รายงานผลการวิจัย

เรื่อง

การศึกษาเมแทบอลิซึมของน้ำในร่างกายสัมพันธ์กับการทำหน้าที่ของไตในกระบือปลักที่ได้รับความเครียดเนื่องจากความร้อนเป็นเวลานาน

(Changes in water metabolism in relation to renal functions of Swamp buffaloes during short term exposed to the solar radiation)

โดย

ณรงค์ศักดิ์ ชัยบุตร

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สมชาย จันทร์ผ่องแสง

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มีนาคม 2532

ทุนวิจัยงบประมาณแผ่นดินปี 2531

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ชื่อโครงการวิจัย การศึกษาเมแทบอลิซึมของน้ำในร่างกายสัมพันธ์กับการทำหน้าที่ของไต ในกระบือปลักที่ได้รับความเครียดเนื่องจากความร้อนเป็นเวลานาน

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เดือนและปีที่วิจัยเสร็จ มีนาคม 2532

บทคัดย่อ

การศึกษาผลของความเครียดเนื่องจากความร้อนเป็นเวลานานต่อการเปลี่ยนแปลงของน้ำในร่างกายและการทำหน้าที่ของไต ทำในกระบือสาว น้ำหนักระหว่าง 174-207 กก. จำนวน 4 ตัว กระบือจะได้รับความร้อนโดยการตากแดด วันละ 8 ชั่วโมง เป็นเวลา 10 วัน ภายหลังจากที่กระบืออยู่ในสภาวะแวดล้อมที่มีอุณหภูมิปกติ (ระยะควบคุม) เมื่อกระบือได้รับความเครียดเนื่องจากความร้อน พบว่า การหมุนเวียนของน้ำในร่างกายเพิ่มขึ้น ในขณะที่ปริมาณน้ำทั้งหมดในร่างกายและปริมาตรเลือดลดลง ในวันที่ 5 หลังจากที่ตากแดดมีการเพิ่มขึ้นของปริมาตรพลาสม่าเล็กน้อยร่วมกับการลดลงของปริมาณน้ำทั้งหมดในร่างกาย ส่วนในวันที่ 10 หลังจากตากแดด พบว่ามีการลดลงของทั้งปริมาตรพลาสม่า และปริมาตรเลือด กระบือที่ได้รับความร้อนจะมีการเพิ่มขึ้นของความเข้มข้นของกลูโคส โปรตีน ยูเรีย และครีอะตินีนในพลาสม่า แต่ไม่พบการเปลี่ยนแปลงของอัตราการกรองผ่านกลอเมอรูลัสและปริมาณของเลือดไหลผ่านไต ในวันที่ 10 หลังจากได้รับความร้อนมีการเพิ่มขึ้นของอัตราการไหลของปัสสาวะควบคู่ไปกับการเพิ่มขึ้นของค่าออสโมล่าเคลียรานซ์ การขับทิ้งของโปตัสเซี่ยมทางไตและค่าพีเอชของปัสสาวะ ความเข้มข้นของคลอไรด์ในพลาสม่าเพิ่มขึ้นในวันที่ 10 หลังจากกระบือได้รับความร้อน ในกระบือที่ตากแดดพบว่าการขับทิ้งของอนินทรีย์ฟอสฟอรัสและคลอไรด์ทางไตลดลง การขับทิ้งของโซเดียมทางไตเพิ่มขึ้นใน 5 วันแรกและลดลงในวันที่ 10 เมื่อได้รับความร้อน จากผลการทดลองสรุปได้ว่าในขณะที่กระบือได้รับความเครียด เนื่องจากความร้อนจะมีการเปลี่ยนแปลงการกระจายของน้ำ

ในร่างกาย ซึ่งแบ่งออกเป็น 2 ระยะ คือในระยะ 5 วันแรกจะมีการเคลื่อนย้ายของน้ำออกจากเซล และในระยะที่ 2 เมื่อสัตว์ได้รับความร้อน 10 วัน ซึ่งจะมีการสูญเสียน้ำออกจากพลาสม่า การเพิ่มขององค์ประกอบในพลาสม่าขณะที่สัตว์ได้รับความร้อนเป็นขบวนการปรับตัวเพื่อที่จะกระตุ้นให้มีการรักษาปริมาณน้ำนอกเซล การศึกษาการทำหน้าที่ของไตบ่งชี้ว่าใน 5 วันแรกของการได้รับความร้อน การเปลี่ยนแปลงของน้ำนอกเซลส่วนหนึ่งถูกกำหนดโดยปริมาณของโซเดียมที่เสียไปทางไต เมื่อสัตว์ได้รับความร้อนเป็นเวลา 10 วัน สัตว์จะอยู่ในภาวะขาดน้ำ ซึ่งควบคู่ไปกับการเพิ่มขึ้นของการสงวนโซเดียมโดยการดูดกลับทางไต การเปลี่ยนแปลงของการขับทิ้งโปตัสเซียมทางไตในวันที่ 10 ของการได้รับความร้อนเป็นผลเนื่องมาจากการควบคุมสมดุลกรด-ด่าง ในขณะที่ร่างกายของสัตว์อยู่ในภาวะด่างเกิน จากการเพิ่มอัตราการหายใจเมื่ออยู่ในสภาวะแวดล้อมที่มีอุณหภูมิสูง

Project Title : Changes in water metabolism in relation to renal functions of swamp buffaloes during short term exposed to the solar radiation.

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Abstract

The effects of short term heat exposure on the changes of body fluid and renal function were studied in four heifer buffaloes weighing between 174-207 kg. The animals were individually exposed to the sun upto 8 h each day over period of ten days (nonshaded period). Before this, they were kept in a normal ambient temperature (control period). Each animal was fed the same diet throughout experimental periods. In short term heat exposure, nonshaded buffaloes increased body water turnover rate while the marked decreases in total body water and blood volume were noted. On the first five days, there was initial increase in plasma volume with the decrease in total body water. On the tenth day of measurement, there were decreases in both plasma and blood volume. Short term heat exposed buffaloes showed increases in plasma concentrations of glucose, protein, urea and creatinine.

During heat exposure, nonshaded buffaloes showed no significant changes of both GFR and E-RPF. On day ten of heat exposure, the increase

in the rate of urine flow was coincided with increases of osmolar clearance, renal excretion of K^+ and urine pH. Plasma chloride concentration significantly increased on the tenth day of heat exposure. The renal excretion of Pi and Cl^- decreased throughout nonshaded period while that of Na^+ increased in the first five day and fell down thereafter. It can be concluded that during heat exposure,the change in body fluid distribution is drawn into two phases of response for evaporative cooling. The first phase occurred within the first five days which was characterized by mobilization of cellular water. A second phase of response occurred on the tenth day of measurement indicated the main loss of plasma water. The increases in plasma constituents during heat expsoure would augment to maintain extracellular water during animals exposed to hot environment. The renal function studies suggested that on the first five days of heat exposure, the maintenance of extracellular fluid was regulated in part by Na^+ loss from the kidney. On the tenth day of heat exposure, the animals let to the state of dehydration which was consistent to increase in renal Na^+ retention. The renal behavior of electrolyte excretion particularly K^+ in the tenth day nonshaded buffaloes was due to acid-base regulation during respiratory alkalosis.

Introduction

It has been well known that control of the turnover of water and electrolytes is important in the survival of mammals in tropical areas. Some aspects of the effect of acute heat exposure on water metabolism in swamp buffaloes have been reported by Chaiyabutr and Co-worker (1987a). These responses entailed changes in body fluids which were reflected in an increase in water turnover rate with an increase in blood volume including both plasma and cell volume. These changes indicate higher water requirement during acute heat exposure for evaporative cooling. However, few experiments on the influence of short term heat exposure on changes in body fluid of buffaloes have been reported.

Unlike many other domestic ruminants, water buffaloes are known to have less sweat gland and are probably less tolerant to solar radiation. The heat dissipation means in buffaloes rely on respiration for evaporative cooling. The roles of body fluid in heat dissipation mechanism are complex. Many bodily components affecting homeostasis play a role in adjusting for losses or gains of water and electrolyte from the body. The way of adjusting for loss, the kidneys are the only system which can control the rate of fluid loss; the extrarenal losses, both G.I. and insensible, would presumably occur with little or no control.

The purpose of the present report was to determine the extent to which the effect of short term exposure to solar radiation on the body fluid and renal function of heifer buffaloes.

Materials and methods

Four heifer swamp buffaloes weighing between 174-207 kg were used in the experiment. They were kept and tethered in the pen under roof-metal shaded or unshaded part which direct exposure to solar radiation. The experiments were performed on unanesthetized animals in standing position on the concrete surface that were openned to air movement.

Animal preparation

On the day of experiment, two polyethylene catheters (i.d. 1.0 mm, o.d. 1.5 m.m.) were inserted into both jugular veins by using a "medicut" intravenous cannula (Argyle, medicut Intravenous Cannula, Sherwood Medical Industries, England). Both catheters were left in place throughout the experiment to facilitate both infusion and blood sampling. The free end balloon catheter (Foley catheter, 2 way, size 26 ch, 60 ml, bulb capacity) was introduced into the bladder and secured with the inflated retaining cuff for collection of urine.

Experimental protocol

The experiments were divided into two series; the first to study water metabolism and renal function in the normal swamp buffalo under roof-metal shades (control period). Measurements of renal function in control period were made in a period of two hours between 0800-1000 h. while those of water metabolism were made until 48 h. of the next day. The second series of experiments were studied during buffaloes exposed to the sun that have the highest ambient temperature during the day around 39:30°C(DB:WB).The measurements of water metabolism and renal functions were performed on the fifth and the tenth day of heat exposure. During the experiment, the animals were fed and allowed to drink water ad libitum.

Total body water and water turnover measurements

Total body water and water turnover were determined using the ^{3}H-radioisotope dilution technique. A single dose of 3000 μCi/animal of carrier-free tritiated water was injected intravenously. The injection was done at the beginning of the first hour of experiments. Blood samples for water turnover measurements were collected ½, 1, 2, 3, 4, 5, 6, 7, 18, 24, 36 and 48 h. subsequent to the injection. Preliminary experiments showed that tritiated water was uniformly distributed in the body water 5 h. after dosing. The preparation for sample counting was achieved by the internal standardization technique as described by Vaughan & Boling (1961). The corrected activity of the samples, in c.p.m., was plotted on a semilogarithmic paper against time, in hours after dosing, and the extrapolated activity at theoretical zero time of complete mixing of radioisotope was used in determining the total body water as the following equation:

$$\text{total body water (V)} = \frac{\text{injection dose}}{\text{sample activity at zero time}}$$

$$\text{water turnover} = \frac{0.693V}{\text{biological half-life}}$$

Determination of plasma volume and plasma solids concentration

Plasma volume was measured by dilution of Evan's blue (T-1824) dye. After the injection of 20 ml of the dye (0.5 g/100 ml normal saline) into the jugular catheter followed by a small quantity of rinsing normal saline, blood samples were taken at 15, 20, 30 and 40 min. Dilution of dye at zero time was determined by extrapolation. Blood volume was calculated from plasma volume and packed cell volume (PCV) (Chaiyabutr, Faulkner and Peaker, 1980).

Renal functions studies

Before the measurement of renal function for one hour, the priming solution containing 1 gm of p-aminohippuric acid (PAH) and 20 ml of isotonic saline (adjusted the pH 7.4) was administered intravenously via left jugular vein followed immediately by the sustaining solution at the rate of 2 ml/min, this contained PAH 40 mg in 2 ml of isotonic saline (pH 7.4). The rate of infusion was kept constant by infusion pump (EYELA, Micro-infusion pump M.P.-3) throughout the course of the experiment. This rate was found to give uniform plasma level of PAH after 30 min which was allowed for equilibration. The rate of infusion maintains the plasma concentration of PAH in the range of 0.6-1.0 mg/100 ml. Plasma concentration of PAH greater than 3 mg/100 ml were unsuitable for renal plasma flow measurement.

After 1 h of infusion and the rate of urine flow stabilized, clearance studies for renal functions were carried out. The bladder was empty and washed out with air before urine collection. The successive double collections for measurement of urine volume were performed at 20 minute interval concomitant to blood sample collection at the mid point of each period.

The measurements of heart rate, respiratory rate and rectal temperature were made on 1400 h. of the experimental days. Heart rate was recorded by palpation the pulse from coccygeal artery. The respiratory rate was recorded from the movement of abdominal wall while rectal

temperature was measured using thermometer. Ambient temperature was recorded by dry bulb thermometer. Relative humidity was calculated from the reading of dry and wet bulb thermometer. The temperature humidity index (THI) was calculated using the equation by Maust et al.(1972).

PAH in plasma and urine were determined by the method of Bratton and Marshall as modified by Smith (1962).

Determination of endogenous creatinine in plasma and urine was carried out according to the method of Kennedy as described by Smith (1962).

Plasma protein free filtrate used in determination of PAH and endogenous creatinine was prepared with trichloroacetic acid.

The solutes content in the plasma and urine were measured as follows : sodium and potasium by flame photometry (Beckman, Klina Flame), Chloride by Chloridometer, calcium by cresolphthalein complexone method in the alkaline solution as described by Varley et al (1980), phosphorus by the method using trichloroacetic acid and molybdate followed by reduction with methyl-p-aminophenol sulfate, plasma and urine osmolality by the freezing-point depression method. Packed cell volume (PCV) was measured by the preparation of the heparinized blood in microcapillary centrifuge. Total plasma solids concentration was determined by a refractometer. Plasma protein concentration was measured by biuret method. Plasma glucose concentration was estimated by a glucose oxidase method. Plasma and urinary urea concentrations were determined by method of Ritcher and Lapointe (1962) using diacetyl mono-oxime reagent for color development. Urine pH was determined using pH meter.

Calculations

The following symbols were used throughout the calculation.

V = urine flow rate (ml/min)

P_{cr} = plasma concentration of endogenous creatinine, ugm/ml

U_{cr} = urine concentration of endogenous creatinine, ugm/ml

C_{cr} = creatinine clearance, ml/min

P_{PAH} = plasma concentration of PAH, ugm/ml

U_{PAH} = urine concentration of PAH, ugm/ml

C_{PAH} = PAH clearance, ml/min

P_{urea} = plasma concentration of urea, mg%

U_{urea} = urine concentration of urea, mg%

C_{urea} = urea clearance, ml/min

P_{osm} = plasma osmolality, mOsm/kg./L

U_{osm} = urine osmolality, mOsm/kg./L

P_{Na} = plasma concentration of sodium (mEq/L)

U_{Na} = urine concentration of sodium (mEq/L)

P_{K} = plasma concentration of potassium, mEq/L

U_{K} = urine concentration of potassium, mEq/L

P_{Cl} = plasma concentration of Chloride, mEq/L

U_{Cl} = urine concentration of Chloride, mEq/L

PCV = packed cell volume, (%)

THI = Temperature humidity index

Using the Fick principle; PAH clearance was used to measure effective renal plasma flow (ERPF), endogenous creatinine clearance was used to measure glomerular filtration rate (GFR).

$$GFR = C_{cr} = \frac{U_{cr}V}{P_{cr}}$$

$$ERPF = C_{PAH} = \frac{U_{PAH}V}{P_{PAH}}$$

$$RBF = \frac{ERPF}{1-PCV}$$

$$\text{Osmolar clearance } (C_{osm}) = \frac{U_{osm}V}{P_{osm}}$$

$$\text{Free water clearance } (C_{H_2O}) = V-C_{osm}$$

$$\text{Urinary sodium excretion} = U_{Na}V$$

$$\text{Urinary potassium excretion} = U_{K}V$$

$$\text{Urinary chloride excretion} = U_{Cl}V$$

$$\text{Urinary urea excretion} = U_{urea}V$$

$$\text{Fractional excretion (\%)} = \frac{UV}{P \times C_{Cr}} \times 100$$

THI = 0.72 (^{o}c dry + ^{o}c wet) + 40.6

T_{db} = dry bulb temperature

RH = relative humidity (%)

Statistics

The conventional paired t-test was used to estimate the statistical significance of differences between values obtained from the same animals under shaded and unshaded conditions.

Results

Changes in heart rate, respiratory rate and rectal temperature, (Table I)

The effects of short term heat exposure on cardiorespiratory frequency and rectal body temperature were studied in heifer buffaloes. The mean dry bulb ambient temperature on the control shaded period was 31:26 (DB:WB) where as the ambient temperature on the fifth and the tenth day of nonshaded period was around 37 : 30 ^{o}C (DB : WB). The calculated temperature humidity index in sun exposed animals were therefore higher than that of normal ambient temperature by 11%. On the fifth day, heat exposed buffaloes showed increase in both cardiorespiratory frequency and rectal temperature. The respiratory rate increased nearly three folds from 58 $\pm$ 14 to 180 $\pm$ 4 breaths/min ($P < 0.01$) while heart rate increased by 6%. When heat exposure was continued upto day 10, the respiratory rate and rectal temperature of buffalo remained at the similar level to that on the fifth day of heat exposure. In contrary to the heart rate, which decreased to control level in this period.

Changes in water turnover rate, total body water and body weight (Table II)

There was no significant change in the body weight of buffaloes during exposure to the sun when compared to animals under shaded condition. On the fifth day of heat exposure, total body water decreased significantly ($P < 0.05$) while blood volume and plasma volume remained constant. The water turnover rate was increased from 204.17 $\pm$ 18.02 during control period to 286.08 $\pm$ 27.37 ml/kg$^{0.82}$/day ($P < 0.05$). The increase in water turnover rate coincided with decrease in biological half life of tritriated water. It has been noted that the packed cell volume of the fifth day

Table I Changes in cardiorespiratory frequency, rectal temperature and ambient temperature in swamp buffaloes during shaded and nonshaded period (mean ± S.E.).

	Control	heat exposure	
		day 5	day 10
Heart rate (beats/min)	72 ± 5	76 ± 5	72 ± 3
Respiratory rate (breaths/min)	58 ± 14	180 ± 4**	175 ± 6*
Rectal temperature (°C)	39.51 ± 0.18	41.92 ± 0.27**	41.76 ± 0.15**
Ambient temperature (dry bulb/wet bulb) (°C)	31 : 27	39 : 31	40 : 30
THI (%)	83.0	92.3*	92.1*

P values with respect to control values ;

* P < 0.05, ** P < 0.01

Table II Changes in water turnover rate, total body water, blood volume and packed cell volume in swamp buffaloes during shaded and nonshaded period (mean ± S.E.).

	Control	heat exposure	
		day 5	day 10
Body weight (kg)	180 ± 10	178 ± 11	178 ± 10
Water turnover (L/24 h)	15.01 ± 1.72	20.27 ± 2.95	26.49 ± 2.01***
Water turnover (ml/kg$^{0.82}$.24 h)	204.17 ± 18.02	286.08 ± 27.37*	376.56 ± 11.38***
Biological T tritiated water (h)	120.3 ± 8.5	77.1 ± 1.8*	57.6 ± 3.3**
Total body water (L)	106.5 ± 9.1	93.1 ± 11.4*	92.7 ± 11.6*
Total body water (L/100 kg)	58.7 ± 2.0	51.8 ± 3.2*	51.5 ± 3.9
Blood volume (ml/kg)	63.62 ± 2.58	62.12 ± 1.50	56.70 ± 2.36**
Plasma volume (ml/kg)	45.95 ± 0.93	46.42 ± 1.43	42.37 ± 1.38*
Packed cell volume (%)	27.6 ± 1.5	25.3 ± 1.4**	25.2 ± 1.0*

P values with respect to control values;

* P < 0.05, ** P < 0.01, *** P < 0.001

of heat exposed buffaloes decreased significantly by 8.3% ($P < 0.01$). On the tenth day of nonshaded period, the total body water decreased in the same level as that measured during 5 day of heat exposure. During the last tenth day, the blood volume and plasma volume decreased significantly by 11% ($P < 0.01$) and 8% ($P < 0.05$) respectively. The stepwise increase in water turnover rate was noted by 84% ($P < 0.001$) when compared with the control value while the decline of biological half life of tritiated water was determined ($P < 0.01$). The degree of decrement of packed cell volume during day 10 was similar to that during day 5 of heat exposure.

Changes in renal functions (Table III)

There were no significant changes in glomerular filtration rate, effective renal plasma flow, renal blood flow and filtration fraction in buffaloes either during 5 days or 10 days of sun exposure. The urine flow rate did not change in the fifth day of heat exposed animals but a marked increase was noted during ten day of heat exposure by 50% when compared to pre-exposure values. The gradual increase in osmolar clearance was noted on the fifth and the tenth day of heat exposure by 7% and 51% respectively ($P < 0.05$). However, the opposit decline in free water clearance in heat exposed buffaloes was determined by the time of heat exposure. The level of plasma osmolarity did not alter throughout the experimental period. It has been noted that the urinary and fractional excretion of urea did not show any significant change after 5 days of heat exposure. From the 5^{th} day to the 10^{th} day of heat exposure, the urinary excretion of urea showed a tendency to increase although the fractional excretion of urea was unaltered. In heat exposed buffaloes, both during on the fifth day and the tenth day, the urine pH had a tendency to increase when compared to pre-exposure values.

Table III Changes in renal functions, renal urea excretion and urine pH in swamp buffaloes during shaded and nonshaded period (mean ± S.E.).

	Control	heat exposure	
		day 5	day 10
V. (ml/min)	2.341 ± 0.473	2.443 ± 0.508	3.516 ± 0.558
GFR (ml/min)	88.54 ± 11.92	85.59 ± 20.39	92.95 ± 8.98
ERPF (ml/min)	586.16 ± 89.37	585.64 ± 33.72	653.83 ± 92.62
RBF (ml/min)	811.61 ± 139.44	786.66 ± 71.34	873.29 ± 129.52
FF (%)	0.152 ± 0.011	0.145 ± 0.027	0.146 ± 0.031
C_{osm} (ml/min)	4.655 ± 0.544	5.00 ± 1.185	7.040 ± 0.749*
C_{H_2O} (ml/min)	-2.311 ± 0.461	-2.560 ± 0.866	-3.524 ± 1.106
P_{osm} (mOsm/L)	278 ± 2.94	280 ± 2.16	280.8 ± 5.85
$U_{urea}V$ (mg/min)	14.47 ± 2.80	15.14 ± 6.16	19.33 ± 6.64
FE_{urea} (%)	62.14 ± 12.54	55.40 ± 24.23	64.61 ± 13.48
urine pH	8.73 ± 0.16	8.91 ± 0.08*	8.81 ± 0.07

P values with respect to control values;

* $P < 0.05$

Changes in renal electrolytes excretion (Table IV)

Data in Table IV showed results for urinary and fractional excretion of electrolytes. During five days of heat exposure, the remarkable increase in urinary and fractional excretion of sodium (230%) without any changes in those of potassium were noticed. Urinary and fractional excretion of chloride decreased significantly by 73% and 72% respectively ($P < 0.05$). No changes of the renal excretion of calcium has been detected while there was a marked reduction in the urinary excretion of inorganic phosphorus by 51%.

In ten day heat exposed buffaloes, the urinary and fractional excretion of sodium decreased below the pre-exposure values by 72%. However, the urinary and fractional excretion of potassium increased nearly twofold from pre-exposure values. The renal excretion of chloride was still lower on the tenth day of heat exposure ($P < 0.05$). The markedly decreased excretion of renal inorganic phosphorus concentration was found by the time of heat exposure.

Changes in plasma electrolyte concentrations and plasma constituents (Table V)

In the present experiment, the plasma concentration of sodium and potassium did not exhibit any significant changes. The plasma concentration of chloride ion showed a gradual increase while that of inorganic phosphorus decreased significantly by the time of heat exposure. Plasma concentration of calcium decreased significanly by 12 % ($P < 0.05$) on the 5^{th} day of heat exposure but returned to pre-exposure value on the tenth day thereafter. The plasma glucose concentration increased significantly on the fifth and tenth day of heat exposure by 28 % and 24% respectively ($P < 0.05$). Plasma protein concentration showed

Table IV Changes in renal electrolyte (Na^+, K^+, Cl^-, Ca^{2+} and P_i) excretions in swamp buffaloes during shaded and nonshaded period (mean $\pm$ S.E.).

	Control	heat exposure	
		day 5	day 10
$U_{Na}V$ (mEq/min)	48.01 $\pm$ 33.51	158.85 $\pm$ 144.07	13.15 $\pm$ 3.69
(FE_{Na}) %	(0.423 $\pm$ 0.283)	(1.374 $\pm$ 1.245)	(0.106 $\pm$ 0.029)
$U_K V$ (mEq/min)	441.38 $\pm$ 123.66	430.26 $\pm$ 182.64	788.16 $\pm$ 139.28
(FE_K) %	(126.19 $\pm$ 35.43)	(140.81 $\pm$ 75.82)	(215.24 $\pm$ 36.56)
$U_{Cl}V$ (mEq/min)	165.70 $\pm$ 50.00	44.21 $\pm$ 10.23	78.20 $\pm$ 46.28*
(FE_{Cl}) %	(1.806 $\pm$ 0.378)	(0.508 $\pm$ 0.135*)	(0.521 $\pm$ 0.307*)
$U_{Ca}V$ (mg/min)	0.062 $\pm$ 0.015	0.047 $\pm$ 0.004	0.067 $\pm$ 0.024
(Fe_{Ca}) %	(0.760 $\pm$ 0.275)	(0.564 $\pm$ 0.139)	(0.769 $\pm$ 0.238)
$U_{Pi}V$ (mg/min)	0.075 $\pm$ 0.086	0.037 $\pm$ 0.022	0.022 $\pm$ 0.015
(FE_{Pi})	(1.195 $\pm$ 1.307)	(1.127 $\pm$ 0.888)	(0.505 $\pm$ 0.259)

P values with respect to control values;

* $P < 0.05$

Table V Change in plasma electrolyte (Na^+, K^+, Cl^-, Ca^{2+} and P_i) concentrations, plasma constituents and total plasma solid in swamp buffaloes during shaded and nonshaded period (mean ± S.E.).

	Control	heat exposure	
		day 5	day 10
Plasma electrolyte concentrations			
P_{Na} (mEq/L)	132.0 ± 4.2	132.75 ± 2.22	132.0 ± 2.16
P_K (mEq/L)	4.0 ± 0.23	3.87 ± 0.26	4.02 ± 0.26
P_{Cl} (mEq/L)	104.5 ± 1.0	108.0 ± 3.9	110.3 ± 4.9
P_{Ca} (mg%)	10.0 ± 0.47	8.85 ± 0.66*	9.40 ± 0.59
P_{Pi} (mg%)	6.55 ± 1.57	4.60 ± 0.52	4.34 ± 0.90*
Plasma glucose concentration (mg%)	79.97 ± 19.91	102.68 ± 27.14*	98.85 ± 13.48*
Plasma protein concentration (gm%)	7.00 ± 0.57	7.52 ± 0.28*	7.84 ± 0.53**
Plasma urea concentration (mg%)	27.17 ± 4.84	34.13 ± 5.56	32.96 ± 8.48
Plasma creatinine concentration (mg%)	1.67 ± 0.07	2.03 ± 0.21	1.97 ± 0.18
Total plasma solid (gm/kg)	3.78 ± 0.28	3.73 ± 0.55	3.65 ± 0.24

P values with respect to control values;

* $P < 0.05$, ** $P < 0.01$

graded increased by the time of heat exposure until it reached 12% from the pre-exposure value. ($P < 0.01$). The plasma concentrations of urea and creatinine had a tendency to increase during heat exposure. Although the remarkable increases in plasma constituents were apparent, it has been noted that the total plasma solid did not change during the time of heat exposure.

Discussion

The effects of short term heat exposure of non-shaded buffaloes on heart rate, respiratory rate and rectal temperature are shown in Table I It is apparent that under the radiant heat load on them, the buffaloes were unable to keep their body temperature constant. The rise in rectal body temperature was accompanied by increases in the heart rate and respiratory rate. The responses to solar radiation on the tenth day of exposure were not different from those on the fifth day. The maximum respiratory rate and rectal temperature (recorded at 1400 h) were kept at the same level on the tenth as compared with the fifth day of exposure to solar radiation indicating that buffaloes have low effeciency for acclimation during intermittent heat exposure. The minimum time for acclimation in buffaloes during exposure to high environmental temperature is still unknown, although the experiments in cattle showed that three weeks of daily exposure to high temperature would induce some degree of acclimation (Bianca 1957).

Short term exposed to solar radiation (up to 8 h of the day for ten days) resulted in more increase in water turnover rate with time of exposure. The change in body water turnover rate coincided with the change in biological half life of tritiated water. Total body water markedly decreased on the fifth day of non-shaded buffaloes and maintained thereafter. These findings can be explained that an increase in body water turnover rate is linked with the use of water for heat dissipation rather than for energy turnover although many studies indicate a relationship between water turnover and either energy metabolism or food intake in many species animals living in tropics (MacFarlane and Howard, 1970; King, 1982). In short term heat exposure, buffaloes spent up throughout the day in the unshaded pen without

wallowing. The heat storage of non-shaded buffaloes from the combination of THI and solar radiation will take much less time for the animal to reach a critical body temperature, at which it must start evaporative cooling. At night it probably take much longer time to radiate body heat and lower its body temperature ready for the heat stressing in the next day. It seems likely that buffaloes were subjected to period of acute heat stress superimposed on period of chronic stress. Thus, on the first five days of non-shaded buffaloes, animals imposed by a day unshade without wallowing have drastically reduction of the ability to conserve body water. A trend for greater consumption of water as well as an increased rate of water consumption to dry matter intake of non-shaded buffaloes is a response of the animals to adaptation to life and maintain the total body water thereafter.

On the fifth day of heat exposure, the blood became more dilute as shown by a slight increase in plasma volume while blood volume slightly decreased. These changes may attributed to decrease in packed cell volume and circulating cell volume. In subsequent exposure on the tenth day, the decrease in circulating cell volume was in the same proportion to the decrease in blood and plasma volume (by 9%). Both blood volume and plasma volume markedly decreased which were mainly from a loss of plasma water. The total plasma solids remained unaffected by short term heat exposure while the plasma concentrations of protein, glucose and creatinine increased with time of heat exposure. These results suggest that the responses of the non-shaded buffalo during short term heat exposure occurred into different phases. The first phase occurred within the first five days. This phase was characterized by initial increase in plasma volume with decreases in circulating cell volume and total body water. The slight increases in plasma volume

of the non-shaded buffalo would be indicative of a mobilization of water for heat dissipation. A marked decrease of total body water was observed on the fifth day of the measurement. Such an excessive water loss to the environment was not reflected in the behavior of blood volume because water was supplied to the blood at a similar rate as it was lost. This water probably come from digestive tract or from the extravascular tissue space as same as animals during acute heat exposure (Chaiyabutr et al, 1987a). Furthermore, an increase in intravascular protein concentration of non-shaded animals will raise plasma oncotic pressure which helping to maintain blood volume by reducing water loss.

A second phase of the responses to short term heat exposure occurred on the tenth day of measurement. In this phase, the non-shaded buffalo showed the decreased both plasma and blood volume. Packed cell volume and circulating cell volume on the tenth day of unshaded period did not differ from those of the fifth day. The decrease in blood volume and plasma volume on day 10 of the non-shaded buffalo may be attributed to adaptive mechanism. However, the water turnover rate of non-shaded buffaloes increased stepwise when wallowing was denied, indicating higher water requirements for evaporative cooling. The body fluid adjustments in this condition occurred as heat exposure continued. The severity and duration of heat exposure are factors affecting water loss from the plasma. Such a decrease in plasma water is harmful to buffaloes in a hot environment because it decreases their ability to dissipate the heat through water vaporization.

The effect of short term of heat exposure on renal hemodynamics was carried out in four heifer non-shaded buffaloes. There were no significant changes of the renal clearance measurements of endogenous creatinine (GFR) and PAH (RPF) taken prior to and during exposed to the sun. According to general concepts, renal circulation is regulated by two hormonal systems: vasoconstrictors and vasodilators. Distribution of blood flow within kidneys is also regulated by these hormonal systems. During prolonged heat exposure, a vasoconstrictor like norepinephrine has been shown to increase in a higher level in plasma of cattle (Alverez and Johnson, 1973). Norepinephrine can affect renal blood flow in addition to local vasoconstriction of both afferent and efferent arterioles. However, these factors responsible for the observed unchange in renal hemodynamics in non-shaded buffaloes in this study are uncertain, since during heat exposure, non-shaded buffaloes showed a marked decrease in packed cell volume. The decrease in packed cell volume may induce a change in blood viscosity which might be expected to reduce the resistance of both afferent and efferent arterioles (Myers et al, 1975). It seems likely that the renovascular adjustment are occurred to a point where normal fluid distribution during heat stress is reestablished.

During short term heat exposure, non-shaded buffaloes showed an increase in the rate of urine flow by 50% on day 10 of exposure. This change coincided with increases of renal osmolar clearance, urinary excretion of potassium and urine pH. The urinary and fractional excretion

of inorganic phosphorus and chloride ion decreased throughout non-shaded period while the urinary and fractional excretion of sodium markedly increased in the first five day of heat exposure and fell thereafter. The urinary excretion of calcium and magnesium showed no alteration throughout the experimental period. According to these findings, the kidneys of non-shaded buffaloes showed to be responsible for retaining both water and electrolytes as much as possible. During experiments, the buffalo received the same diet. Therefore, changes in electrolytes excretion during heat stress are of course to be related with changes of bodily status.

On the day 5 of non-shaded buffaloes, urinary sodium excretion markedly increased nearly three folds concomitant with an increase in plasma volume. The renal loss of sodium seems to be carefully controlled to maintain optimum sodium concentration in the extracellular fluid. In this period both plasma sodium concentration and plasma osmolality remained constant. In general, the principal controlling factor which influences renal sodium reabsorption is the hormone aldosterone. Aldosterone also promotes excretion of potassium ion in the renal tubular cell. According to these results, whether the reduction in plasma aldosterone occurred on the fifth day of heat exposed buffaloes since there is evidence, obtained by El-Nouty and co-workers from the study in cattle that prolonged heat exposure can reduce plasma aldosterone level (El-Nouty et al, 1980). At present it seems to us that the reduction of plasma potassium concentration while elevation of plasma volume on the fifth day of non-shaded buffaloes may account for the reduction of plasma aldesterone. Therefore, if a reduction of plasma aldosterone is occurred in non-shaded buffaloes, sodium will be excreted via the kidney

rather than being retained. This may be an important adaptive mechanism due to the sodium buffalo can not excrete via sweating which differ from man exposed to heat (Collins and Weiner, 1968).

On the day 10 of non-shaded buffaloes, the urinary and fractional excretion of sodium markedly decreased while the marked increase was noted for potassium. These changes were related to a marked reduction of total body water and plasma volume which led animals to the state of dehydration. The dislocation of body fluids during heat stress may stimulate the kidneys to conserve salt and water until plasma volume is increased to a steady state. The kidney is much less able to conserve potassium than sodium which probably relate to increase aldosterone level in plasma during dehydrated period. A possible secretion of aldosterone may be accompanied with an elevation of plasma cortisol which have been demonstrated in non-shaded buffaloes exposed to heat for 10 days (Loypetjra et al, 1987).

During exposure to heat, non-shaded buffaloes showed signs of distress with rapid shallow breathing which usually produced alkalosis (Hale and Webster, 1967). It is possible that increases in urine pH and plasma chloride concentration in non-shaded buffaloes could be due to respiratory alkalosis. A likely explanation might be that compensation for this disturbance during non-shaded period is by increased renal excretion of base instead of chloride, resulting in an increase in plasma chloride concentration to replace lost base from the body (Johnson and Selkurt, 1966). An increase in either urinary or fractional excretion of potassium could be explained by the well known fact that during alkalosis the kidney plays a significant roïe in acid-base regulation by increased exchange of potassium ions for hydrogen ions in the renal

tubular fluid (Johnson and Selkurt 1966). This renal behaviour has also been demonstrated in acute heat stressed pigs (Chaiyabutr et al, 1987b). However, an increase in urinary potassium excretion of non-shaded buffaloes may also be superimposed on the effect of either aldosterone or antidiuretic hormone (ADH) (MacFarlane et al, 1967).

Upto day 10 of non-shaded period in the buffaloes, total body water decreased while no alteration of either plasma sodium or plasma osmolality were noted. This result can be referred to as "isotonic contraction" of the extracellular fluid. This findings should be interpreted as sodium losses of the same magnitude as the losses of extracellular water.

During heat exposure, non-shaded buffaloes showed a marked reduction of the plasma concentration of inorganic phosphorus. This reduction is similar to the effect of acute heat exposure. The process of cellular trapping of phosphorus for elevation of the metabolic rate and ATP production during heat stress may be responsible for the decrease in the plasma level of inorganic phosphorus. Therefore, the reduction of the urinary excretion of inorganic phosphorus might be attributed to a reduction of glomerular filtered load. However, renal handling of inorganic phosphorus was not similar to that of calcium and magnesium ions which were not altered in non-shaded period although all of these ions are under control of circulating parathyroid hormone. It seems likely that prolong heat exposure did not influence the renal handling of both calcium and magnesium ions which probably share a common intratubular transport mechanism.

On day 10 of non-shaded buffaloes, an increase in the rate of urine flow in buffaloes exposed to heat is not due to a lack of antidiuretic hormone (ADH) since free water clearance (C_{H_2O}) showed

decreased under this condition. An increase in electrolyte excretion particularly potassium ion can create osmotic diuretic effect (increased osmolar clearance) which contribute to an increase in urine output in buffaloes.

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