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HUMAN CIRCULATORY AND THERMOREGULATORY ADAPTATIONS WITH HEAT ACCLIMATION AND EXERCISE IN A HOT, DRY ENVIRONMENT

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SUMMARY

1. Heat acclimation was induced in eight subjects by asking them to exercise until exhaustion at 60% of maximum oxygen consumption rate ($\dot{V}_{O_2}$) for 9–12 consecutive days at an ambient temperature of 40 °C, with 10% relative humidity (RH). Five control subjects exercised similarly in a cool environment, 20 °C, for 90 min for 9–12 days; of these, three were exposed to exercise at 40 °C on the first and last day.

2. Acclimation had occurred as seen by the increased average endurance from 48 min to 80 min, the lower rate of rise in the heart rate (HR) and core temperature and the increased sweating.

3. Cardiac output increased significantly from the first to the final heat exposure from 19·6 to 21·4 l min^{-1}; this was possibly due to an increased plasma volume and stroke volume.

4. The mechanism for the increased plasma volume may be an isosmotic volume expansion caused by influx of protein to the vascular compartment, and a sodium retention induced by a significant increase in aldosterone.

5. The exhaustion coincided with, or was elicited when, core temperature reached 39·7 ± 0·15 °C; with progressing acclimation processes it took progressively longer to reach this level. However, at this point we found no reduction in cardiac output, muscle (leg) blood flow, no changes in substrate utilization or availability, and no recognized accumulated 'fatigue' substances.

6. It is concluded that the high core temperature *per se*, and not circulatory failure, is the critical factor for the exhaustion during exercise in heat stress.

INTRODUCTION

In hot, compared to cool, environments exercise capacity is reduced. With acclimatization, the ability to withstand the heat stress is augmented. In acute heat exposure, the increased demand for skin circulation during exercise in hot

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environments, together with the requirements for blood flow to the exercising muscles, is met by a reduction in renal, splanchnic, hepatic and non-exercising muscle blood flow (Radigan & Robinson, 1949; Rowell, Blackmon, Martin, Mazzarella & Bruce, 1965; Rowell, Brengelmann, Blackmon, Twiss & Kusumi, 1968; Rowell, 1986). At low to moderate exercise intensities cardiac output may be increased (Williams, Bredell, Wyndham, Strydom, Morrison, Peter & Fleming, 1962; Klausen, Dill, Philips & McGregor, 1967; McDougall, Reddan, Layton & Dempsey, 1974; Nadel, Cafarelli, Roberts & Wenger, 1979). In addition, it has been suggested that blood flow to the exercising muscles is reduced, and this could be a major contributor to the reduced performance in hot conditions (Rowell, 1974; Fink, Costill & Van Handel, 1975; Kosłowski, Brzezinska, Kruk, Kaciuba-Uschilko, Greenleaf & Nazar, 1985). Quantitative microsphere measurements of muscle blood flow have shown that this occurs in sheep (Bell, Hales, King & Fawcett, 1983). However, direct measurements of human leg blood flow during acute heat stress, imposed by a water-perfused suit, with exercise up to 40–50% of $\dot{V}_{O_2,max}$, showed no significant changes in leg blood flow (Savard, Nielsen, Laszczynska, Larsen & Saltin, 1988). Also in uphill walking at 60% of $\dot{V}_{O_2,max}$ to exhaustion (Nielsen, Savard, Richter, Hargreaves & Saltin, 1990), the muscle blood flow was the same as in neutral environment.

The changes which occur with repeated heat exposure are still under debate, because the earlier studies on heat acclimation are ambiguous. Cardiac output is reported to decrease, increase, or remain unchanged (e.g. Wyndham, 1951; Rowell, Kraning, Kennedy & Evans, 1967; Wyndham, Benade, Williams, Strydom, Goldin & Heyns, 1968; Wyndham, Rogers, Senay & Mitchell, 1976). The circulatory changes have been explained as being due to changes in blood volume (Senay, Mitchell & Wyndham, 1976; Senay, 1986), or due to changes in venous tone and cardiac filling pressure (Rowell *et al.* 1967; Rowell, 1974, 1983; Kirsch, Röcker, v. Ameln & Hrynyschyn, 1986). Blood volume changes can be caused both by heat exposure and by exercise, training and posture (Senay, 1972; Harrison, 1985).

Further, the contribution of enhanced sweating to acclimation is a matter for discussion, since in hot wet conditions, sweating rate is often greater than that which can be evaporated even during the first exposure (Mitchell, Senay, Wyndham, van Rensburg, Rogers & Strydom, 1976; Candas, Libert & Voigt, 1980).

In the present investigation we studied the effects of 9–12 days acclimation to dry heat (about 41 °C, with 12% RH), in order to explain the increased ability to withstand the stress, i.e., exercise in the heat. The questions approached were: in endurance trained subjects (1) is the blood flow to major vascular beds changed with acclimation, and (2) what is the ultimate cause of exhaustion during exercise in a hot environment? The measurements included thermoregulatory, circulatory, metabolic and hormonal parameters, in an attempt to evaluate not only whole-body reactions, but also local conditions in exercising muscle.

METHODS

Measurements

Exercise was performed while seated on a bicycle ergometer, in which the saddle was replaced by an armchair, suspended in a weighing balance (Krogh & Trolle, 1936).

A continuous registration of weight loss (sweat loss) was obtained on the Krogh balance, with

the exception of the catheter experiments (see procedure p. 470). Further, total weight loss was checked by weighing on a medical balance (Secca) before and after each experiment.

Temperatures (oesophageal, T_c, and air, T_a) were measured by thermocouples on an electrical thermometer (Ellab CTF-84) and printed out every 5 min during the experiment. Mean skin temperature ($\overline{T}_{sk}$) of the chest and face was measured regularly with a radiation receiver (Aga Thermopoint 80).

Whole-body oxygen consumption rate ($\dot{V}_{O_2}$) was determined by the Douglas bag method. Volume measurements were made on a Collins wet spirometer, and O_2 and CO_2 contents of the bags were measured with paramagnetic (Servomex) and infrared (Beckman LB-2) analysers, respectively. Cardiac output ($\dot{Q}$) was measured by dye dilution, i.e. 2 ml of Cardiogreen was flushed into the right brachial vein, and arterial blood was withdrawn through a densitometer (Waters) at a rate of 22 ml min^{-1} by a Harvard pump. The dye curve was recorded and the area was measured by planimetry. Arterial blood pressures were continuously followed, and the electrocardiogram and heart rate (HR) (Simonsen & Weel cardioscope) was recorded (Gould, 8 channel recorder).

Leg blood flow (LBF) was determined by a constant-infusion thermodilution technique (Andersen & Saltin, 1985) as described in a previous paper (Savard *et al.* 1988).

The skin circulation was monitored by three different methods. Forearm blood flow (FBF) was measured by venous occlusion plethysmography with a Whitney mercury-in-Silastic strain gauge. Movement artifacts during exercise were eliminated by suspending the arm at the level of the heart, in a sling fastened to the frame of the bicycle ergometer. Skin blood flow was measured with a laser Doppler probe mounted on the upper side of the forearm close to the strain gauge (Perimed PF2B), and with spectrophotometric probes (tissue perfusion monitor; Hales, Stephens, Fawcett, Daniel, Sheahan, Westerman & James, 1989) fixed with double-sided tape on forearm, finger, chest and forehead. These measurements and results will be described elsewhere (Hales, Nielsen & Yanase, manuscript in preparation).

Blood samples were drawn simultaneously from the femoral artery and femoral vein during rest and at 10, 30, 40, 60 min and then every 10 min until exhaustion during the experiment. Arterial and venous haemoglobin concentrations ([Hb]) and O_2 saturation were measured (Radiometer, OSM-2) as were P_{O_2}, P_{CO_2} and pH (Radiometer, ABL[10]). Haematocrit (Hct) determinations were made, in triplicate, using microcentrifugation. Plasma protein (PP) was determined by the biuret reaction (Boehringer kit). Glucose was measured using whole blood by the hexokinase method (Yellow Springs, OH, USA). Free fatty acids (FFAs) were determined by a fluorometric method (B. Kiens, B. Essen-Gustavsson, N. J. Christensen & B. Saltin, unpublished observations). Arterial plasma catecholamines were measured by a radio enzymatic assay (Christensen, Vestergaard, Sørensen & Rafaelsen, 1980). Plasma K^+ and Na^+ were measured with flame photometry (Radiometer). Arginine vasopressin (AVP) was extracted from plasma by means of C-18 SEP-Pak cartridges (Waters Associates) and measured by radioimmunoassay as previously described (Bie & Warberg, 1983); synthetic AVP (Ferring) served as the reference preparation, and the least detectable quantity of AVP was 0·1–0·3 pg $tube^{-1}$. Within-assay coefficient of variation at the middle-sensitivity range of the standard curve was 7%, and between-assay coefficient of variation at the same range was 12%. Plasma renin activity (PRA) was determined by use of the antibody-trapping method described by Poulsen & Jørgensen (1974). Synthetic angiotensin I (Bio-Schwartz), which was tested against Research Standard A (Institute for Medical Research, Holy Hill, London) served as the standard, and the within-assay and between-assay coefficients of variation were 3·2 and 8·5%, respectively. Aldosterone (ALDO) was extracted from plasma using dichloromethane and quantified by radioimmunoassay as previously described (Sander-Jensen, Secher, Astrup, Christensen, Giese, Schwartz, Warberg & Bie, 1986); D-ALDO (Sigma) served as reference preparation; the least detectable quantity of ALDO was 2–3 pg $tube^{-1}$ and the within-assay and between-assay coefficients of variation were 6·1 and 12·3%, respectively. Growth hormone (GH) concentration was determined in arterial plasma by ELISA, an enzymatic immunoassay, with monoclonal antibody bound to polystyrene microtitration plates. GH is bound to this followed by a binding of peroxidase-marked polyclonal guinea-pig antibody, with tetramethylbenzidin as colour substrate for peroxidase (Novo Nordisk kit).

Pre-exercise plasma volume (PV) was determined from resting blood samples by measuring radio-labelled human serum albumin (^{131}I-RISA) activity (Selectronic counter) and extrapolating to zero from the 10 and 20 min blood samples. Further, relative changes in PV were estimated from changes in Hct and [Hb] (Dill & Costill, 1974).

In the control subjects plasma volume, both measured with RISA and calculated from the percentage change in plasma volume (ΔPV) from Hct and [Hb], was the same before and after 9–12 consecutive days of exercise in cool condition, while in the heat-acclimated subjects we found a significant, 10·5%, *decrease* in PV measured with RISA, but calculated from Hct and [Hb], the percentage ΔPV was *increased* +13·1% after acclimation. We have no explanation for this striking difference. However, the calculated percentage ΔPV seems most plausible, because loss of 500 ml of blood cells would have to be accounted for if the blood volume decrease indicated by the RISA were real. We have therefore only used the calculated values in the Discussion.

Maximal force. The maximal force which could be exerted (maximum voluntary contraction, MVC) was taken as the best of three trials with the knee-extensor muscle group and the elbow flexors in a standardized 90 deg limb position before and after the exercise; four subjects in cool, and three of them also in hot conditions, were examined.

Statistics

Non-parametric methods (Willcoxon's test and Mann–Whitney test (Siegel, 1956) were used for testing differences between related samples.

Procedure

Thirteen well-trained male endurance athletes were studied wearing only shorts and shoes. Their average $\dot{V}_{O_2,max}$ (estimated from submaximal HR and $\dot{V}_{O_2}$) was 59 (49–74) ml min^{-1} kg^{-1}. Their data (average and range) were: height 182 (174–189) cm, weight 75·7 (68–82) kg, age 24·8 (19–28) years for the eight acclimating subjects, and height 184 (175–190 cm, weight 79·0 (69–87) kg, age 26·4 (22–30) years for the control subjects. An initial experiment was performed in cool conditions (20 °C), in which the work intensity was adjusted to induce a heart rate of 125–130 beats min^{-1} corresponding to approximately 50% of their maximum $\dot{V}_{O_2}$. The subjects could exercise at this intensity for 90 min in the cool environment. Thereafter, eight of the subjects exercised daily for 9–12 days in a hot, dry environment (40–42 °C ambient air and wall temperature, with 10–15% RH) at the same predetermined work load either until they became exhausted, or for 90 min. Five (control) subjects exercised likewise, but at 18–20 °C for 90 min each day. Three of the control subjects exercised at 42 °C on the first and the last day.

In the heat acclimation subjects a complete set of measurements was taken during rest and exercise in the first and the final heat exposure. The day of these 'full' experiments (1st and final test) the subject arrived in the morning, after a light meal. While they were supine and thermally comfortable, catheters were placed in a femoral artery and vein, and in the right brachial vein. Radio-labelled human serum albumin (^{131}I-RISA, < 0·25 mSv) was injected intravenously for determination of plasma volume after they had been in the supine position for at least 45 min. After approximately 10 and 20 min, blood samples were drawn for determination of γ-activity. An oesophageal thermometer was then inserted through the nose, and the subject walked into the climatic chamber and sat down on the bicycle ergometer. The cuffs, strain gauge, laser probe, and tissue perfusion monitors were applied to the left forearm for peripheral circulation measurements, and the catheters were connected to the recording equipment. This setting-up, including the resting blood samples and measurements, usually took 10–20 min. The subject then started to exercise at the predetermined intensity. Measurements during exercise were made at 10, 30 and 40, and then, depending on the endurance of the subject, approximately every 10th min until exhaustion.

During the remainder of the exercise days only non-invasive measurements were performed. In the control subjects plasma volume was measured as described above on the 1st and the final day; apart from that, only the non-invasive procedures were performed in the control experiments.

Due to the complexity of the experiments, involving several catheters, infusions and frequent blood samples and measurements, some data are incomplete and therefore a variable number (n) is seen in the tables and figures.

The protocol was approved by the Copenhagen Ethics Committee. Before giving his consent, each subject was fully informed of the risks involved and of the fact that he was free to withdraw from the experiments at any time.

RESULTS

Daily exercise in dry heat for 9–12 days resulted in approximately a doubling of endurance time (48 ± 1·9 to 80 ± 3·3 min). In contrast, three control subjects

TABLE 1. Final values of variables during exercise in the 1st, 2nd, last but one, and final experiment

		1st day	2nd day	d − 1	d (final)
Duration work (min)	Accl.	48·0 ± 1·9	54·0 ± 2·2	74·0 ± 4·7	80·0 ± 3·3*
	Control	47·0 ± 2·2 (3)	90·0	90·0	47·0 ± 3·6 (3)
T_c (°C)	Accl.	39·8 ± 0·13	39·9 ± 0·14	39·8 ± 0·14	39·7 ± 0·15 (6)
	Control	39·6 ± 0·20 (3)	37·8 ± 0·15	37·8 ± 0·10	39·5 ± 0·09 (3)
HR (beats min^{-1})	Accl.	164·0 ± 5·7	163·0 ± 6·2	159·0 ± 6·2	153·0 ± 6·3 (6)
	Control	165·0 ± 3·7 (3)	133·0 ± 1·9	124·0 ± 2·4	159·0 ± 2·4 (3)
V_{O_2} (l min^{-1})	Accl.	2·42 ± 0·04	2·40 ± 0·09	2·31 ± 0·06	2·45 ± 0·08 (6)
	Control	2·63 (3)	2·57	2·54	2·63 (3)
Rate of weight loss (g min^{-1})	Accl.	—	16·1 ± 0·95	18·9 ± 1·31*	—
for 10–40 min	Control	17·7 (3) (14–21)	12·9 ± 1·40	12·6 ± 1·01	20·6 (3) (15–24)
Sweat sensitivity	Accl.	—	368·0 ± 42·9	473·0 ± 50·9	—
(g °C^{-1})	Control	426·0 (3) (309–534)	—	—	495·0 (3) (280–691)

Significant changes from 1st day are marked with *. All values are means ± S.E.M. or range. Eight acclimating subjects and five controls, unless a smaller number is given in parentheses. Three controls exercised at 40–42 °C on the 1st and last day only.

achieved almost identical endurance times ($47 \pm 2{\cdot}2$ and $47 \pm 3{\cdot}2$ min) when they exercised in the heat before and after 6–9 days of exercising in cool conditions (Table 1).

Oesophageal temperature

This increased during exercise. In the cool environment it attained a steady state after about 15–20 min and remained constant at approximately 37·8 °C throughout

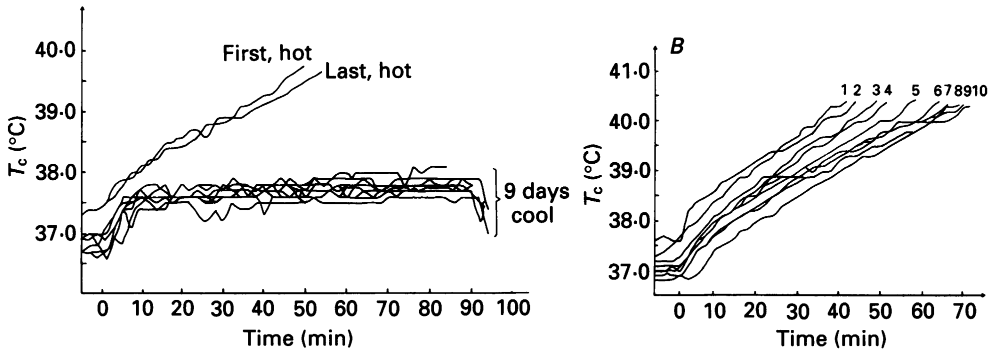

Fig. 1. Oesophageal temperature plotted against time. *A*, one control subject during exercise at 40 °C in the first and final experiments, and at (18–20 °C) for 90 min for the 9 days in between. *B*, one acclimating subject during ten consecutive days of exercise until exhaustion at 40 °C.

the 90 min; but during heat stress it rose continually to nearly 40 °C each day. In the heat acclimated subjects the final value at exhaustion was $39{\cdot}8 \pm 0{\cdot}13$ on the first and $39{\cdot}7 \pm 0{\cdot}15$ °C on the last day. In the control subjects, T_c reached 39·5 °C both in the first and the last heat exposure 10 days later; none of the levels at exhaustion differed significantly (Table 1, Fig. 1).

Sweating

Sweating increased with acclimation, expressed both as rate of weight loss (sweat loss by evaporation and dripping) and the sweat sensitivity (the rate of evaporation per °C change in oesophageal temperature). There was no change in the control subjects (Table 1).

Oxygen consumption

$\dot{V}_{O_2}$ remained unchanged, both in the acclimated, and in the control group (Table 1).

Heart rate

HR in the cool experiments reached a steady state of $133 \pm 1{\cdot}9$ to $124 \pm 2{\cdot}4$ beats min^{-1}. During the heat exposures HR increased gradually, reaching a final mean value in the acclimated of 164 ± 6 in the first *vs.* 153 ± 6 beats min^{-1} in the last exposure (Table 1).

Cardiac output

($\dot{Q}$) had increased after acclimation (Fig. 2). It was $17{\cdot}8 \pm 1{\cdot}7$ at 10 min, and $19{\cdot}6 \pm 1{\cdot}3$ l min^{-1} at exhaustion in the first, and $19{\cdot}4 \pm 2{\cdot}0$ at 10 min, while $21{\cdot}4 \pm 1{\cdot}6$ l min^{-1} at exhaustion in the last heat test after acclimation. The values at

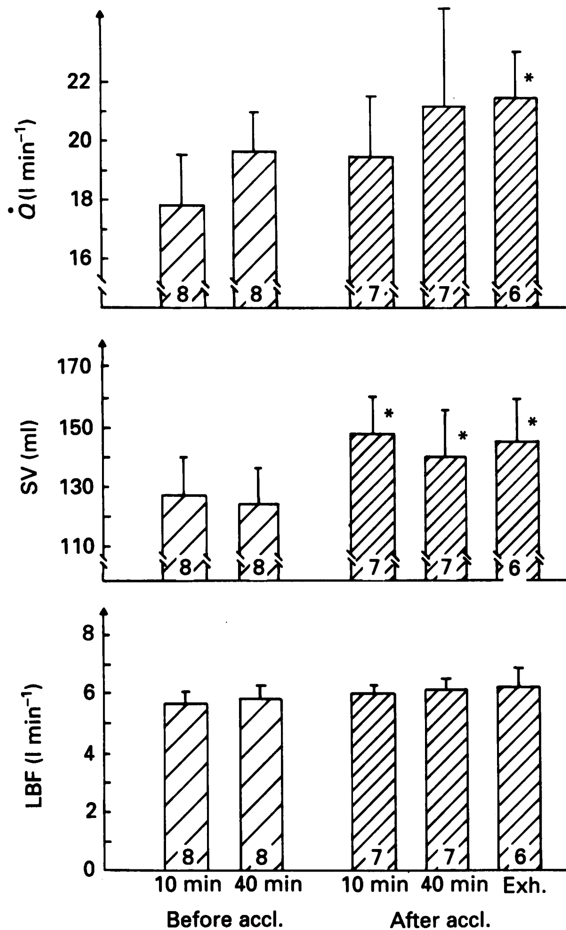

Fig. 2. Cardiac output ($\dot{Q}$), stroke volume (SV) and leg blood flow (LBF) measured at 10 min exercise, 40 min exercise, and just before stopping. Open hatching, before acclimation; close hatching, after 9–12 days of acclimation. Before acclimation the 40 min values were those taken just before exhaustion (mean ± S.E.M.). Number of measurements indicated. Exh = at exhaustion; accl. = acclimation. Measurements that are significantly different from the final measurements before acclimation are marked by *.

exhaustion differed significantly between the two tests ($P < 0{\cdot}05$) in the six subjects in whom paired measurement were obtained.

This was the result of an enhanced *stroke volume* ($P < 0{\cdot}05$) which reached 124 ± 11 and 145 ± 14 ml at exhaustion in the first and last heat test, respectively (Fig. 2).

Leg blood flow (LBF)

LBF was 5·71 ± 0·34 at 10 min, and 5·78 ± 0·39 l min^{-1} at exhaustion in the first heat exposure, and essentially unchanged, i.e. 5·95 ± 0·20 at 10 min, 6·09 ± 0·33 at 40 min, and 6·17 ± 0·60 l min^{-1} at exhaustion after acclimation (Fig. 2).

Forearm blood flow (FBF)

At exhaustion FBF showed an (insignificant) 15 % increase after heat acclimation, and a significant 25 % decrease in the cool condition for the control group in their experiment ($P < 0·05$, Table 2).

TABLE 2. Forearm blood flow, FBF (ml (100 ml)$^{-1}$ min^{-1})

	First experiment		Last experiment		
	Rest	Final	Rest	40 min	Final
Heat accl., hot ($n = 8$)	4·5 ± 0·68	15·8 ± 2·78	4·9 ± 1·02	16·1 ± 2·43	18·1 ± 2·59†
Control, cool ($n = 5$)	1·8 ± 0·29	9·6 ± 1·56	1·6 ± 0·36	7·0 ± 0·97	7·2 ± 0·90*
Control, hot ($n = 3$)	3·1 (1·3–5·6)	14·4 (12·0–17·8)	3·0 (1·8–4·6)	—	14·8 (11·8–16·7)

* Significantly different from final value in the first experiment.
† $P = 0·15$.
Measurements from eight acclimating and five control subjects. Three of the latter were exposed to heat twice at 10 days interval. All measurements are means ± S.E.M., or the range.

Skin temperature

At 30 min exercise the mean skin temperature of face and chest ($\overline{T}_{sk}$) was significantly lower in the last than in the first experiment in the heat acclimated subjects (37·6 ± 0·3 on the first *vs* 37·1 ± 0·2 °C ($P < 0·05$) on the last day). However, the final $\overline{T}_{sk}$ values at exhaustion were not significantly different: first day 38 ± 1 ± 0·2 (at approximately 48 min) and last day 38·2 ± 0·2 °C (at approximately 80 min). In the control subjects $\overline{T}_{sk}$ measured at 30 min in the first and final experiments (32·8 ± 0·3 *vs.* 32·5 ± 0·8) and at 90 min (32·6 ± 0·3 *vs.* 32·3 ± 0·3 °C), respectively, were not different.

Plasma volume (PV)

The percentage change in PV between the first and the final experimental day, was calculated from resting arterial Hct and [Hb] (Dill & Costill, 1974). Percentage ΔPV was + 2·7 ± 1·8 % in the control subjects and + 13·1 ± 3·1 % in the acclimated group.

Blood constituents

There were significant changes after acclimation in Hct, and Hb, glucose and lactate concentrations as indicated in Table 3.

Hormone analyses

The final values at exhaustion, in the catecholamines, growth hormone (GH), renin activity, AVP or aldosterone concentrations were not significantly different when values measured after and before acclimation were compared; but the hormone

TABLE 3. Blood constituents

			First test experiment		Last test experiment	
			Rest	Final	Rest	Final
HCT (%)	Accl.	a	43·5 ± 1·2	44·8 ± 0·8	41·8 ± 0·8*	42·2 ± 0·7
	(n = 8)	v	45·0 ± 1·3	44·3 ± 1·1	41·8 ± 0·9*	43·1 ± 0·4
	Control (n = 5)	v	43·6 ± 1·2		42·9 ± 0·8	
Hb (g (100 ml)$^{-1}$)	Accl.	a	14·1 ± 0·4	14·7 ± 0·3	13·4 ± 0·3*	13·8 ± 0·4
	(n = 8)	v	14·3 ± 0·4	14·8 ± 0·6	13·6 ± 0·2*	13·9 ± 0·4
	Control (n = 5)	v	14·7 ± 0·4		14·4 ± 0·3	
PP (g (100 ml)$^{-1}$)	Accl.	a	6·9 ± 0·14	7·2 ± 0·1	6·7 ± 0·2	7·1 ± 0·1
	(n = 8)	v	6·8 ± 0·13	7·3 ± 0·1	6·9 ± 0·1	7·3 ± 0·1
	Control (n = 3)	v	6·4 (0·1–0·8)		6·5 (0·1–0·7)	
Na^+ (mM l^{-1})	Accl.	a	139 ± 2	142 ± 2	141 ± 3	147 ± 3
	(n = 8)	v	140 ± 1	143 ± 2	139 ± 3	147 ± 2
K^+ (mM l^{-1})	Accl.	a	3·8 ± 0·1	4·6 ± 0·2	4·0 ± 0·2	4·8 ± 0·2
	(n = 8)	v	3·9 ± 0·1	4·5 ± 0·1	4·1 ± 0·2	4·8 ± 0·2
Glucose (mM l^{-1})	Accl.	a	5·1 ± 0·2	4·9 ± 0·2	4·9 ± 0·2	4·3 ± 0·1†
	(n = 8)	v	4·8 ± 0·1	4·7 ± 0·2	4·6 ± 0·3	4·1 ± 0·2
FFA (μm l^{-1})	Accl.	a	906 ± 207	1303 ± 479	487 ± 125	1081 ± 199
	(n = 8)	v	901 ± 194	1175 ± 169	519 ± 128	1011 ± 202
Lactate (mM l^{-1})	Accl.	a	1·1 ± 0·1	2·2 ± 0·3	1·2 ± 0·1	1·6 ± 0·2†
	(n = 8)	v	1·3 ± 0·2	2·6 ± 0·3	1·4 ± 0·2	1·6 ± 0·2†

Arterial (a) and venous (v), taken at rest and just before stopping. Values are means ± S.E.M. or the range. n = number of subjects. Eight acclimating and five to three control subjects.

* Significantly different from rest in the first test; † significantly different from final value in the first test.

TABLE 4. Effect of the hormones: noradrenaline (NA), adrenaline (A), growth hormone (GH), arginine-vasopressin (AVP), aldosterone (ALDO), and renin activity

		First experiment			Last experiment		
		Rest	30 min	Final *ca* 40 min	Rest	30 min	Final *ca* 80 min
NA (ng ml^{-1})	a	0·206 ± 0·032 (5)	—	1·302 ± 0·264 (6)	0·195 ± 0·027 (6)	0·840 ± 0·115 (6)‡	1·085 ± 0·175 (6)*
	v	0·213 ± 0·079 (3)	—	1·360 ± 0·562 (3)	0·215 ± 0·039 (4)	0·902 ± 0·174 (4)	1·180 ± 0·177 (4)
A (ng ml^{-1})	a	0·080 ± 0·014 (5)	0·116 ± 0·016 (5)	0·120 ± 0·020 (6)	0·062 ± 0·011 (6)	0·082 ± 0·016 (6)‡	0·092 ± 0·009 (6)
	v	0·04 ± 0·00 (3)	0·087 ± 0·017 (3)	0·097 ± 0·027 (3)	0·030 ± 0·004 (4)	0·080 ± 0·024 (4)	0·082 ± 0·014 (4)
GH (ng ml^{-1})	a	5·695 ± 1·925 (8)	—	10·226 ± 2·332 (7)*	4·417 ± 1·213 (8)	—	8·853 ± 2·011 (6)*
AVP (pg ml^{-1})	v	1·2 ± 0·35 (5)	3·6 ± 1·13 (5)	6·3 ± 1·56 (5)	0·9 ± 0·29 (6)	2·1 ± 0·30 (6)	7·4 ± 2·00 (4)
ALDO (pg ml^{-1})	v	96·6 ± 12·8 (5)	178·5 ± 41·6 (4)	286·5 ± 30·4 (4)	156·0 ± 15·3 (6)†	226·2 ± 45·9 (5)	321·5 ± 65·4 (6)
Renin (ng ml^{-1} h^{-1})	v	2·32 ± 1·01 (5)	6·26 ± 1·84 (5)	8·70 ± 1·04 (5)*	2·37 ± 0·76 (6)	5·55 ± 1·17 (6)	7·63 ± 1·49 (4)

All values are the mean ± S.E.M. with the number of measurements indicated in parentheses. a = arterial, v = femoral venous sample.
* Significantly different from rest.
† Significantly different between rest values in 1st and 2nd experiment.
‡ Significantly different from final value in 1st experiment.

concentrations increased more slowly as the endurance time was prolonged (see Table 4). The arterial adrenaline (A) and noradrenaline (NA), and GH concentrations increased significantly from the resting value both in the first and last heat test ($P < 0.05$). The Na and A concentrations were significantly lower at 30 min in the final compared to the first heat exposure. Resting aldosterone concentration was increased after acclimation (Table 4).

TABLE 5. Muscle strength: maximal voluntary contraction (MVC) (in Newtons) measured before and after 90 min exercise in cool, and in hot conditions at exhaustion

		Before	After exercise
Knee extension			
Four subjects, six measurements	Cool	534 ± 30	529 ± 39 (90 min)
Three subjects, four measurements	Hot	593 ± 41	625 ± 34 (*ca* 45 min)
Arm flexion			
Four subjects, six measurements	Cool	192 ± 13	182 ± 7 (90 min)
Three subjects, four measurements	Hot	177 (138–220)	176 (*ca* 45 min) (150–213)

All values are the mean ± S.E.M. with the range given in parentheses.

Maximum voluntary contraction (MVC)

Measurements of MVC in the knee extensor muscles and the arm flexors before and after 90 min exercise in cool condition, and at exhaustion in hot condition, are presented in Table 5. No significant changes were observed in these measurements.

DISCUSSION

Acclimation to heat may take a different course depending upon the type of environment, e.g. hot and dry *versus* hot and humid, and effects of physical training may interact further with the acclimation process. We used endurance-trained subjects so that the exercise included in the acclimation was only a small fraction of their continuing daily training regime. Further, our protocol was unique in that the daily physiological strain was kept constant, as the highly motivated subjects exercised until exhaustion each time. We allowed them no clues as to how long they had worked, i.e. HR, T_c or time (with measurements taken at irregular intervals). This protocol produced pronounced acclimation to exercise in heat, as evidenced by: (1) the increased sweating rate, (2) lower rate of rise in core temperature (Fig. 1) and heart rate, and (3) the prolonged endurance time. In seeking mechanisms explaining these adaptations, we focused on the cardiovascular system.

Cardiac output

As we found previously (Savard *et al.* 1988; Nielsen *et al.* 1990) $\dot{Q}$ increased insignificantly with time during an individual period of heat exposure, due to the very rapid rise with onset of exercise. However, it was higher at exhaustion after acclimation than before, and the increase in $\dot{Q}$ during exercise (19·4 to 21·4 l min^{-1} = 10%) between the first and the final heat stress test, was due to a larger stroke volume (Fig. 2). The results of earlier studies appear to be equivocal. Firstly, in

addition to the methodological and environmental differences, which could be part of the explanation, the type of exercise may be critical. It varied from bench stepping, (Wyndham (1951); Wyndham *et al.* (1968)) to upright bicycling (Wyndham *et al.* 1976; *Senay et al.* 1976; Mitchell *et al.* 1976) and to uphill walking on a treadmill (Rowell *et al.* (1967)) *vs.* our seated bicycling; this would create different hydrostatic conditions in the cardiovascular system and thereby varying baroreceptor inputs and plasma volume changes (Rowell, 1974; Harrison, 1985) as part of the acclimation process. Secondly, the training status of subjects differed between studies, a factor which could influence responses. Finally, and we believe most importantly, is the fact that our subjects were stressed to exhaustion with the same strain level at each heat exposure, compared with the other studies in which exposure time was kept constant and therefore the strain gradually declined due to the acclimation process. This could explain our greater response in $\dot{Q}$ and SV to the repeated heat stress.

Stroke volume and plasma volume

The effect of acclimation on plasma volume is an extremely important question because of its significance for cardiovascular stability, and we found it to be elevated by 13%. An expanded plasma volume would be the basis for our observed significant increases in stroke volume with heat acclimation, a process regarded by Wyndham and co-workers (1976) as crucial to the central circulatory adaptations. However, the latter observed a decline after 6–8 days of acclimation, in association with a decrease in $\overline{T}_{sk}$ and rectal temperature, T_{re}. We did not follow the day-by-day changes; however, our significantly increased SV values after 9–12 days may be the result of our 'constant strain' procedure (see p. 470). These data do not support the hypothesis of Rowell and co-workers (1967, 1974, 1983) that the gradual lowering of core temperature decreases heart rate and thereby leads to an increased stroke volume. We found that after 40 min exercise core temperature and HR were lower whereas SV was higher following acclimation. However, SV remained high while core temperature continued to rise until it reached exactly the same high level at exhaustion, but now after 80 min (see Figs 1 and 2).

Although there have been some wide contrasts in reported effects of exercise and heat on PV (Bass, Kleeman, Quinn, Henschel & Hegnauer, 1955; Wyndham *et al.* 1968; Senay *et al.* 1976; Shapiro, Hubbard, Kimbrough & Pandolf, 1981; Harrison, Edwards, Graveney, Cochrane & Davies, 1981), just as with $\dot{Q}$ above, it is likely that variations in experimental protocol, fitness and posture have critical influences, e.g. $\pm$10 to 15% depending upon posture, as discussed by Harrison (1985) in his review on plasma volume. Our measurements, taken 9–12 days apart, were all obtained from subjects who were very well trained, so that the exercise involved was a small part of their daily training. Blood was sampled with the subject in a supine position, after 45 min lying in a thermoneutral room temperature. Under these conditions the control subjects had the same plasma volume in the first and second measurement, while in the heat acclimated subjects a significant 13% increase was found in the percentage ΔPV.

The mechanism for the plasma volume expansion remains speculative. It may well be initiated through an influx of protein from the cutaneous interstitial space to the vascular compartments, as suggested by Senay and co-workers (1972, 1976, 1986) and Harrison *et al.* (1981). In our study, the plasma protein concentration was the

same before and after, and the PP/Hct ratio was increased after acclimation. Also, plasma Na^+ measured during rest was unchanged. However, resting aldosterone had increased significantly, perhaps signifying a sodium and water retention as background for isosmotic plasma volume expansion. During exercise, at the time when exhaustion occurred in the first heat test, i.e. about 48 min, the aldosterone concentration and also the AVP and renin levels were lower after acclimation; this conforms with previous studies (Finberg & Berlyne, 1977; Francesconi, Sawka & Pandolf, 1983).

Muscle blood flow

As discussed previously (Savard *et al.* 1988; Nielsen *et al.* 1990), LBF appears to be an adequate index of major shifts in muscle blood flow under our experimental conditions, if it is accepted that an estimated contribution of leg skin blood flow of 300–500 ml min^{-1} to the femoral vein blood flow is close to the 10% accuracy of our (thermodilution) method. Rowell (1974, 1983) has proposed that blood flow in active muscle might be compromised during heat stress, with this being the main cause for fatigue and exhaustion. This is supported by quantitative measurements on sheep (Bell *et al.* 1983). However, neither the present nor any of the three other studies on humans have found any supportive data for that.

Leg blood flow in our study was similar at 10 min of exercise and at exhaustion, and was unaffected by acclimation; this agrees with Kirwan, Costill, Kuipers, Burrell, Fink, Kowaleski & Fielding (1987), Savard *et al.* (1988), and Nielsen *et al.* (1990). That the blood supply remains adequate is substantiated by the finding that the metabolic requirements were met, and no signs of metabolic disturbances could be observed from the arterial–venous (a–v) measurements across the exercising leg, either before, or after acclimation (Fig. 3; Table 3). Plasma FFA levels at exhaustion were not altered by acclimation, but glucose and lactate concentrations were reduced. The decline in glucose could be due to the longer duration of exercise (48 increased to 80 min) and the lowering of lactate could reflect improved blood supply to non-exercising tissues, made possible by the increased $\dot{Q}$. Calculations of glucose and FFA uptake showed that utilization of the blood-borne substances by the exercising leg was not changed by acclimation in our study. This is in contrast to findings of Kirwan *et al.* (1987), who observed an increased FFA utilization after acclimation.

The skin circulation

This was estimated as changes in FBF and was about 15% higher at exhaustion in the final heat exposure. Earlier studies (e.g. Johnson & Rowell, 1975) have shown that increases in FBF during leg exercise are confined to the skin with any changes in blood flow to other tissues being insignificant or in the opposite direction. A release of vasoconstriction in non-active (forearm) muscles due to the high $\dot{Q}$ after acclimation, might also contribute to an increase in FBF. However, the estimated muscle blood flow of the resting forearm is less than 2 ml $(100\ ml)^{-1}\ min^{-1}$ (Johnson, Brengelmann, Hales & Vanhoutte, 1986), so that any small change will be of minor importance relative to the total FBF of up to 18·1 ml $(100\ ml)^{-1}\ min^{-1}$ (mean). Although our 15% increase in FBF after acclimation did not reach statistical significance, it is in line with our indices of skin microvascular perfusion measured

with laser Doppler and photoelectric plethysmography which increased by (respectively) similar or greater magnitudes (J. R. S. Hales, B. Nielsen & M. Yanase, in preparation).

Human studies in which skin blood flow was calculated from conductance have reported a decrease (Eichna, Park, Nelson, Horvath & Palmes, 1950; Wyndham

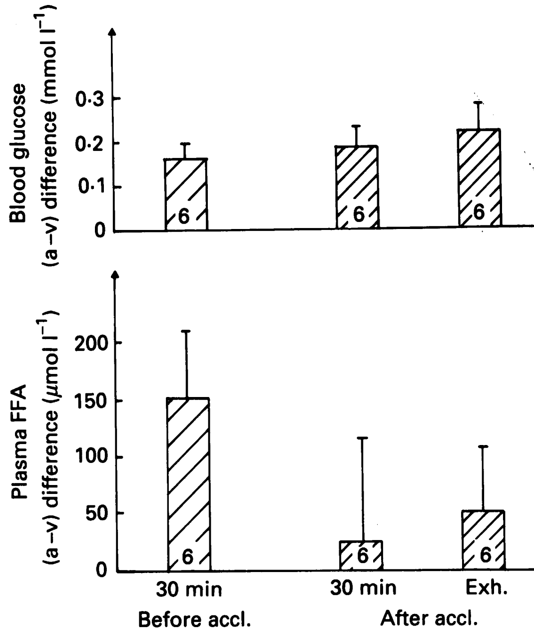

Fig. 3. The arterial–venous (a–v) differences across the exercising leg of blood glucose (above) and plasma free fatty acids (below) after 30 min of exercise and final measurements before and after acclimation. (The 30 min value was the final measurement before acclimation.) Exh. = at exhaustion. Open hatching, before acclimation, close hatching, after acclimation.

et al. 1976), or inconsistent change (Whitney, 1954) with acclimation to heat. The only absolute measurements have been in sheep (Alexander, Hales, Stevens & Donnelly, 1987), but support our present data by finding increased skin blood flow with heat acclimation. Nadel, Pandolf, Roberts & Stolwijk (1974) and Roberts, Wenger, Stolwijk & Nadel (1977) found no change in sensitivity of the FBF/T_c relationship, but an earlier onset of vasodilatation, which is in accordance with our general results. Unfortunately, many experimental factors are likely to be responsible for the seemingly conflicting data, as discussed above.

Thus, our results point to an improved cardiovascular capacity enabling a larger heat transfer to the skin as one benefit of acclimation, linking closely with the increased sweating discussed below.

Sweating

In a sense, the increased evaporative rate and 'sensitivity' (Table 1) is the key reason for the improved endurance after heat acclimation in our study, occurring gradually, day by day. Sweat rate elevations following heat acclimation have been

consistently reported with exercise in both dry and humid heat, and by passive whole-body heating (Robinson, Turrell, Belding & Horvath, 1943; Adolph, Brown, Goddard, Gosselin, Kelly, Molnar, Rahn, Rothstein, Towbin, Wills & Wolf, 1947; Eichna *et al.* 1950; Fox, Goldsmith, Kidd & Lewis, 1963; Fox, Goldsmith, Hampton & Lewis, 1964; Robinson, Belding, Consolazio, Horvath & Turrell, 1965; Chen & Elizondo, 1974; Mitchell *et al.* 1976; Avellini, Kamon & Krajewski, 1980; Candas *et al.* 1980; Candas, 1987).

Although increased sensitivity and earlier onset of sweating with heat acclimation are well-established phenomena (Nadel *et al.* 1974; Gonzalez, Pandolf & Gagge, 1974; Roberts *et al.* 1977; Libert, Candas & Vogt, 1983), the mechanisms responsible for peripheral and central modifications of sweating remain incompletely understood. Mitchell *et al.* (1976) spoke of 'temperature-induced changes in sweat gland function', and there has been evidence of increased size of the sweat duct (Ogawa, Asayama & Miyagawa, 1982) and sweat gland (Sato & Sato, 1983). Peripheral cholinergic and adrenergic mechanisms are involved in sweat gland control, and aldosterone and ADH modify duct absorption of sodium (Sato, Kang, Saga & Sato, 1989). However, we found no changes in the final concentrations of NA, ALDO, or ADH at exhaustion after acclimation. At 30 min exercise NA and adrenalin levels in the second test were significantly lower than *at this time* during the first heat exposure; this could be indicative of reduced overall sympathetic activity.

Also growth hormone has been associated with the ability for sweating (Main, Nilsson & Skakkebæk, 1991), but we observed no changes in GH due to acclimation, neither in resting nor in the final values at exhaustion. Thus, the increased sweating is unlikely to be attributable to increased stimulation by the measured hormones; an increased sensitivity, such as due to increased receptor density, remains a possible explanation.

What causes exhaustion with exercise in a hot environment, and particularly with acclimation?

The endurance for exercise in the hot environment increased gradually from 48 to 80 min in the 9–12 days acclimation period, i.e. it showed a remarkable doubling. Clearly, the decreasing rate of rise in T_c was achieved by an increasing heat loss due to enhanced skin blood flow and sweating, but the subjects always stopped when they reached the same high T_c, approximately 39·5 °C (Fig. 1). There is considerable evidence of marked changes in numerous biochemical and physiological functions at critical temperature *in vivo* and *in vitro*, and differences in thermosensitivity exist between tissues (see Brinnel, Cabanac & Hales, 1987). The level of core temperature which becomes limiting for exercise performance may depend on many factors (e.g. duration and intensity of the exercise, dehydration, nutrition, fitness and motivation), and therefore in absolute terms would be expected to vary markedly. Further, core temperature depends on the site of measurement; there are temperature gradients within the body core, especially during exercise and during heat storage. We measured oesophageal temperature, which is lower than muscle temperature in these conditions (Nielsen *et al.* 1990), and perhaps higher than brain temperature, since heat is liberated in the muscles and then distributed via the circulation.

At the point of exhaustion, there were no reductions in muscle or skin blood flow, no lack of substrate and no accumulation of lactate, K^+ or other recognized 'fatigue

substances'. Although the final skin blood flow estimated from FBF was not reduced, our essentially continuous photoelectric plethysmographic index of skin blood flow did indicate a significant fall as exhaustion approached, having reached a peak earlier in exposure (J. R. S. Hales, B. Nielsen & M. Yanase, in preparation). However, as discussed elsewhere (Hales, 1987), we regard this as aggravating the hyperthermia rather than specifically being the cause of exhaustion. No change in the ability to recruit motor units in a maximal contraction was detected. To obtain an index of local, central or general factor involvement in fatigue the maximal force which could be exerted (MVC) with the elbow flexor and the knee extensor group was measured in a standardized 90 deg position. Both the rested arm flexors and the leg muscles (knee extensors) that had 'worked until exhaustion' in the warm environment could mobilize the same maximal isometric force after exercise as before (Table 5).

In conclusion, the physical endurance for exercise in hot, dry environments appears to be limited by the attainment of a critical level of core temperature, perhaps due to temperature, reducing motivation (Brück & Olschewski, 1987); the endurance can be substantially prolonged by heat acclimation, due to enhanced cardiovascular and thermoregulatory capacity. High core temperature, and not circulatory failure, was therefore the critical factor for fatigue in heat stress, both before and after acclimation.

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