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PLASMA ALDOSTERONE AND SWEAT SODIUM CONCENTRATIONS AFTER
EXERCISE AND HEAT ACCLIMATION

The University of Arizona

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PLASMA ALDOSTERONE AND SWEAT SODIUM CONCENTRATIONS

AFTER

EXERCISE AND HEAT ACCLIMATION

by

CHRISTOPHER ROBIN KIRBY

A Thesis Submitted to the Faculty of the

DEPARTMENT OF EXERCISE AND SPORT SCIENCES

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

1 9 8 5

STATEMENT BY AUTHOR

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APPROVAL BY THESIS DIRECTOR

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5/6/85
Date

DEDICATION

This thesis is dedicated to my loving family and
to the glory of the Lord. (Proverbs 3:5-6).

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ABSTRACT

This investigation was designed to determine the relationship between the levels of plasma aldosterone and eccrine sweat gland activity following exercise and heat acclimation. Ten subjects exercised at 45% of their maximal oxygen uptake in a hot (40 C) and moderately humid environment (45%rh) for 2h/day on ten consecutive days. Acclimation was verified by significant reductions in peak heart rate, peak rectal temperature, and heat storage, and by significant increases in plasma volume ($\Delta PV=12\%$) and sweat rate on day 10 vs. day 1 ($P<0.05$). The concentration and total content of sodium in sweat samples decreased significantly ($P<0.05$) from day 1 to day 10. Plasma aldosterone levels after 1 hr. were significantly decreased on day 1 vs. day 10. The ratio of sweat sodium reabsorbed (mmol/l) to plasma aldosterone concentration (ng/dl), was used as an index of the sweat gland responsiveness to aldosterone (SRI). SRI values were significantly increased from day 1 to 10, after both hours 1 and 2 of exercise. These data indicate that 1) plasma aldosterone concentrations decrease following heat acclimation; and 2) eccrine gland sensitivity to aldosterone, as represented by sweat sodium reabsorption, can be augmented through exercise and heat acclimation.

CHAPTER 1

INTRODUCTION

Aldosterone, the final effector of the renin-angiotensin-aldosterone (RAA) system, has been studied in an effort to isolate and describe its actions as a hormone. Two active sites which are receptive to changes in plasma aldosterone concentration are found in the kidney and the sweat gland (7,40,86,100). Both areas are affected in such a way as to increase Na^+ reabsorption, in an effort to maintain plasma osmolality. This effect at the distal renal tubules has been demonstrated by several studies including the 1969 study by Blair-West, Coghlan, Denton, Scott and Wright (7). Acute aldosterone-induced sodium conservation at the sweat gland has also been reported by several investigators (44,93).

Although the response by renal and sweat gland tissues to aldosterone have been well documented for acute exposure, there are very few studies describing the effects of aldosterone on chronic exercise-trained or heat-acclimated subjects. A recent study by Skipka (100) utilized a subject population which was exercise trained at various levels and compared their renal sodium retention following the same absolute dose of aldosterone. An augmented renal sensitivity to aldosterone with exercise training was suggested by an increased renal sodium conservation for the same amount of aldosterone injected.

Greater hypotonicity of sweat following heat acclimation is well documented (81,87) and is due primarily to reductions in sweat sodium output. In the few studies examining aldosterone levels following chronic exercise and heat acclimation, both increased and decreased levels of plasma aldosterone have been reported for the same absolute workrate (26,77). In addition, the reported effects of aldosterone concentration changes on sweat sodium concentrations following acclimation are mixed (42,67,74). Therefore, it remains unclear as to the mechanism which precipitates this change in sweat osmolality and the possible association between changes in sweat gland sodium reabsorption and their relationship to plasma aldosterone concentrations. Can the augmented renal sensitivity to aldosterone, as suggested by the work of Skipka, be likewise apparent at the eccrine sweat gland?

CHAPTER 2

REVIEW OF RELATED LITERATURE

The thermoregulatory system of man has the capability of dealing with large changes in ambient environment as well as the subtle internal changes associated with normal and increased metabolism. Of particular interest and importance to the understanding of thermoregulation are those stimuli, environmental and metabolic, acute and chronic, which result in the physiological adaptation to heat stress. With homeostasis as the ultimate criterion for evaluating physiological control, this review will emphasize those thermoregulatory changes which enhance the dissipation of large quantities of heat in order to maintain core temperature, plasma osmolality and total plasma volume within the normal physiological range. More specifically, investigations on the progressive decrease of sweat electrolyte concentrations will be examined in order to establish the mechanism currently associated with this thermoregulatory adaptation.

The two most significant changes which augment the heat dissipating capacity following heat acclimation are increases in plasma volume (97) and overall sweating capacity (84). Increases in plasma volume as a result of exercise training and heat acclimation are advantageous to the cardiovascular system specifically by increasing exercise stroke volume (80). This heterometric response to an increased preload allows for greater perfusion of metabolically active tissue. Convertino (23) has reported a correlation of -0.89 between increases in

plasma volume and decreased heart rate resulting from exercise training in the heat. Increased delivery may also be aided by a decrease in resistance to flow via decreases in blood viscosity.

These changes in plasma volume have been shown to be highly correlated to, increased sweating capacity, the second major physiological adaptation to heat stress. A correlation of +0.93 was found between increased plasma volume and elevations in sweat rate (23). These results tend to suggest that increases in plasma volume, from which sweat is generated, allow for greater absolute sweat losses without sacrificing the cardiovascular enhancement of increased stroke volume following exercise training and heat acclimation.

In addition to the greater total sweat output gained through exercise/heat acclimation, earlier onset of the sweating response (53) and a decrease in sweat osmolality (56) may also take place. Since differences in heat storage between trained and untrained individuals are associated with training-induced adaptations of the sweating mechanism, further investigation of eccrine glandular activity will better illuminate the potential thermoregulatory advantages of these adaptations.

Increased sweat rate during heat stress following exercise training and heat acclimation has been documented by several studies (16,23,40). Fox, Goldsmith, Hampton and Lewis (40) showed increases of up to 130% in total body sweating following heat acclimation, at the same absolute workrate, a response which seems to hold for both males and females (62). These increases in sweat rate have been generally

attributed to local training of the sweat gland apparatus and show no relationship to core temperature (T_{re}) or mean skin temperature (T_{sk}) when the local areas are not subjected to peripheral sweat gland training (40). Collins, Crockford and Weiner (16) induced sweating with drugs, immersion, and radiation in an effort to investigate the peripheral nature of sweating control and also found no significant relationship to T_{re} . Although these results indicate a peripheral training adaptation, other researchers have proposed a central control via increased central nervous system (CNS) sensitivity. This central control is thought to exist in the pre-optic anterior hypothalamus. In relation to this proposed central control site, Nadel, Pandolf, Roberts and Stolwijk (79) hypothesized a zero point control for sweating during progressive heat acclimation. A change in zero point reflected a change in the esophageal temperature per unit mean skin temperature. Peripheral sensitivity was thought to increase per unit of metabolic (central) drive. That is, a progressively lower zero point is required to cause the CNS to initiate heat dissipation mechanisms during acclimation. Chen and Elizondo (14) tried to determine whether the controls for changes in the sweating response were peripheral or central in their investigation of the effects of various sweating stimuli. They concluded that increased sweat output following acclimation is due primarily to an increased capacity of the glandular apparatus which is modified only by exposing the whole body to heat.

The earlier onset of sweating in trained subjects was also demonstrated by Chen and Elizondo (14), who postulated this phenomenon

to be related to the increased total sweat output also associated with training. This was thought to be due to the overflow of sweat above that which could be reabsorbed at the sweat gland tubules. Henane, Flandrois and Charbonnier (53) further substantiated this shorter onset time in their study comparing sedentary and active individuals. Increased neural activity was the factor attributed to this difference in a study by Collins et al. (16). In general, earlier onset of sweating may allow the body to dissipate heat progressively from the onset of thermal stress and thereby mediate any large rise in core temperature.

In addition to the earlier onset of sweating and overall sweat rate increase during exercise in the heat, acclimation is also known to enhance the ability to produce a more dilute sweat (81). As early as 1912, Hunt (57) suggested this adaptation in Indian subjects who were producing up to 13 L/day of total sweat. Since that time, numerous studies have been done in an effort to isolate the chemical composition of sweat and the changes in sweat constituents associated with acclimation.

Two studies by Dill, Hall, Edwards and van Beaumont (28,29) supported the overall dilution of sweat with chronic acclimation proposed by Hunt. They also indicated that sweat chlorine (Cl^-) concentrations changed acutely in direct relation to total sweat rate. However, the changes in Cl^- concentration were not found to be related to changes in T_{sk} or T_{re} . Robinson, Kinkaid and Rhamy (85) suggested that lower sweat Cl^- concentrations during heat exposure following

acclimation are directly dependent upon a progressive salt deficit incurred in acclimating subjects and are not related to increases in secretory practice by the sweat gland. It was their interpretation that the principal factor in dilution was increased adrenal cortex activity elicited by a salt deficiency. In contrast to the studies by Dill et al., these investigators reported a direct relationship between Tsk and Cl⁻ concentration in sweat. They postulated a neurohormonal control which is augmented by a direct feedback system. Therefore, if a reduction in Tsk occurred during acclimation, this temperature effect would be additive with increased adrenal activity and thereby decrease salt excretion in sweat. Efforts by Brubacher and Vander (11) suggested a hormonal control during salt depletion via extrarenal sensory receptors possibly located in venous or cardiac chambers. These receptors may alter Na⁺ reabsorption proximal to the macula densa, alter intrarenal baroreceptor sensitivity or directly stimulate the juxtaglomerular apparatus (JGA).

Due to the importance of Na⁺ in the maintenance of extracellular fluid (ECF) osmotic pressure, much effort has gone into the research of this solute with regards to its concentration in sweat. Similar to the earlier studies on Cl⁻ concentrations in sweat, Na⁺ was found to increase acutely in direct relation to increases in total sweat rate (56,104). This was also demonstrated by Schwartz and Thaysen (96) when they induced sweat with local injections of acetyl-B-methylcholine. After several injections, Na⁺ output by the sweat gland decreased with subsequent doses, while sweat rate continued to increase. This was

thought to suggest classic desensitization which is found in many tissues controlled by neurochemical receptors (59).

Other studies have directly investigated the influence of heat acclimation and exercise training on sweat Na^+ concentrations. These studies, as did those concerning Cl^- , indicated that a primary factor in decreasing sweat Na^+ output was the creation of a salt deficit. Robinson, Maletich, Robinson, Rohrer and Kunz (86) demonstrated increased dilution during salt depletion, but found it to be enhanced by the addition of dehydration. McCance (73) found that ad libitum replacement of salt and water produced no changes in sweat NaCl concentration following heat acclimation. Smiles and Robinson (104) demonstrated no decrease in sweat Na^+ concentrations without a subsequent plasma Na^+ deficit. However, decreases in plasma volume and ECF along with increases in plasma renin activity were also implicated, making it difficult to separate out which factor played the primary role in initiating the Na^+ conservation response. Somewhat in contrast, Amatruda and Welt (1) demonstrated no relationship between ECF tonicity and NaCl concentration in sweat.

Several recent studies have dealt with these same adaptations in sweat response over a chronic period of acclimation. Johnson, Pitts and Consolazio (58) studied the factors influencing Cl^- concentrations in human sweat and found no change in post acclimation sweat Cl^- concentration, with sweating stimulated at the same T_{re} and T_{sk} . In contrast, a study of the thermoregulatory differences between tropical natives of Japan and their immigrant counterparts supported a decrease

in sweat salt concentration with acclimatization (56). Hori and Yoshimura (56) reported similar results when investigating seasonal dilution within these same two groups. The acclimatized subjects had significantly decreased Na^+ concentrations in sweat during both winter and summer seasons as compared to the unacclimatized group. Interestingly, both groups showed greater salt losses per unit sweat during the winter months, which was attributed to changes in the reabsorption of precursor sweat sodium in the ducts. These changes, also reproduced by Ohara (81), were associated with systemic factors mediating the salt excretion of sweat at the glandular level.

The mechanism by which this dilution is accomplished has been the subject of several investigations (66,70,77). All studies showed decreases in sweat salt concentrations with general body heating and each author suggested reabsorption as the mechanism for lower osmolality in sweat versus plasma. Sweat NaCl concentrations were reported as 1/25th the concentration of those found in plasma by Hensel (54). Lloyd (69) gave evidence for reabsorption by sweat glands by indicating a threshold stimulation which occurs between the formation and emergence of sweat. The rate at which reabsorption equals formation is only 1.65% of the total sweating capacity. In other words, the sweating power is up to 60 times greater than the reabsorptive capacity. Further work by Slegers (103) has determined the maximal capacity for retention to be approximately 140 meq of sodium per liter of sweat. Through microslice analyzing of the eccrine gland this dilution was demonstrated to decrease progressively from the secretory coil to the duct of the sweat

gland (102). Although most evidence in this area supports a strict relationship between rate of sweating and salt concentrations, Ohara (81) has presented results showing an increase in reabsorption in conjunction with lower sweat rates in chronically acclimatized Japanese islanders.

A quantitative analysis by Elizondo (35) supported increases in neuroglandular transmission and the potentiation of synchronous subliminal impulses as two potential factors for increased sweat gland functional capacity. The renin-angiotensin-aldosterone (RAA) system is thought to be the primary neuroglandular controller for both renal and eccrine gland sodium conservation.

Research on the effects of aldosterone has clearly substantiated its role as the primary controlling hormone in initiating renal sodium conservation. Blair-West et al. (7) attributed increased sodium retention to 3 possible factors affecting receptor sensitivity; 1) a lowering of the threshold for inception of aldosterone effect, 2) an increased effect per given concentration of aldosterone, and 3) an increase in the dimension of the maximal effect associated with aldosterone. This explanation has been recently investigated by Skipka (100), who demonstrated decreased renal sodium excretion associated with the level of endurance training. These results were associated with increased sensitivity to a given aldosterone injection which was comensurate with the level of training. Wyndham (114) has noted that changes in thermoregulatory capacity can be elicited by chronic regular increases in T_{re} resulting from endurance training. Therefore, these

changes in renal aldosterone sensitivity may have been due to the partial acclimation achieved through the various levels of endurance training. Direct evidence for renal sodium conservation has been presented in two studies which infused exogenous aldosterone directly into the renal arteries (5,90). Both investigations reported decreased renal Na⁺ excretion during and for 2-6 hours post injection.

Numerous studies have investigated the effect of both systemic and local aldosterone levels on the Na⁺ conservation response at the sweat gland. In 1949 Conn (19) first produced results suggesting increased sweat sodium reabsorption via the effects of aldosterone. Conn (21) again published support for these results showing decreased sweat gland salt excretions of up to 95% with increased aldosterone concentrations. By the tenth day of heat exposure salt output by the sweat glands had decreased from 3 to 1 gm/day. In fact, the reabsorptive efficiency of the glands was so great, that renal escape of Na⁺ took place in order to maintain normal plasma osmolality.

Grand, di Sant'Agnese, Talamo and Pallavicini (43) studied the effects of exogenous aldosterone on the sweat electrolytes by intravenous infusion of 1.0 or 2.0 mg of aldosterone/day. They observed decreases in sweat sodium concentrations within 48 hr of injection and maximal decreases after 5 days. Similar results were reported by Siegenthaler and de Haller (98) and Grandchamp, Scherrer, Veyrat and Muller (44). This same response was observed by Collins, Foster and Hubbard (17) in the palmar and forearm sweat of cats where sweating was induced by 10 μ g injections of intradermal acetylcholine. Another study

by Collins (15) reported decreases in both sweat sodium concentrations and sweat rates in response to aldosterone injections. A study by Sato and Dobson (92) demonstrated increased sodium reabsorption in sweat glands following d-aldosterone injections. The sodium conservation began at 5-10 hr, was maximal at approximately 24 hr, and returned to normal after 72 hr. Additionally, sodium was found to decrease significantly ($P < .001$) at two different sweat rates. As in the Collins study, aldosterone also seemed to reduce the sweat rates, both mean and maximal, in virtually all subjects. Dixon and Schwarz (30) have reported increases in RNA synthesis in sweat glands perfused with exogenous aldosterone. Most recently, Sato (91) has suggested the duct cells to be the primary site of action for aldosterone in sweat glands. Here and in the secretory coil, Na/K ATP-ase linked active transport induces reabsorption.

Further evidence concerning support of the dependance of sweat sodium excretion on aldosterone is found in three studies which investigated the effects of aldosterone antagonists. Ladell and Shepard (67) demonstrated increases in sweat Na^+ excretion during acclimation when aldosterone production was blocked with spironolactone. Siegenthaler, de Haller, Veyrat and Muller (99) abolished the sodium conservation response in sweat glands by administering aldosterone inhibitors. Furman and Beer (41) were able to simulate a non-acclimated state, with regards to sweat sodium reabsorption, in acclimated subjects by injecting the aldosterone inhibitor aldactone.

Several studies of a similar nature and design have produced conflicting results regarding the relationship of aldosterone and sweat sodium concentrations. Gibinski, Nowak, Giec and Kokut (42) studied three groups exposed to various plasma aldosterone concentrations; 1) group 1; with increased aldosterone concentration via acute heat exposure, 2) same as group 1, with the addition of exogenous aldosterone, 3) same as group 1, with aldosterone blockade. Their findings suggested no differences in the NaCl concentrating capacity of sweat glands, following the three treatments. McConahay, Robinson and Newton (74) found no effect on sweat electrolyte composition with intravenous/intraarterial injections of up to 5-6 times the normal level of aldosterone. A similar study by Braun, Maher and Byron (10) looked at the effects of exogenous d-aldosterone on the process of acclimation in man. Although they found no differences in sweat sodium conservation with or without the drug, cessation of treatment following acclimation greatly increased both urine and sweat sodium excretions.

Actually, these studies may be exhibiting the fact the sodium conservation response is not controlled by the total amount of aldosterone present, but is also related to the sensitivity of receptor tissue in the eccrine sweat gland. A study by Rosemberg, Demany, Bunditz, Underwood and Leard (89) supplied evidence for initial sodium conservation after administration of large amounts of aldosterone, with a return to normal excretion after 3 days in non-acclimated subjects. This transient retention of sodium during prolonged aldosterone administration has been documented by several investigators, including

August, Nelson and Thorn (2) and Zierler and Lilienthal (117). A comparison of 1.0 versus 2.0 mg doses in non-acclimated subjects demonstrated no relationship between sweat sodium output and total dosage (43). Such results tend to indicate that in the absence of physiological adaptations produced by thermal acclimation, aldosterone has both a transient and limited effect on the sodium conservation response of the eccrine sweat gland. Data from the work of Furman and Beer (41) lead them to suggest that a progressive rise in sodium output by the sweat gland does not imply simultaneous rapid aldosterone increases. However, they proposed a sweat gland conditioning, i.e., change in sensitivity, to be the mechanism for acclimation. If sensitivity changes are comensurate with acclimation, the results of Braun et al. (10) and McConahay et al. (74) would provide support for this contention. That is, post-acclimation, when aldosterone sensitivity would be at its greatest, cessation of aldosterone treatments produced marked effects of sodium conservation at the sweat gland. This is supported by the work of Robinson, Nicholas, Smith, Daly and Percy (87), who found that chronic acclimation took a minimum of approximately 20 hr of intermittent exposure to heat stress.

Summary

Research has isolated many adaptations of the human thermoregulatory system in response to exercise training and thermal stress. These adaptations enhance the body's capability to dissipate thermal loads and thereby regulate core temperature within an optimal range. One such adaptation is the decrease in sweat osmolality associated with chronic acclimation. Possible advantages inherent in this increased dilution include maintenance of plasma osmotic pressure via decrease in plasma ion loss and maintenance of plasma volume via water retention associated with plasma solutes.

The one component of sweat which exerts the single greatest influence on total sweat osmolality is sodium. Conservation of sweat sodium has been associated with plasma aldosterone levels in response to both acute and chronic exercise training in the heat. Plasma aldosterone is known to increase in direct relation to exercise and heat intensity, however chronic training may produce decreases in plasma aldosterone. An apparent discrepancy develops between this decrease in aldosterone and the subsequent or progressive decrease in sweat sodium concentration. Some studies have suggested an increased sensitivity to aldosterone by the kidney to account for enhanced renal sodium conservation. Likewise, there is a small body of research which lends support to a similar increase in sensitivity at the eccrine sweat gland.

Statement of the Problem

This investigation was designed to determine the relationship between the hormone aldosterone and eccrine sweat gland sodium reabsorption. More specifically, this experiment attempts to determine whether sweat sodium conservation per unit aldosterone is enhanced following exercise and heat acclimation.

CHAPTER 3

METHODS AND PROCEDURES

Ten healthy college-aged males were recruited from Tucson, Arizona to serve as subjects for this study. Informed written consent was obtained from each subject after all procedures and associated risks had been thoroughly explained. Each subject underwent a graded exercise test on a cycle ergometer (Monarch, Quinton Instruments, Seattle, WA.) to determine maximal oxygen uptake ($\text{VO}_{2\text{max}}$) prior to the acclimation period. A branching protocol utilizing heart rate response was used to establish $\text{VO}_{2\text{max}}$ on the cycle ergometer (Figure 2). Demonstration of $\text{VO}_{2\text{max}}$ was determined by a lack of increase in VO_2 after a subsequent increase in workrate. The acclimation period consisted of 10 consecutive days, during which each participant performed continuous exercise on the bicycle ergometer at 45% $\text{VO}_{2\text{max}}$ for two hours. The ambient environment was maintained at 40°C and 45% relative humidity. Pre-acclimation blood samples were taken on the first day of the control period. Subsequent samples were taken on days 1 and 10 of the acclimation period and on day 1 of the recovery phase. Each blood sample underwent assays for plasma aldosterone and sodium concentration. Sweat samples were taken on days 1 and 10 of the acclimation period and were evaluated for sodium concentration and content. Additional measurements included: 1) Pre and post-exercise nude body weights for the determination of total sweat loss; 2) heart rates (resting and exercise) in order to substantiate a normal acclimation response; 3)

core (T_{re}) and skin (T_{sk}) temperatures to evaluate changes in thermoregulatory capacity; and 4) oxygen uptake for use in calculating sweat loss and metabolic cost of work.

Oxygen Uptake Measurement

Expired gases were collected in meteorological weather balloons through a Hans Rudolph breathing valve and large bore tubing. Compositional analyses for oxygen and carbon dioxide were done with Applied Electrochemistry analyzers (S-3A and CD-3A) calibrated with standard gases analyzed by the Scholander technique (96). Expired volumes were measured with a 120 liter Tissot gasometer (W. Collins Inc., Braintree, MA). Oxygen uptake and carbon dioxide output were calculated from the following equations:

$$\dot{V}O_2 = V_e (\text{STPD}) \times [((1 - FEO_2 - FECO_2) \times .265) - FEO_2]$$

$$VCO_2 = V_e (\text{STPD}) \times (FECO_2 - FICO_2)$$

where:

$\dot{V}O_2$ = oxygen uptake (L/min)

VCO_2 = carbon dioxide output (L/min)

V_e = rate of expired gas volume (L/min)

STPD = correction factor to convert gas
volume to standard temperature (0°C)
and pressure (760 mm Hg dry)

FEO_2 = fraction of oxygen in expired gas (%)

FECO_2 = fraction of carbon dioxide in expired
gas (%)

FICO_2 = fraction of carbon dioxide in inspired
gas (%)

Heart Rate Measurement

Exercise and resting heart rates were determined by a 10-s manual palpation of the carotid artery.

Dietary Analysis

Dietary and fluid recall was recorded by each subject during the five days of control. This menu was then repeated twice during the acclimation period in an effort to standardize exogenous sodium intake during data collection. Dietary recall was analyzed with the Quick Entry of Food program (112) on a Digital LA-180 computer.

Ambient Environment Measurements

Ambient dry-bulb (T_{db}) and wet-bulb (T_{wb}) temperatures were measured with an aspirated sling psychrometer (Taylor Instruments Co. Rochester, NY). Radiant heat was measured with a calibrated YSI thermistor (No. 409, Yellow Springs Instrument Co., Inc., Yellow Springs, OH) placed in a black painted copper bulb. Relative humidity was determined from a standard Cartesian graph.

Body Temperature Measurements

Rectal temperature (Tre) and six skin temperatures (Tsk) (arm, forearm, chest, back, thigh and calf) were measured with individually calibrated YSI series 400 thermistors and with a YSI model 46TUC telethermometer. The rectal probe was inserted approximately 20 cm. Skin thermistors were attached to the skin with holders which allowed essentially free air movement (YSI PH09) (50). The skin and rectal temperatures were used to calculate mean skin (Tsk) and mean body (Tmb) temperatures according to the weighted scheme of Hardy and Dubois (52):

$$Tsk = .06 \text{ arm} + .13 \text{ forearm} + .19 \text{ chest} + .20 \text{ back}$$

$$+ .21 \text{ thigh} + .21 \text{ calf}$$

$$Tmb = .80 \text{ Tre} + .20 \text{ Tsk}$$

Tsk and Tmb were then used to calculate the total heat exchange for rest and exercise using the heat balance equation:

$$M \pm C \pm R - W - E \pm S = 0$$

where:

M= heat gained via metabolism (kcal)

$$= VO_2 \text{ (L/min)} \times \text{min} \times \text{kcal}/LO_2$$

(caloric equivalent for O_2)

$$\text{kcal}/LO_2 = 3.884 + 1.2 \times R$$

R= respiratory exchange ratio

C= heat gained or lost via convection (kcal)

$$= 7.14 (Tsk - Tdb)$$

Tdb= dry bulb temperature

$$m = wt^{.73} (kg) \times ht^{.43} (cm) \times (.7184 \times 10^{-2}) (\text{Body surface area})$$

R= heat gained or lost via radiation (kcal)

$$= -5.58 (T_{sk} - T_r) AR/A$$

T_r= mean radiant temperature

(Black Globe °C)

AR/A= porportion of projected total body

surface area (assume AR/A= 1)

W= heat lost via external work

$$= K_p \times \text{min} \times \text{rpm} \times m$$

K_p= kilograms of resistance

rpm= revolutions per min

m= circumference of bicycle wheel (meters)

S= heat gained or lost by tissues of the body

$$= .83 BW (.8 T_{re} + .2 T_{sk}) = \text{kcal}$$

.83= specific heat content of body tissue

(kcal)

BW= body weight (kg)

E= heat lost via evaporation

$$= M \pm C \pm R - W \pm S$$

Sweat Rate Measurement

Sweat rate was calculated from changes in body weight with the equations of Ekblom et al. (35) :

$$1) \text{ TWL} = \Delta \text{BW} - (1.977 \text{ VCO}_2 - 1.429 \text{ VO}_2) \times 60$$

$$2) \text{ RWL} = \text{Ve (BTPS)} \text{ Dw @ Te} - (\text{Dw @ Ta} - \text{rh}) \times 60$$

$$3) \text{ SR} = \text{TWL} - (\text{RWL} + \text{IWL} + \text{BVL})$$

where:

ΔBW = change in body weight (kg)

SR= sweat rate (g/hr)

TWL= total water loss (g/hr)

RWL= respiratory water loss (g/hr)

IWL= insensible water loss (g/hr),

(assume IWL= 40g/hr)

BVL= volume loss from blood withdrawal (g)

VCO_2 = carbon dioxide output (L/min)

VO_2 = oxygen uptake (L/min)

1.977= density of carbon dioxide (g/L)

1.429= density of oxygen (g/L)

60= min per hour

Ve= rate of expired gas volume (L/min)

BTPS= correction factor to convert gas volume

to body temperature, pressure and saturation.

Dw= density of water (g/L)

Te= temperature of expired air (°C)

Ta= dry bulb temperature of ambient air (°C)

rh= relative humidity of ambient air (%)

Sweat Sodium Measurement

A filter paper method similar to that of Dole et al. (33) was used for the measurement of sweat sodium concentration and content. This method is recommended by Ohara (82) in his review of the various methods of sweat collection and sampling due to its greater accuracy and minimal disturbance of the true sweating response caused by capsule application.

The skin test areas were thoroughly cleaned and dried with surgical soap, alcohol, distilled water and sterile gauze. The pectoral area of the chest (supra-nipple) was used as the collection site, as it has been found to correlate consistently with other areas of the body and represents well the mean sodium concentration of total body sweat (82). An air-tight stainless steel capsule was affixed to the skin with standard toupe' tape. Sweat samples were absorbed on dried and pre-weighed filter paper discs (Whatman, No. 541m analytical grade, 4.5cm diameter). Discs were carefully handled with surgical forceps so as to avoid contamination. Pre- and post-testing storage of discs in silicone coated glassware minimized exogenous sodium absorption.

After absorption, the discs were weighed on an analytical balance (Mettler AE160) for the determination of sweat volume (specific gravity of sweat assumed to be 1). Samples were then dried for 2 hr at 100 °C and eluted with 10 ml of distilled water. The resulting solution was decanted and analyzed for sodium concentration with a NOVA 1 ion sensitive electrode system (Nova Biomedical, Newton, MA).

Chemical Analysis of Blood Samples

Approximately 30 ml of blood was drawn without stasis from an antecubital vein and delivered into tubes coated with lithium heparin. Samples were centrifuged and 1.5 ml of the separated plasma was stored at -20 C for aldosterone analysis at a later date, while the remaining plasma was immediately analyzed for plasma sodium and osmolality. Plasma sodium was analyzed with the Nova 1 system. Plasma osmolality was determined by freezing point depression (Precision Instruments, Sudbury, MA).

Plasma aldosterone levels were analyzed with the Coat-a-Count method solid phase radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). A calibration curve was derived using duplicate tubes coated with a known quantity of aldosterone antibody (0 pg/ml - 1,200 pg/ml). To these tubes and those containing control and subject samples, 220 µl of zero calibrator and 1.0 ml of buffered (I^{125}) aldosterone was added. After gentle and brief vortexing, samples were incubated at 37 °C for three hours. Thorough decanting was followed by

one minute of counting in a gamma counter. Average net counts per minute were corrected for nonspecific binding to yield net counts. Percent bound was then determined with the following equation:

$$\text{Percent Bound} = \frac{\text{Net Counts}}{\text{Net Maximum Binding Counts}} \times 100$$

The calibration curve with percent bound plotted on the vertical axis and concentration of aldosterone plotted on the horizontal axis was then consulted in order to interpolate aldosterone concentrations for the unknowns.

Analysis of Results

Statistical evaluation of the data was performed using a Student's dependent t-test for paired data. In addition, aldosterone values were analyzed via a repeated measures ANOVA (BMDP 2V.6).

300 *kgm (0-3 min)

600 kgm (4-6 min)

900 kgm (7-9 min)

HR<120	120<HR<135	HR>135
1350 kgm	1200 kgm (10-12min)	1050 kgm
HR<150	150<HR<180	HR>180
1800 1650 1500	1650 1500 1350	1500 1350 1200

Add 150 kgm/3 min to max

Recover 5 min

Supra-max 150 kgm to volitional fatigue

* Given 6 meter wheel circumference at 60 rpm.

Figure 2- Cycle Ergometer VO max Protocol

(Modified from: Golding, L.A., C.R. Myers and W.E. Sinning, editors, The Y's Way to Physical Fitness, revised, Chicago: The YMCA of the USA, 1982.)

CHAPTER 4

MANUSCRIPT

Abstract

This investigation was designed to determine the relationship between the levels of plasma aldosterone and eccrine gland activity following exercise training and heat acclimation. Ten subjects exercised at 45% of their maximal oxygen uptake in a hot (40°C) and moderately humid environment (45%rh) for 2h/day on ten consecutive days. Acclimation was verified by significant reductions in peak heart rate, peak rectal temperature, and heat storage, and by significant increases in plasma volume ($\Delta PV=12\%$) and sweat rate on day 10 vs. day 1 ($P<0.05$). The concentration and total content of sodium in sweat samples decreased significantly ($P<0.05$) from day 1 to day 10. Plasma aldosterone levels after 1 hr. were significantly decreased on day 1 vs. day 10. The ratio of sweat sodium reabsorbed (mmol/l) to plasma aldosterone concentration (ng/dl), sodium reabsorption index (SRI), was used as an index of the sweat sodium conservation response per unit aldosterone. SRI values were significantly increased from day 1 to 10, after both hours 1 and 2 of exercise in the heat. These data indicate that 1) plasma aldosterone concentrations decrease following heat acclimation; and 2) eccrine sweat gland sensitivity to aldosterone, as represented by sweat sodium concentrations, can be augmented through exercise training and heat acclimation.

Introduction

Increased sweat solute retention as a result of chronic thermal acclimation was first suggested in 1912 by Hunt (57). Since that time numerous investigations have established decreases in sweat sodium output by sweat glands following prolonged exposure to thermal stress (66,70,77). Enhanced glandular reabsorption was the proposed mechanism for this increased sodium conservation.

As a result of its role in renal sodium conservation, plasma aldosterone has been of primary interest as a possible hormonal regulator of sweat sodium excretion. Conn (19) first produced results linking increased sweat sodium reabsorption to the effects of aldosterone and later reported decreased sodium secretion in sweat of up to 95% with concomitant increases in aldosterone (21). The effect of exogenous aldosterone on sweat electrolyte composition was studied by Grand et al. (43), who reported reductions in sweat sodium levels within 48 hr of injection, with maximal decreases after 5 days. Similar results have been reported by Siegenthaller and de Haller (98), Grandchamp et al. (44) and Collins (15).

In further consideration of the relationship of aldosterone to sweat sodium concentration, Ladell and Shepard (67) demonstrated increases in sweat sodium concentration following aldosterone blockade with spironolactone. Two studies found that aldosterone inhibitors in acclimated subjects abolished the sweat gland sodium conservation response and lead to a simulated pre-acclimation state (42,98).

More recently, work by Sato and Dobson (92) on isolated sweat glands has lent further support to the action of aldosterone in the increase of eccrine gland sodium conservation. Dixon and Schwarz (30) produced evidence suggesting increases in RNA synthesis in sweat glands perfused with exogenous aldosterone. Sato has speculated that the duct cells are the primary site of action for aldosterone, where Na/K-ATPase linked active transport induces reabsorption in both the duct cells and the secretory coil (91).

Based on the interaction between plasma aldosterone concentrations and eccrine sweat gland activity, this investigation was designed to determine the relationship between plasma aldosterone levels and sweat sodium excretion. More specifically, this experiment was an attempt to demonstrate a greater sweat sodium conservation response to aldosterone after chronic exercise in the heat.

Methods and Procedures

Ten healthy male college students volunteered to serve as subjects for this investigation. Written consent was obtained after all participants were apprised of the rationale, methods, and potential risks of the study. Subject descriptive data is as follows; Age 24 ± 2 yrs., Ht. 177 ± 2 cm, Wt. 73.3 ± 2.9 kg, BSA 1.99 ± 0.16 m², $VO_{2\max}$ 3.7 ± 0.12 L/min. The experimental protocol consisted of a 5-day pre-acclimation control period, a 10-day acclimation phase and 2 days of recovery. On the first day of control, each subject reported to the lab in a fasted state for

determinations of baseline levels of plasma aldosterone, sodium, and plasma volume. Pre-acclimation maximal oxygen uptake (VO_2max) values were obtained on the final control day and relative workrates used during heat exposure were determined from these data. Dietary and fluid recall was recorded by each subject during the five days of control. This menu was then repeated twice during the acclimation period in an effort to standardize exogenous sodium intake during data collection. All subjects were encouraged to euhydrate prior to entering the heat chamber, as they were not allowed liquids during the 2-hr period. Measurements for sweat sodium (40 and 100 min), plasma sodium and plasma aldosterone (pre, 1 hr, and post) were taken at rest and during exercise-heat exposure on days 1 and 10 of the acclimation period. Post-acclimation VO_2max , resting plasma volume, plasma aldosterone, and plasma sodium were measured on the second day of the recovery phase.

The exercise and heat exposure protocol consisted of a 2-hr workout in which each subject pedalled (60 rpm) on a cycle ergometer at 45% VO_2max , with a dry-bulb temperature of 40 °C and 45% relative humidity. The same workrates were used for each individual subject on days 1 and 10, while on days 2-9 workrates were adjusted so as to maximize the exercise and heat stress, such that peak rectal temperatures did not exceed 39.5°C at any time during the 2 hours of exposure. Skin (6 sites) and rectal temperatures were taken periodically throughout exercise. Nude body weights were taken prior to, and after 1 and 2 hours of exercise. Heart rates were measured by manual palpation of the carotid artery at 20-min intervals. Expired gas

samples were collected via a Hans Rudolph breathing valve, large bore tubing, and meteorological weather balloons. Fractional expired oxygen and carbon dioxide was measured with Applied Electrochemistry O and CO analyzers and volumes were obtained from a 120-liter Tissot gasometer. Blood samples were taken pre, at 1 hr and immediately post exercise, without stasis from an antecubital vein and delivered into chilled lithium heparin-coated tubes. Samples were then centrifuged and separated for analysis of plasma sodium (Nova 1 ion sensitive electrode system) and plasma aldosterone (Diagnostic Products RIA, Coat-a-count method). The characteristics of the aldosterone assay were as follows; Sensitivity- 15 pg/ml, Between Assay Coefficient of Variability- 8.3% (low standard), 12.6% (normal standard), 3.6% (high standard), Within Assay Coefficient of Variability- 7.3%. Plasma volumes were determined using Evans Blue dye dilution (115). Sweat samples were taken from both sides of the chest via a modification of the filter paper absorption method described by Dole et al. (32), at 40 and 100 minutes of the heat and exercise regimen. The skin was cleaned with surgical soap, alcohol, and distilled water and the collecting capsule was affixed with standard toupe' tape. During sweat collection, dried and pre-weighed filter paper was sealed in the capsule for a period of five minutes. Samples were then replaced in their silicone coated glass jars and weighed on an analytical balance to determine volume (specific gravity of sweat assumed to be 1). After drying for 2 hours at 100 °C, sweat sodium concentrations were determined by saturating the dehydrated filter paper

in 10 ml of distilled water. The resulting solution was decanted and analyzed on the Nova 1 system.

Sweat sodium reabsorption index (SRI) was calculated using the difference between plasma sodium concentration (PS) and sweat sodium concentration (SS), divided by the plasma aldosterone concentration (PA). Precursor sweat, derived from interstitial fluid, has a sodium concentration virtually identical to that of plasma (102). SRI was derived as a means of evaluating changes in sweat sodium reabsorption following exercise and heat acclimation.

Statistical evaluation of pre vs post acclimation data was performed using a Student's t-test for paired data. In addition, aldosterone values were analyzed via a repeated measures ANOVA. All values are reported as mean standard deviation. The Null hypothesis was rejected when $P < 0.05$ and nonsignificant differences were denoted NS.

Results

Changes reflecting heat acclimation

Following ten days of exercise and heat exposure, peak rectal temperature (T_{re}) during the 2-hr exercise and heat exposure decreased significantly from $39.02 \pm .20$ C on day 1 to $38.31 \pm .19$ C on day 10. Likewise, there was a significant reduction in peak heart rates (HR) from 165 ± 4 to 138 ± 5 beats per minute on days 1 and 10, respectively. Heat storage (S) decreased significantly from day 1 (84 ± 7.9 kcal) to day 10 (57 ± 10 kcal). Resting plasma volumes were significantly expanded by 12% after ten days of exercise in the heat, while there was no change in maximal oxygen consumption.

Dietary Analysis

Dietary recall was analyzed with the Quick Entry of Food program (111) on a Digital LA-180 computer. Repetition of the 5-day menu for each subject resulted in no significant differences in total caloric intake or exogenous sodium consumption, when values for the three 5-day periods were compared. Caloric intake was 2268 ± 229 kcal during the control period, and 2166 ± 251 kcal and 2116 ± 201 kcal during the two 5-day periods of experimentation (NS). Sodium intake during control was 159 ± 17 meq, compared with 152 ± 19 meq and 141 ± 16 meq during data collection (NS).

Sweat gland responses to chronic exercise in the heat

Significant increases in whole body sweat loss occurred from day 1 (2434 ± 176 g/2hr) to day 10 (2781 ± 244 g/2hr). Total sweat rates during the first hour (1186 ± 82 g/hr) were not statistically different from those in the second hour (1327 ± 75 g/hr). Local sweat rates were not significantly altered on either collection day. However, local sweat production during the second hour ($8.03 \pm .73$ mg/min/cm²) was significantly greater than that found in the first hour ($4.79 \pm .58$ mg/min/cm²). The concentration and total content of sodium in sweat samples decreased significantly from days 1 to 10 (Figure 1). Sweat sodium concentration in hour one was not statistically different than in hour two, whereas sweat sodium content increased significantly in the second hour of exposure.

Humoral and hormonal changes with acclimation

Acute increases in plasma sodium concentrations during the 2-hr work period were noted on days 1 and 10 (Figure 1). Resting plasma sodium concentrations increased significantly from day 1 to day 10 ($P < 0.05$). However, the levels of plasma sodium were not significantly different when values from day 1 and 10 were compared for hour 1 and hour 2 samples.

Plasma aldosterone increased markedly throughout the exercise and heat exposure on both collection days (Figure 1). Aldosterone concentration of samples taken prior to exercise were not statistically

different from control values for either collection day. Comparison of samples taken after one hour of exercise, for day 1 and 10, revealed a significant decrease in plasma aldosterone concentration. Post-exercise (2HR) samples were not significantly different on days 1 and 10. However, 8 of 10 subjects showed marked decreases in peak aldosterone levels (hour 2) over the course of ten days. This decreasing trend is borne out by comparison of the mean (SD) plasma aldosterone concentrations (post-exercise) on days 1 and 10, 136.3 ± 22.3 ng/dl and 90.0 ± 15.9 ng/dl, respectively.

Endocrine-Eccrine interrelationship

Sweat sodium reabsorption index (SRI) values after chronic heat acclimation were significantly greater than those found prior to exercise training in the heat (Figure 1). Hour 1 SRI values increased from 10.3 ± 6.3 (mm/ng) on day 1 to 28.9 ± 18.4 (mm/ng) on day 10. Likewise, SRI for the second hour on the first day (4.05 ± 2.4 mm/ng) increased to 21.5 ± 27.9 (mm/ng) by the tenth day of the acclimation period.

		Day 1	Day 10
Plasma Sodium (mmol/L)	PRE	146.3±.48	150.6±.45*
	HR1	146.7±1.0	148.6±.50
	HR2	149.3±.87	151.9±.65
Sweat Sodium (mmol/L)	HR1	76.2±36.0	42.8±20.8*
	HR2	103.3±6.9	45.8±8.2*
Aldosterone (ng/dl)	PRE	23.2±3.6	31.1±3.2
	HR1	74.1±15.0	50.9±10.6*
	HR2	136.3±22.0	90.0±15.9
SRI (mm/ng)	HR1	10.3±6.3	28.9±18.4*
	HR2	4.05±2.4	21.5±27.9*

* P<0.05 Day 1 vs 10

Figure 1- Humoral, Hormonal and Sweat Changes with Acclimation

Discussion

The physiological adaptation to chronic exercise and thermal stress, as represented by decreases in heat storage, peak T_{re} , and peak HR, as well as increased plasma volume, were evident by the final day of the acclimation period. Total body sweat rates also increased significantly from day 1 to day 10. Previous studies (15,92) have indicated decreased sweat rates in response to increased exogenous aldosterone. Our results did not support this relationship between plasma aldosterone and sweat rate. When comparisons were made between the first and second hour sweat rates, no significant differences were noted, whereas aldosterone levels over the same period increased almost 100%. Even more dramatic are the local sweat rates, which increased significantly from hour 1 to hour 2, while aldosterone also increased. Results from this investigation suggest an uncoupling of the relationship between sweat rate and plasma aldosterone concentration in chronically acclimated subjects.

The decreases in sweat sodium concentration after chronic heat acclimation found in the present study, have been noted in several previous investigations (18,41). The acute increases observed in plasma sodium have also been documented previously (80). Chronically, plasma sodium is highly regulated and without the effects of a sodium deficit, significant changes would not be expected.

Plasma aldosterone concentrations, both acute and chronic, appear to increase during exercise and heat acclimation (25,77). The acute changes observed in this study are consistent with values previously

reported in magnitude of increase during heat stress, but are considerably higher in absolute level (ng/dl). However, the decreases in plasma aldosterone levels following exercise and heat acclimation have not been previously reported. Studies by Hellman et al. (52) and Crabbe' et al. (26) have shown decreases in urinary aldosterone with acclimation and sodium deprivation, respectively. However, these decreases in urinary aldosterone occurred after peak aldosterone levels had been established after approximately five days of acclimation or sodium deprivation. Our results may be due to the very high heat stress which our subjects endured and the fact that they were exposed to the same absolute workrate on day 1 and day 10 of the exercise-heat regimen and therefore a lower relative stress as they became acclimated.

The most interesting aspect of the data is the relationship between sweat sodium concentration and plasma aldosterone levels (SRI). Increased sweat sodium reabsorption in non-acclimated subjects following increased aldosterone concentrations has been well documented in previous investigations (44,92). However, our results demonstrate these same increases in sodium conservation with marked decreases in plasma aldosterone concentrations. Increased SRI values from pre to post acclimation for both hour one and hour two may indicate a greater eccrine gland aldosterone sensitivity following ten days of exercise in the heat. Similar adaptations in sodium conservation response to aldosterone have been demonstrated at the renal tubules of subjects exposed to chronic intermittent increases in core temperature via endurance training. An investigation by Skipka et al. (100) studied the

correlation between renal sodium excretion and $\dot{V}O_2$ max following 0.5 mg aldosterone injections. The increased renal sensitivity reported by that investigation could in part be due to the physiological adaptation to the dissipation of metabolic heat. Partial heat acclimation in humans has been associated with increases in metabolic heat production as a result of exercise training (114).

Other studies directly investigating the role of aldosterone in mediating sweat sodium reabsorption have produced mixed results. Sato and Dobson (92) demonstrated increased sodium reabsorption in sweat glands following d-aldosterone injections in resting subjects. Furthermore, aldosterone blockade with spironolactone has been shown to increase sweat sodium concentrations during acclimation (67). In contrast, McConahay et al. (74) reported no effect on sweat sodium concentrations with d-aldosterone injections of 5-6 times the normal level. Similarly, Braun et al. (10) observed no differences in sweat sodium conservation or acclimation time in subjects receiving 1.0 mg/day of d-aldosterone. However, cessation of the drug greatly increased both urinary and eccrine gland sodium excretion. Our results indicate that these studies may in fact provide evidence showing that the sodium conservation response in sweat glands is not dependent merely upon the total amount of aldosterone present, but the sensitivity of eccrine glandular receptor tissue to aldosterone. This was supported in a study by Grand et al. (43), where the comparison of 1.0 and 2.0 mg aldosterone doses demonstrated no relationship between sweat sodium output and total dosage in non-acclimated subjects. Furthermore, Rosenberg et al. (90)

supplied evidence showing initial sweat sodium conservation in non-acclimated subjects following large doses of aldosterone, with a return to normal excretion after 3 days. Such results suggest that in the absence of physiological adaptations produced by thermal acclimation, aldosterone has both a transient and limited effect on the eccrine sweat gland.

Furman and Beer (41) have proposed sweat gland conditioning (e.g., increased sensitivity) to be a normal response in thermal acclimation. If glandular sensitivity to aldosterone is commensurate with the state of acclimation, the results of Braun et al. (10) would provide support for this hypothesis. That is, post-acclimation when aldosterone sensitivity is greatest, cessation of aldosterone treatment initiated marked increases in sweat sodium secretion. In a similar fashion, without eccrine glandular adaptations to heat stress, subjects in the McConahay et al. (74) investigation would not be expected to respond to supra-normal aldosterone doses. Our results, showing a statistically significant increase in the amount of sweat sodium reabsorbed per unit aldosterone following acclimation, are consistent with this hypothesis.

Summary

Results from the present investigation lend support to the hypothesis that eccrine gland sensitivity to aldosterone may be augmented through exercise and heat acclimation. Combined with increased renal sodium conservation, this adaptation could serve as a mechanism for electrolyte maintenance during prolonged exposure to high thermal stress. Chronic conservation of solutes, especially sodium, is critical for maintaining plasma osmolality, and the physiological benefits associated with increased plasma volume. Further investigation of the relationship between sweat sodium conservation and plasma aldosterone levels is necessary in order to determine the exact nature and location of sensitivity changes associated with thermal acclimation.

REFERENCES

1. Amatruda, T. and L. Welt. Secretion of electrolytes in thermal sweat. *J. Appl. Physiol.* 5: 759-772, 1953.
2. August T. J., D.H. Nelson and G.W. Thorn. Response of normal subjects to large amounts of aldosterone. *J. Clin. Inv.* 37: 1549-1555, 1958.
3. Aurell, M. and P. Viskren. Plasma renin activity in supine muscular activity. *J. Appl. Physiol.* 31: 839-859, 1971.
4. Baclar, J.O., W.D. Swanson and R.T. Woodyatt. Fever and the water reserve of the body. *Arch. Int. Med.* 24: 116-128, 1919.
5. Barger, A.C., R.D. Berlin and J.F. Tulenko. Intrarenal arterial infusion of aldosterone and diuretic hormone in normal and adrenalectomized dogs; electrolytes and water excretion. *Fed. Proc.* 16: 6, 1975.
6. Bartter, F. The role of extracellular fluid in the control of aldosterone secretion in man. *J. Clin. Inv.* 35: 688, 1956.
7. Blair-West, J.R., J.P. Coghlan, D.A. Denton, D. Scott and R.D. Wright. The role of aldosterone in renal sodium conservation during sodium depletion. *Austr. J. Expt. Biol. Med. Sci.* 46: 525-539, 1968.
8. Bozovic, L. and J. Castenfors. Effect of dihydralazine on plasma renin activity and renal function during supine exercise in normal subjects. *Acta. Physiol. Scand.* 70: 281-289, 1967.
9. Bozovic, L. and J. Castenfors. Effect of ganglionic blocking on plasma renin activity in exercise and pain stressed rats. *Acta. Physiol. Scand.* 70: 290-292, 1967.
10. Braun, W.E., J.T. Maher and R.F. Byron. Effect of exogenous d-aldosterone on heat acclimation in man. *J. Appl. Physiol.* 23: 341-361, 1967.
11. Brubacher, E.S. and A.J. Vander. Sodium deprivation and renin excretion in unanesthetized dogs. *Am. J. Physiol.* 214(1): 15-21, 1968.

12. Bulmer, M. and G. Forwell. The concentration of sodium in thermal sweat. *J. Physiol.* 13: 1115-1122, 1956.
13. Castenfors, J. Effect of ethracynic acid on plasma renin activity during supine exercise in normal subjects. *Acta. Physiol. Scand.* 70: 215-220, 1967.
14. Chen, W.Y. and R.S. Elizondo. Peripheral modification of thermoregulatory function during heat acclimatization. *J. Appl. Physiol.* 37: 367-373, 1974.
15. Collins, K.J. Action of exogenous aldosterone on the secretion and composition of drug-induced sweat. *Clin Sci.* 30: 207, 1969.
16. Collins, K.J., G.W. Crockford and J.S. Weiner. The local training of secretory activity on the response of eccrine sweat glands. *J. Physiol.* 184: 203-214, 1966.
17. Collins, K.J., K.G. Foster and J.L. Hubbard. Effect of aldosterone on mammalian eccrine sweat glands. *Experientia* 12: 1313-1314, 1970.
18. Conn, J.W., L.H. Louis, M.W. Johnston and B.J. Johnson. The electrolyte content of thermal sweat as an index of adrenocortical function. *J. Clin. Inv.* 27: 529-530, 1948.
19. Conn, J.W. The mechanism of acclimation to heat. *Adv. Intern. Med.* 3: 373, 1949.
20. Conn, J.W. and L.H. Lavis. Production of endogenous "salt-active" corticoids as reflected in the concentrations of sodium and chloride of thermal sweat. *J. Clin. End.* 10: 12, 1950.
21. Conn, J.W. Aldosteronism in man., *JAMA* 183: 135-140, 1963.
22. Consolazio, C.F., R.E. Johnson and L.J. Pecora. Physiological measurements of metabolic functions in man. McGraw-Hill Co., N.Y., 1963.
23. Convertino, V.A. Heart rate and sweat rate responses associated with exercise induced hypovolemia. *Med. Sci. Sp. Ex.* 16: 77-82, 1983.
24. Convertino, V.A., L.C. Keil, E.M. Bernauer and J.E. Greenleaf. Plasma volume, osmolality, vasopressin and renin activity during graded exercise in man. *J. Appl. Physiol.* 50: 123-128, 1981.

25. Costill, D.L., G. Branam, W. Fink and R. Nelson. Exercise induced sodium conservation: changes in renin and aldosterone. *Med. Sci. Sp. Ex.* 8: 209-213, 1976.
26. Crabbe', J., W.J. Reddy, F. Ross and G. Thorn. The role of adrenal cortex in the normal adaptation to dietary sodium deprivation. *J. Clin. End.* 18 1147-1157, 1958.
27. Crandall, F.M. Inanition fever. *Arch. Pediat. NY.* 16: 174-180, 1899.
28. Dill, D.B., F.G. Hall and H.T. Edwards. Changes in sweat composition during acclimatization to heat. *Am. J. Physiol.* 123: 412-419, 1938.
29. Dill, D.B., F.G. Hall and W. van Beaumont. Sweat chloride concentration, sweat rate, metabolic rate, skin temperature and age. *J. Appl. Physiol.* 21: 99-106, 1966.
30. Dixon, K. and V. Schwarz. Uptake of aldosterone by human skin and stimulation of RNA synthesis in the gland in vitro. *J. End.* 45: 231-244, 1969.
31. Dobson, R.L. and J.F.R. Slegers. The effect of aldosterone on sweating in the cat. *J. Inv. Derm.* 56: 337-339, 1971.
32. Dole, V.P., B.G. Stall and I.L. Schwartz. Methods for local induction and quantitative ananlysis of human sweat. *Proc. Soc. Exp. Biol. Med.* 77: 412-415, 1951.
33. Dontas, S. Uber den mechanismus der warmeregulation. *Arch. t.d. ges Physiol.* 241: 612-629, 1939.
34. Ekblom, B., C.S. Greenleaf, J.E. Greenleaf and L. Hermansen. Temperature regulation during continous and intermittent exercise in man. *Acta. Physiol. Scand.* 81: 1-10, 1971.
35. Elizondo, R. Local control of eccrine sweat gland function. *Fed. Proc.* 32: 1583-1587, 1973.
36. Faggard, R., A. Amery, T. Reybrouck, P. Linsen, E. Moerman, M. Bogart and A. de Schaepdryver. Effects of angiotensin antagonism on hemodynamics, renin and catecholamines during exercise. *J. Appl. Physiol. Resp. Env. Ex. Physiol.* 43: 440-444, 1977.
37. Few, J.D., G.C. Cashmore and G. Turton. Adrenocortical response to 1-leg and 2-leg exercise on a bicycle ergometer. *Eur. J. Appl. Physiol.* 44: 167-174, 1980.

38. Fletcher, K., C. Leithead, T. Deegan, M. Pallister, A. Lind and B. Maegraith. Aldosterone excretion in acclimatization to heat. *Ann. Trop. Med. Par.* 55: 498-504, 1961.
39. Ford, H., H. Peiters and R. Bailey. Aldosterone and sodium conservation: The effect of acute dietary sodium deprivation on plasma concentration, the metabolic clearance, secretion, and excretion rates of aldosterone in normal subjects. *J. Clin. End. Met.* 28: 481-491, 1968.
40. Fox, R.H., R. Goldsmith, I. Hampton and H.E. Lewis. The nature of the increase in sweating capacity produced by heat acclimatization. *J. Physiol.* 171: 368-376, 1964.
41. Furman, K.I. and G. Beer. Dynamic changes in sweat electrolyte composition induced by heat stress as an indication of acclimatization and aldosterone activity. *Clin. Sci.* 24: 7-12, 1963.
42. Gibinski, K., S. Nowak, L. Giec and F. Kokut. Aldosterone and sweat gland function. *Acta. Med. Polona* IV 3: 313-320, 1963.
43. Grand, R.J., P.A. di Sant'Agnese, R.C. Talamo and J.C. Pallavicini. The effect of exogenous aldosterone on sweat electrolytes. *J. Pediatrics* 70: 346-356, 1967.
44. Grandchamp, R.J., J.R. Scherrer, R. Veyrat and A.F. Muller. Measurement of sweat sodium and potassium excretion for evaluation of mineralocorticoid activity in normal subjects. *Helv. Med. Acta.* 34: 367-385, 1968.
45. Greene, C.H. and L.G. Rowntree. The effect of experimental administration of excessive amounts of water on body temperature. *Am. J. Physiol.* 80: 229-235, 1927.
46. Greenleaf, J.E., P.S. Brock, J.T. Morse, W. van Beaumont, L.D. Montgomery, V.A. Convertino and G.R. Mangseth. Effect of sodium and calcium ingestion on thermoregulation during exercise in man. In: *New trends in thermal physiology*, edited by Y. Houdas and J.D. Guieu, Paris, 1975, pp. 157-160.
47. Greenleaf, J.E. and B.L. Castle. Exercise temperature regulation during hypohydration and hyperhydration. *J. Appl. Physiol.* 30: 847-853, 1971.
48. Greenleaf, J.E., W. van Beaumont, P.S. Brock, L.D. Montgomery, J.T. Morse, E. Schwartz and S. Kravik. Fluid electrolyte shifts and thermoregulation: Rest and work in the heat with head cooling. *Av. Sp. Env. Med.* 51: 747-753, 1980.

49. Greenleaf, J.E. and B.A. Williams. Thermistor holder for skin temperature measurements. NASA Tech. Brief B7-10119, 1974.
50. Haight, J.S. and W.R. Keatinge. Elevation in set point for body temperature regulation after prolonged exercise. *J. Physiol.* 22: 29-77, 1973.
51. Hardy, J.D. and E.B. Dubois. The technique of measuring radiation and convection. *J. Nutr.* 15: 461-475, 1938.
52. Hellman, K., K. Collin, C. Gray, R. James, J. Lunnion and J. Weiner. The excretion of urinary adrenocortical steroids during heat stress. *J. End.* 14: 209-216, 1956.
53. Henane, R., R. Flandrois and J.P. Charbonnier. Increase in sweating sensitivity by endurance conditioning in man. *J. Appl. Physiol.* 43: 822-828, 1977.
54. Hensel, J. Cutaneous thermoreceptors. In: "Handbook of sensory physiology". A. Iggo, Editor, vol. 2, pp. 79-110, Springer, NY.
55. Hori, S., A. Inouye and H. Ihzuka. Indices of sweating patterns for the assesment of heat tolerance. *Jap. J. Physiol.* 24: 263-275, 1974.
56. Hori, S. and H. Yoshimura. Comparison of changes in sweating reaction between short term and long term heat acclimatization. In: New Trends in thermal physiology. Proceedings of the satellite symposium on thermal regulation, Lille, France. July, 1977, XXVII Int. Congr. of Physiol. Sci. Edited by Y. Houdas and J. Guieu, NY 1978.
57. Hunt, J. J. Hyg. 12: 479-488, 1912.
58. Johnson, R., G. Pitts and F. Consolazio. Factors influencing chloride concentration in human sweat. *Am. J. Physiol.* 128: 575-589, 1944.
59. Katz, B. and S. Thesleff. A study of the desensitization produced by ACH at the motor end-plate. *J. Physiol.* 138: 63-80, 1957.
60. Keys, A. and L. Addison. Calcium changes in plasma resulting from brief severe work and questions as to the permiability of capillaries to calcium. *Am. J. Physiol.* 115: 539-547, 1936.
61. Keys, A. and H. Taylor. The behavior of plasma colliods in response to breif severe work and the question as to the permiability of the capallaries to protiens. *J. Biol. Chem.* 109: 55-67, 1935.

62. Koboyashi, Y., Y. Ando, N. Okuda, S. Takaba and K. Ohara. Effects of endurance training on thermoregulation in females. *Med. Sci. Sp. Ex.* 12: 361-364, 1980.
63. Kosunen, K.J. and A.J. Parkarinen. Plasma renin, angiotensin II and plasma and urinary aldosterone in running exercise. *J. Appl. Physiol.* 41: 26-29, 1976.
64. Kotchen, T., L. Hartley, T. Rice, E. Mougey, L. Jones and J. Mason. Renin, norepinephrine, epinephrine, responses to exercise. *J. Appl. Physiol.* 31: 178-184, 1971.
65. Kozlowski, J., J.E. Greenleaf, E. Turlejka and K. Nazar. Extracellular hyperosmolality and body temperature during physical exercise in dogs. *Am. J. Physiol. Reg. Integ. Comp. Physiol.* 23: R180- R183, 1980.
66. Kuno, Y. Human Perspiration, Charles C. Thomas, Springfield, IL. 1954.
67. Ladell, W.S.S. and R.J. Shepard. Aldosterone inhibition and acclimatization to heat. *J. Physiol.* 160: 19-20P, 1961 (Abstract).
68. Leon, A., W. Pettinger and M. Saviano. Enhancement of serum renin activity by exercise in rats. *Med. Sci. Sp. Ex.* 5: 31-40, 1972.
69. Lloyd, D.P. Secretion and reabsorption in sweat glands. *Proc. Natl. Acad. Sci. U.S.* 45: 405-409, 1959.
70. Locke, W., N. Talbot, H. Jones and J. Worcester. Studies on the combined use of measurements of sweat electrolyte composition and rate of sweating as an index of adrenocortical activity. *J. Clin. Inv.* 30: 325-337, 1971.
71. Luetscher, J. and R. Curtis. Aldosterone: regulation of sodium and potassium balance. *Ann. Int. Med.* 43: 658, 1955.
72. Marriot, W. Anhydremia. *Physiol. Rev.* 3: 275-295, 1923.
73. McCance, R. The effect of salt deficiency in man on the volume of extracellular fluids and a comparison of sweat saliva, gastric juice and cerebrospinal fluid. *J. Physiol.* 92: 208-218, 1938.
74. McConahay, T., S. Robinson and J. Newton. d-Aldosterone and sweat electrolytes. *J. Appl. Physiol.* 19: 575-579, 1964.
75. Michelakis, A.M. and R. Horton. The relationship between plasma renin activity and aldosterone in normal man. *Circ. Res. Suppl.*, Suppl. I 185: 194, 1970.

76. Mitchell, D., L.C. Senay, C.H. Wyndham, A.J. Van Rensburg, G.G. Rogers and N. B. Strydom. Acclimatization in a hot humid environment, energy exchange, body temperature and sweating. *J. Appl. Physiol.* 40: 768-778, 1976.
77. Moshimura, M. Role of aldosterone in acclimatization. *J. Phys. Soc. Jap.* 26: 403-421, 1964.
78. Myers, R.D. and W.L. Veale. Body temperature: possible ionic mechanism in the hypothalamus controlling the set point. *Science* 170: 95-97, 1970.
79. Nadel, E.R., K.B. Pandolf, M.F. Roberts and J.A. Stolwijk. Sweating and skin blood flow control during progressive acclimatization. *Isr. J. Med.* 12(8): 812-814, 1976.
80. Nielsen, B. Effects of changes in plasma volume and osmolality on thermoregulation during exercise. *Acta. Physiol. Scand.* 90: 725, 1974.
81. Ohara, K. Chloride concentration in sweat: its individual, regional, seasonal, and some other variations and interrelations between them. *Jap. J. Physiol.* 16: 274-298, 1966.
82. Peart, W.J. The renin-angiotensin system. *Pharm Rev.* 17: 143-182, 1965.
83. Peter, J. and C. Wyndham. Activity of human eccrine sweat gland during exercise in a hot humid environment: before and after acclimatization. *J. Physiol.* 187: 583-594, 1966.
84. Roberts, M.F., C.B. Wenger, J.A. Stolwijk and E.R. Nadel. Skin blood flow and sweating changes following exercise training and heat acclimatization. *J. Appl. Physiol.* 43: 133-138, 1977.
85. Robinson, S., R.K. Kinkaid and R.K. Rhamy. Effect of salt deficiency on salt concentration in sweat. *J. Appl. Physiol.* 3: 55-62, 1950.
86. Robinson, S., R. Maletich, W. Robinson, B. Rohrer and A. Kunz. Output of sodium chloride by sweat glands and kidneys in relation to dehydration and salt depletion. *J. Appl. Physiol.* 8: 615-620, 1956.
87. Robinson, S., J. Nicholas, J. Smith, W. Daly and M. Pearcy. Time relation of renal and sweat gland adjustments to salt deficiency in men. *J. Appl. Physiol.* 8: 159-165, 1955.

88. Romero, J., R. Standon, M. Dufau, R. Dohmen, A. Binia, B. Killman and J. Fasciolo. Changes in fluid compartments, renal hemodynamics, plasma renin and aldosterone secretion induced by low sodium intake. *Metabolism* 17: 10-19, 1968.
89. Rosenberg, E., M. Demany, E. Bunditz, R. Underwood and R.S. Leard. Effects of administration of large amounts of d-aldosterone in normal subjects and subjects with sheehan's syndrome. *J. Clin. End.* 22: 465, 1962.
90. Ross, E.J., W.I. Reddy, A. Riveria and G.W. Thorn. Effects of intravenous infusion of d-aldosterone acetate on sodium and potassium excretion in man. *J. Appl. Physiol.* 23: 341, 1967.
91. Sato, K. The physiology, pharmacology and biochemistry of the eccrine sweat gland, In: *Rev. Physiol. Biochem. Pharmacol.*, Vol. 79 Springer Verlag, 1977.
92. Sato, K. and R.L. Dobson. The effect of intracutaneous d-aldosterone and hydrocortisone on human eccrine sweat gland function. *J. Inv. Derm.* 54: 450-460, 1970.
93. Sato, K. and R.L. Dobson. Enzymatic basis for the active transport of sodium in the duct and secretory portion of the eccrine sweat gland. *J. Inv. Derm.* 55: 53-56, 1970.
94. Schneider, E., J. Davis and J. Bambers. Hepatic metabolism of renin and aldosterone. *Circ. Res. Suppl. I* 1: 175, 1970.
95. Scholander, P.F. Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. *J. Biol. Chem.* 167: 235-250, 1947.
96. Schwartz, I. and J. Thaysen. Excretion of sodium and potassium in thermal sweat. *J. Clin. Inv.* 35: 114-120, 1956.
97. Senay, L.C., D. Mitchell and C.H. Wyndham. Acclimatization in a hot humid environment: body fluid adjustments. *J. Appl. Physiol.* 40: 786-796, 1976.
98. Siegenthaller, P. and R. de Haller. Consideration of diagnostic problems of cystic fibrosis and of pancreatic mucoviscoidosis in adults. *Helvet. Med. Acta.* 32: 1, 1965.
99. Siegenthaller, P., R. de Haller, R. Veyrat and F. Muller. *Helvet. Med. Acta.* 29: 550, 1962.
100. Skipka, W. Augmented renal sensitivity to aldosterone in endurance trained subjects. *Med. Sci. Sp. Ex.* 14: 118, 1982 (Abstract).

101. Slegers, J.F.G. The mechanism of eccrine gland function in normal subjects and patients with mucoviscidosis. *Dermatologica* 127: 242, 1963.
102. Slegers, J.F.G. The mechanism of sweat secretion. *Pflugers Arc.* 279: 256-273, 1964.
103. Slegers, J.F.G. The influx and outflux of sodium in the sweat gland. *Dermatologica* 132: 152-174, 1966.
104. Smiles, K.A. and S. Robinson. Sodium ion conservation during acclimatization of man to work in the heat. *J. Appl. Physiol.* 31: 63-69, 1971.
105. Sobocinska, J. and J.E. Greenleaf. Cerebrospinal fluid calcium and rectal temperature response during exercise in dogs. *Am. J. Physiol.* 230: 1416-1419, 1976.
106. Streeten, D., J. Conn, H. Lewis, S. Fajan, H. Seltzer, R. Johnson, R. Gittler and A. Dube. Secondary aldosteronism: Metabolic and adrenocortical responses of normal men to high environmental temperatures. *Met. Clin. Exptl.* 9: 1071, 1960.
107. Sundsfjord, J., S. Stromme, H. Retsum and A. Aakvaag. Plasma aldosterone, plasma renin activity and cortisol in exercise. *Acta. End. Suppl.* 177: 184, 1973.
108. Udea, H., H. Tagawa, M. Ishti and Y. Kaneko. Effect of renal denervation on the release and content of renin in unanesthetized dogs. *Jap. Heart J.* 8: 156-167, 1967.
109. Walker, W.G., J.S. Horvath, M.A. Moore and P.K. Whelton. Relation of plasma renin activity and plasma concentration and response to postural and volume changes. *Circ. Res.* 38: 477-483, 1976.
110. Winer, N., D. Choski, M. Sun Yoon and A. Freedman. Adrenergic receptor mediation of renin. *J. Clin. End. Met.* 29: 1168-1178, 1969.
111. Witchie, J., H. Kowaloff, S. Bloom and W.V. Flack. The analysis of dietary data: An interactive computer method for storage and retrieval. *J. Am. Diet. Assoc.* 78: 609, 1981.
112. Wright, D. Control of secretion of aldosterone. *Br. Med. Bull.* 18: 159-163, 1962.
113. Wyndham, C.H. Effect of acclimatization on the sweat rate rectal temperature relationship. *J. Appl. Physiol.* 22: 27-30, 1967.

114. Wyndham, C.H. The physiology of exercise under heat stress. Ann. Rev. Physiol. 35: 193-200, 1973.
115. Young, H.L., L. Juhos, B.L. Castle, J. Yusken and J.E. Greenleaf. Body water compartments during bed rest: evaluation of analytical methods. NASA Tech. Rep. R-406, pp. 1-19, 1973.
116. Zambraski, E.J. and M. Tucker. Decreased renin release in guanethidine treated dogs. Med. Sci. Sp. Ex. 12: 140, 1980 (Abstract).
117. Zierler, K.L. and J.L. Lilienthal. Sodium loss in man induced by desoxycorticosterone acetate. Am. J. Med. 4: 186, 1948.