PV; BV was calculated as the sum of PV and RCV.

Whole-body haematocrit (Hct\( _w \)) was then calculated as the ratio of RCV to BV, and the \( f \)-ratio was considered as the ratio between Hct\( _w \) and the haematocrit in the
corresponding venous blood sample (Hct). Relative changes in BV, PV, and RCV were calculated, with respect to values immediately preceding each environmental exposure, from measured volumes, and also from changes in Hct, and [Hb] (after Dill & Costill, 1974; see Section 4.1.5.4).

6.1.7 Analysis

Analysis was restricted to the first 50 min of exercise, because, on three occasions in the heat, exercise was terminated after 50 min. On two such occasions, exercise was terminated because the subject felt faint and nauseous, while on the other occasion, T_c rose above 39.0°C, which was the predetermined safety margin for T_c. Within the 50-min period, T_c, T_sk, and f_c were averaged for the minute surrounding each 10-min interval, such that values were derived for minutes 0, 10, 20, 30, 40, and 50 of each exercise period.

Expired water in the 5, 15, and 45 min collections was averaged to give a mean rate of respiratory water loss, which was then extrapolated to determine the volume of water expired during each 10-min interval*. The resultant expired water volumes, and urine volumes, displayed proportional variance, and were therefore transformed to natural logarithms prior to further analysis (Montgomery & Peck, 1982).

Body-fluid volumes were normalised to their initial values, to negate physiological variations occurring over the course of the study. Considerable physiological variations were possible over the minimum course of 56 d, which would have been unrelated to the experimental manipulations. It was therefore considered prudent to normalise the fluid volumes, and hence to consider absolute changes in volumes, during subsequent analysis.

Factorial analysis of variance was used to determine differences related to environmental

*The volume of water expired did not differ between the 5, 15, and 45 min collections, regardless of environmental condition (p=0.251, power(φ)=0.260), which was not surprising given the dependence of expired water on ventilatory rate (V_B). V_B has previously been shown to be stable during cycle exercise at 55% of maximal aerobic power, for up to 60 min (Solomon, 1991).
condition and time, while one-way analysis of variance was used to determine differences when environmental condition was the only independent variable (Statistical Package for Social Scientists [SPSS], 1988). In the event of a significant result, specific differences were determined using Tukey's test of Wholly Significant Difference (Tukey's WSD; Howell, 1992).

Differences were considered significant if the probability that the difference was due to chance was less than 5% (\( \alpha = 0.05 \)). When differences were not significant, the power (\( \phi \)) of the analysis was computed (SPSS, 1988). Analysis of variance summary tables are included in Appendix H, while data in the text are means with standard errors of the means (SEM), unless otherwise stated as standard deviations (\( \sigma \)).

6.2 Results

Prior to exercise, \( T_{ac} \) ranged between 36.4° and 38.2°C, with a mean of 37.0°C (\( \pm 0.4^\circ \); mean \( \pm \sigma \)). Preexercise \( T_{sk} \) was between 28.7° and 34.4°C, with a mean of 30.9°C (\( \pm 0.7^\circ \)), and \( f_c \) averaged 75 (\( \pm 20 \)) b·min\(^{-1} \) (means \( \pm \sigma \)).

6.2.1 A Case Study of Exercise in the Control Environment

\( T_{ac} \), \( T_{sk} \), and \( f_c \) all subsequently rose during exercise in all three environments, with the response of one subject, N5, illustrating the effect of exercise in the control environment (Figure 6.1). The subject was 27.8 y old, 183.9 cm tall, and weighed 90.90 kg. His initial \( T_{ac} \), \( T_{sk} \), and \( f_c \) were 37.6°C, 30.0°C, and 58 b·min\(^{-1} \), respectively. A short time after commencing exercise, \( T_{ac} \) and \( T_{sk} \) began to rise, reaching 38.1° and 33.1°C, respectively, after 30 min, and then stabilising for the remainder of the period. His \( f_c \) rose immediately on commencing exercise, reaching 129 b·min\(^{-1} \) at minute 10, and then increasing only slightly during the remaining time.

The response of N5’s fluid volumes (Figure 6.2) were also fairly typical of those of the remaining subjects, with BV responding rapidly to the onset of exercise. Prior to exercise, his BV was 7335 ml, while his TBW, ICW, ECFV, IFV, PV, and RCV were 56935, 36400, 21965, 17725, 4235, and 3100 ml, respectively. BV then declined with
**Figure 6.1** Aural ($T_{ac}$) and mean skin ($T_{sk}$) temperatures, and cardiac frequency ($f_c$) for Subject N5 during 50 min of cycle exercise at 50% of maximal work-rate in a control environment (22°C).
Figure 6.2 Total body water (TBW), intracellular water (ICW), extracellular fluid (ECFV), interstitial fluid (IFV), blood (BV), plasma (PV), and erythrocyte (RCV) volumes for Subject N5 during 50 min of cycle exercise at 50% of maximal work-rate in a control environment (22°C).
the onset of exercise, decreasing by 170 ml during the initial 10 min of exercise, including losses of 90 ml from PV and 80 ml from RCV. ICW concurrently decreased by 480 ml and IFV increased by 310 ml, suggesting that fluid collected in the interstitium from both intravascular and intracellular sources. Consequently, ECFV rose by 220 ml, while TBW decreased by 280 ml. As exercise progressed, BV stabilised, reflecting a continued decrease in PV accompanied by an expansion of RCV. PV decreased by a total of 240 ml during 50 min of exercise, while RCV increased by 105 ml. It therefore appeared that plasma fluid passed into both erythrocytes and the interstitium, where it probably helped defend IFV against dehydration. IFV still declined by 215 ml during the 50 min, making a total decrease in ECFV of 455 ml. In comparison, ICW decreased by just 60 ml, while TBW decreased by 490 ml. Thus, most of the fluid lost from the body appeared to be drawn from the extracellular compartment, with the intracellular compartment being preferentially defended. The fluid lost from the body included 300 ml of sweat, 50 ml of respiratory water, and 125 ml of urine produced postexercise.

6.2.2 Thermoregulatory Responses to Exercise and Thermal Stress

The increase in $T_{ac}$ during exercise was common to all subjects and to all three environments ($p=0.001$), with the magnitude of the increase being independent of environmental condition (Figure 6.3). $T_{ac}$ rose by 1.3° (± 0.1°), 1.3° (± 0.2°), and 1.4°C (± 0.2°) during the cool, control, and hot exposures, respectively, remaining similar between conditions throughout exercise ($p=0.336, \phi=0.213$). However, it appeared that the dynamics of $T_{ac}$ may have differed between conditions, with $T_{ac}$ rising rapidly to a plateau during exercise in the cool, but rising more slowly and failing to plateau during exercise in the heat. $T_{ac}$ reached 38.0°C within 20 min in the cool, and then plateaued at 38.4°C, while it required more than 30 min to reach 38.0°C in the heat, but exceeded 38.5°C at the end of the period.

$T_{sk}$ also rose during exercise in all three environments ($p=0.001$), but was consistently higher during exercise in the heat than during the other two exposures (Figure 6.3). In turn, $T_{sk}$ was higher in the control condition than in the cool ($p=0.001$). $T_{sk}$ reached 35.8° (± 0.2°), 31.7° (± 0.4°), and 31.1°C (± 0.3°) after 10, 20, and 40 min in the hot, cool, and
Figure 6.3 Aural ($T_{ac}$) and mean skin ($T_{sk}$) temperatures, and cardiac frequency ($f_c$) during 50 min of cycle exercise at 50% of maximal work-rate in hot (36°C), control (22°C), and cool (14°C) environments (means ± SEM).
Similarly, $f_c$ was higher during exercise in the heat than in either the control or the cool conditions ($p=0.003$), but, in turn, there was no difference between $f_c$ in the latter two environments (Figure 6.3). The dynamics of $f_c$ did not differ between any of the conditions, with the elevation of $f_c$ in the heat being described by a displacement from the other two conditions, which was constant regardless of the duration of exercise ($p=0.110$, $\varphi=0.739$). Averaged throughout exercise, $f_c$ in the heat was elevated by 14 and 13 b·min$^{-1}$, compared to $f_c$ in the control and cool environments, respectively ($p=0.001$), reaching 169 (± 4) b·min$^{-1}$ by minute 40, compared to 151 (± 4) and 149 ± 5 b·min$^{-1}$ at the corresponding time in the control and cool conditions. No significant changes were evident in $f_c$ after minute 40, regardless of environmental condition**.

6.2.3 Acute Body-Fluid Responses to Exercise and Thermal Stress

On average, preexercise TBW, ECFV, PV, and RCV, averaged across conditions, were 50975 (± 4665), 20316 (± 1671), 3488 (± 360), and 2698 (± 403) ml, respectively (means ± σ). Hence, corresponding ICW, IFV, and BV were 30853 (± 3353), 16828 (± 1372), and 6186 (± 682) ml, respectively (means ± σ).

The onset of exercise caused a decrease in BV in all three environments ($p=0.001$), with the decrease being greater in the heat and cool than during exercise in the control condition (Figure 6.4). During the initial 10 min of exercise in the heat and cool, BV decreased by 470 (± 192) and 287 (± 60) ml, respectively, compared to a decrease of 114 (± 86) ml during the corresponding time in the control condition ($p=0.002$). These initial decreases in BV were largely accounted for by plasma shifts, with PV decreasing during the initial 10 min of all three exposures ($p=0.001$; Figure 6.4). Again the loss in the heat (356 ± 128 ml) was similar to that in the cool (243 ± 42 ml), but greater than that in the control environment (110 ± 46 ml; $p=0.001$).

**Cardiac frequency was seen to decrease intermittently during exercise in all three environments, coinciding with the brief pause necessary to measure body mass following each blood sample.
Figure 6.4 Blood (BV), plasma (PV), and erythrocyte (RCV) volumes during 50 min of cycle exercise at 50% of maximal work-rate in hot (36°C), control (22°C), and cool (14°C) environments (means ± SEM; normalised to mean preexercise levels).
Decreases in RCV also appeared to contribute to the loss of BV during the initial 10 min of exercise, but these changes were slight and did not reach significance until minute 20 (Figure 6.4). At that time RCV had decreased in all three environments \((p=0.010\)\), by 114 (± 62), 76 (± 55), and 75 (± 37) ml in the hot, control, and cool conditions, respectively. The magnitude of the decrease did not differ between conditions \((p=0.874, \phi=0.239)\).

Neither IFV, ECFV, or ICW were significantly affected during the initial 10 min of exercise, regardless of environmental condition \((p=0.541, \phi=0.422; p=0.731, \phi=0.321; \text{ and } p=0.350, \phi=0.535, \text{ respectively}; \text{ Figure 6.5})\). Similarly, TBW did not change during the initial 10 min period \((p=0.776, \phi=0.296; \text{ Figure 6.6})\), although fluid was noticeably lost from the body in all three environments. Forty-six (± 18), 49 (± 23), and 79 (± 46) ml of sweat were lost during the initial 10 min of exercise in the hot, control, and cool conditions, respectively, as determined from changes in body mass.

**6.2.4 Body-Fluid Responses to Prolonged Exercise and Thermal Stress**

As exercise progressed, BV recovered in the control and cool conditions, but remained depleted throughout the hot exposure (Figure 6.4). BV had recovered to preexercise levels by minute 30 in both the control and cool environments, but was depleted by 589 (± 240) ml at the same time in the heat \((p=0.002)\). BV lost a total of 635 (± 230) ml during 50 min of exercise in the heat \((p=0.002)\).

Changes in BV were largely accounted for by changes in PV, such that PV also recovered during exercise in the control and cool environments, but remained depleted throughout exercise in the heat (Figure 6.4). PV had recovered to preexercise level by minute 30 in the control and cool conditions, but was depleted by 532 (± 138) ml after 50 min in the heat \((p=0.001)\). In contrast, RCV was depleted from minute 20 onwards in all three environments \((p=0.010; \text{ Figure 6.4})\), losing a total of 103 (± 101), 20 (± 40), and 106 (± 52) ml during the hot, control, and cool periods, respectively. The extent of this cellular depletion did not differ between conditions \((p=0.874; \phi=0.239)\).

Similarly, both IFV and ECFV decreased during all three exercise periods (both
Figure 6.5 Intracellular water (ICW), extracellular fluid (ECFV), and interstitial fluid (IFV) volumes during 50 min of cycle exercise at 50% of maximal work-rate in hot (36°C), control (22°C), and cool (14°C) environments (means ± SEM; normalised to mean preexercise levels).
Figure 6.6  Body-fluid losses during 50 min of cycle exercise at 50% of maximal work-rate in hot (36°C), control (22°C), and cool (14°C) environments, determined from changes in total body water (using tritium dilution) and from changes in body mass (means ± SEM).
with changes in both volumes being independent of environment (Figure 6.5). IFV decreased by 122 (± 254), 473 (± 214), and 409 (± 194) ml, in the heat, control, and cool, respectively (p=0.731, $\phi$=0.321), while ECFV, combining changes in both IFV and PV, decreased by 654 (± 167), 483 (± 259), and 427 (± 84) ml, respectively (p=0.541, $\phi$=0.422).

No consistent changes were detected in ICW during any of the exercise periods (p=0.350, $\phi$=0.535; Figure 6.5). However, this probably reflected the insensitivity of ICW measurement, as much as it did the stability of the intracellular compartment. For example, the biggest change in ECFV, a decrease of 654 ml during exercise in the heat, equated to less than 2.5% of ICW, which was probably within the error of ICW measurement. The error of ICW measurement is a combination of those for the measurements of TBW and ECFV, thus making it difficult to detect the relatively small changes in ICW that might have occurred during exercise.

Similarly, TBW apparently did not change during exercise regardless of environment (Figure 6.6), despite the noticeable loss of fluid from the body. Again, it was considered that this reflected the insensitivity of TBW measurement, rather than a true stability of total body fluid volume. For example, body fluid losses determined from changes in body mass totalled 861 (± 100), 563 (± 61), and 520 (± 70) ml during the hot, control, and cool periods, respectively, which equated to less than 2% of TBW. Fluid losses were therefore probably within the error of TBW measurement. However, the difference between TBW before and after the hot, control, and cool exposures was 845 (± 448), 504 (± 520), and 491 (± 345) ml, respectively (p=0.776, $\phi$=0.296), which was similar to the losses determined from changes in body mass (Figure 6.6). Hence, it was possible that tritium dilution was able to detect small losses of body fluid, but that individual variations prevented the change in TBW from reaching statistical significance.

Losses of body fluid, determined from changes in body mass, were greater during exercise in the heat than during exercise in the control and cool environments, totalling 861 (± 100) ml during the 50 min compared to 563 (± 61) and 520 (± 70) ml, respectively, during the control and cool periods (p=0.012; Figure 6.6). The increased
fluid loss in the heat was primarily due to increased sweat loss, which totalled 788 (± 98) ml during exercise in the heat compared to 453 (± 71) and 383 (± 81) ml in the control and cool, respectively (p=0.001). Total respiratory water loss was also greater in the heat (60 ± 3 ml) than in the cool (55 ± 3 ml; p=0.014), but was similar to that in the control environment (59 ± 2 ml). In contrast, urine loss was depressed in the heat (13 ± 9 ml) compared to both the control (51 ± 18 ml) and cool (83 ± 33 ml) conditions (p=0.014). Total fluid losses during the hot, control, and cool exposures were respectively equivalent to 1.10%, 0.72%, and 0.67% of initial body mass.

On average, throughout the three exercise periods, 49% of body-fluid losses were accounted for by changes in ECFV. Thus, it appeared that the extracellular fluid compartment was depleted during exercise out of proportion to its initial volume in the body. Extracellular fluid initially accounted for 40% of TBW, with the remainder being in the intracellular phase. It was therefore considered that ICW was partially defended during exercise in each of the hot, control, and cool environments.

6.2.5 Indirect Determination of Changes in Intravascular Volumes

Relative changes in BV calculated from changes in Hct, and [Hb] did not differ from those measured using radionuclide dilution, regardless of the environment or the duration of exercise (p=0.478, ψ=0.457). For example, the decreases in BV calculated using changes in Hct, and [Hb] during the initial 10 min of exercise were equivalent to losses of 6.8% (± 0.4%), 3.4% (± 1.2%), and 4.8% (± 0.6%) in the hot, control, and cool conditions, respectively, compared to equivalent losses of 6.7% (± 2.1%), 1.5% (± 1.3%), and 4.8% (± 0.9%) measured using radionuclide dilution.

Similarly, the method of calculation did not affect the magnitude of relative changes in RCV, regardless of environment or exercise duration (p=0.818, ψ=0.273). For example, relative changes in RCV, calculated using Hct, and [Hb], equated to 0.6% (± 0.2%), 0.4% (± 0.2%), and -0.1% (± 0.4%) during 20 min in the hot, control, and cool, respectively, compared to equivalent decreases of 3.3% (± 1.3%), 2.5% (± 1.9%), and 2.7% (± 1.4%) measured using radionuclide dilution.
Relative changes in PV, on the other hand, were significantly greater at minute 10 of all three exercise periods, when determined using Hct, and [Hb] compared to those measured using radionuclide dilution ($p=0.018$; Figure 6.7). The losses calculated using Hct, and [Hb] were 11.8% ($\pm$ 1.0%), 6.3% ($\pm$ 2.4%), and 9.0% ($\pm$ 0.9%), during 10 min in the heat, control, and cool, respectively, compared to losses of 9.4% ($\pm$ 2.8%), 3.0% ($\pm$ 1.2%), and 7.3% ($\pm$ 1.0%) measured using radionuclide dilution.

The discrepancy between the two derivations of relative changes in PV coincided with a change in the ratio of Hct to Hct (f-ratio). The f-ratio, which initially averaged 0.966 ($\pm$ 0.014), decreased during the initial 10 min of exercise in all three environments, to a mean value of 0.943 ($\pm$ 0.019; $p=0.002$; Figure 6.8). The ratio subsequently recovered to resting level by the end of exercise.

6.3 Discussion

Preexercise $T_{ac}$, $T_{sk}$, and fC were within normal ranges (Gagge et al., 1967), and initial body-fluid volumes, while higher than reference norms (International Commission on Radiological Protection, 1975; International Committee for Standardisation in Haematology, 1980), were appropriate for endurance trained males; comparable volumes have previously been reported for similar populations (Sjöstrand, 1962; see Section 3.3 for a complete discussion of resting fluid volumes).

6.3.1 Thermoregulatory Responses to Exercise and Thermal Stress

Thermoregulatory responses during exercise were also similar to those previously reported for exercise in diverse environments. For example, $T_{ac}$ rose throughout exercise in all three environments, reflecting the thermal stress imposed by physical activity, but did not differ between environments, supporting the proposal that exercising deep-body temperature is independent of environmental temperature. Both rectal and oesophageal temperatures have previously been shown to be independent of environmental temperature during exercise, in environments ranging from 5° to 32°C (Nielsen & Nielsen, 1938, 1962; Lind, 1963), and to be dependent instead on the relative rate of work (Saltin & Hermansen, 1966). With work-rate in the present study constant between
Figure 6.7 Relative changes in plasma volume during 50 min of cycle exercise at 50% of maximal work-rate, determined using the dilution of radioiodinated fibrinogen (RISF) and from changes in venous haematocrit (Hct,) and haemoglobin concentration ([Hb]); after Dill & Costill, 1974; means ± SEM; averaged across hot (36°C), control (22°C), and cool (14°C) environments.)
Figure 6.8 The f-ratio during 50 min of cycle exercise at 50% of maximal work-rate in hot (36°C), control (22°C), and cool (14°C) environments (means ± SEM; normalised to mean preexercise level).
environments, it was not surprising that $T_{ac}$ did not differ between conditions.

The environmental independence of exercising deep-body temperature has not, however, been universally corroborated. For example, when ambient temperature has exceeded what might be termed a "prescriptive zone", deep-body temperature has become elevated beyond that experienced during equivalent tasks in cooler conditions (Lind, 1963). Exceeding the "prescriptive zone" has generally required environments in excess of 40°C (Wilson et al., 1975; Wells & Horvath, 1974), and therefore hotter than the temperature of deep-body tissues. Hence, cooling has been limited to evaporation, which apparently was insufficient to cope with the thermal stress imposed by exercise. Deep-body temperature therefore rose beyond the level achieved during cooler tasks. However, in the present study, the hot environment was fractionally cooler than $T_{ac}$, and therefore body temperature was effectively regulated by conduction and convection, as well as evaporation.

At the same time, it is possible that the effect of the environment on exercising $T_{ac}$ was partially masked by rapid changes in $T_{ac}$ immediately on entering the Environmental Laboratory. It was shown in Chapter Five that entering the same hot and cool environments respectively caused rapid depression and elevation of resting $T_{ac}$, which subsequently lasted throughout the 30-min periods. Similar responses at the start of the present exposures would perhaps have blunted or exaggerated the subsequent response of $T_{ac}$ during exercise, perhaps equalising the temperature during the respective exercise periods. Had the subjects been equilibrated to the prevailing environment prior to commencing exercise, or had exercise been continued beyond 50 min, $T_{ac}$ may then have become dissociated between the hot and cool conditions.

$T_{sk}$ was different between the hot and cool conditions, regardless of exercise duration, reflecting its relationship with both environmental temperature and cutaneous blood flow. In the hot environment, $T_{sk}$ was elevated, indicating the presence of increased cutaneous blood flow in an attempt to overcome the concurrent reductions in conductive and convective cooling. Savard et al. (1988) previously showed that cutaneous blood flow increased during exercise in hot conditions, without detracting from muscle
perfusion. Hence, cutaneous blood flow was able to contribute to thermal regulation even as exercise increased the temperature of the deep tissues. The increase in $T_{sk}$ during the present cool and control exposures therefore primarily reflected the presence of cutaneous vasodilation as exercise increased $T_{ac}$.

The proximity of $T_{sk}$ to $T_{ac}$ during exercise in the heat limited the extent of heat flux from the body's core to the periphery, necessitating an increase in evaporative heat loss to achieve thermal equilibrium. Hence, both sweat and respiratory water losses were increased during exercise in the heat compared to the two cooler conditions. The increased sweat loss was apparently peripherally, rather than centrally mediated, as $T_{ac}$ was similar between all three conditions. Indeed, the role of skin temperature in the regulation of sweating has previously been demonstrated (Nadel et al., 1971; McCaffrey et al., 1979). The increased respiratory water loss, on the other hand, was probably only indirectly related to the hot environment, through changes in ventilation, rather than by direct influence of the environment on the water content of expired air. Mitchell et al. (1972) suggested that the mass of water expired during exercise was directly dependent on the prevailing environment. However, it is known that it is absolute humidity that determines the rate of evaporation (Seeley, 1940), and, in the present study, absolute humidity was similar between conditions. In contrast, minute ventilation increased during exercise in the heat (Wells & Horvath, 1974), consequently increasing the total volume of both air and water expired during the exercise bout.

At the same time, increased cutaneous blood flow, indicated by increased $T_{sk}$ in the heat, would have presented a challenge to the circulation to provide both cutaneous and muscle perfusions. To meet the challenge, it would have been necessary to either redistribute blood from other dormant vasculature, or to increase the outflow of blood from the heart. Savard et al. (1988) suggested that, during exercise in the heat, blood was redirected to the skin from the splanchnic and renal vasculature with little increase in cardiac output. However, Rowell et al. (1969) found that cardiac output increased when skin heating was acutely applied during steady-state exercise. In addition, it has been consistently shown that stroke volume is reduced during exercise in the heat compared to cooler conditions (Rowell et al., 1969; Oddershede et al., 1977; Suzuki et
al., 1980). Hence, to increase or maintain cardiac output during the present heat stress, a compensatory increase in cardiac frequency would have been necessary. Indeed, $f_c$ was elevated during exercise in the heat compared to the two cooler conditions, supporting the idea that cardiac output increased, to satisfy the competing demands of exercise and thermoregulation. Similar increases in cardiac frequency have previously been reported during various exercises in a range of hot environments (Wilson et al., 1975; Suzuki et al., 1980; Maw et al., 1993 [Appendix D]). In contrast, the similarity between $f_c$ in the cool and control conditions was contrary to previous reports that cardiac frequency is depressed during exercise in the cold (Suzuki et al., 1980; Maw et al., 1993 [Appendix D]), and it was therefore considered that the present cool stress was insufficient to alleviate the circulatory strain of exercise.

6.3.2 Acute Body-Fluid Responses to Exercise and Thermal Stress

In all three environments, the strain of exercise was exacerbated by a rapid decrease in BV, probably caused by both hydrostatic and osmotic forces. Capillary hydrostatic pressure increases during exercise, forcing an extravasation of fluid through the capillary pores, thus reducing circulating blood volume (Björnberg, 1990). However, it is the concurrent increase in intramuscular tonicity that is thought to provide the first stimulus for intravascular depletion, creating an osmotic gradient between the tissues and the plasma, and hence drawing fluid across the vascular membrane (Sejersted et al., 1986; Björnberg, 1990; see Senay & Pivarnik, 1985, for review). Thus, blood volume has consistently been found to decrease with the onset of seated cycle exercise, regardless of environmental condition (Kaltreider & Meneely, 1940; Edwards et al., 1983; see Harrison, 1985, and Senay & Pivarnik, 1985, for reviews).

The decrease in BV was greater in the heat than in the control condition, suggesting that increased cutaneous blood flow in the heat further increased the rate of intravascular fluid loss initially stimulated by the onset of exercise. This was in contrast to the effect of heat during seated rest, when blood volume increased, probably due to rapid venodilation causing a fall in capillary hydrostatic pressure (see Section 5.3). Thus, it appeared that the increased thermal stress imposed by exercise caused sufficient cutaneous vasodilation to outweigh the concurrent venomotor response, and therefore to
supplement the extravasation of intravascular fluid occurring in the musculature.

The decrease in BV with the onset of exercise in the cool environment was also greater than that during the initial 10 min of the control period, although this probably represented the superimposition of the exercise effect on the effect of cold seen at rest. During 15 min of seated rest in the same cool environment, blood volume decreased by 166 ml (see Section 5.2), compared to a decrease of 287 ml during 10 min of exercise in the cool; the difference, 121 ml, was similar to the decrease in BV seen during exercise in the control condition. Thus, it appeared that rapid cutaneous venoconstriction and muscular vasodilation occurred simultaneously to additively decrease the volume of intravascular fluid.

Regardless of the environmental condition, the efflux of fluid from the capillaries must have consisted solely of plasma, as capillary membranes are impermeable to the intravascular cells. Thus, it can be assumed that the immediate decrease in BV was entirely a shift of plasma fluid. Indeed, the volume of plasma crossing the vascular membrane was probably greater than the measured decrease in PV, due to the concurrent flux of fluid into the plasma from a number of sources. For example, Lundvall et al. (1972) suggested that, during exercise, fluid was drawn into the plasma from inactive musculature, while plasma was concurrently filtered into the active tissues in response to increasing intramuscular tonicity. Similarly, in the present study, fluid was apparently drawn from the erythrocytes into the plasma, probably in response to increasing plasma tonicity as plasma filtrate was passed into the interstitium. Diaz et al. (1979) previously showed that the volume of erythrocyte water decreased by 1.5% during 45 min of cycle exercise, while others have shown that erythrocyte volume can also decrease during both treadmill running and cross-country skiing (Åstrand & Saltin, 1964; Sawka et al., 1984). Decreases in erythrocyte volume during exercise have not, however, been universally corroborated. For example, both Myhre and Robinson (1969) and Wilkerson et al. (1977) found no change in erythrocyte volume during prolonged exercise, despite significant decreases in plasma volume. While it is difficult to reconcile these conflicting reports, it is apparent that exercise-induced changes in erythrocyte volume are small, and may, therefore, frequently fall within the error of volume measurement. For example,
although Costill and Saltin (1974) found no significant change in erythrocyte volume following intermittent running exercise, four of the six subjects experienced a decrease in volume, and the mean for the group was a loss of 2.1%. It is therefore likely that prolonged exercise does deplete erythrocyte volume, but that such changes are relatively small in comparison to changes in plasma volume.

The decrease in erythrocyte volume during exercise could be attributed to a decrease in either the total number of cells in circulation or the volume of individual cells. Several previous reports have described haemolysis during exercise (Poortmans & Haralambie, 1979; Selby & Eichner, 1985), which would decrease the number of existing cells; however, it is unlikely that sufficient haemolysis had occurred to account for the decrease in erythrocyte volume by minute 20 of the present study. Indeed, there is even evidence to suggest that the number of circulating erythrocytes can increase during the early stages of exercise, due to the emptying into the circulation of splenic blood (Laub et al., 1993). The present erythrocyte depletion can therefore probably be attributed to cellular dehydration rather than destruction, with the dehydration resulting from increased plasma tonicity during the early stages of exercise. It has been shown previously that plasma tonicity increases during exercise (Nose et al., 1991; Gore et al., 1992), and it is further known that erythrocytes are responsive to the tonicity of their surrounds (Costill et al., 1974). Thus, it appeared that the onset of exercise forced an extravasation of plasma fluid, which, in turn, produced an increase in plasma tonicity and so dehydrated the circulating erythrocytes.

It is difficult, from the present data, to determine the destination of the fluid lost from the vascular space during the initial 10 min of exercise, as IFV, ECFV, and ICW were all apparently unchanged during the period, regardless of environment. However, Sjøgaard and Saltin (1982) have shown that intramuscular water content increases during short periods of cycle exercise, with the majority of the increase occurring in the extracellular phase. Thus, it is probable that, in the present study, the plasma filtrate was drawn into the extracellular compartment of active muscles, in response to an increase in the compartments osmotic concentration (Björnberg, 1990). At the same time, averaged across conditions, 69 ml of fluid were lost from the body through sweat and
respiration, implying that fluid from the intravascular space helped defend interstitial fluid volume during the early stages of exercise.

6.3.3 Body-Fluid Responses to Prolonged Exercise and Thermal Stress

As exercise progressed in the control and cool environments, PV, and hence BV, recovered, possibly due to a relaxation of veno-motor tone, but more likely due to concurrent increases in interstitial hydrostatic and capillary osmotic pressures (Jacobsson & Kjellmer, 1964). As indicated by increases in $T_{re}$, cutaneous blood flow increased during exercise in both the cool and control environments, implying that the initial vasoconstriction was removed, potentially decreasing capillary hydrostatic pressure and facilitating the influx of interstitial fluid (Harrison, 1985). However, the effect was probably outweighed by an increase in capillary pressure caused by increased cutaneous perfusion, and hence intravascular expansion would have been opposed. In contrast, the initial efflux of intravascular fluid would have increased the hydrostatic pressure in the interstitium, and hence, in itself, deterred continued extravasation (see Aukland & Nicolaysen, 1981). At the same time, rising plasma tonicity would have created an osmotic gradient, attracting fluid from the interstitium back into the capillaries, and hence helping to restore PV.

That BV was not restored during exercise in the heat can probably be attributed to lesser increases in capillary osmotic and interstitial hydrostatic pressures, compared to the responses during exercise in the cooler conditions. Both Senay et al. (1980) and Edwards et al. (1983) showed that total circulating albumin decreased during cycle exercise in the heat, due to increased capillary filtration, and hence capillary tonicity may not rise to the same extent in the heat as in the cool. At the same time, the fluid being filtered from the vasculature to the interstitium was probably removed to a greater degree by progressive dehydration, preventing the aggregation of interstitial hydrostasis. Thus, the regulation of vascular volume was less effective during exercise in the heat, and both BV and PV remained depleted throughout the period.

The continued depletion of BV may also have contributed to a decrease in stroke volume, which has previously been identified during exercise in the heat (Oddershede
et al., 1977; Suzuki et al., 1980). It is therefore possible that intravascular depletion contributed to the elevation of $f_c$ in the heat, attributed earlier to the competition for perfusion between muscle and skin. Similarly, the stability of BV in the control and cool environments may have been important in ameliorating the circulatory strain of exercise, and hence in preventing cardiovascular drift.

The removal of plasma filtrate from the interstitium was indicated by progressive decreases in both IFV and ECFV during the latter stages of exercise in all three environments. The magnitude of these decreases did not differ between environments, although the volume of fluid lost from the body was noticeably greater during exercise in the heat compared to the two cooler conditions. Thus, it appeared that IFV was relatively defended during heat exposure, probably by the increased plasma filtration concurrently occurring. The loss of fluid from the body, however, prevented the accumulation of interstitial fluid, and therefore also encouraged the depletion of PV.

As already discussed, fluid loss from the body was increased during heat exposure as a result of increased sweating, probably mediated by elevated $T_{ak}$ in the heat compared to the cool and control conditions (Nadel et al., 1971; McCaffrey et al., 1979). However, at the same time, urine production decreased, although the decrease was insufficient to compensate for the increased fluid lost by evaporation. Urine production has previously been found to decrease during exercise, regardless of the environment, due to increased levels of antidiuretic hormones and decreased glomerular filtration rate following decreased renal blood flow (Smith et al., 1952; Francesconi et al., 1985), and it would appear that these effects are most marked in the heat. Indeed, Francesconi (1988) suggested that urine production was at its lowest during exercise in hot environments, potentially falling below 25 ml·h$^{-1}$ during 6 h of continuous exercise. In the present study, urine production averaged just 13 ml during 50 min of exercise in the heat, compared to excretions of 51 and 83 ml during the control and cool periods, respectively.

Total fluid losses, determined from changes in body mass, averaged 861, 563, and 520 ml during exercise in the hot, control, and cool environments, respectively, which was...
similar or marginally greater than the corresponding changes in TBW. While the
difference was marginal, it has previously been shown that losses of body fluid exceed
the depletion of total body water during exercise, due to the concurrent production of
water from the breakdown of carbohydrate (Shephard & Kavanagh, 1978; Noakes et al.,
1988). Hence, the true depletion of body fluid during exercise may actually be less than
previously thought, and less than that indicated by concurrent changes in body mass.

Throughout exercise in all three environments, extracellular fluid losses accounted for
an average of 49% of total fluid losses, with the remaining 51% being drawn from
intracellular sources. Initially, prior to exercise, ECFV contributed 40% to TBW, with
ICW providing the remaining 60%; hence, it appeared as though the extra- and
intracellular compartments contributed almost proportionately to exercise-induced fluid
losses, with only partial defence of the intracellular space. The intracellular compartment
was probably partially defended by an increase in its tonicity, preferentially retaining
water within the active muscle cells (Sjøgaard & Saltin, 1982). Previously, Nose et al.
(1988) suggested that intracellular fluid volume was gradually depleted during exercise,
in response to escalating extracellular tonicity, and ultimately provided 42% of body-
fluid losses. Similarly, Costill et al. (1976) showed that the intracellular compartment
provided 30% of the body's fluid losses during moderate dehydration, with its
contribution increasing as dehydration became more severe. Thus, it would appear that
exercise-induced fluid losses affect the whole body, regardless of environment, and that
the distinction between fluid shifts in hot and cool conditions is confined to the
intravascular volumes. Hence, the defence of intracellular volume was only partial, and
the effects of exercise and its associated fluid losses were manifested almost
proportionately throughout the body.

6.3.4 Methodological Considerations
In the present study, relative changes in BV calculated using the indirect method, from
changes in Hct, and [Hb] (after Dill & Costill, 1974), were similar to those measured
using radionuclide dilution. Similarly, there was no difference in the two derivations of
changes in RCV, regardless of environment or exercise duration. Thus, it appeared that
changes in BV and RCV could be determined from changes in Hct, and [Hb], without
the need for radionuclide dilutions. This was perhaps not surprising, as the particular application of Hct, and [Hb] was considered to account for the possibility of erythrocyte dehydration in conjunction with changes in plasma volume (Dill & Costill, 1974).

However, relative changes in PV during the initial 10 min of exercise were greater when calculated from changes in Hct, and [Hb] than those measured using radionuclide dilution. It appeared that the difference could be attributed to changes in Hct, which tended to exaggerate the actual extent of haemoconcentration. Thus, such a discrepancy would not have been evident in the calculation of relative changes in BV or RCV, as those calculations did not involve the application of Hct, (see Section 4.1.5.4).

Harrison and Edwards (1976) previously found that changes in plasma volume during exercise in the heat were greater when calculated from Hct, and [Hb] than when determined using the dilution of radioiodinated albumin, and Harrison et al. (1982) later concluded that the former method would be in error if the plasma shift was accompanied by a change in the ratio between the whole-body (Hctw) and venous (Hctv) haematocrits. In the present study, the discrepancy between the two measures of plasma volume coincided with a decrease in the ratio between Hctw and Hctv (f-ratio). This decrease, although difficult to explain, confirmed the disproportionate increase in Hct, compared to changes in blood volumes throughout the body. It is possible that the decrease was due to the emptying from the spleen of highly erythrocytic blood during the early stages of exercise. However, the human spleen does not possess the musculature necessary to actively contract (Ebert & Stead, 1941), and, although it has recently been suggested that it empties during exercise as a result of reduced visceral circulation (Laub et al., 1993), the volume of splenic blood would probably not be sufficient to cause the present decrease in the f-ratio.

A more likely explanation is that a redistribution of blood to the microcirculation disproportionately reduced central plasma volume during exercise. The microcirculation is thought to provide something of a reservoir for plasma, such that its haematocrit is lower than that in the larger vessels (Gibson et al., 1946). Hence, a redistribution of blood to the microcirculation would tend to store plasma and exaggerate the increase in
Hct. If correct, however, this effect should have been more marked in the heat than in the cooler environments, reflecting the increased skin blood flow, and should have been maintained throughout exercise, as muscle perfusion was maintained and $T_{re}$ increased as exercise progressed. In fact, the $f$-ratio decreased a similar amount in all three environments and subsequently recovered to resting level during the course of exercise.

Finally, it is possible that, in increasing cardiac output, the onset of exercise affected the dynamics of blood circulation, such that the speed of erythrocyte flow increased out of proportion to the flow of plasma. Erythrocytes are known to flow faster than plasma through the resting circulation, and their disproportionate flow has been offered as an explanation for the $f$-ratio itself (Fåhræus, 1929; see Chapter Three for a complete discussion of the resting $f$-ratio). Whether the flow rates become further separated during the early stages of exercise is presently unknown, and so this explanation must remain as supposition. Regardless of the mechanism, however, the change in the $f$-ratio during the early stages of cycle exercise makes the application of Hct, uncertain, and hence questions the validity of the use of the indirect method to monitor changes in plasma volume during exercise.

### 6.3.5 Conclusion

In conclusion, it was shown that cycle exercise reduced the body’s fluid reserves, regardless of the prevailing environment. However, fluid losses were greater during exercise in the heat than in the cooler conditions. Thus, Hypothesis 1.1.4 (see Section 1.1) was partially accepted, although no difference was found between fluid losses in the control and cool environments. Regardless of environment, fluid lost during cycle exercise was drawn almost equally from extra- and intracellular reserves, with only partial protection of the intracellular compartment. Intravascular volume, on the other hand, was defended in all but the hot environment, when it was progressively depleted throughout exercise, probably due to a combination of peripheral vasodilation and thermal dehydration. Thus, Hypothesis 1.1.3 (see Section 1.1) was largely rejected, as intravascular, plasma, and erythrocyte volumes were ultimately maintained during cycle exercise in the control and cool environments. Intravascular volume was, however, depleted during the early stages of cycle exercise in all the environments, probably due
to a combination of hydrostatic and osmotic forces. The early vascular depletion included a decrease in both erythrocyte and plasma volumes. Changes in erythrocyte volume could be detected equally using both direct and indirect measurement methods; however, changes in plasma volume calculated from Hct, and [Hb] did not accurately reflect exercise-induced changes in vascular volumes throughout the body. The discrepancy was attributed to a change in the $f$-ratio, which decreased during the early stages of exercise. The indirect method of calculating changes in plasma volume should therefore be viewed with caution.
6.4 References


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Ebert, R.V. & Stead, E.A. (1941). Demonstration that in normal man no reserves of blood are mobilized by exercise, epinephrine, and hemorrhage. *American Journal of Medical Sciences*, 201, 655-664.


Chapter Seven
Conclusions, Implications, and Future Research

Throughout the history of body-fluid measurement, it has been apparent that fluid distribution is dependent on posture, environmental temperature, and exercise state. The distribution of intravascular fluid has consistently been shown to change during postural and thermal manipulation (Thompson et al. 1928; Bazett et al., 1940; Hagan et al., 1978), and has also been found to respond to changes in exercise state (Schneider & Havens, 1915; Sawka et al., 1984). Furthermore, the latter changes are dependent on both posture and environmental condition (Diaz et al., 1979; Gore et al., 1992). In all circumstances, the redistribution of intravascular fluid has been attributed to plasma shifts, instigated and regulated by changes in the balance of hydrostatic, osmotic, and oncotic forces across vascular and cellular membranes (Starling, 1896). However, little regard has been paid to the response of the remaining, extravascular, fluids during acute stress, although it has been suggested that fluid accumulates within muscles during cold exposure and exercise (Barbour et al., 1943; Sjøgaard & Saltin, 1982). In contrast, Costill et al. (1976) and Nose et al. (1988) found that intra- and extracellular fluid volumes decreased during exercise and heat stress, although their calculations were based on changes in the tissue chloride concentration, rather than on measurements of the respective compartmental volumes. Hence, it was the purpose of the present investigation to measure the volume of fluid throughout the body, using a method that would accurately determine acute changes in distribution during postural, thermal, and exercise stress.

Body-fluid distribution was measured using the simultaneous dilution of four radionuclides, to measure or derive the volumes of fluid throughout the intra- and extravascular compartments. Thus, using tritiated water, radiobromine, radioiodinated fibrinogen, and radiochromated erythrocytes, the volumes of total body water, intracellular water, extracellular fluid, interstitial fluid, blood, plasma, and erythrocytes were determined simultaneously. The method proved to be reliable between repeat assessments, and to accurately determine the body-fluid distribution of physically conditioned, adult males. In so doing, it showed that physically conditioned males
maintain a general hyperhydration, which was characterised by a proportionate expansion of all the body's fluid compartments and was probably related to habitual physical activity and low adiposity. Similar exercise-induced expansions have previously been demonstrated for intravascular fluid volumes (Sjöstrand, 1962; Magnusson et al., 1984); however, this was apparently the first report of hyperhydration throughout the body in physically conditioned males.

The radionuclide dilution method also proved suitable for the determination of serial changes in body-fluid distribution, and was thus applicable to the study of acute fluid shifts during postural, thermal, and exercise stress. The overall outcome was the repeated finding that body-fluid distribution was disturbed by acute stress, implying that fluid distribution is a dynamic concept, rather than a series of static volumes. Thus, previous knowledge of intravascular dynamics was extended to include the distribution of fluid throughout the body, and it was apparent that fluid distribution could only be discussed in terms of the prevailing circumstances.

It was shown in Chapter Four that, on assuming the upright posture, intravascular volume decreased, probably due to an increase in capillary hydrostatic pressure causing the extravasation of circulating plasma. The plasma filtration would have continued until interstitial hydrostatic and plasma oncotic forces had increased sufficiently to balance the rise in capillary pressure, and thus to prevent the further extravasation of intravascular fluid. At the same time, the accumulation of plasma oncotic pressure would have created an osmotic gradient which dehydrated the circulating erythrocytes, and thus partially defended plasma volume. The redistribution of fluid was slow, making it likely that fluid distribution had not stabilised within the present 30 min of standing. Thus, it is recommended that a posture is maintained for at least 30 min if a stable fluid distribution is desired.

Exposure to a cool environment (Chapter Five) caused a similar plasma filtration in response to an increase in capillary hydrostatic pressure, this time probably mediated by rapid venoconstriction. The cool-induced extravasation was not, however, as great as that provoked during upright rest (Chapter Four), and erythrocyte volume was essentially
maintained. It therefore appeared that the increase in capillary hydrostatic pressure during cool exposure was less than that associated with standing upright, and therefore probably less than the 69-mmHg increase reported by Stick et al. (1993) for the response in the saphenous vein during upright rest in a control environment. Thus, the challenge to body-fluid distribution and the resultant cardiovascular strain were less during cool exposure than during upright rest, and fluid distribution had probably stabilised within the present 30-min period. This may not, however, have been the case had the cold stress been more severe, and it is therefore essential to achieve thermal equilibrium prior to body-fluid measurements.

The response of body-fluid distribution to heat stress (Chapter Five) was variable, and may have been dependent on the subjects’ individual tolerance to heat. In general, there was a trend for plasma volume to expand during heat stress, probably due to a decrease in capillary hydrostatic pressure caused by venodilation. However, some subjects experienced a decrease in plasma volume, probably caused by increased cutaneous blood flow, in an attempt to regulate deep-body temperature. It was unclear, in either case, whether fluid distribution had equilibrated within the 30-min period, as some subjects who first experienced a plasma expansion later displayed a plasma contraction, which may have continued beyond the experimental period. Senay and Christensen (1965) previously showed that, during rest in 36°C, plasma volume contracted following an initial expansion, and it therefore appears that, in environments approaching deep-body temperature, the final response of intravascular fluids may be a contraction caused by continued vasodilation and progressive dehydration. As the present hot exposure was also associated with elevated cardiac frequency, it appears that the maintenance of intravascular volume is desirable, to help minimise both cardiovascular and thermoregulatory heat-strains.

The response of intravascular volume to cycle exercise (Chapter Six) was similarly variable, with blood volume first decreasing and then recovering to preexercise level as exercise continued. The initial decrease, in the control condition, was attributed to increased capillary hydrostatic and tissue osmotic pressures, such that plasma was filtered into the interstitium and possibly then into the extravascular cells. The
intravascular depletion was, however, smaller than that incurred during upright rest in the control environment (Chapter Four), implying that cycle exercise induced less cardiovascular strain than did standing upright. This may account in part for the acknowledged stability of intravascular volume during standing exercise, when rapid, posturally-induced fluid shifts may overwhelm those effected by exercise (Gore et al., 1992). Indeed, Stick et al. (1993) showed that venous pressure decreased during exercise following upright rest, potentially decreasing capillary pressure and reducing the rate of capillary filtration.

The imposition of cold, however, increased the immediate cardiovascular strain of exercise, such that changes in intravascular volume combined the thermal and exercise depletions. Indeed, the initial intravascular depletion amounted to an exact addition of the control exercise effect and the resting cold-induced extravasation reported in Chapter Five. Thus, further confirmation was provided of the interactive effect of exercise and the environment on body-fluid distribution.

As exercise progressed in both the control and cool conditions, intravascular volume recovered, probably due to the accumulation of plasma oncotic and tissue hydrostatic forces reversing the outflow of intravascular fluid (Jacobsson & Kjellmer, 1964). However, the consequence of intravascular recovery was the depletion of interstitial fluid volume, and it is worth contemplating whether this would be detrimental to physiological function. It has previously been shown that an imbalance of fluid between the interstitium and the intracellular compartment can change muscle membrane potential (Sjøgaard et al., 1985), and it is therefore possible that the depletion of interstitial fluid volume might similarly impair muscle function.

In contrast to the control and cool conditions, cycle exercise in the heat constantly decreased intravascular volume, creating the most severe cardiovascular strain of all those imposed in the present investigation. The decrease in intravascular volume at the onset of exercise was greater than that seen at the onset of exercise in the control condition, implying that the combination of cycling and heat stress increased capillary filtration beyond that produced by cycling alone. In addition, it appeared that cycle
exercise and heat produced greater cardiovascular and thermoregulatory strains than did rest in the same hot environment, when intravascular volume was essentially maintained. Thus, plasma filtration was facilitated by increased cutaneous perfusion probably caused by widespread vasodilation.

As cycling progressed in the heat, intravascular volume continued to decline, reflecting both continued vasodilation and progressive whole-body fluid loss. In general, the fluid loss affected all compartments of body fluid, with little defence of either the intra- or the extracellular spaces, or indeed of the intravascular volume. Thus, the plasma filtrate was probably rapidly removed from the interstitium, preventing the accumulation of tissue pressure, and therefore encouraging further extravasation. Costill et al. (1976) previously suggested that the intracellular compartment was partially defended during moderate dehydration. However, it was later shown that the intra- and extracellular spaces contributed almost equally to dehydration as fluid losses became more severe (Nose et al., 1988). Thus, it would appear that exercise-induced fluid losses are spread throughout the body, and that, without appropriate fluid replacement, the intra- and extravascular spaces will contract and exacerbate the cardiovascular and thermoregulatory strains associated with prolonged exercise per se.

Increased cardiovascular strain was evident in the increased cardiac frequency during exercise in the heat. In comparison to the control and cool conditions, where intravascular volume recovered during exercise, cardiac frequency was elevated by 14 and 13 b·min⁻¹, respectively. It therefore appeared as though the autoregulation of intravascular volume during the control and cool periods was important in ameliorating the cardiovascular strain associated with prolonged exercise. Thus, it is possible that judicious use of fluid replacement or acclimation may be similarly useful in decreasing exercise-induced cardiovascular strain in all thermal conditions.

7.1 Future Research
Further investigation is needed into the maintenance of intravascular fluid volume during exercise, and during postural and thermal stress, and it can be suggested that similar
radionuclide dilution methods to that used in the present investigation may be useful in quantifying the accompanying fluid shifts. The use of radiolabelled exogenous water, in combination with dilution tracers to measure plasma and erythrocyte volumes, for example, may help determine the applicability of procedures such as preexercise hyperhydration or heat-induced plasma expansion.

The measurement of acute changes in plasma volume must, however, be quantified using the dilution of a large plasma tracer, such as radioiodinated fibrinogen, as volume changes determined from changes in the venous haematocrit may not always be accurate. It was shown in Chapter Six, for example, that, during cycle exercise, the ratio between the whole-body and venous haematocrits (f-ratio) changed, and consequently the venous haematocrit exaggerated changes in plasma volume. Further investigation is required to determine the nature of the change in the f-ratio, but it is suggested that appropriate radionuclide dilution methods may disclose a concurrent change in the dynamics of blood flow. The use of radiolabelled plasma and erythrocyte tracers might facilitate a thorough examination of blood-flow dynamics and the f-ratio during a variety of acute stresses. In the meantime, however, it is recommended that the determination of intravascular volume changes through changes in venous haematocrit should only be made cautiously when cardiovascular dynamics are disturbed.

It is further recommended that future research into the distribution or composition of body fluid should carefully control for extraneous variables, such as posture and environmental temperature, which may co-act with the desired experimental condition. For example, Tan et al. (1973) previously showed that the concentration of serum lipids was dependent on the patients posture, and the present data suggest that the concentration would be further affected by the environment and by the patients state of rest. Thus, any measurement of serum lipids, or any other haematologic concentration, must ultimately be reported with reference to the prevailing circumstances.

The patient’s level of physical conditioning must also be borne in mind, as those with a history of habitual physical training may well present as abnormal in comparison to more sedentary populations. For example, it was shown in Chapter Three that the
present physically trained individuals possessed intravascular fluid volumes considerably larger than those considered normal by the International Committee for Standardization in Haematology (1980), which was attributed to their habitual exercise and their low adiposity. Further investigation is needed into the respective influences of exercise and adiposity, as current methods do not allow accurate determination of body fatness but lean body mass may be the best relative measure of body-fluid volumes.

7.2 Conclusion

In conclusion, it was clearly apparent that the distribution of body fluid was dependent on posture, environmental temperature, and exercise state. It was shown that fluid distribution required up to 30 min to equilibrate following a change in posture, and it was suggested that a similar period may be required following a change in environment, depending on the severity of the imposed or preceding thermal stress. The effect of cycle exercise on fluid distribution was evident within just a few minutes, and hence, if a stable fluid distribution is desired, it is recommended that the subject’s circumstances are not disturbed for 30 min or more prior to fluid measurement. In a steady state, it appears that the venous haematocrit can be used to indicate changes in intravascular fluid volume; however, during circumstances that might affect the dynamics of blood flow, application of the venous haematocrit becomes vague and may result in erroneous haematological diagnoses.
7.3 References


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Appendix A

SOUTH COAST BLOOD BANK & HAEMATOLOGY SERVICE

January 1993

Director: Dr. I.L. MacKenzie.

Senior Hospital Scientist: Mr. D.A.M. Comer.

Prepared by: Graeme J. Maw* and Des A.M. Comer.

MULTIPLE RADIONUCLIDE MEASUREMENT OF HUMAN BODY FLUIDS

Principle

The method is essentially that described by Spears et al. (1974), and is based on the dilution principle for measurement of unknown volumes. Known quantities of four radionuclides are injected simultaneously into the patient, allowed to reach equilibria, and subsequently measured in 10 ml blood samples. By comparing the concentrations of the nuclides in the samples with those in prepared standards, the unknown volumes are derived.

Tritiated water ($^3$H) is used to measure total body water, radiobromine ($^82$Br) is used to measure extracellular water, radioiodinated ($^{125}$I) human serum fibrinogen or albumin is used to measure plasma volume, and radiochromate ($^{51}$Cr) is used to measure erythrocyte volume. Crucially, the four nuclides are distinguishable in the counting process.

Although Spears et al. (1974) dismissed the need for correction for nuclide losses in urine, accuracies of the volume measurements are enhanced if such corrections are made. Correction is also made for penetration of $^82$Br into erythrocytes, while plasma volume is derived in the usual way, by extrapolation of $^{125}$I concentration to time-zero, to account for the continual exchange of plasma proteins with interstitial fluid.

Reagents and Equipment

1. Tritiated water ($^3$H$_2$O) for injection; TRS7, Amersham Australia, 100 mCi ml$^{-1}$.
   Sodium-radiobromine (Na$^{82}$Br) for injection; BR2M2, Australian Radioisotopes, 1.08 mCi ml$^{-1}$.
   Radioiodinated ($^{125}$I) human serum fibrinogen for injection; IM.53P, Amersham Australia, freeze-dried, reconstituted to 100 μCi ml$^{-1}$.
   Sodium-radiochromate (Na$^{51}$Cr) for injection; CJS1, Amersham Australia, 1 mCi ml$^{-1}$.
   Liquid Scintillation cocktail suitable for human serum; Emulsifier Safe, Packard Instruments.

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*Department of Biomedical Science, University of Wollongong.
2% xylocaine solution.
1N hydrochloric acid.
Saponin powder.
100 ml vials of sterile saline (4).
Sterile saline.
Distilled water.

2. Well-type γ counter, calibrated for 82Br, 125I, and 51Cr; Auto-LOGIC, Abbott Laboratories.
Liquid scintillation β counter, calibrated for 3H; 1219 Rackbeta, LKB Wallac.
Apparatus for measuring haematocrit and haemoglobin concentrations; S-Plus IV, Coulter Electronics.
Apparatus for measuring plasma protein concentration; Refractometer 93032, Atago.
Scientific electronic balance, with precision of 0.001 g.
Centrifuge capable of generating 1700g.
Water bath at 37° C.
Calibrated 3 ml autopipette with tips.
Safe working cabinet.
Stop watch.

3. Calibrated 500 ml glass flasks (2).
Calibrated 250 ml glass flasks (2).
Large, calibrated measuring cylinder.
Sterile McCartney bottle, prepared with 2 ml of citrate-phosphate-dextrose-adenine solution.
12 x 75 mm glass tubes with caps, suitable for γ counting.
Glass vials with caps, suitable for β counting.

4. 20 ml syringe and 21 gauge needle.
10 ml syringes.
2.5 ml syringes and 21 gauge needles.
Graduated 1 ml syringes and needles.
16 gauge teflon cannula, extension tube, and three-way tap.
10 ml ethylenediamine tetra-acetic acid (EDTA) tubes.
Urine specimen jars.
Disposable pipettes.

5. Semilogarithmic graph paper.

**Preparation of Nuclides**

All preparations, except centrifuging, are conducted under sterile conditions, in a safe working cabinet, maintaining the sterility of all vials with isopropyl alcohol swabs.

**Tritiated water.**

Total body water is measured using 500 μCi of 3H. The 3H₂O is diluted to a
concentration of 0.25 mCi ml⁻¹:

1. Remove 0.25 ml from a 100 ml vial of sterile saline.
2. Add 0.25 ml of ³H₂O to the saline vial and mix thoroughly.

**Radiobromine.**

Extracellular water is measured using 20 μCi of ⁸²Br. The Na⁸²Br is diluted to a concentration of 10.8 μCi ml⁻¹:

1. Remove 1 ml from a 100 ml vial of sterile saline.
2. Add 1 ml of Na⁸²Br to the saline vial and mix thoroughly.

**Radioiodinated fibrinogen.**

Plasma volume is measured using 2 μCi of ¹²⁵I-fibrinogen. The ¹²⁵I-fibrinogen is reconstituted as directed in the product information, and diluted to a concentration of 1 μCi ml⁻¹:

1. Add 1.1 ml of sterile water to the dried fibrinogen and agitate for 10 min.
2. Remove 1 ml from a 100 ml vial of sterile saline.
3. Add 1 ml of the reconstituted fibrinogen to the saline vial and mix thoroughly.

**Radiochromate.**

Erythrocyte volume is measured using 8 μCi of ⁵¹Cr, tagged to autologous erythrocytes. The Na⁵¹Cr is diluted to a concentration of 10 μCi ml⁻¹:

1. Remove 1 ml from a 100 ml vial of sterile saline.
2. Add 1 ml (whole contents) of Na⁵¹Cr to the saline vial and mix thoroughly.

**Procedure**

The patient is seated at rest throughout assessment. The hour between drawing the initial blood sample and injecting the nuclides is necessary for preparation of the injections. The volumes of all injections and standards are determined gravimetrically.

**Preliminaries.**

1. Collect 20 ml of blood from the patient approximately 1 hour before the nuclide injection is intended.
2. Dispense 10 ml of this blood into an EDTA tube, to use as a background
reference during radioactivity counting.

3. Dispense the remaining 10 ml in to the McCartney, for preparation of the $^{51}$Cr injection.

4. Centrifuge the McCartney bottle for 10 min at 1700g and subsequently remove the plasma, leaving the erythrocytes for $^{51}$Cr labelling.

5. Collect a urine sample from the patient, to use as a background reference during radioactivity counting.

**Erythrocyte labelling.**

1. Add approximately 0.8 ml of the $^{51}$Cr preparation to the erythrocytes in the McCartney bottle and mix gently.

4. Incubate the mixture at 37° C for 20 min, to allow secure labelling of the cells.

5. Wash the mixture three times with sterile saline, centrifuging for 10 min at 1700g each time.

6. Reconstitute the preparation with sterile saline to a haematocrit of approximately 50%.

**Preparation of injections.**

1. Aspirate approximately 2.1 ml of the $^3$H preparation in to a sterile syringe and record the mass of the filled syringe.

2. Dilute approximately 0.1 ml from the syringe to 500 ml by volume in distilled water, from which to prepare the $^3$H standard. Record the new mass of the syringe and determine the exact dilution by subtraction from the previous mass.

3. Aspirate approximately 2.1 ml* of the $^{82}$Br preparation in to a sterile syringe and record the mass of the filled syringe.

4. Dilute approximately 0.1 ml* from the syringe to 500 ml by volume in distilled water, from which to prepare the $^{82}$Br standard. Record the new mass of the syringe and determine the exact dilution by subtraction, as above.

5. Aspirate approximately 2.2 ml of the diluted $^{125}$I in to a sterile syringe and record the mass of the filled syringe.

---

*The volume of the injection necessary to include 20 $\mu$Ci of $^{82}$Br will vary as the nuclide decays. The figure listed here is for the day of product reference. On the second day a dose of 3 ml, plus 0.15 ml for the standard, will be required, increasing to 5 plus 0.25 ml and 8 plus 0.4 ml on the third and fourth days.
6. Dilute approximately 0.2 ml from the syringe to 250 ml by volume in distilled water, from which to prepare the $^{125}$I standard. Record the new mass of the syringe and determine the exact dilution by subtraction, as above.

7. Aspirate the entire contents of the $^{51}$Cr-labelled erythrocyte mixture into a sterile syringe and record the mass of the filled syringe.

8. Dilute approximately 0.5 ml from the syringe to 250 ml by volume in distilled water, from which to prepare the $^{51}$Cr standard. Record the new mass of the syringe and determine the exact dilution by subtraction, as above.

Injection.

The four injections are given through an indwelling cannula or butterfly needle, without pause, in the sequence $^{125}$I, $^{51}$Cr, $^{82}$Br, $^{3}$H.

Record the mid-time of the fibrinogen injection. Following infusion, thoroughly flush the indwelling needle with sterile saline. Record the new masses of the four emptied syringes, and determine the exact volumes of the injections by subtraction of the respective previous masses.

Sampling and handling.

All blood and urine handling is in glassware to avoid nuclide absorption during the necessary storage periods. The blood handling procedures must be performed carefully, as 10 ml of blood is only just sufficient for the process.

Collect 10 ml blood samples into EDTA tubes 15, 30, 60, and 180 min after the noted start-time (note the exact timing of the collections). Collect a urine sample immediately after the 180 min sample; the subject should fully void. Measure the volume of urine and retain a sample for radioactivity counting.

1. Measure the haematocrit and haemoglobin concentrations of all blood samples, including the background reference.

2. Centrifuge all blood samples for 40 min at 1700g.

3. Measure the plasma protein concentrations of all blood samples.

4. Dispense exactly 3 ml of plasma from the samples into separate glass tubes; cap and set aside.

5. Dispense a further 0.5 ml of plasma from the samples into the glass vials.

---

*Forty minutes is recommended in order to reduce trapped plasma to an absolute minimum (approximately 2%), and hence leave the erythrocytes free from extracellular $^{82}$Br and $^{125}$I.*
6. Remove the remaining plasma and buffy coat from the samples.

7. Lace separate glass tubes with Saponin, followed by a drop of water. Add exactly 3 ml of erythrocytes from the samples, and agitate gently; cap and set aside.

8. Dispense exactly 3 ml of the urine sample in to a glass tube, and 0.5 ml in to a glass vial.

9. Add 50 µl of HCl to each glass vial, including standards and backgrounds (see below) and urine, as a tissue solubiliser, followed by 9 ml of the liquid scintillation cocktail; cap and shake vigorously.

**Standards and backgrounds.**

1. Dispense exactly 3 ml from each of the diluted ³H, ⁸²Br, ¹²⁵I, and ⁵¹Cr flasks in to separate glass tubes; cap and set aside.

2. Dispense a further 0.5 ml from each flask, except ⁵¹Cr, in to separate glass vials.

3. Dispense exactly 3 ml of distilled water in to a glass tube, and 0.5 ml in to a glass vial, to use as background references during radioactivity counting.

4. Dispense exactly 3 ml of the original urine sample in to a glass tube, and 0.5 ml in to a glass vial, to use as background references during radioactivity counting.

**Radioactivity Counting**

The counting process makes allowance for the multiple interactions that occur between the nuclides in the first few days. Ten days after the reference date, only 0.97% of the original ⁸²Br activity will be remaining, diminishing to 0.38% after 12 days and 0.14% after 14 days. Therefore, counting of ³H, ¹²⁵I, and ⁵¹Cr is delayed until approximately 2 weeks after the ⁸²Br reference date.

1. Count all glass tubes twice in the ⁸²Br channel of the γ-counter, on the day of sampling, reversing the order of successive counts.

2. Refrigerate all tubes and vials at 4° C for approximately 14 days.

3. Count all standards, backgrounds, and plasma tubes three times in the ¹²⁵I channel of the γ-counter, and all standards, backgrounds, and erythrocyte tubes three times in the ⁵¹Cr channel, reversing the order of successive counts.

4. Count all glass vials three times in the β-counter.

**Calculations**

Calculation of respective fluid volumes follows the general principle of dilution: dividing
the infused nuclide concentration by that of the sample to give the unknown volume. However, as mentioned in introduction, several corrections must be made to derive the respective volumes.

1. Average the sum of the radioactivity counts relevant to each nuclide and express the means as counts min⁻¹ ml⁻¹.

2. Subtract the respective (ie. plasma, erythrocytes, distilled water or urine) background reference from the resultant means, to leave respective nuclide concentrations ([³H], [¹³Br], [¹²⁵I], [⁵¹Cr]).

**Total body water.**

The plasma and urine vials measured for ³H on the β-counter are corrected for the presence of ¹²⁵I. This is done by calculating the ratio (i), for the ¹²⁵I standard, of radioactivity in the ³H channel to that in the ¹²⁵I channel, and subtracting this percentage of all [¹²⁵I] from the respective [³H]. The resultant [³H] is then be used to calculate total body water:

1. Calculate the infused ³H from the ³H standard measured in the ³H channel (S₃H):
   \[
   \text{dose} = S_3H \times \text{dilution} \times \text{injection volume}.
   \]

2. Calculate the ³H loss in urine from the urine sample counted in the ³H channel (U₃H) and counted in the ¹²⁵I channel (U₁₂⁵I):
   \[
   \text{urine} = (U_{3H} - iU_{125I}) \times \text{urine volume}.
   \]

3. Calculate the [³H] of protein-free plasma from the plasma samples counted in the ³H channel (P₃H) and counted in the ¹²⁵I channel (P₁₂⁵I), and from the plasma protein concentration ([PP]) (after Chien & Gregersen, 1962):
   \[
   \text{sample} = (P_{3H} - iP_{125I}) \times \left( 100 - 0.73[PP] \right) \div 100.
   \]

4. Calculate total body water:
   \[
   \text{TBW} = (\text{dose} - \text{urine}) \div \text{sample}.
   \]

**Extracellular water.**

The plasma and urine tubes measured for ⁸²Br in the ⁸²Br channel are not corrected for the presence of ¹²⁵I, as ¹²⁵I radioactivity is not of sufficient energy to be detected in the ⁸²Br channel. However, the erythrocyte and urine tubes measured for ⁸²Br in the ⁸²Br channel are corrected for the presence of ⁵¹Cr. This is done by calculating the ratio (c), for the ⁵¹Cr standard, of radioactivity in the ⁸²Br channel to that in the ⁵¹Cr channel, and subtracting this percentage of all [⁵¹Cr] from the respective [⁸²Br]. The resultant [⁸²Br] is then be used to calculate extracellular water:
1. Calculate the infused $^{82}\text{Br}$ from the $^{82}\text{Br}$ standard measured in the $^{82}\text{Br}$ channel ($S_{\text{Br}}$):

$$\text{dose} = S_{\text{Br}} \times \text{dilution} \times \text{injection volume}.$$ 

2. Calculate the $^{82}\text{Br}$ loss in urine from the urine sample counted in the $^{82}\text{Br}$ channel ($U_{\text{Br}}$) and counted in the $^{51}\text{Cr}$ channel ($U_{\text{Cr}}$):

$$\text{urine} = (U_{\text{Br}} - cU_{\text{Cr}}) \times \text{urine volume}.$$ 

3. Calculate the penetration of $^{82}\text{Br}$ into erythrocytes from the erythrocyte samples counted in the $^{82}\text{Br}$ channel ($E_{\text{Br}}$) and counted in the $^{51}\text{Cr}$ channel ($E_{\text{Cr}}$), and from erythrocyte volume (RCV; see below):

$$\text{rbc} = (E_{\text{Br}} - cE_{\text{Cr}}) \times \text{RCV}.$$ 

4. Calculate the $[^{82}\text{Br}]$ of protein-free plasma from the plasma samples counted in the $^{82}\text{Br}$ channel ($P_{\text{Br}}$) (after Chien & Gregersen, 1962):

$$\text{sample} = P_{\text{Br}} \times (100 - 0.73[\text{PP}]) \div 100.$$ 

5. Calculate the volume of protein-free plasma from plasma volume (PV; see below):

$$\text{PW} = PV \times (100 - 0.73[\text{PP}]) \div 100.$$ 

6. Calculate extracellular water, adjusting for the Gibbs-Donnan electrolyte balance (1.02 for anions; Manery, 1954):

$$\text{ECW} = (0.98(((\text{dose} - \text{urine} - \text{rbc}) \div \text{sample}) - \text{PV})) + \text{PW}.$$ 

**Plasma volume.**

1. Calculate the infused $^{125}\text{I}$ from the $^{125}\text{I}$ standard measured in the $^{125}\text{I}$ channel ($S_I$):

$$\text{dose} = S_I \times \text{dilution} \times \text{injection volume}.$$ 

2. Accurately plot the $[^{125}\text{I}]$, counted in the $^{125}\text{I}$ channel, of the 15, 30, and 60 min (exact time needed) plasma samples on semilogarithmic graph paper; omit the 15 min sample if prolonged mixing is suspected. Extrapolate the line to determine the theoretical $[^{125}\text{I}]$ of a plasma sample collected at time-zero (sample).

3. Calculate plasma volume:

$$\text{PV} = \text{dose} \div \text{sample}.$$
Erythrocyte volume.

1. Calculate the infused $^{51}$Cr from the $^{51}$Cr standard measured in the $^{51}$Cr channel ($S_{cr}$):

   $$dose = S_{cr} \times \text{dilution} \times \text{injection volume}.$$

2. Calculate the $^{51}$Cr loss in urine from the urine sample counted in the $^{51}$Cr channel ($U_{cr}$):

   $$\text{urine} = U_{cr} \times \text{urine volume}.$$

3. Note the [$^{51}$Cr] of the 30, 60, or 180 min erythrocyte sample, or calculate the mean of a combination of these (sample).

4. Calculate erythrocyte volume:

   $$\text{RCV} = (\text{dose} - \text{urine}) \div \text{sample}.$$

Normal Values

<table>
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<tr>
<th></th>
<th>Males (ml kg$^{-1}$)</th>
<th>Females (ml kg$^{-1}$)</th>
<th>Source</th>
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<tbody>
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<td>500</td>
<td>ICRP (1975)</td>
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<tr>
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<tr>
<td>RCV</td>
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<td>25</td>
<td>ICSH (1980)</td>
</tr>
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</table>

References


Appendix B

EPRL Technical Report 92-1

CLIMATE CHAMBER
COMPUTER CONTROL AND CALIBRATION

GRAEME J. MAW
with technical assistance from
PETER WILLIAMSON

Environmental Physiology Research Laboratory
Dept. Human Movement Science
University of Wollongong

P.O. Box 1144
Wollongong
NSW 2500

Phone: 042-213881
Fax: 042-214096
ABSTRACT

The climate chamber in the Department of Human Movement Science, University of Wollongong, can be controlled either manually or automatically through a personal computer. A controlling computer program has been developed in Microsoft FORTRAN-77 to enable a stable environment, regulated humidity, and/or a constant rate of change of temperature. Temperature and humidity readings were calibrated against a reference thermometer and a wide-range hygrometer, respectively. The status of the chamber can be displayed on screen, disk or printer.
ACKNOWLEDGEMENTS

The FORTRAN programming of the climate chamber would not have been possible without the impetus of Dr. Nigel A.S. Taylor, or the patience of Mr. Peter Williamson. Mr. Williamson must be specially recognised for his assistance on the fiddly bits and contribution of the necessary assembler routines.

Dr. Tony Hulbert, of the Biology Department, and the Biological and Chemical Analysis Service (BACAS) at the University of Wollongong are gratefully thanked for the loan of a hygrometer and reference thermometer, respectively.
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1. INTRODUCTION

1.1 General Description

The climate chamber at the University of Wollongong is housed within the Environmental Physiology Research Laboratory, in the Department of Human Movement Science (Building 8, room G16). It was built in 1989 to enable scientific research to be conducted under simulated environmental conditions.

The chamber consists of an insulated room serviced by an air-conditioner, a dehumidifier, three heaters and two humidifiers. The system was designed to operate between 15° and 45°C, with a humidity range from 30% to 80% at 35°C. However, the plant are such that temperatures outside this range can be achieved. For example, temperatures as low as 4° and as high as 50°C have been recorded.

The chamber can be controlled either manually or through a personal computer located in the adjoining ante-room. The choice between manual and computer control is made at the MAN/OFF/AUTO switch on the main control panel in the ante-room. Manual control involves adjustment of slow response thermostats and humidistats in the control panel inside the chamber. These regulators are disabled if AUTO is chosen, and control is diverted to the computer terminal."

1.2 Automatic Control

Automatic (computer) operation enables temperature and humidity to be monitored through a thermistor and humidity sensor, located in the recess of the exhaust port of the chamber. Signals are relayed to the computer in the ante-room by means of an A-D interface. The computer therefore detects voltages relating to temperature and humidity.

Computer operation has traditionally been controlled through a PASCAL

program, which allows the user to set target temperature and relative humidity and observe the environmental response, displayed on the monitor or as a printed record. The status of specific hardware is also displayed, and can be toggled on or off using respective function keys. However, individual choices are overridden as the computer resumed control.

The PASCAL program was considered inconvenient in that predetermined cooling or heating protocols could not be controlled to proceed automatically. Alternative software was therefore developed to enable either stable environmental control or changes in temperature at constant, user-defined rates. This report documents the resultant Microsoft FORTRAN-77 program.
2. DEVELOPMENT OF THE PROGRAM

2.1 MS Fortran-77 for Personal Computers

A computer program, CHAMBER.FOR, was written and compiled using Microsoft FORTRAN-77* to meet the requirements for either stable or transient environmental control (Attachment A). The program was compiled in a medium memory model under mathematical emulation without optimization. In communicating with the chamber hardware, it interfaces with an assembly language routine, for which the source is in the file PORTM.ASM (Attachment B). Thus, CHAMBER.FOR must be linked with PORTM.OBJ. It must also be linked with GRAPHICS.LIB, and include the Microsoft FORTRAN-77 files FGRAPH.FI and FGRAPH.FD, to enable the CLEARSCREEN subroutine. Although the program contains extensive comments to facilitate understanding by subsequent users, the following sections of this report elaborate on its development.

2.2 User-Defined Variables

Stable, constant cooling or constant heating protocols dictated by the user can be executed through CHAMBER.EXE. Current temperatures can be maintained, or desired temperatures reached through specified rates of change. Thus, the user is asked to convey the desired target and rate of change of temperature. As far as possible, targets will be achieved at a relative humidity determined by the user.

A typical protocol will have a finite duration. Hence, the program requires this length to be specified. If the target temperature is reached before the time has elapsed, the environment is maintained in a steady state. Targets cannot be modified in the interim without escaping and rerunning the program. On completion of the protocol, the option is presented to continue with the established targets, to redefine the protocol or to terminate the program.

Output can be directed to any combination of monitor, disc or printer.

Regardless of destination, information is initialized by the date and real start-time, and continues with the elapsed time, the temperature and relative humidity. Data files include real values to one decimal place (F5.1) in ASCII format. Report updates are generated at the interval specified by the user. Reports can be directed to the screen or disk up to every 2 seconds. However, it is advisable to allow up to 3 seconds for combinations requiring printed records.

2.3 Plant Operation

The personal computer in the ante-room communicates with chamber hardware through an A-D conversion interface. The temperature and humidity sensors are accessed through the subroutine READ_ADC, with the channels having addresses of 0 and 1 respectively. The subroutine contains a delaying loop after the initial approach to the hardware to enable the output to be prepared.

The heaters, air-conditioners, humidifier and dehumidifier are controlled through the SWITCH subroutine. The heaters are addressed 1, 2 and 3, the air-conditioners are 4 and 5, with the humidifier and dehumidifier being 6 and 7 respectively. The INIT_OUTPUT_HARDWARE subroutine is called at the beginning of the program to initialize the input and output channels to the hardware. Similarly, the SWITCH_ALL_OFF subroutine is called before exiting the program, to ensure that all devices are shut down.

2.4 Plant Protection

The thermal range of the chamber is limited by the capabilities of its hardware. In attempting to exceed these limits, it is possible to overload individual components, and hence disable chamber operation. It is thought that the system will overheat at approximately 50°C. Heater control is therefore progressively disabled from 47° to a maximum of 49°C. Similarly, the air-conditioners will freeze below 15°C so are disabled at that point. Temperatures below 15°C can be achieved by continued dehumidification. However, the dehumidifier is limited to a maximum of six on/off cycles in any 60 minutes, restricting the ability to "super-cool". The humidifier is disabled whenever the dehumidifier is active.
3. CHAMBER CALIBRATION

3.1 Methods

Temperature and humidity signals are received by the personal computer as voltages. It was therefore necessary to calibrate these readings to degrees Celsius and relative humidity respectively.

An initial version of CHAMBER.FOR wrote raw voltage data to the monitor and/or to a data file. This raw information was collected during trial operations, simultaneously with the real temperature and relative humidity in the exhaust port of the chamber. A Grant ambient thermistor and American Instruments wide-range Hygrosensor (L15-2011A) were placed in the exhaust next to the integral sensors, and recorded to a Grant Squirrel Meter/Logger (1200 series), and Hitachi QPD-22 chart recorder respectively. The thermistor had itself been calibrated against a Dobros reference thermometer, through a range from 12° to 60°C, during the preceding month. The hygrometer was on loan from the Biology Department at the University of Wollongong, and had not been calibrated for some time. Although chart recordings were carefully corrected for ambient temperature, some uncertainty must exist over the validity of humidity readings. Respective data from the integral sensors, thermistor and hygrometer were regressed using Lotus 3-2-1 spreadsheet software.

3.2 Temperature

Temperature calibration was established by operating the chamber at constant relative humidity while temperature was varied between 40° and 10°C. The temperature was held at 40°C for 20 minutes, followed by 18 minutes of constant cooling to 22°, 20 minutes at 22°, and 40 minutes cooling to 10°C. Data analysis established the equation:

\[ y = (0.184 \times x) - 0.019 \]

where \( x \) is the input voltage, and \( y \) is actual temperature in degrees Celsius (Figure A1).
The protocol confirmed the ability of CHAMBER.FOR to perform ramp cooling, and to stabilise relative humidity (Figure A2).

3.3 Humidity

Information on correcting Hygrosensor readings to relative humidity was available for an ambient temperature of 26.7°C. Humidity calibration was therefore performed with the chamber at a constant temperature of 26° to 27°C, while humidity was progressively reduced from 80% to 30%. The following regression equation was derived:

\[ y = (0.545 \times x) - 24.230 \]

where \( x \) is the input voltage, and \( y \) is actual relative humidity corrected from the Hygrosensor (Figure A3).

The protocol confirmed the ability of the program to hold a constant temperature across a range of humidities (Figure A4).
Figure A1. Relationship between A-D voltage and actual temperature during a cooling protocol at constant humidity.
Figure A2. Changes in temperature and humidity, compared to respective targets, during a cooling protocol at constant humidity.
Figure A3. Relationship between A-D voltage and actual humidity during dehumidification at constant temperature.

Regression: 
\[ y = (0.545 \times x) - 24.230 \]
Figure A4. Changes in humidity and temperature, compared to respective targets, during dehumidification at constant temperature.
This program controls the climate chamber in the Department of Human Movement Science, University of Wollongong: permitting either a stable temperature and humidity, or a linear change in temperature. It should be compiled by Microsoft Fortran Compiler with optimization switched off (to allow program delays to operate), with math emulation, and medium memory model. It should be linked, with PORTM.OBJ, to *GRAPHICS.LIB. Thus, compile and link command line is:

```
FL /FPi /AM /Od CHAMBER.FOR PORTM.OBJ /link GRAPHICS.LIB
```

The program interfaces with an assembly language routine: The assembler source for the routines READPORT and WRITEPORT is held in the file PORTM.ASM, compiled by Professional Officer, Mr. Peter Williamson (thanks Pete). Any modifications to this file will require reassembly with TASM or MASM prior to linking.

```
INTERFACE TO SUBROUTINE READ_PORT (IPORT_ADDRESS, IREAD_VALUE)
INTEGER*2 IPORT_ADDRESS, IREAD_VALUE
END

INTERFACE TO SUBROUTINE WRITE_PORT (IPORT_ADDRESS, IWRITE_VALUE)
INTEGER*2 IPORT_ADDRESS, IWRITE_VALUE
END
```

IPORT_ADDRESS is a 16 bit value which uniquely specifies the port to be accessed. Specifying INTEGER*2 simplifies the ASM interface. The ASM routine zeros the high order byte before passing it back, and uses low order byte in writing to port.

Enable use of graphics routine to periodically clear the screen. The INCLUDE statements import the following interface:

```
INTERFACE TO SUBROUTINE
+clearscreen[FAR,C,ALIAS: "clearscreen"]($GCLEARSCREEN)
INTEGER*2 $GCLEARSCREEN
END
```

```
INCLUDE 'FGRAPH.FT'
INCLUDE 'FGRAPH.FD'
```
*Define variables for main program.

REAL DESTEMP, DESHUM, RATE, TARGOLD, TARGNEW
REAL TEMPAV, HUMAV, RTEMP, RHUM
INTEGER*2 I, K
INTEGER*2 STARTHR, STARTMIN, STARTSEC, ST100TH
INTEGER*2 ITEMP, IHUM, ITEMTOT, IHUMTOT, TEMPCHAN, HUMCHAN
INTEGER*2 IYEAR, IMONTH, IDAY, IHOUR, IMIN, ISEC, I100TH
INTEGER*2 HEATER1, HEATER2, HEATER3, COOLER1, COOLER2
INTEGER*2 HUMID, DEHUMID
INTEGER*2 ON, OFF
INTEGER*2 PAUSE, INHR, INMIN, INSEC, IN100
INTEGER*2 OUTHR, OUTMIN, OUTSEC, OUT100
INTEGER NEWTIM, RUNTIM, TOTTIM, STARTTIM, RESTART, OLDTIM
INTEGER USERMIN, USERSEC
INTEGER DURATION, LENGTH, FREQ, OUT, ACTION
CHARACTER FILENAME*14, BELL*2

!Dehumidifier control needs to incorporate a logical flag
!so that humidification and cooling can not occur simultaneously
!below 15 degrees.
LOGICAL DEHUM
DEHUM=.FALSE.

!Define BELL as C String character.
BELL = 'a'

!Initialise the channels to access temperature and humidity.
TEMPCHAN=0
HUMCHAN=1

!Initialise devices with their respective control numbers.
HEATER1=1
HEATER2=2
HEATER3=3
COOLER1=4
COOLER2=5
HUMID=6
DEHUMID=7
ON=1
OFF=0

!Enable devices to be switched on and off, by calling SUBROUTINE
to initialise 8255 I/O hardware.
CALL INIT_OUTPUT_HARDWARE

*---------

*Determine destination for output, and open relevant files.
*Initialise each destination with the current date and time.

CALL clearsreen ($GCLEARSCREEN)
PRINT*
PRINT*, '**************************************************'
PRINT*
PRINT*, 'YOU HAVE ENTERED'
PRINT* 'THE ENVIRONMENTAL ZONE'
PRINT* '*******************************************************'
PRINT* Designed exclusively and fastidiously'
PRINT* for The University of Wollongong'
PRINT* by'
PRINT* Graeme J. Maw'
PRINT* with assistance on the fiddly bits from'
PRINT* Peter Williamson'
PRINT* PAUSE 'Press ENTER to continue'
CALL clearsreen (SGCLEARSCREEN)
PRINT* PRINT* PRINT* PRINT* PRINT* PRINT* PRINT* PRINT* PRINT* PRINT* PRINT* PRINT* PRINT* PRINT*, 'DONT MESS WITH IT OR HE WILL KILL YOU !'
CALL GETTIM (INHR, INMIN, INSEC, IN100)
PAUSE=0
DO 2, WHILE (PAUSE.LE.2)
   CALL GETTIM (OUTHR, OUTMIN, OUTSEC, OUT100)
   PAUSE=OUTSEC-INSEC
2 CONTINUE
CALL clearsreen (SGCLEARSCREEN)
PRINT* PRINT* PRINT*, 'WELCOME TO THE ENVIRONMENTAL ZONE'
PRINT* '**********************************************************'
PRINT* PRINT*, 'Where do you want the output directed?'
PRINT*, 1 = Screen'
PRINT*, 2 = File'
PRINT*, 3 = Printer'
PRINT*, 4 = Screen & File'
PRINT*, 5 = Screen & Printer'
PRINT*, 6 = File & Printer'
PRINT*, 7 = All'
PRINT*, 'Enter a number between 1 and 7:'
READ*, OUT
PRINT*
IF (OUT.GE.1.AND.OUT.LE.7) THEN
    GO TO 10
ELSE
    GO TO 5
END IF

10 IF (OUT.EQ.2.OR.OUT.EQ.4.OR.OUT.EQ.6.OR.OUT.EQ.7) THEN
    PRINT*, 'Enter filename (P:ABCDEFGH.XYZ) in inverted commas:
    READ*, FILENAME
    OPEN (UNIT=1, FILE=FILENAME, STATUS='NEW')
END IF

IF (OUT.EQ.3.OR.OUT.EQ.5.OR.OUT.EQ.6.OR.OUT.EQ.7) THEN
    PRINT*, 'Ensure printer is connected to LPT1 and is on-line.'
    PAUSE 'Press "ENTER" to continue.
    OPEN (UNIT=2, FILE='LPT1', STATUS='NEW')
END IF

CALL clearsaeen ($GCLEARSCREEN)

*Display current temperature and humidity on the screen.
*

ITEMPTOT=0
IHUMTOT=0
DO 15 I=1,10
    CALL READ_ADC (TEMPCHAN, ITEMP)
    CALL READ_ADC (HUMCHAN, IHUM)
    ITEMPTOT=ITEMPTOT+ITEMP
    IHUMTOT=IHUMTOT+IHUM
15 CONTINUE

TEMPAV=REAL (ITEMPTOT/10)
HUMAV=REAL (IHUMTOT/10)
RTEMP=(0.1835*T-0.0194)
RHUM=(0.5454*HUMAV-24.2299
WRITE (*,16) RTEMP, RHUM
16 FORMAT (1X, 'The chamber is currently at ', F5.1, ' degrees and ', F5.1, ' percent humidity.')
*

*Ascertain target variables.
*

PRINT*
20 PRINT*, 'Enter the target temperature in degrees Celsius:
    PRINT*, '(The maximum temperature is 50 degrees.)'
    READ*, DESTEMP
    PRINT*, 'Enter the target relative humidity percentage:
    READ*, DESHUM
    PRINT*, 'Enter the desired rate of change in degrees per minute:
    PRINT*, '(Enter a positive value regardless of direction.)'
    READ*, RATE
    PRINT*, 'How many minutes will this protocol last?
    READ*, DURATION
    LENGTH=DURATION*60
    PRINT*, 'How many seconds between data collections?
    READ*, FREQ
    PRINT*
Initialise the clock at zero for the start of the protocol, and print date and start time to relevant outputs.

CALL GETTIM (STARTHR, STARTMIN, STARTSEC, ST100TH)
STARTTIM=(60*60*STARTHR)+(60*STARTMIN)+STARTSEC
TOTTIM=0
CALL GETDAT (IYEAR, IMONTH, IDAY)
IF (OUT.EQ.1.OR.OUT.EQ.4.OR.OUT.EQ.5.OR.OUT.EQ.7) THEN
WRITE (*,21) IDAY, IMONTH, IYEAR
WRITE (*,22) STARTHR, STARTMIN, STARTSEC
END IF
IF (OUT.EQ.2.OR.OUT.EQ.4.OR.OUT.EQ.6.OR.OUT.EQ.7) THEN
IF (IDAY.LT.10.AND.IMONTH.LT.10) THEN
WRITE (1,23) IDAY, IMONTH, IYEAR
ELSEIF (IDAY.LT.10) THEN
WRITE (1,24) IDAY, IMONTH, IYEAR
ELSEIF (IMONTH.LT.10) THEN
WRITE (1,25) IDAY, IMONTH, IYEAR
ELSE
WRITE (1,26) IDAY, IMONTH, IYEAR
END IF
IF (STARTHR.LT.10.AND.STARTMIN.LT.10.AND.STARTSEC.LT.10) THEN
WRITE (1,27) STARTHR, STARTMIN, STARTSEC
ELSEIF (STARTHR.LT.10.AND.STARTMIN.LT.10) THEN
WRITE (1,28) STARTHR, STARTMIN, STARTSEC
ELSEIF (STARTHR.LT.10.AND.STARTSEC.LT.10) THEN
WRITE (1,29) STARTHR, STARTMIN, STARTSEC
ELSEIF (STARTMIN.LT.10.AND.STARTSEC.LT.10) THEN
WRITE (1,30) STARTHR, STARTMIN, STARTSEC
ELSEIF (STARTHR.LT.10) THEN
WRITE (1,31) STARTHR, STARTMIN, STARTSEC
ELSEIF (STARTMIN.LT.10) THEN
WRITE (1,32) STARTHR, STARTMIN, STARTSEC
ELSEIF (STARTSEC.LT.10) THEN
WRITE (1,33) STARTHR, STARTMIN, STARTSEC
ELSE
WRITE (1,34) STARTHR, STARTMIN, STARTSEC
END IF
WRITE (1,35)
END IF
IF (OUT.EQ.3.OR.OUT.EQ.5.OR.OUT.EQ.6.OR.OUT.EQ.7) THEN
WRITE (2,36) IDAY, IMONTH, IYEAR
WRITE (2,37) STARTHR, STARTMIN, STARTSEC
WRITE (2,38)
36  FORMAT (1X,'Date: ' ,I2,'/',I2,'/',I4)
37  FORMAT (1X,'Start time: ' ,I2,'.',I2)
38  FORMAT (1X,'Duration',3X,'Temp','5X','RH')
END IF

*At specified interval, FREQ, assess the status of the chamber, using
*the subroutine READ_ADC, and convert the inputs ITTEMP and IHUM
*into degrees celcius and relative humidity, using the calibrations:
* Degrees  = 0.1835*ITMP - 0.0194
* Humidity  = 0.5454*IHUM - 24.2299
*(These equations were derived from pilot studies in January 1992.
*Temperature was measured between 40° and 8° C at a constant humidity of
*50% using a Grant thermistor calibrated from a Dobros reference
*thermometer. Humidity was measured between 90 and 30% at a constant
*temperature of 30° C using a wide-range American Instruments
*Hygrosensor. Respective data are stored in Lotus 1-2-3 files
*TEMPCAL.WKS and HUMCAL.WKS - G.Maw, 1992.)
*Send the information to the predetermined output(s).
*
50 CALL GETTIM (IHOUR, IMIN, ISEC, I100TH)
RESTART=(60*60*IHOUR)+(60*IMIN)+(ISEC)
OLDTM=0
RUNTIM=0
ITEMPTOT=0
IHUMTOT=0
TARGOLD=RTEMP
DO 80, WHILE (RUNTTM.LE.LENGTH)
CALL GETTIM (IHOUR, IMIN, ISEC, I100TH)
NEWTIM=(60*60*IHOUR)+(60*IMIN)+(ISEC)
IF (NEWTIM.NE.OLDTJM) THEN
IF (MOD(TOTTIM,FREQ).EQ.0) THEN
DO 52 K=1,10
CALL READ_ADC (TEMPCHAN, ITEMP)
CALL READ_ADC (HUMCHAN, IHUM)
ITEMPTOT=ITEMPTOT+ITEMP
IHUMTOT=IHUMTOT+IHUM
52  CONTINUE
TEMPAV=REAL(ITEMPTOT/10)
HUMAV=REAL(IHUMTOT/10)
RTEMP=(0.1835*TEMPAV)-0.0194
RHUM=(0.5454*HUMAV)-24.2299
USERMIN=INT((NEWTIM-STARTTIM)/60)
USERSEC=MOD((NEWTIM-STARTTIM),60)
IF (OUT.EQ.1.OR.OUT.EQ.4.OR.OUT.EQ.5.OR.OUT.EQ.7) THEN
WRITE (*,60) USERMIN, USERSEC, RTEMP, RHUM
60  FORMAT (1X,I4,'.',I2,3X,F4.1,3X,F4.1)
END IF
IF (OUT.EQ.3.OR.OUT.EQ.5.OR.OUT.EQ.6.OR.OUT.EQ.7) THEN
WRITE (2,61) USERMIN, USERSEC, RTEMP, RHUM
61  FORMAT (1X,I4,'.',I2,3X,F5.1,3X,F5.1)
END IF
IF (OUT.EQ.2.OR.OUT.EQ.4.OR.OUT.EQ.6.OR.OUT.EQ.7) THEN
WRITE (3,61) USERMIN, USERSEC, RTEMP, RHUM
61  FORMAT (1X,I4,'.',I2,3X,F5.1,3X,F5.1)
END IF
WRITE (1,62) USERMIN, USERSEC, RTEMP, RHUM
   FORMAT (1X,I4,'.',I1,4X,F5.1,3X,F5.1)
ELSE
   WRITE (1,63) USERMIN, USERSEC, RTEMP, RHUM
   FORMAT (1X,I4,'.',I2,3X,F5.1,3X,F5.1)
END IF
ENDIF
ITEMPTOT=0
IHUMTOT=0
TOTTIM=NEWTIM-STARTTIM
ENDIF

*-----------------------------------------------
*Every 15 seconds, compare the current status of the chamber with
*desired status and make any necessary responses using the 3 heaters,
*2 coolers, humidifier and dehumidifier. Notes: (i) the coolers cannot
*operate below 15 degrees - cooling then must be achieved by the
*dehumidifier; (ii) Heating the chamber to 50 degrees may cause the
*heaters to trip - when approaching this temperature, further heating
*is progressively disabled.
*
*-----------------------------------------------

IF (MOD(TOTTIM,15).EQ.0) THEN
   IF (ABS(DESTEMP-RTEMP).GT.0.5) THEN
      IF (RTEMP.GT.DESTEMP) THEN
         RATE=RATE*(-1.0)
      END IF
      ELSE
         TARGNEW=TARGOLD+(RATE/4)
      END IF
   ELSE
      CALL SWITCH (COOLER1, OFF)
      CALL SWITCH (COOLER2, OFF)
      CALL SWITCH (HEATER1, OFF)
      CALL SWITCH (HEATER2, OFF)
      CALL SWITCH (HEATER3, OFF)
   END IF
ENDIF
IF (((RHUM-DESHUM).GT.1.0).OR.((RTEMP-TARGNEW).GT.0.5.AND.
+ RTEMP.LE.15.0)) THEN
   DEHUM=.TRUE.
   CALL SWITCH (DEHUMID, ON)
ELSE
   DEHUM=.FALSE.
   CALL SWITCH (DEHUMID, OFF)
END IF
IF (DEHUM) THEN
   CALL SWITCH (HUMID, OFF)
   GO TO 75
ELSEIF ((DESHUM-RHUM).GT.1.0) THEN
   CALL SWITCH (HUMID, ON)
ELSE
   CALL SWITCH (HUME), OFF)
END IF
75 END IF
END IF
TARGOLD=TARGNEW
OLDTIM=NEWTIM
RUNTIM=NEWTIM-RESTART
80 CONTINUE
*
*-----------------------------------------------
* Determine next course of action: to continue with same protocol
* to reset targets, or to exit the program.
*
*-----------------------------------------------
PRINT*
PRINT*, BELL
PRINT*, BELL
PRINT*, BELL
PRINT*, 'Your protocol time has elapsed.'
PRINT*
PRINT*, 'How do you wish to proceed?'
PRINT*, ' 1 = Continue with present targets'
PRINT*, ' 2 = Reset target values'
PRINT*, ' 3 = Leave the environmental zone'
98 PRINT*
PRINT*, 'Enter a number between 1 and 3:'
READ*, ACTION
IF (ACTION.EQ.1) THEN
   GO TO 50
ELSEIF (ACTION.EQ.2) THEN
   GO TO 20
ELSEIF (ACTION.EQ.3) THEN
   CLOSE (UNIT=1)
   CLOSE (UNIT=2)
   !switch off all devices using the subroutine SWITCH_ALL_OFF
   CALL SWITCH_ALL_OFF
   CALL clearscreen ($GCLEARSCREEN)
   PRINT*
   PRINT*
   PRINT*
   PRINT*
   PRINT*
   PRINT*
206
PRINT*, ' AWESOME DUDE!'
CALL GETTIM (INHR, INMIN, INSEC, IN100)
PAUSE=0
DO 100, WHILE (PAUSE.LE.2)
   CALL GETTIM (OUTHR, OUTMIN, OUTSEC, OUT100)
   PAUSE=OUTSEC-INSEC
100 CONTINUE
CALL clearscreen ($GCLEARSCREEN)
PRINT*
PRINT*
PRINT*
PRINT*, ' REMEMBER TO SWITCH OFF THE CHAMBER CONTROLS'
PRINT*
PRINT*
PRINT*, ' AND RETURN TO DRIVE C:'
PRINT*, ' TO PARK THE HARD DRIVE'
CALL GETTIM (INHR, INMIN, INSEC, IN100)
PAUSE=0
DO 101, WHILE (PAUSE.LE.1)
   CALL GETTIM (OUTHR, OUTMIN, OUTSEC, OUT100)
   PAUSE=OUTSEC-INSEC
101 CONTINUE
PRINT*
PRINT*
PRINT*
PRINT*
PRINT*
PRINT*, ' THANK YOU FOR PLAYING'
ELSE
   GO TO 98
END IF
END

*---------------------------------------------------------------------
*Define subroutine GET_ADC which reads input from the chamber channel
*status of the chamber using the file PORTM.ASM.
*
*---------------------------------------------------------------------

SUBROUTINE READ_ADC (WHAT_CHAN, IVAL)
INTEGER*2 WHAT_CHAN, IVAL, ITRIGGER, IADDR1, IADDR2, K, TIMWASTE
IADDR1=#0170
IADDR2=#0171
ITRIGGER=#0000
TIMWASTE=0
CALL WRITE_PORT (IADDR1, WHAT_CHAN)
CALL WRITE_PORT (IADDR2, ITRIGGER)
DO 2, K=0,5000
   TIMWASTE=TIMWASTE+1
2 CONTINUE
CALL READ_PORT (IADDR2, IVAL)
RETURN
END
Define subroutine INIT_OUTPUT_HARDWARE to initialise 8255 I/O hardware. This subroutine enables devices to be switched on and off.

```
SUBROUTINE INIT_OUTPUT_HARDWARE
INTEGER*2 ICTRL_PORT, ICTRL_DATA
ICTRL_PORT=#017F
ICTRL_DATA=#80
!set 8255 port a to output
CALL WRITE_PORT (ICTRL_PORT, ICTRL_DATA)
RETURN
END
```

Define subroutine SWITCH to switch devices on or off.

```
SUBROUTINE SWITCH (IDEVICE, ISTATE)
INTEGER*2 IDEVICE, ISTATE
INTEGER*2 IDEVICE_OPADDR  
!output port address
INTEGER*2 IDEVICE_OUTDATA  
data to write to port
INTEGER*2 IMASK(7)  
!bit masks
INTEGER*2 IDEVICE_STATUS  
!value of device output port

DATA IMASK /#01, #02, #20, #04, #08, #10, #40/  !data for masks
IDEVICE_OPADDR=#17C

!Check the range of subroutine parameters: The device number
!IDEVICE, must be between 1 and 7 inclusive, and the device state
!ISTATE must be either 1 or 0. If they are outside these limits,
!do nothing and return to main program.

IF ((IDEVICE.LT.1).OR.(IDEVICE.GT.7)) THEN
   GOTO 500
ENDIF
IF ((ISTATE.LT.0).OR.(ISTATE.GT.1)) THEN
   GOTO 500
ENDIF

!Read the current status (i.e. what’s switched on now).

CALL READ_PORT (IDEVICE_OPADDR, IDEVICE_STATUS)

!If necessary, turn the device on.

IF (ISTATE.EQ.1) THEN
   IDEVICE_OUTDATA=IDEVICE_STATUS.OR.IMASK(IDEVICE)
ENDIF

!If necessary, turn the device off.

IF (ISTATE.EQ.0) THEN
   IDEVICE_OUTDATA=IDEVICE_STATUS.AND.IMASK(IDEVICE)
   IDEVICE_OUTDATA=IDEVICE_STATUS-IDEVICE_OUTDATA
ENDIF
```
!Perform switching.

* CALL WRITE_PORT (DEVICE_OPADDR, DEVICE_OUTDATA)
  500 RETURN
* END

*--------------------------------------------------------
* Define subroutine SWITCH_ALL_OFF to switch off all devices before
* exiting the program.
*--------------------------------------------------------

SUBROUTINE SWITCH_ALL_OFF
  INTEGER*2 DEVICE_OPADDR, IOFF_VALUE
  DEVICE_OPADDR=#17C
  IOFF_VALUE=#00
  CALL WRITE_PORT (DEVICE_OPADDR, IOFF_VALUE)
  RETURN
END
Assembler routines to allow MS FORTRAN 5.0 to read and write PC's I/O ports.
This file must be assembled using TASM or MASM and the resulting OBJ file linked to the required FORTRAN program.
Refer FL documentation in reference manual.
Written by Peter Williamson 1991

.PROGRAM
MODEL MEDIUM
.CODE
PUBLIC read_port, write_port

read_port proc
; fortran call is
CALL READ_PORT (IPORT_ADDRESS, IREAD_VALUE)
; where:
IPORT_ADDRESS is the address of the required port, declared as INTEGER*2
IREAD_VALUE is the data read from the port declared as INTEGER*2
read_port
push bp
; save BP and
mov bp, sp
; set stack frame pointer
mov bx, [bp+8]
; DX
mov dx, [bx]
; read port, value into AL
mov ah, 0
; zero high byte
mov bx, [bp+6]
mov [bx], ax
; put BP back
pop bp
; clean up stack and return
read_port endp

write_port proc
; fortran call is
CALL WRITE_PORT (IPORT_ADDRESS, IWRITE_VALUE)
; where:
IPORT_ADDRESS is the address of the required port, declared as INTEGER*2
IWRITE_VALUE is the data to write to the port declared as INTEGER*2
write_port
push bp
; save BP and
mov bp, sp
; set stack frame pointer
mov bx, [bp+8]
; DX
mov dx, [bx]
; AX
mov bx, [bp+6]
mov ax, [bx]
; write to port this is an 8 bit write:
out dx, al
; only use low byte in AL
pop bp
; all done
write_port endp

210
Appendix C


This study compared oesophageal (T_oe), rectal (T_re) and aural (T_au) temperatures in 6 healthy males at rest and during cycle ergometry (150 W) in thermoneutral (TN: 24°C, RH=40%), cold (CO: 13°C, RH=40%) and hot (HO: 36°C, RH=40%) environments. Subjects were monitored at rest in TN for 30 mins, and later during 30 mins cycling in TN, followed by 30 mins cycling in either CO or HO. At rest, T_oe (37.48±0.30°C) and T_re (37.46±0.26°C) were within 0.10°C in all cases, and exceeded T_au (36.86±0.35°C) by 0.60°C. During the first 20 mins of exercise in TN, T_oe, T_re and T_au rose in parallel by 0.40°C. From 20 to 30 mins, T_oe and T_au plateaued, while T_re rose a further 0.15°C. Continued exercise in CO caused T_oe and T_au to rise a further 0.15° and 0.20°C respectively, while T_re rose by 0.30°C. When exercise continued in HO, T_oe, T_re and T_au rose in parallel by 0.60°C without achieving equilibrium. During lower-body exercise in TN and CO, T_re may not accurately reflect central temperature changes measured elsewhere, becoming disproportionately elevated perhaps due to local blood flow from active muscle. T_au approximates changes in T_re. Fluctuations in T_oe, attributed to increased ventilation and saliva, may make a fully insulated aural thermometer a more practical measure of core temperature during lower-body exercise.
### Comparison of Three Repeated Measurements of Body Mass

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### Comparison of Three Repeated Measurements of Total Body Water

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### Comparison of Three Repeated Measurements of Intracellular Water Volume

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### Comparison of Three Repeated Measurements of Extracellular Fluid Volume

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### Comparison of Three Repeated Measurements of Interstitial Fluid Volume

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### Comparison of Three Repeated Measurements of Blood Volume

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### Comparison of Three Repeated Measurements of Plasma Volume

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<td>12</td>
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### Comparison of Three Repeated Measurements of Erythrocyte Volume

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<td>0.633</td>
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<td>20</td>
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### Appendix F

**Analysis of Variance Summary Tables for Chapter Four**

#### Analysis of Total Body Water During 30 Min of Supine, Seated, and Upright Rest

<table>
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<tr>
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<td>Posture</td>
<td>195511</td>
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<td>Time</td>
<td>314348</td>
<td>2</td>
<td>157174</td>
<td>0.45</td>
<td>0.644</td>
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<tr>
<td>Interaction</td>
<td>664908</td>
<td>4</td>
<td>166227</td>
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#### Analysis of Intracellular Water Volume During 30 Min of Supine, Seated, and Upright Rest

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<tr>
<td>Time</td>
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<td>2</td>
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#### Analysis of Extracellular Fluid Volume During 30 Min of Supine, Seated, and Upright Rest

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### Analysis of Blood Volume During 30 Min of Supine, Seated, and Upright Rest

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### Comparison of Relative Changes in Blood Volume During 30 Min of Supine, Seated, and Upright Rest, Calculated Using Hct, and [Hb] or Measured Using Radionuclide Dilution

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Comparison of Relative Changes in Erythrocyte Volume During 30 Min of Supine, Seated, and Upright Rest, Calculated Using Hct. and [Hb] or Measured Using Radionuclide Dilution

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Analysis of the f-Ratio During 30 Min of Supine, Seated, and Upright Rest

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Analysis of Urine Production During 30 Min of Supine, Seated, and Upright Rest

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## Analysis of Cardiac Frequency During 30 Min of Supine, Seated, and Upright Rest

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Appendix G
Analysis of Variance Summary Tables for Chapter Five

Analysis of Total Body Water During 30 Min of Exposure to Cool, Control, and Hot Environments

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Analysis of Intracellular Water Volume During 30 Min of Exposure to Cool, Control, and Hot Environments

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Analysis of Extracellular Fluid Volume During 30 Min of Exposure to Cool, Control, and Hot Environments

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### Analysis of Blood Volume During 30 Min of Exposure to Cool, Control, and Hot Environments

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Analysis of Erythrocyte Volume During 30 Min of Exposure to Cool, Control, and Hot Environments

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Comparison of Relative Changes in Blood Volume During 30 Min of Exposure to Cool, Control, and Hot Environments, Calculated Using Hct, and [Hb] or Measured Using Radionuclide Dilution

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### Comparison of Relative Changes in Erythrocyte Volume During 30 Min of Exposure to Cool, Control, and Hot Environments, Calculated Using Hct, and [Hb] or Measured Using Radionuclide Dilution

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**Analysis of Evaporative Water Loss During 30 Min of Exposure to Cool, Control, and Hot Environments**

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**Analysis of Aural Temperature During 30 Min of Exposure to Cool, Control, and Hot Environments**

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### Analysis of Cardiac Frequency During 30 Min of Exposure to Cool, Control, and Hot Environments

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Appendix H
Analysis of Variance Summary Tables for Chapter Six

Analysis of Total Body Water During 50 Min of Exercise in Cool, Control, and Hot Environments

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Analysis of Intracellular Water Volume During 50 Min of Exercise in Cool, Control, and Hot Environments

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Analysis of Extracellular Fluid Volume During 50 Min of Exercise in Cool, Control, and Hot Environments

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### Analysis of Blood Volume During 50 Min of Exercise in Cool, Control, and Hot Environments

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Analysis of Erythrocyte Volume During 50 Min of Exercise in Cool, Control, and Hot Environments

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Comparison of Relative Changes in Blood Volume During 50 Min of Exercise in Cool, Control, and Hot Environments, Calculated Using Hct, and [Hb] or Measured Using Radionuclide Dilution

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Comparison of Relative Changes in Erythrocyte Volume During 50 Min of Exercise in Cool, Control, and Hot Environments, Calculated Using Hct, and [Hb] or Measured Using Radionuclide Dilution

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### Analysis of Body-Fluid Loss During 50 Min of Exercise in Cool, Control, and Hot Environments

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### Analysis of Sweat Loss During 50 Min of Exercise in Cool, Control, and Hot Environments

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<td>60</td>
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### Analysis of Urine Production During 50 Min of Exercise in Cool, Control, and Hot Environments

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### Analysis of Aural Temperature During 50 Min of Exercise in Cool, Control, and Hot Environments

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### Analysis of Mean Skin Temperature During 50 Min of Exercise in Cool, Control, and Hot Environments

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### Analysis of Cardiac Frequency During 50 Min of Exercise in Cool, Control, and Hot Environments

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