รายงานผลการวิจัย

เรื่อง

ผลของการฉีดสารละลายยูเรียต่อยูเรียเมตาบอลิสมของกระบือที่อยู่ในภาวะเครียดจากความร้อน
(Effect of exogenous urea on urea metabolism in heat stressed swamp buffalo)

โดย

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ตุลาคม 2530

ทุนวิจัยงบประมาณแผ่นดิน ปี 2528

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ผลของการฉีดสารละลายยูเรียต่อยูเรียเมตาบอลิสมของกระบือที่อยู่ในภาวะเครียดจากความร้อน

กระบือที่ได้รับการฉีดยูเรียเข้าหลอดเลือดดำ ในขณะที่อยู่ในอุณหภูมิแวดล้อมปกติ ไม่พบการเปลี่ยนแปลงอัตราการเต้นของหัวใจ อัตราการหายใจ ปริมาตรเม็ดเลือดแดงอัดแน่น อัตราการกรองผ่านไต ความเข้มข้นของอิเลคทรอลัยท์ โปรตีน และครีอะตินีนในพลาสม่า แต่พบว่า อัตราการไหลของปัสสาวะ สัดส่วนการขับทิ้งของยูเรีย อัตราการขับทิ้งโปตัสเซี่ยมทางไต และค่าออสโมล่าเคลียรานซ์ ลดลงอย่างมีนัยสำคัญในชั่วโมงที่ 3 ของการฉีดยูเรีย พบว่ามีการลดลงของสัดส่วนการขับทิ้งของโปตัสเซี่ยม ร่วมไปกับการลดลงของอัตราการไหลของปัสสาวะ และปัสสาวะเป็นกรดมากขึ้น อัตราการดูดกลับของยูเรียทางไตเพิ่มขึ้นอย่างเด่นชัดในขณะที่อัตราการไหลของปัสสาวะลดลง เมื่อกระบือได้รับความร้อน พบมีการเพิ่มขึ้นอย่างมีนัยสำคัญของอุณหภูมิร่างกายผ่านทางทวารหนักรวมทั้งอัตราการเต้นของหัวใจและอัตราการหายใจ ในขณะที่อัตราการกรองผ่านไตและปริมาณเลือดที่ไหลผ่านไตไม่มีการเปลี่ยนแปลง เมื่อกระบือได้รับการฉีดยูเรีย อัตราการขับปัสสาวะลดลง แต่ไม่พบการเปลี่ยนแปลงของอัตราการดูดกลับของยูเรียทางไต พีเอชของปัสสาวะและสัดส่วนการขับทิ้งของอิเลคทรอลัยท์ ขณะที่สัตว์ได้รับความร้อน ค่าโปรตีน อัลบูมินและครีอะตินีนในพลาสม่าเพิ่มขึ้นอย่างมีนัยสำคัญ ในขณะที่ ความเข้มข้นของฟอสฟอรัสในพลาสม่าลดลง จากผลการทดลองสรุปว่าการเพิ่มอัตราการดูดกลับของยูเรียที่ไตหลังการฉีดยูเรีย เมื่อสัตว์อยู่ในสภาพอุณหภูมิปกติ เป็นผลจากการเปลี่ยนแปลงการขับทิ้งของอิเลคทรอลัยท์ทางไต การลดลงของอัตราการขับทิ้งของอิเลคทรอลัยท์โดยเฉพาะโปตัสเซี่ยม จะทำให้อัตราการไหลของปัสสาวะลดลง มีผลทำให้อัตราการขับทิ้งของยูเรียลดลง ด้วยแม้ว่าปริมาณยูเรียที่กรองผ่านไตจะเพิ่มขึ้น เมื่อสัตว์ได้รับความร้อน อัตราการดูดกลับของยูเรียจะไม่เปลี่ยนแปลงตามการลดลงของอัตราการขับปัสสาวะ แต่จะขึ้นกับการเพิ่มขึ้นของปริมาณไนโตรเจนในร่างกายทั้งหมดซึ่งเป็นตัวจำกัดการดูดกลับยูเรียภายในไต

Abstract

Effect of exogenous urea on urea metabolism in heat stressed swamp buffalo.

In buffaloes during normal ambient temperature given exogenous urea infusion intravenously; the heart rate, respiratory rate, packed cell volume, glomerular filtration rate, plasma concentration of electrolyte, protein and creatinine were not significantly affected. The rate of urine flow, fractional urea excretion, urinary potassium excretion and osmolar clearance significantly decreased on the third hour of urea infusion. The decrease of fractional potassium excretion was concomitant with the reduction of the rate of urine flow and urine pH. The renal urea reabsorption markedly increased after urea infusion which had the negative correlation to urine flow rate. Before and during urea infusion in heat exposed animals, the rectal temperature, heart rate and respiratory rate significantly increased. No significant changes in GFR, RBF were noted. After urea infusion, the urine flow rate slightly decreased while renal urea reabsorption, urine pH and fractional electrolyte excretion kept constant. During heat exposure, there were marked increases in concentrations of total plasma protein, albumin and plasma creatinine whereas plasma inorganic phosphorus concentration significantly decreased. It is concluded that an increase in renal urea reabsorption during urea infusion in normal ambient temperature depends on urinary electrolyte excretion. Urea alone has limited diuretic ability. The decrease in urinary electrolytes

excretion (e.g. K^+) will affect to the reduction of the rate of urine flow by an osmotic diuretic effect. These changes are insufficient to aid renal urea excretion although an increase in the filtered urea has been determined. In heat stressed animals, renal urea reabsorption is not affected by the decrease in the rate of urine flow, but it depends on an increase in the body pool size of nitrogenous substance which will limit renal urea retention.

Introduction

In general, the conservation mechanism of urea appears to be of primary importance in the ruminant. The utilization of urea for protein synthesis has been known in this species animal whenever the nitrogen intake is low. Addition of urea together with starch has been shown to increase the amount of protein synthesis in sheep and cattle (Pierce, 1951). It has been demonstrated that kidney should be able to conserve urea by decreasing the renal urea excretion in some physiological condition eg. growth, lactation and during protein shortage (Macfarlane, 1964). Very few data are available in urea metabolism during heat stress particularly in buffalo, although increases of plasma creatinine and protein concentration have been reported in acute heat exposure (Chaiyabutr et al, 1983). The data also showed a slight increase in plasma urea concentration without the change in renal urea excretion. These results did not provide enough information on the intrarenal handling of urea in animal exposed to heat.

An attempt to elucidate the mechanism by which the role of kidney in regulation of urea excretion is brought about; the urea clearance, renal hemodynamic and electrolyte excretion are studied in buffalo given exogenous urea infusion during in either normal environmental temperature or acute heat exposure.

Materials and Methods

The experiment was conducted in five healthy female swamp buffaloes weighing between 330 to 440 kgs. The animals were fed paragrass and water hyacinth ad lib throughout the experimental period. On the day of experiments, the animal was withheld of food and drinking water while it was tethered with standing position in the control climatic room.

Animal preparation :

Before the start of the experiment the polyethylene catheter (PE 200) was inserted into jugular vein by using Medicut intravenous canula as the site for both infusion and blood sampling. The S-shape rubber catheter was inserted into urinary bladder for urine collection.

Experimental proceduce :

The experiment was divided into two series, the first series, animal was housed in a room held at an normal ambient temperature ($28 \pm 3^{\circ}C$), the second it was exposed to high ambient temperature which was controlled the temperature and relative humidity around $42 \pm 1^{\circ}C$ and 60% respectively by using thermostatically electric heating unit. Constant air was blown out to stimulate ventilation of the room by electric fan. All animals were elapsed for 1 month before the second series was performed. Each series was divided into two periods, before and after urea infusion. During, the first period, the animal was studied kidney functions and urea metabolism without exogenous

urea administration for 3 hours. In the second period, administration of exogenous urea was performed with the priming dose of 3 gm/animal followed by sustaining infusion of 50 mg/4 ml/min. of urea diluted in normal saline solution (NSS) via the jugular catheter. Approximately one hour after equilibration, the studies on the effect of exogenous urea infusion were performed for 3 hours.

On the beginning of the experiment of both series, the buffalo was given by a bolus injection of PAH 1 gm as a priming dose and was sustained by continuous intravenous infusion of 25 mg/4 ml/min of PAH diluted in NSS throughout the experimental study. After one hour equilibration, successive double collection and measurement of urine volume were conducted at 10 minutes interval which was consistent to blood sample collection at the midpoint of each urine collection period. Blood sample for determination of electrolytes and other constituents were collected every 30 mins interval while heart rate, respiratory rate, rectal body temperature and temperature/humidity of surrounding environment were measured every one hour interval throughout the experimental run.

Determination of renal function

Glomerular filtration rate (GFR) was obtained using the clearance of endogenous creatinine expressed in ml/min as described by Chaiyabutr et al (1982). Endogenous creatinine concentration was determined by the method of Kennedy as described by Smith (1962).

Effective renal plasma flow (ERPF) was obtained by clearance of PAH using Fick Principle expressed in ml/min. The PAH concentration was determined by the method of Bratton and Marshall as described by Smith (1962).

Plasma filtrate for determination of creatinine and PAH was deproteinized by Trichloroacetic acid

Plasma and urinary urea concentrations were determined by the method of Ritcher & Lapoint (1962) using diacetyl mono-oxime reagent.

Urinary nitrogen concentration was obtained by Kjeldhal method. Total plasma protein was analysed using biuret method, globulin by forming the complex with glyoxylic acid and albumin by the difference.

Electrolyte concentrations in plasma and urine sample were analysed by the following procedures; Sodium and potassium by flame photometry, chloride by chloridometer, calcium by cresolphthalein complexone method as described by Varley et al (1980) and phosphorus by the method using trichloroacetic acid, molybdate followed by reduction with methyl-p-aminophenol sulfate. Plasma and urine osmolarity were measured using freezing point depression method.

Packed cell volume was determined by microcapillary method. Heart rate was measurement by palpation pulse of coccygeal artery while respiratory rate was recorded from the movement of abdominal wall. Rectal body temperature was obtained using thermometer and the ambeint temperature was recorded from dry bulb thermometer. Relative humidity

was calculated by the difference of dry and wet bulb temperature. Temperature humidity index (THI) was calculated using the equation of Maust, et al (1972) as follows;

$$THI = 0.72\ (^{o}C\ dry + {}^{o}C\ wet) + 40.6$$

Calculation. The following symbols are used throughout the calculation.

V = urine flow rate (ml/min)

C_{cr} = creatinine clearance (ml/min)

P_{cr} = plasma creatinine concentration (mg/100 ml)

U_{cr} = urinary creatinine concentration (mg/100 ml)

C_{PAH} = PAH clearance (ml/min)

P_{PAH} = plasma PAH concentration (mg/100 ml)

U_{PAH} = urinary PAH concentration (mg/100 ml)

C_{Osm} = osmolar clearance (ml/min)

P_{Osm} = plasma osmolality (mOsm/kg)

U_{Osm} = urine osmolality (mOsm/kg)

C_{H_2O} = free water clearance (ml/min)

C_{urea} = urea clearance (ml/min)

P_{urea} = plasma urea concentration (mg/100 ml)

U_{urea} = urinary urea concentration (mg/100 ml)

U_{N} = urinary total nitrogen concentration (mg/ml)

U_{ureaN} = urinary urea nitrogen concentration (mg/ml)

$U_{non\ ureaN}$ = urinary non urea nitrogen concentration (mg/ml)

P_{E} = plasma concentration of electrolyte (mEq/l)

U_{E} = urinary concentration of electrolyte (mEq/l)

Hct = packed cell volume (%)

Renal function studies were calculated by the following equation.

Glomerular filtration rate (GFR) = $C_{cr} = \frac{U_{cr}V}{P_{cr}}$

Effective renal plasma flow (ERPF) = $C_{PAH} = \frac{U_{PAH}V}{P_{PAH}}$

Filtration fraction (FF) = $\frac{GFR}{ERPF} \times 100$

Renal blood flow (RBF) = $\frac{ERPF \times 100}{100 - Hct}$

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

Free water clearance (C_{H_2O}) = $V-C_{Osm}$

Renal urea clearance (C_{urea}) = $\frac{U_{urea}V}{P_{urea}}$

Renal electrolyte excretion = $U_E V$

Renal urea excretion = $U_{urea}V$

Urinary non-urea-nitrogen excretion ($U_{nonureaN}V$)

= Total Urinary nitrogen excretion ($U_N V$) - Urinary urea nitrogen excretion (U_{ureaN}

Fractional electrolyte excretion (FE_E) = $\frac{U_E/P_E}{U_{cr}/P_{cr}} \times 100$

Fractional urea excretion (FE_{urea}) = $\frac{U_{urea}/P_{urea}}{U_{cr}/P_{cr}} \times 100$

Statistic

The data are expressed as mean $\pm$ SD. Their statistical significances were evaluated using student paired t-test compared with control values (values obtained on the first period before urea infusion). If the P-value is less than 0.05 the difference was considered significant.

Results

Changes of ambient temperature, rectal body temperature, heart rate, respiratory rate and packed cell volume during control and heat exposure period. (Table I, Fig. 1)

The results in Table I and Figure 1 show that there were no changes of rectal body temperature, heart rate respiratory rate and packed cell volume throughout experimental run in the normal ambient temperature. In the first series of the experiment, the mean value of surrounding ambient temperature slightly increased by approximately 2.5% during urea infusion which was due to the variation of diurnal ambient temperature. After 3 h. of heat exposure, the rectal body temperature increased significantly with the rate of $0.005^{\circ}F$/min. The further increase in rectal temperature was observed during urea infusion. Moreover, the heat-exposed animal given exogenous urea showed a marked elevation of heart rate and respiratory rate which increased by 14% and 120% respectively at the 7^{th} h. of exposure. The packed cell volume slightly decreased during heat exposure. The temperature humidity index (THI) was higher during heat exposure compared with preexposed value ($P < 0.001$) whereas no significant change in THI was observed in the first series of the experiment.

Changes of renal function during control and heat exposure period (Table II, Fig. 2, 3)

After 4 h. of urea infusion during normal ambient temperature, there was a significant decrease in urine flow rate by approximately

Table I Effect of exogenous urea infusion on rectal temperature, heart rate, respiration rate and packed cell volume of normal and acute heat stressed buffaloes. (Mean±S.D.)

	Normal ambient tesmperature		Heat exposure		
	Before urea infusion	3 h.after urea infusion	Pre-heat exposure	3 h.heat exposure	3 h.after urea infusion
Ambient temp. (°C, D.B)	27.8±3.1	28.5±3.3	32.1±3.4	41.1±0.5**	40.6±0.9**
Humidity (%)	55.8±14.8	53.6±18.0	70.0±8.8	42.0±1.7**	41.0±0**
THI (%)	76.4±4.1	76.9±3.3	84.2±2.6	92.8±0.7**	91.9±1.3**
Rectal temperature (°F)	100.5±1.5	100.8±0.9	101.1±0.9	102.0±0.6**	102.7±0.3**
Heart rate (beats/min)	43±6	43±6	43±7	47±8	49±5**
Respiratory rate (breaths/min)	23±6	21±7	25±9	58±14*	55±20*
Packed cell volume (%)	26.9±2.0	27.3±1.9	28.2±3.2	27.1±2.5	27.3±3.3

* $P < 0.05$

** $P < 0.01$

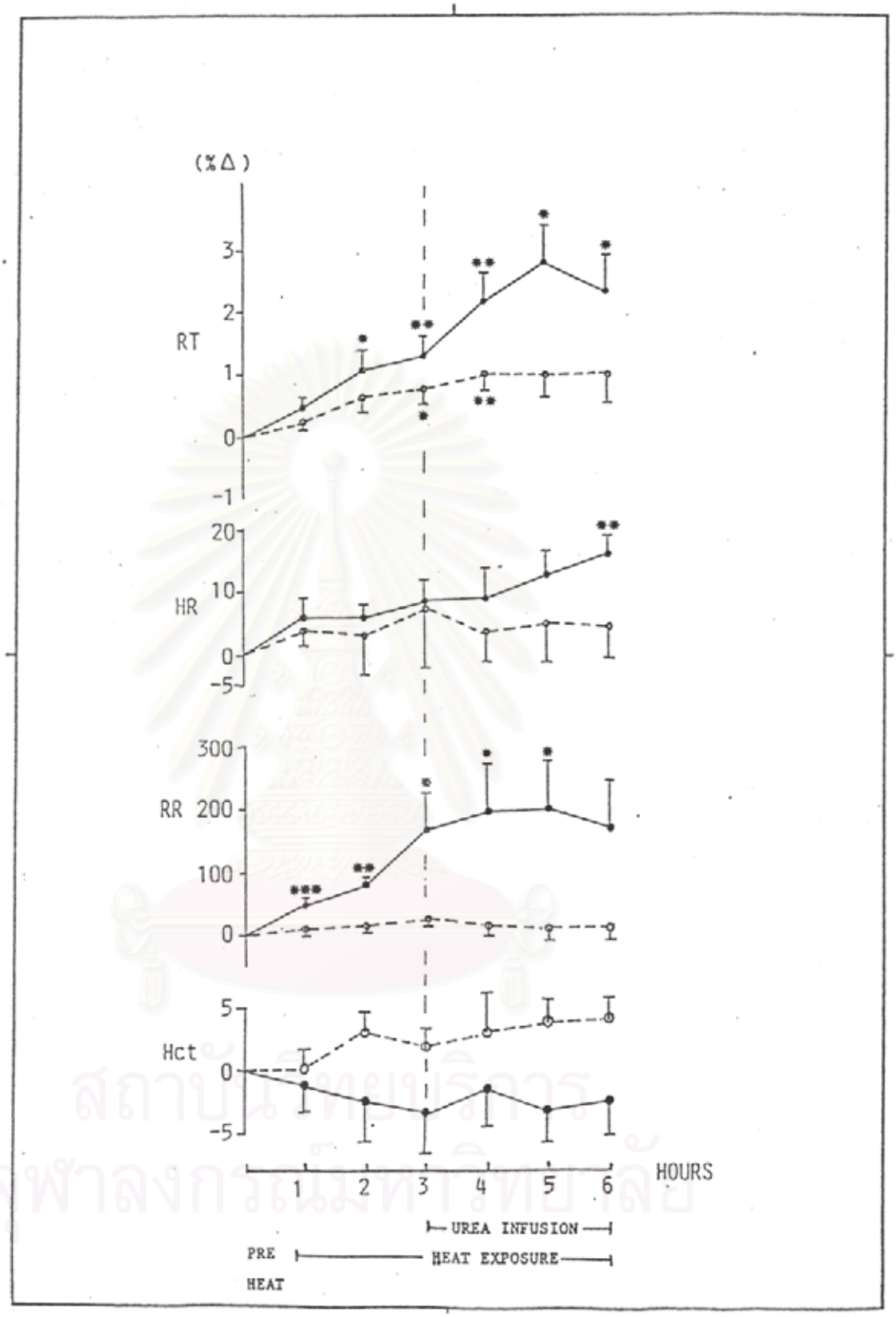

Fig 1 Mean percent changes for rectal temperature, heart rate, respiratory rate and packed cell volume of five swamp buffaloes given exogenous urea infusion during normal ambient temperature (o--o) and acute heat exposure (●—●) period. The values are mean±S.E., P-values with respect to the first hour value and preexposed value for animals during normal ambient temperature and heat exposure period respectively.

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

37% in comparison to the value obtained before urea insusion. The marked reduction of urine flow rate was also noted in animal exposed to heat for 3 h. and it was more obvious after urea insusion which decreased by approximately 33% from preexposed value. There was positive correlation between the rate of urine flow and fractional urea excretion ($P < 0.001$, Fig. 4). Urea infusion had no effect on glomerular filtration rate (GFR) of animal during control period while a marked reduction was apparent in heat exposed animal. Renal plasma flow (RPF) and renal blood flow (RBF) did not alter in either control or heat exposure period. Changes of GFR and RPF were not proportionate which caused a significant decrease in calculated filtration fraction. Renal urea excretion showed no alteration throughout the experiment of both series while the decreases in renal urea clearance and fractional urea excretion were apparent in animals given urea infusion in normal ambient temperature. It has been noted that urinary total nitrogen excretion decreased markedly by 57% after urea infusion in normal animals while no changes were apparent in heat exposed animals. These reduction coincided with the decrease in urinary non urea nitrogen excretion by approximately 18.5% of the mean value obtained before urea infusion. It was found that the rate of urine flow also showed a positive correlation with urinary non-urea nitrogen excretion ($P < 0.001$, Fig. 5). The calculated renal urea reabsorption showed significant increased by approximately 46% after urea infusion in animals kept in normal ambient temperature ($P < 0.05$) while a slight decrease was recorded during heat exposure.

Table II Effect of exogenous urea infusion on renal hemodynamic and urea excretion of normal and acute heat stressed buffaloes. (Mean±S.D.)

	Normal ambient temperature		Heat exposure		
	before urea infusion	3 h.after urea infusion	Pre-heat exposure	3 h.heat exposure	3 h.after urea infusion
Urine flow rate (ml/min)	9.37±7.18	5.87±4.77*	7.08±2.68	5.87±2.2	4.73±2.08
Glomerular filtration rate (ml/min)	280.4±64.8	278.3±89.2	302.2±56.7	308.9±29.7	233.5±51.8
Effective renal plasma flow (ml /min)	1237.8±323.1	1091.0±401.9	1255.8±307.7	1321.9±221.2	1165.4±322.3
Renal blood flow (ml/min)	1732.0±452.1	1501.6±545.7	1751.1±434.8	1812.7±278.1	1609.6±466.7
Filtration fraction (%)	23.0±3.49	26.91±8.71	25.10±6.73	23.76±3.72	20.39±3.76*
Renal urea clearance (ml/min)	197.12±80.79	166.14±97.84	194.6±52.9	190.4±26.2	156.9±43.6
Renal urea excretion (mg/min)	89.4±41.1	84.3±50.5	91.0±27.1	88.9±16.9	81.1±17.5
Fractional urea excretion (%)	67.94±14.10	56.17±15.24**	63.81±9.61	62.11±10.29	68.16±15.45
Urinary total nitrogen excretion (mg/min)	128.5±110.9	55.5±26.6	55.5±9.8	58.9±13.4	55.5±20.5
Urinary non urea nitrogen excretion (mg/min)	86.8±93.4	16.1±6.6	12.9±7.3	17.4±8.6	17.7±14.9
Urinary urea nitrogen excretion (mg/min)	41.6±19.3	39.4±23.5	42.5±12.5	41.5±7.8	37.8±8.2
Urea reabsorption (mg/min)	41.8±20.1	61.1±22.5*	52.7±20.1	57.3±25.3	42.2±25.7

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

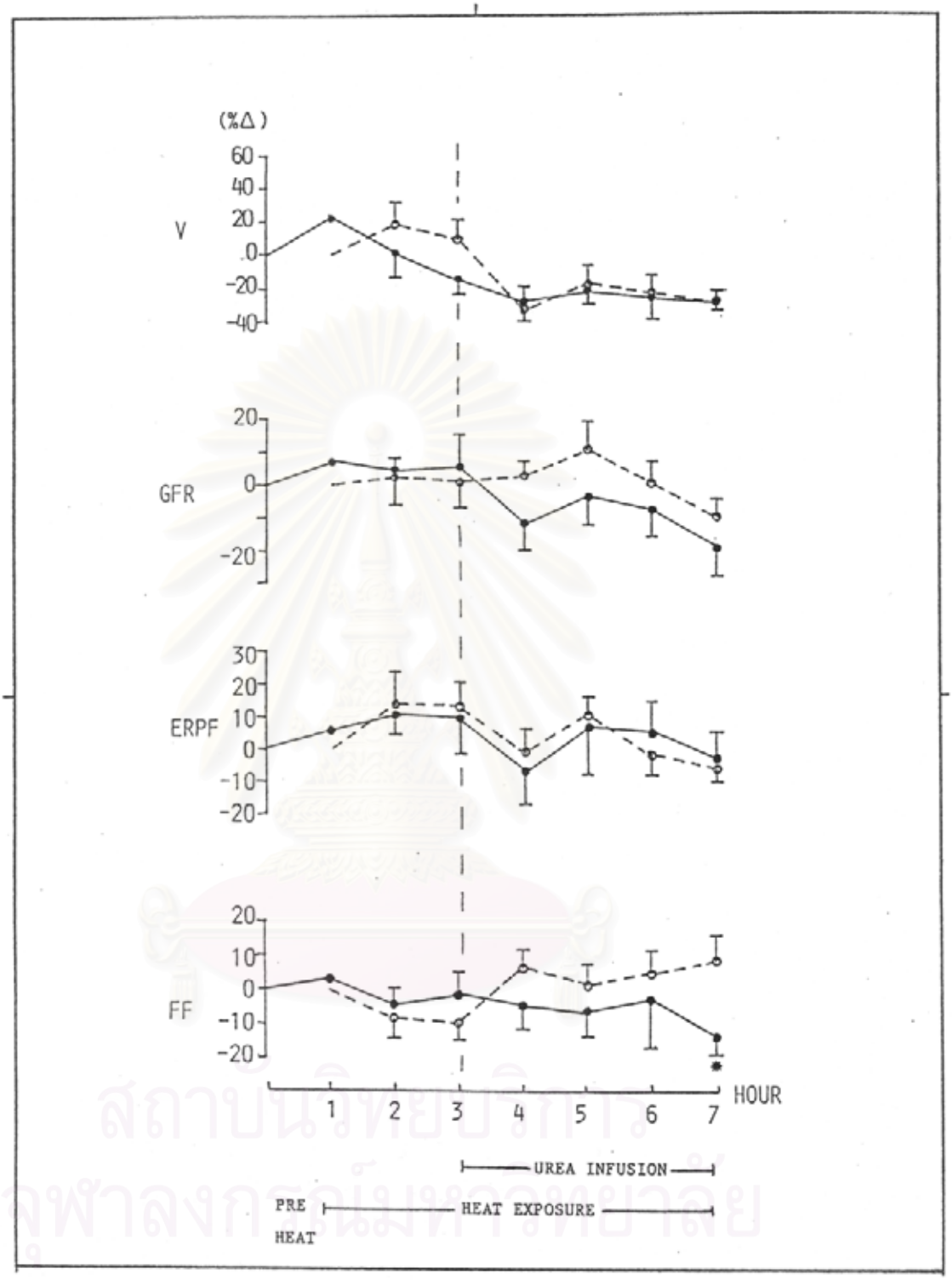

Fig 2 Mean percent changes for urine flow rate, glomerular filtration rate, effective renal plasma flow and filtration fraction of five swamp buffaloes given exogenous urea infusion during normal ambient temperature (o---o) and acute heat exposure (●——●) period. The values are mean$\pm$S.E., P-values with respect to the first hour value and preexposed value for animals during normal ambient temperature and heat exposure period respectively.

* $P < 0.05$

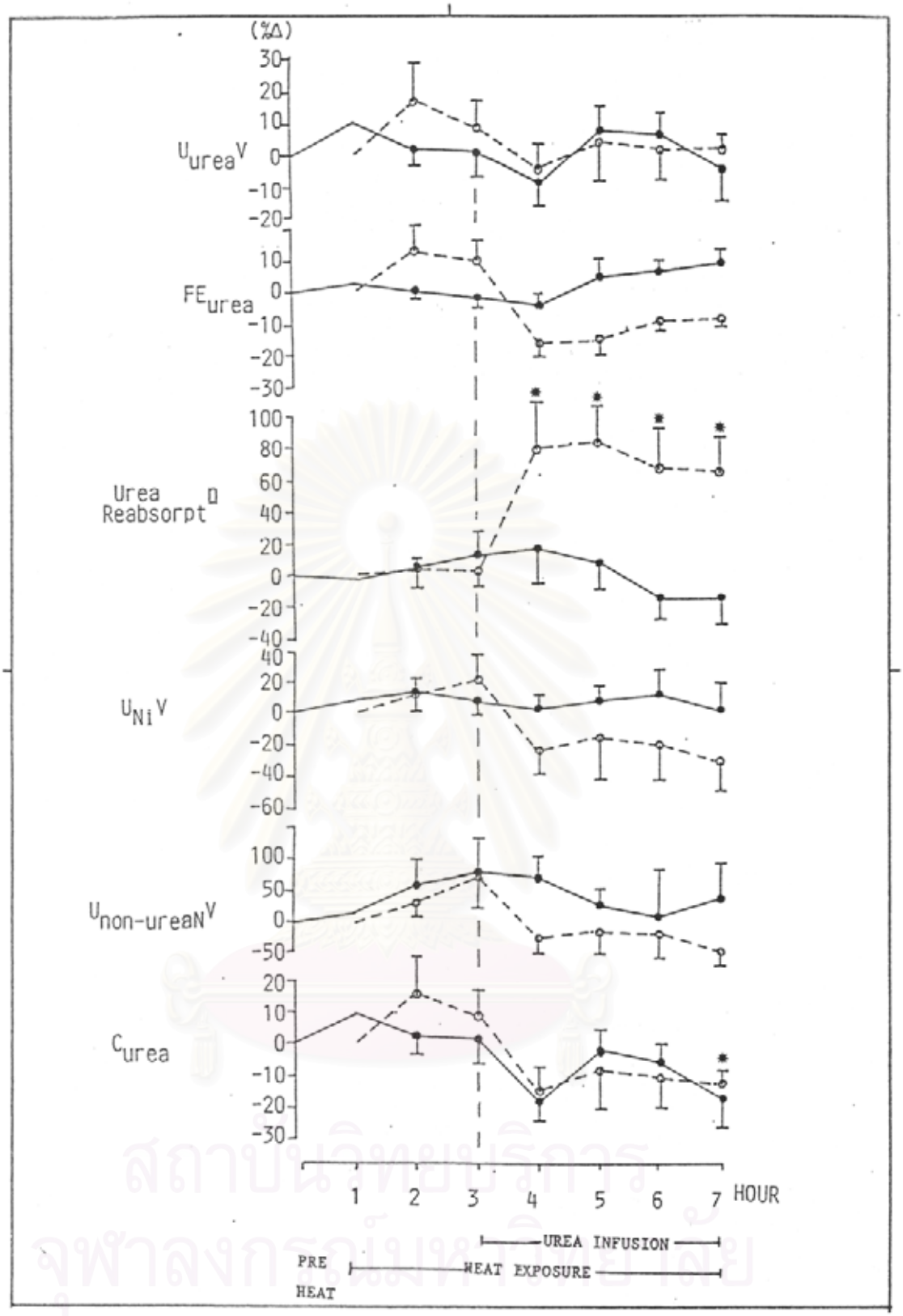

Fig 3 Mean percent changes for urinary urea excretion, fractional urea excretion, renal urea reabsorption, urinary total nitrogen excretion, urinary non-urea nitrogen excretion and renal urea clearance of five swamp buffaloes given exogenous urea infusion during normal ambient temperature (o---o) and acute heat exposure (●——●) period. The values are mean$\pm$S.E., P-values with respect to the first hour value and preexposed value for animals during normal ambient temperature and heat exposure period respectively.

* $P < 0.05$

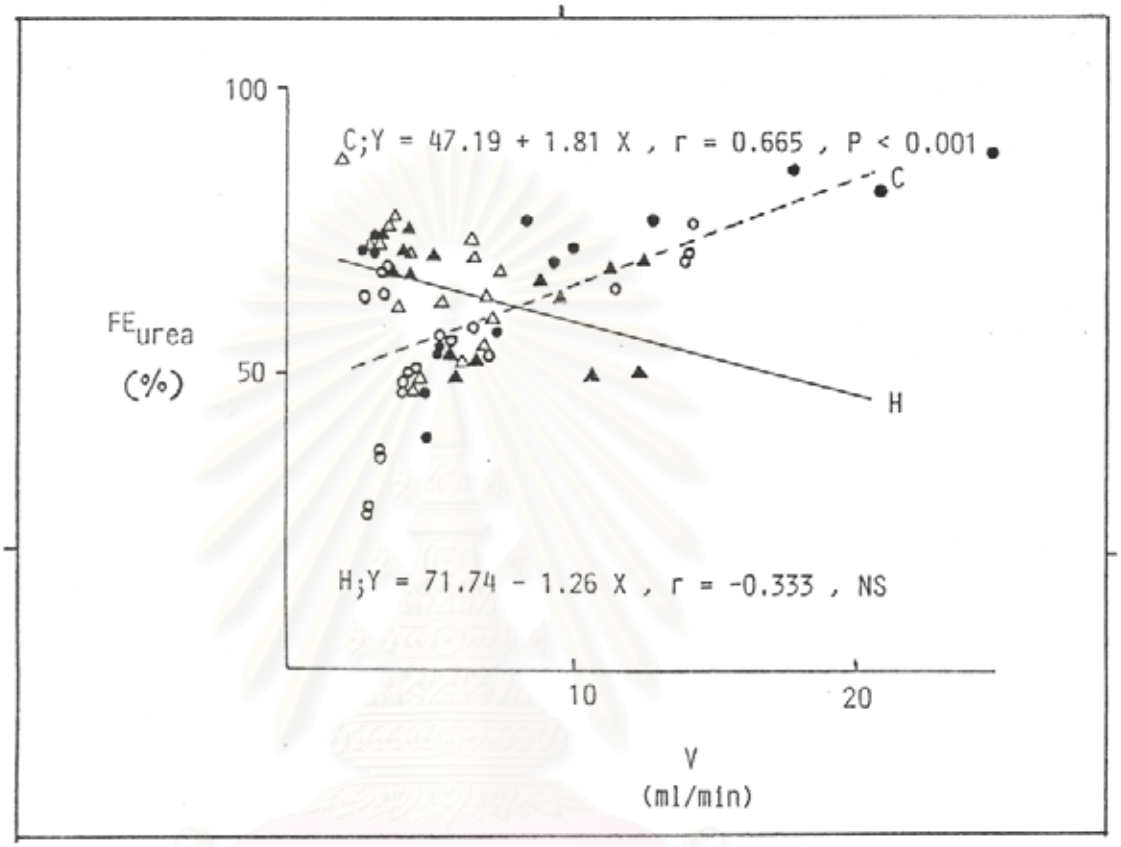

Fig 4 The relationship between urine flow rate (V) and fractional urea excretion (FE_{urea}) of five swamp buffalces given exogenous urea infusion during normal ambient temperature and acute heat exposure period.

o normal temperature without urea ● normal temperature with urea

Δ heat exposure without urea ▲ heat exposure with urea

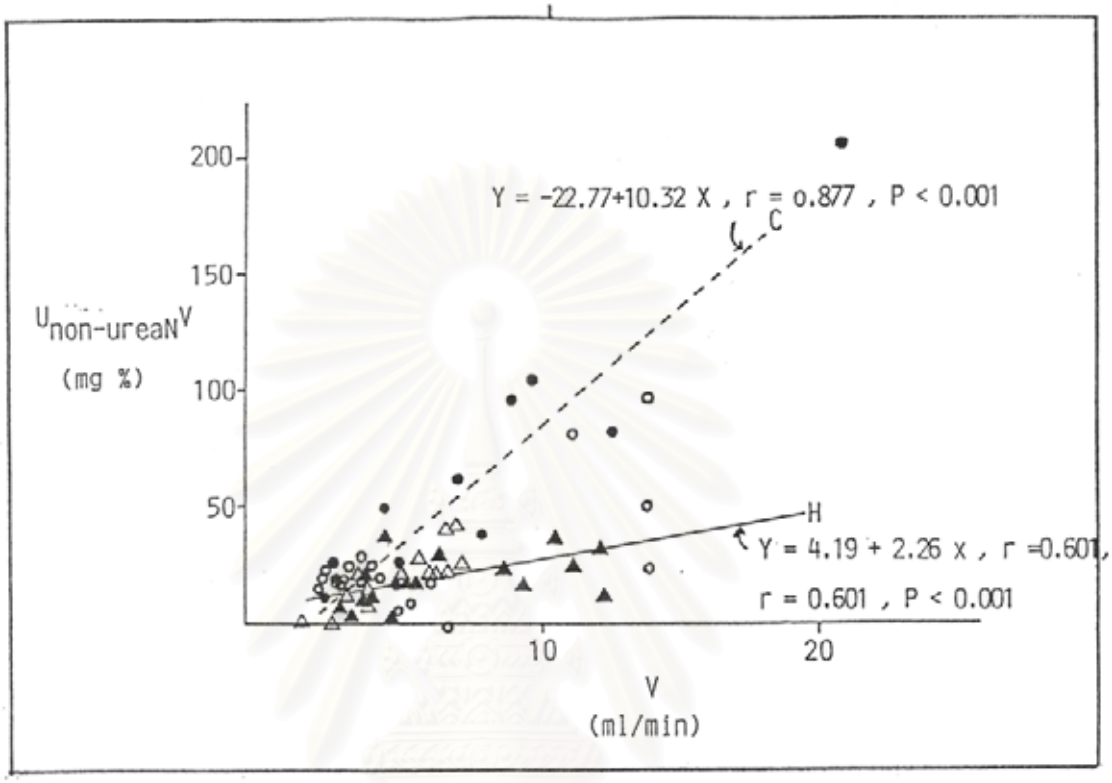

Fig 5 The relationship between urine flow rate (V) and urinary non-urea nitrogen excretion ($U_{non-ureaN}V$) of five swamp buffalces given exogenous urea infusion during normal ambient temperature and acute heat exposure period.

o normal temperature without urea • normal temperature with urea

△ heat exposure without urea ▲ heat exposure with urea

Changes of renal electrolyte excretion, Osmolar clearance free water clearance and urine pH during control and heat exposure period.

(Table III, Fig. 7, 8)

The results show that the urinary and fractional sodium excretion slightly increased after urea infusion in animals kept in normal ambient temperature. However after 3 h. of heat expossure, the marked decrease was detected by 32% and 42% respectively. The tendency of an increase in either urinary and fractional sodium excretion was exhibited thereafter during infusion of urea in heat stressed animals.

Urinary and fractional K^+ excretion decreased significantly by approximately 43% after urea infusion in animals kept in normal ambient temperature ($P < 0.05$), whereas slight reduction was detected during heat exposure. A positive correlation between urinary flow rate and fractional excretion of potassium ($P < 0.001$, Fig. 6), were observed in either heat exposure or during urea infusion. The pattern of excretion of Cl^- was similar to that of K^+. No significant changes of the excretion of Ca^{++} and P_i were noted throughout the study.

The osmolar clearance of animals kept in either normal ambient temperature or exposed to heat showed marked decrease after urea infusion. However, no remarkably changes of free water clearance by the effect of urea infusion were apparent. Urine pH in animals kept in normal ambient temperature markedly decreased after urea infusion whereas there were no changes in animals exposed to heat.

Table III Effect of exogenous urea infusion on renal electrolyte excretion, osmolar clearance, free water clearance and urine pH of normal and acute heat stressed buffaloes. (Mean±S.D.)

	Normal ambient temperature		Heat exposure		
	before urea infusion	3 h.after urea infusion	Pre-heat exposure	3 h. heat exposure	3 h. after urea infusion
Urinary Na^+ excretion (μEq/min)	186.3±195.3	212.9±158.5	320.2±301.8	217.2±136.3	280.2±267.4
Urinary K^+ excretion (μEq/min)	1898.1±1000.4	1093.8±704.5*	1181.3±647.9	1172.7±455.1	738.9±418.9
Urinary Cl^- excretion (μEq/min)	1337.9±928.9	973.0±727.4*	1207.8±398.7	1051.7±614.3	699.2±430.1
Urinary Ca^{2+} excretion (mg/min)	0.413±0.436	0.728±0.575	0.929±0.391	0.730±0.448	0.551±0.496
Urinary $PO_4^{=}$ excretion (mg/min)	0.032±0.013	0.028±0.015	0.046±0.028	0.048±0.033	0.040±0.032
Fractional Na^+ excretion (%)	0.492±0.468	0.623±0.460	0.923±1.008	0.538±0.315	0.911±0.886
Fractional K^+ excretion (%)	162.3±67.6	90.9±24.6*	87.5±26.9	93.4±20.6	80.5±33.9
Fractional Cl^- excretion (%)	4.531±2.301	3.433±1.392	4.130±1.445	3.378±1.822	2.860±1.734
Fractional Ca^{++} excretion (%)	1.642±1.666	3.145±1.790	4.061±2.167	2.946±1.807	2.758±2.390
Fractional $PO_4^{=}$ excretion (%)	0.245±0.069	0.251±0.067	0.305±0.142	0.340±0.143	0.435±0.268
Osmolar clearance (ml/min)	21.63±10.97	16.27±9.36*	17.07±5.50	17.38±6.04	13.40±4.64
Free water clearance (ml/min)	-12.176±4.099	-10.398±4.646	-9.996±2.940	-11.512±4.021	-8.551±2.682
Urine pH	9.52±0.20	9.14±0.40	8.83±0.79	8.86±0.90	8.79±0.9

* $P < 0.05$

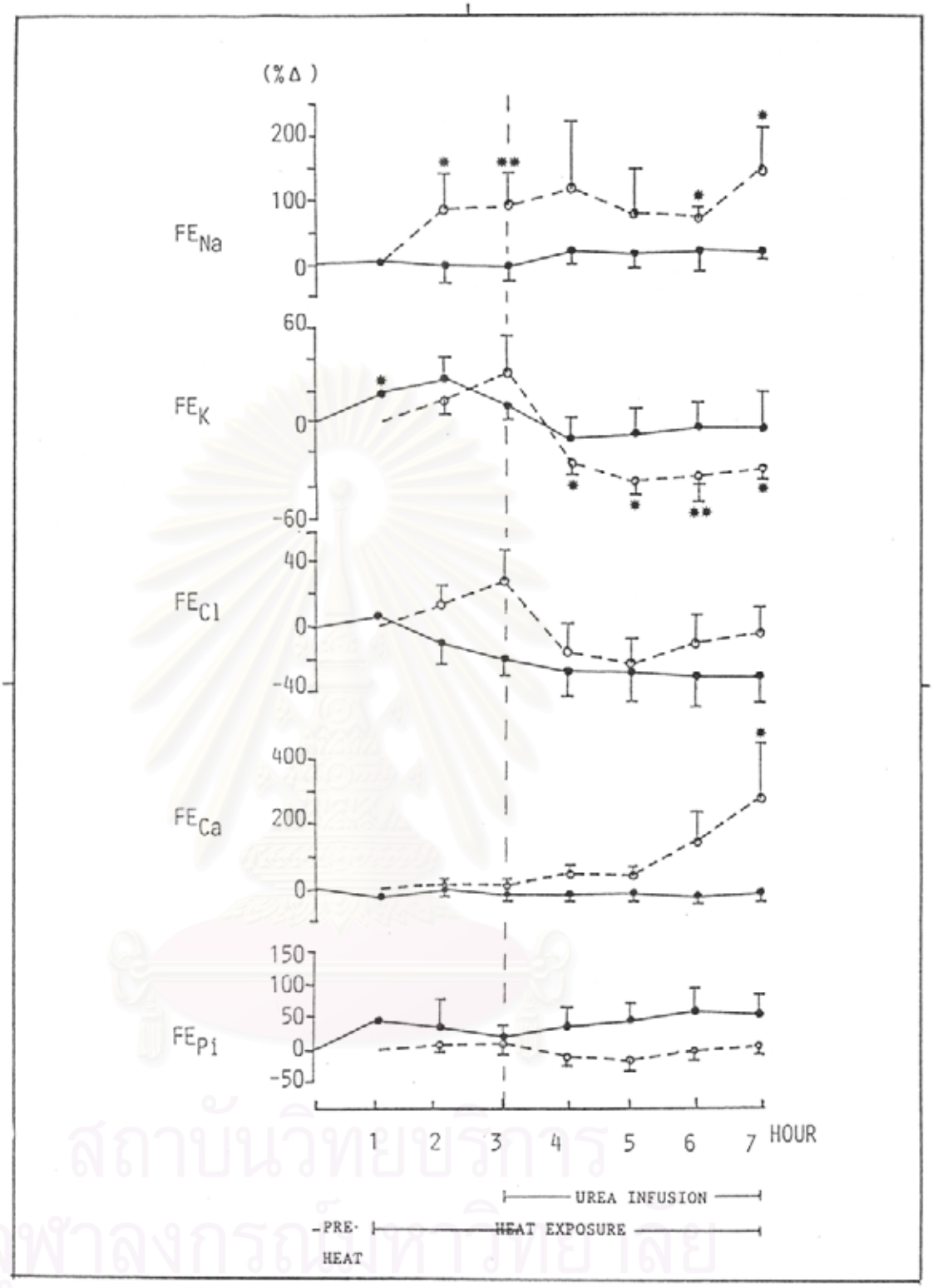

Fig 7 Mean percent changes for fractional excretion of sodium, potassium, chloride, calcium and phosphorus of five swamp buffaloes given exogenous urea infusion during normal ambient temperature (o---o) and acute heat exposure (●——●) period. The values are mean±S.E., P-values with respect to the first hour value and preexposed value for animals during normal ambient temperature and heat exposure period respectively.

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

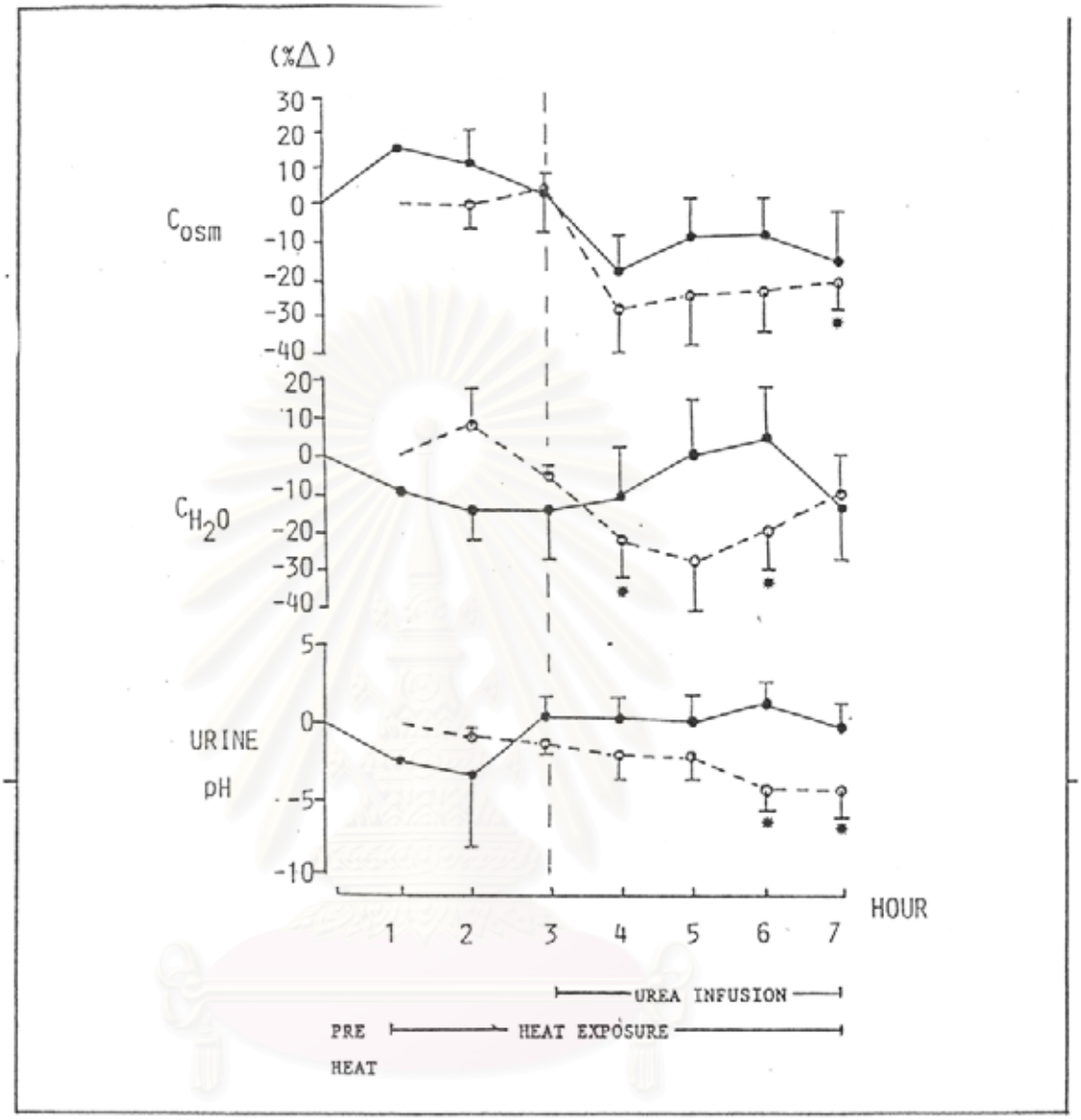

Fig 8 Mean percent changes for osmolar clearance, free water clearance and urine pH of five swamp buffaloes given exogenous urea infusion during normal ambient temperature (o---o) and acute heat exposure (●——●) period. The values are mean±S.E., P-values with respect to the first hour value and preexposed value for animals during normal ambient temperature and heat exposure period respectively.

* $P < 0.05$

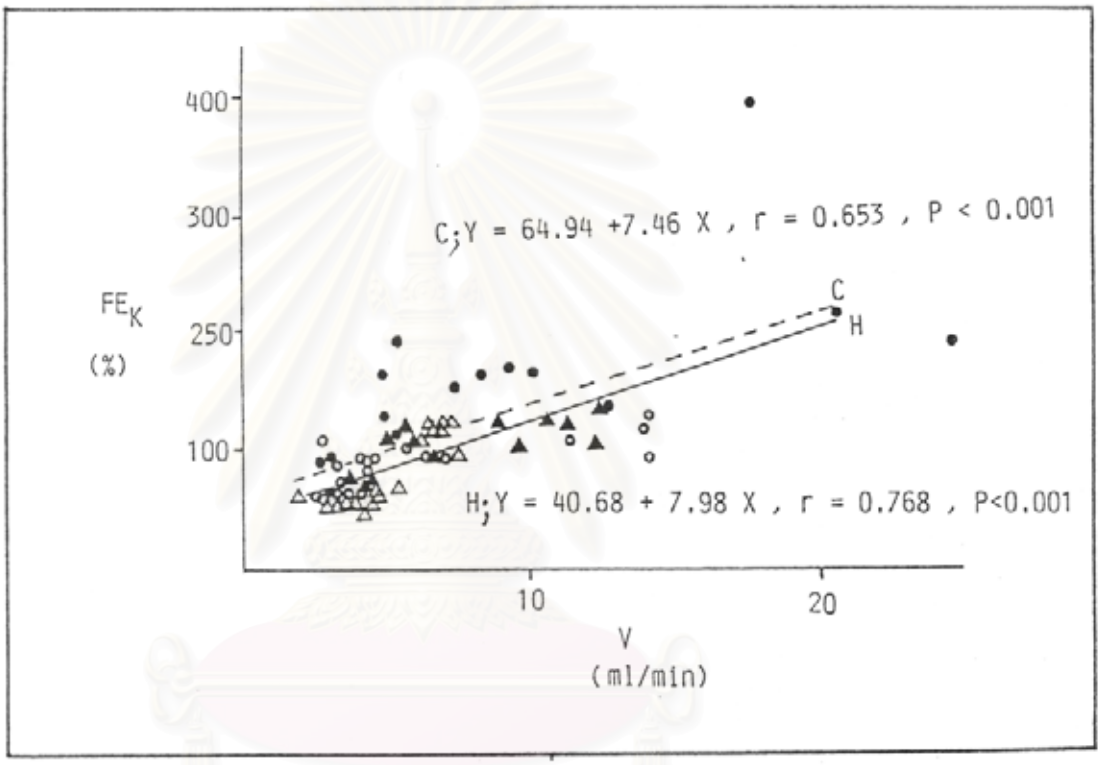

Fig 6 The relationship between urine flow rate (V) and fractional potassium excretion (FE_K) of five swamp buffaloes given exogenous urea infusion during normal ambient temperature and acute heat exposure period.

o normal temperature without urea ● normal temperature with urea

Δ heat exposure without urea ▲ heat exposure with urea

Changes of plasma electrolytes and plasma constituents during control and heat exposure period (Table IV, Fig. 9, 10)

The results indicate that no significant changes of the concentration of plasma constituents were apparent in animals kept in normal ambient temperature. During heat exposure, plasma sodium concentration significantly decreased after 3 h. of heat exposure ($P < 0.05$) and it returned to preexposed value after urea infusion. Plasma concentrations of Cl^- and Ca^{++} remained constant throughout the period of study. It has been shown that plasma inorganic phosphate concentration decreased gradually by 10% and 23% during heat exposure and after urea infusion respectively ($P < 0.05$). During heat exposure, there was a rise of plasma creatinine concentration with the concomitant increase in total plasma protein concentration. An elevation was obviously coincided with the time course of heat exposure. Plasma protein concentration increased nearly 13% during 7^{th} h. of heat exposure after urea infusion ($P < 0.001$). The increased values of plasma albumin concentration was higher than that of globulin. Therefore, the albumin globulin ratio exhibited a tendency to increase. Plasma osmolarity decreased significantly during animals exposed to heat for 3 h. ($P < 0.05$) and it returned to the preexposed value during urea infusion.

Table IV Effect of exogenous urea infusion on plasma electrolytes and plasma constituents of normal and acute heat stressed buffaloes. (Mean$\pm$S.D.)

	Normal ambient temperature		Heat exposure		
	before urea infusion	3 h.after urea infusion	Pre-heat exposure	3 h. heat exposure	3 h. after urea infusion
Plasma sodium (mEq/L)	133.4$\pm$6.4	134.3$\pm$8.3	138.2$\pm$4.0	136.2$\pm$3.9*	137.1$\pm$3.0
Plasma potassium (mEq/L)	4.44$\pm$0.38	4.32$\pm$0.22	4.62$\pm$0.34	4.27$\pm$0.35	4.14$\pm$0.34
Plasma chloride (mEq/L)	95.8$\pm$5.0	99.1$\pm$7.0	99.6$\pm$5.7	98.4$\pm$5.0	100.1$\pm$2.9
Plasma calcium (mg%)	7.90$\pm$0.50	7.87$\pm$0.36	8.63$\pm$0.44	8.36$\pm$0.26	8.58$\pm$0.28
Plasma inorganic phosphate (mg%)	4.34$\pm$1.05	4.10$\pm$1.21	4.68$\pm$0.82	4.19$\pm$0.67	3.59$\pm$0.45*
Plasma Protein (gm%)	9.77$\pm$0.9	9.90$\pm$0.91	9.12$\pm$0.23	9.57$\pm$0.22**	10.27$\pm$0.25***
Albumin (gm%)	4.47$\pm$0.51	4.60$\pm$0.63	4.0$\pm$0.38	4.38$\pm$0.27	4.76$\pm$0.38*
Globulin (gm%)	5.30$\pm$0.63	5.30$\pm$0.49	5.12$\pm$0.46	5.20$\pm$0.42	5.51$\pm$0.27
A/G ratio	0.854$\pm$0.127	0.873$\pm$0.129	0.791$\pm$0.147	0.851$\pm$0.111	0.870$\pm$0.100
Plasma creatinine (mg%)	1.39$\pm$0.25	1.43$\pm$0.29	1.53$\pm$0.29	1.63$\pm$2.7	1.82$\pm$0.3*
Posm (mOsm/kgH_2O)	264$\pm$8	263$\pm$10	274$\pm$6	263$\pm$8*	275$\pm$4
Plasma urea concentration (mg%)	46.3$\pm$12.5	53.2$\pm$15.0**	47.4$\pm$8.6	47.1$\pm$8.6	53.0$\pm$9.1***

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

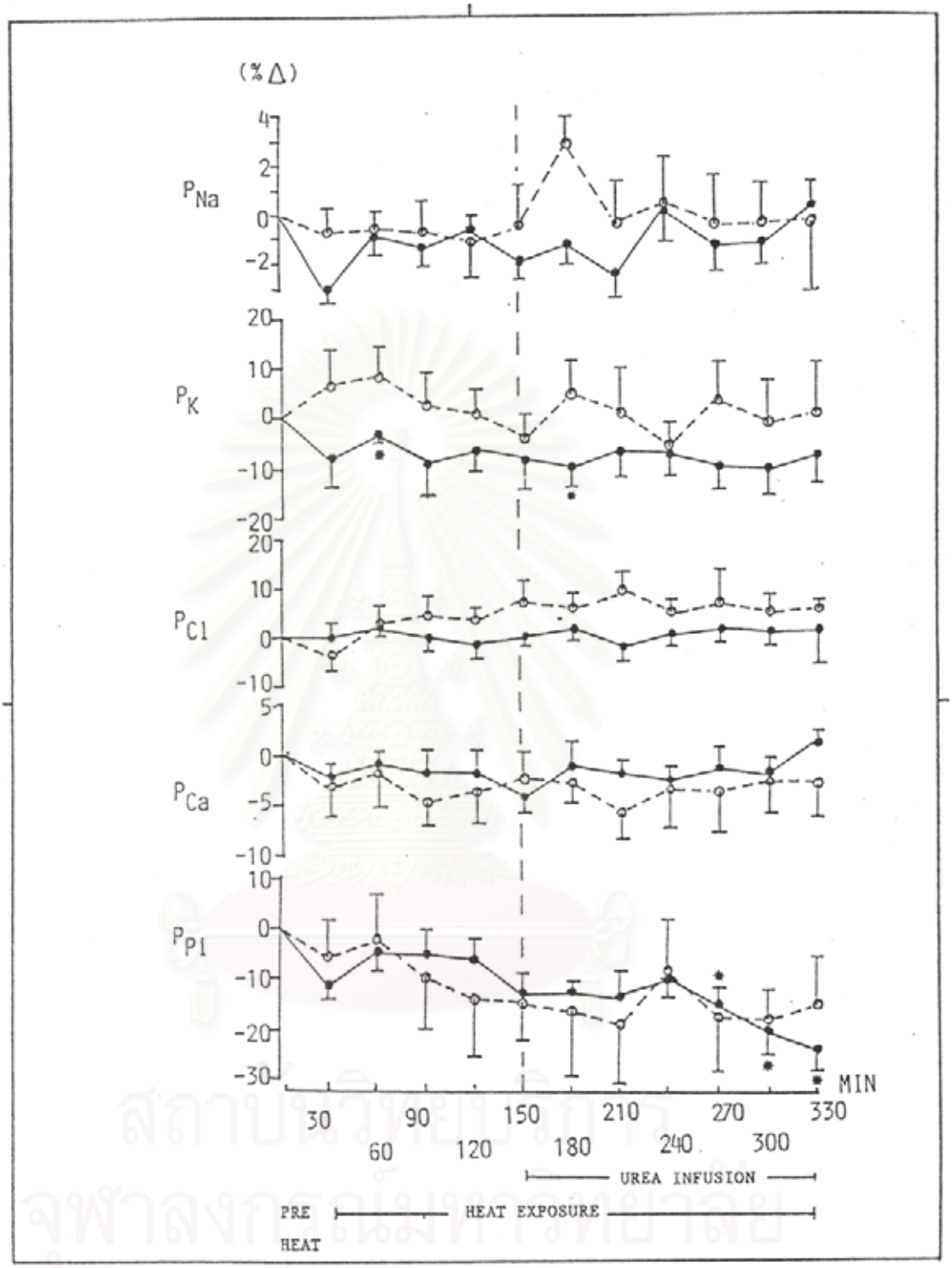

Fig 9 Mean percent changes for plasma concentrations of sodium, potassium, chloride, calcium and inorganic phosphorus of five swamp buffaloes given exogenous urea infusion during normal ambient temperature (o---o) and acute heat exposure (●——●) period. The values are mean±S.E., P-values with respect to the first hour value and preexposed value for animals during normal ambient temperature and heat exposure period respectively.

* $P < 0.05$

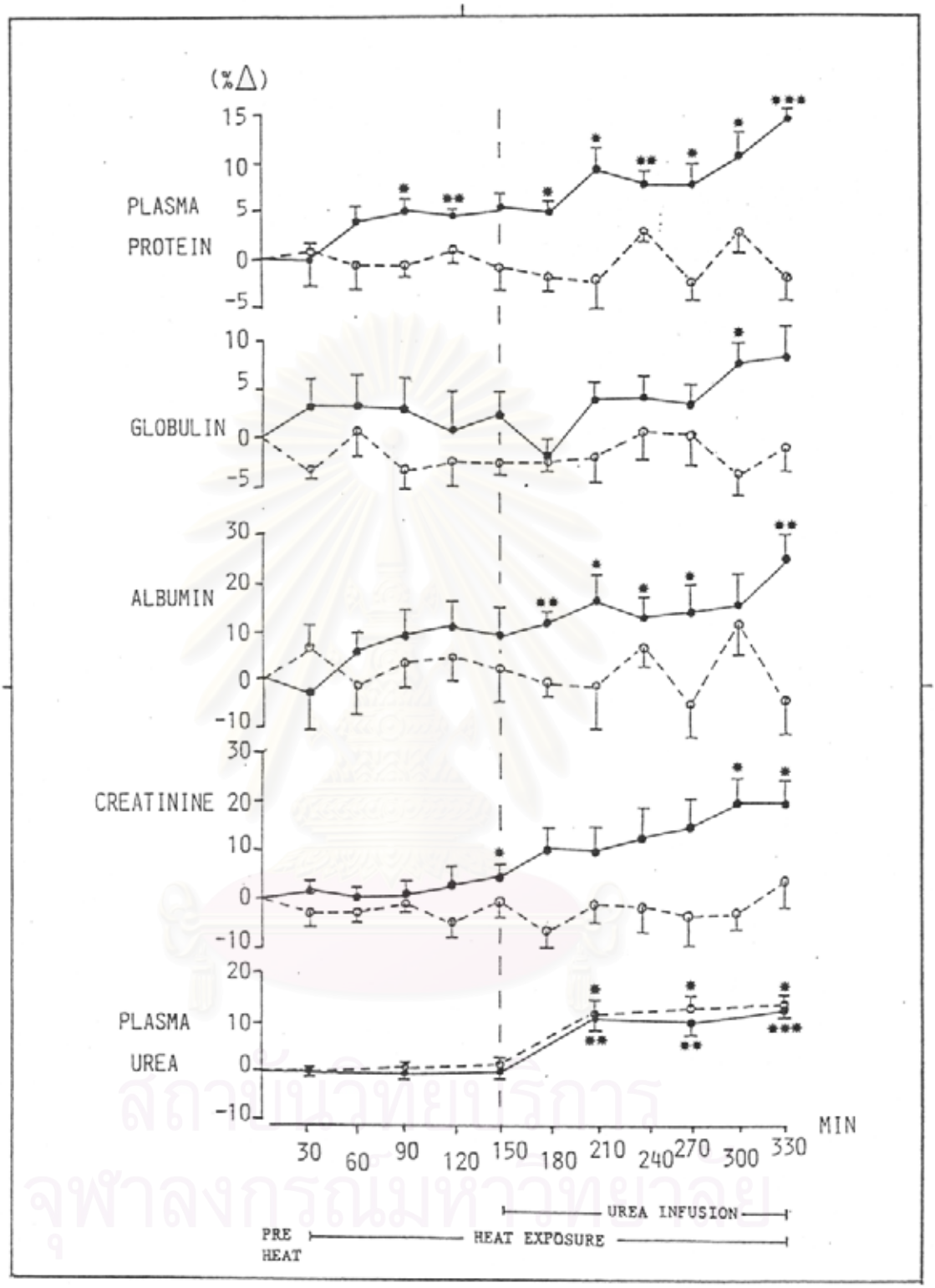

Fig 10 Mean percent changes for plasma protein, globulin, albumin, creatinine and urea concentrations of five swamp buffaloes given exogenous urea infusion during normal ambeint temperature (o---o) and acute heat exposure (●——●) period. The values are mean±S.E., P-values with respect to the first hour value and preexposed value for animals during normal ambient temperature and heat exposure period respectively.

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

Discussion

The amount of urea excreted in the urine of mammals is known by the determination of both the amount of urea filtered at the glomerulus and by the extent to which this urea is reabsorbed. The present experiments show that buffalo at normal ambient temperature excreted urea in the urine by approximately 68% of glomerular filtered urea. These results indicate that buffaloes could maintain on adequate dietary protein, since it was shown that very low percentage of glomerular filtered urea could be detected in ruminating animals fed low protein diet (Gans, 1966; Schmidt-Nielsen et al, 1957). However, in the present experiment, only 56.9% glomerular filtered urea was excreted in buffaloes given exogenous urea intravenously. The marked increase in renal urea reabsorption in this period indicates urea retention which was independent of glomerular filtration rate and the level of protein in the diet. (Schmidt-Nielsen, 1958).

No increase in GFR was observed, despite a marked increase in plasma urea concentration which was similar to the experiment in sheep given short term urea infusion (Ergene and Pickering, 1978). There were significant positive correlations between the rate of urine flow and fractional urea excretion which indicates reabsorption or back diffusion of urea through renal tubule. This relationship has been shown in domestic animals including the sheep (Gans, 1966; Cocimano & Leng, 1967).

In the present experiment the decrease in the non-urea nitrogen excretion coincided with the decrease in the rate of urine flow. This phenomenon indicates that the decrease in the total nitrogen excretion during exogenous urea infusion depends not only on the nitrogen intake but also on nutritional condition (Houpt, 1959).

The decrease in the urine flow rate during urea infusion was related to the decrease in electrolyte excretion particularly potassium ion which cannot create osmotic diuretic effect resulting in the decline of osmolar clearance. Therefore, effect of urea alone had limited diuretic ability (Godwin & William, 1984). The decrease of urine pH was concomitant with the decrease of K^+ excretion during urea infusion which could be explained by the well known fact that kidney play a significant role in acid-base regulation by an attempt to ensure hydrogen ions with a reciprocal secretion of potassium ions (Johnson & Selkert, 1966). The similar decrease in urinary K excretion was also reported in sheep after, increase urea level in the blood (Juhasz and Szegedi, 1969). Whether the decrease in urinary potassium excretion by the effect of metabolic acidosis during exogenous urea administration in the present experiment would be further investigated. In the present investigation, the antidiuretic effect after urea infusion was not apparently due to the action of antidiuretic hermone since the free water clearance kept constant. This finding is apparently due to the fact that urea readily penetrates cell membranes and thus does not provide an effective osmotic stimulus. Hypertonic urea has also been reported to provide a poor osmotic stimulus for ADH. (Robertson et al, 1977).

During heat exposure for 3 h., there were no significant changes in renal hemodynamics. These results are not similar to the previous study in buffaloes after 4 h. exposed to solar radiation in which the elevation of renal blood flow has been apparent (Chaiyabutr et al, 1983).

In the present study, total plasma protein increased significantly by the time of heat exposure. An elevation has also been reported in both man (Senay, 1970) and buffalo exposed to severe heat (Chaiyabutr et al, 1983; 1987). An increase in albumin fraction was exceed than that of globulin causing a tendency of increase in A/G ratio. The change in albumin had a large role in determining total protein behavior (Senay & Christensen, 1968). The mechanism of increase in plasma protein concentration would be due to the activation of endogenous nitrogen catabolism (Blincoe & Brody, 1951) or due to the muscle protein breakdown. However, neither the change of plasma urea concentration nor the urinary total nitrogen excretion were observed. Therefore, the elevation of plasma protein concentration during heat exposure would be due to muscle protein breakdown since plasma creatinine concentration increased indicating skeletal muscle cell degeneration (Terui et al, 1979). An exogenous urea infusion during heat exposure could not be accounted for an increase in renal urea reabsorption in comparison to the buffalo study in normal ambient temperature even though the decrease of urine flow has been observed. These findings suggest that the body pool size of nitrogenous substances would be

a factor influencing the relation of blood urea level to its renal retention of N-substance rather than dietary protein status during acute heat exposure. Since, In acute heat stressed buffalo there was an increase in body water turnover and blood volume (Chaiyabutr et al, 1987) concomitant with an increase in plasma concentration of protein and creatinine. In this case either urea and non-urea nitrogen may be transported from the tissue into the blood and distribution in the body fluid. The decrease in plasma potassium concentration in the present study is complex. During acute heat exposure the sympathetic outflow could be responsible for increase cardiorespiratory frequency. Significant increase of norepinephrine and dopamine have been reported in cattle exposed to severe heat (Yousef, 1979) which will cause the reduction of plasma K^+ concentration (Brown, 1984). The previous experiment has also been shown markedly increase of plasma glucose concentration in acute heat stressed buffalo (Chaiyabutr et al, 1987). This effect may induce to release endogenous insulin and can cause hypokalemia by a shift of K^+ into cell (Rose, 1977). Another possibility for the decrease in plasma K^+ concentration might be due to the shift of potassium ion moving intracellularly during alkalotic states which usually occur in panting animals. The decrease in pK was consistent to the decrease in P_{pi} which has also been demonstrated in heat exposed steer (Terui et al, 1979). The reduction of P_{pi} might result in increased rates of glucolytic enzyme activity and accumulation of phosphorylative glycolytic intermediates by trapping more P intracellularly during alkalotic states (Knochel & Caskey, 1977). During exogenous urea infusion, both urinary and fractional K^+ excretion

markedly decreased in buffaloes kept in normal ambient temperature. However, no apparent change during heat exposure might be due to the effect of exogenous urea infusion which was superimposed by respiratory alkalosis since urine pH kept constant. In conclusion, an increase in renal urea reabsorption during urea infusion in normal ambient temperature depends on urinary electrolyte excretion. Urea alone has limited diuretic ability. The decrease in urinary electrolytes excretion (e.g. K^+) will affect to the reduction of the rate of urine flow by an osmotic diuretic effect. These changes are insufficient to aid renal urea excretion although an increase in the filtered urea has been determined. In heat stressed animal, renal urea reabsorption is not affected by the decrease in the rate of urine flow, but it depends on an increase in the body pool size of nitrogenous substance which will limit renal urea retention.

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