รายงานผลการวิจัย

เรื่อง

ผลของการฉีดยูเรียต่อกลูโคสเมตาบอลิสมในกระบือปลักที่ได้รับความเครียดเนื่องจากความร้อน

(Effects of exogenous urea infusion on glucose metabolism in acute heat stressed swamp buffaloes)

โดย

ณรงศักดิ์ ชัยบุตร

ชลลดา บูรณกาล

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พฤษภาคม 2531

ทุนวิจัยงบประมาณแผ่นดินปี 2530

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ผลของการฉีดยูเรียต่อกลูโคสเมตาบอลิสมในกระบือปลักที่ได้รับความเครียดเนื่องจากความร้อน

กระบือที่ได้รับการฉีดยูเรียเข้าหลอดเลือดดำเป็นเวลา 4 ช.ม. ในขณะที่อยู่ในอุณหภูมิแวดล้อมปกติ ไม่พบการเปลี่ยนแปลงของอัตราการเต้นของหัวใจ อัตราการหายใจ ค่าเม็ดเลือดแดงอัดแน่น อัตราการหมุนเวียนกลูโคส ปริมาณกลูโคสทั้งหมด เคลียรานซ์ของกลูโคส อัตราการกรองผ่านกลอเมอรูลัส อัตราการไหลของพลาสม่าผ่านไต รวมทั้งความเข้มข้นของอิเล็กโทรไลต์ โปรตีน และครีอะตินีนในพลาสม่า ส่วนอัตราการขับปัสสาวะ สัดส่วนการขับทิ้งยูเรีย การขับทิ้งโปตัสเซี่ยมทางปัสสาวะ และออสโมล่าเคลียรานซ์จะลดลง ในขณะที่การดูดกลับยูเรียที่ไตจะเพิ่มขึ้น ในกระบือที่ได้รับความเครียดเนื่องจากความร้อน อุณหภูมิร่างกาย อัตราการเต้นของหัวใจ และอัตราการหายใจจะเพิ่มขึ้นอย่างมีนัยสำคัญ อัตราการหมุนเวียนของ U-^{14}C- กลูโคส และ 3-^{3}H-กลูโคส เพิ่มขึ้นอย่างเด่นชัดในขณะที่การนำอะตอมคาร์บอนของกลูโคสกลับมาใช้ลดลง ความเข้มข้นของกลูโคสในพลาสม่าเพิ่มขึ้นอย่างมีนัยสำคัญเมื่อสัตว์ได้รับความร้อน แต่อัตราการกรองและอัตราการไหลของพลาสม่าผ่านไตไม่เปลี่ยนแปลง ในการฉีดยูเรียในกระบือปลักที่ได้รับความร้อนพบว่าอัตราการหมุนเวียนของกลูโคสลดลงอย่างเด่นชัด แม้ว่าความเข้มข้นของกลูโคสในพลาสม่าจะเพิ่มขึ้นอย่างมาก อัตราการขับปัสสาวะจะลดลง แต่การดูดกลับยูเรีย พีเอชของปัสสาวะและสัดส่วนการขับทิ้งของอิเล็กโทรไลต์ทางปัสสาวะพบว่าไม่มีการเปลี่ยนแปลง ในขณะที่สัตว์ได้รับความเครียดจากความร้อนระดับโปรตีนและครีอะตินีนในพลาสม่าเพิ่มขึ้นอย่างเด่นชัด แต่ความเข้มข้นของอนินทรีย์ฟอสฟอรัสในพลาสม่าลดลง จากการศึกษานี้สรุปได้ว่า การสร้างและการใช้กลูโคสจะเพิ่มขึ้นในสัตว์ที่ได้รับความเครียดจากความร้อน แต่ขบวนการใช้จะถูกขัดขวางเมื่อมีการเพิ่มระดับยูเรียในพลาสม่า การเพิ่มการดูดกลับยูเรียที่ไตในขณะที่มีการฉีดยูเรียในสัตว์ที่อยู่ในอุณหภูมิแวดล้อมปกติขึ้นอยู่กับอัตราการขับปัสสาวะ ซึ่งเป็นผลมาจากการเปลี่ยนแปลงการขับทิ้งของอิเล็กโทรไลต์ การดูดกลับของยูเรียในสัตว์ที่ได้รับความเครียดจากความร้อนถูกจำกัดโดยการเพิ่มปริมาณของสารไนโตรเจนในร่างกาย และการเปลี่ยนแปลงของเมตาบอลิสมของร่างกาย

Abstract

Effects of exogenous urea infusion on glucose metabolism in acute heat stressed swamp buffaloes.

Five buffaloes kept in normal ambient temperature showed no significant changes in the heart rate, respiratory rate, packed cell volume, glucose turnover rate, glucose pool size, glucose clearance, glomerular filtration rate (GFR), effective renal plasma flow (ERPF), plasma concentration of electrolytes, protein and creatinine during intravenous infusion of urea for 4 h. The rate of urine flow, fractional urea excretion, urinary potassium excretion and osmolar clearance significantly decreased on the 4 h. 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 during urea infusion. In animals exposed to heat, the rectal temperature, heart rate and respiratory rate significantly increased. The turnover rate of both U-^{14}C glucose and 3-^{3}H glucose markedly increased while the reduction of glucose carbon recycling was observed. Plasma glucose concentration significantly increased during heat exposure but no significant changes in GFR and ERPF were noted. An intravenous infusion of urea in heat exposed animals caused the reduction of glucose turnover rate and glucose clearance while plasma glucose concentration progressively increased. Glucose carbon recycling slightly increased during exogenous urea infusion. The rate of urine flow decreased while renal urea reabsorption, urine pH

and fractional electrolyte excretions showed no significant changes. During heat exposure, there were marked increases in concentrations of total plasma protein and plasma creatinine whereas plasma inorganic phosphorus concentration significantly decreased. It is concluded that glucose production and utilization increased in animals exposed to heat but the interference of utilization occurred during urea infusion. An increase in renal urea reabsorption during urea infusion in buffaloes kept in normal ambient temperature depends on the rate of urine flow which affect by an osmotic diuretic effect of electrolytes. The limitation of renal urea reabsorption in heat stressed animals would be attributed to an increases in either plasma pool size of nitrogenous substance or body metabolism.

Introduction

It is known that a provision of glucose in the ruminant is entirely by endogenous synthesis from non-carbohydrate sources in the liver and kidney (Bergman, 1973). A wide variety of factors; for example, lactation, pregnancy and starvation, can affect blood glucose concentration by changing the rates of entry and removal of glucose in the circulation (Chaiyabutr et al, 1982). In acute heat exposure, an increase in glucose concentrations has been reported in either calves (Bianca & Findley, 1962) and buffaloes (Chaiyabutr et al, 1987). The mechanisms for rapid change in plasma glucose conentration particularly in buffalo have not been determined, although the water turnover rate and changes in renal hemodynamics have been reported (Chaiyabutr et al, 1983; 1987). However, in chronic heat exposed sheep, a decrease in glucose pool size, turnover rate and rate of gluconeogenesis without any changes in plasma glucose concentration have been described (Sano et al, 1979). In addition, acute heat exposed buffaloes showed increases in concentrations of plasma protein, creatinine and urea (Chaiyabutr et al,. 1983; 1987).

The mechanism responsible for the intrarenal handling of urea in the buffalo exposed to heat was unclear although increase in urea reabsorption was observed after urea infusion in normal buffalo. The relationship between metabolic substrate such as urea and glucose during heat stressed condition have not been determined. An attempt to elucidate the mechanism by which the animal regulated the bodily glucose metabolism during high plasma nitrogenous substance is brought about, glucose turnover rate, glucose recycling, renal urea reabsorption, renal hemodynamic and electrolyte excretion were studied in buffalo given exogenous urea infusion in either normal environmental temperature or acute heat exposure.

Materials and Methods

The experiment was performed in five adult female swamp buffaloes weighing between 334 to 439 kgs. All animals were fed with paragrass, rice straw and water hyacinth under natural condition. On the day of the experiment, each of the animal was tethered with standing position in the room while food and drinking water were withheld throughout the study.

Preparation

Before the experimental period, polyethylene catheter (PE 200) were inserted into both jugular veins by using medicut intravenous cannular to facilitate both infusion and blood sampling. A rubber urethral catheter with a coiled, multiperforate tip (9.0 mm o.d., 6 mm i.d.) was inserted into urinary bladder for urine collection as described by Anderson & Pickering (1961).

Experimental procedures

The experiment was divided into two series. First, the animal was housed in the room held at normal ambient temperature 30 $\pm$ 1 ^{o}C (dry bulb) and relative humidity was 60 $\pm$ 4%. In the second series of experiments, the animal was exposed to high ambient temperature of 41 $\pm$ 0.4 ^{o}C (dry bulb) and relative humidity of 42 $\pm$ 2% by using thermostatically electric heating unit as described by Chaiyabutr et al (1987). In each series of experiments the measurements of renal functions and glucose metabolism were performed both on 3 h before exogenous urea infusion and on 4 h after.

At the begining of the experiment of both series, the buffalo received a priming dose of p-aminohippurate (PAH) 1 gm in 40 ml of normal saline followed immediately by a sustaining solution of PAH 25 mg/4 ml/min. The solution was infused at a constant rate throughout the experimental study using peristaltic pump (EYELA, MP-3, Tokyo Rikakikai Co. Japan). Clearance measurements were started about an hour after injection of the priming dose. In each hour of clearance measurement, urine sample

was collected over an accurately timed period about 15-20 min. To ensure each accurate collection, the urine sample was started after voidness the bladder. Samples for determination of electrolytes and other constituents were collected every 30 min. interval while heart rate, respiratory rate and rectal body temperature were measured very one hour interval throughout the experimental runs. On both series of experiments after the first 3 h periods when PAH solution was infused alone, intravenous infusion of urea was started and continued for up to 4 h. The effects of short term infusion of urea were studied by intravenous infusion of exogenous urea with the priming dose of solution containing 3 gm of urea in 30 ml of normal saline followed immediately by continuous infusion of urea 50 mg with 4 ml of PAH solution.

To determine glucose metabolism, the animal was administered a bolus of 50 µCi 3-^{3}H glucose and 50 µCi of U-^{14}C glucose via another jugular catheter either on 3 h before exogenous urea infusion and 4 h after. Sequence of blood were collected at 10, 30, 45, 60, 75, 90, 105, 120, 135 and 150 min after isotope administration to determine glucose specific activity. The determinations of plasma volume using dye T-1824 were performed before and after exogeneous urea infusion on both series of experiments.

Analytical procedures

Glomerular filtration rate (GFR) was obtained using the clearance of endogenous creatinine. In the present study, the endogenous creatinine clearance (C_{cr}) can be accepted as an index of GFR in swamp buffaloes. Comparison of 54 simultaneous measurements of renal clearances of inulin (C_{in}) and endogenous creatinine showed a significant positive correlations ($C_{in} = 126 + 0.64\ C_{cr}$), $r = 0.75$, $p < 0.001$) (Chaiyabutr, unpublished data). Effective renal plasma flow (ERPF) was obtained using the

clearance of PAH. Urinary and plasma creatinine concentrations were determined by the method of Kennedy and the PAH concentration were determined by the method of Bratton and Marshall as described by Smith (1962). Plasma and urine urea were analyzed by the method of Ritcher & Lapointe (1962). Plasma total protein concentration was analyzed by biuret method. Plasma glucose concentration was determined by enzymatic colorimetric method.

Electrolyte concentrations in plasma and urine samples were analysed by the following procedures; sodium and potassium by flame photometry (KLiNa Flame Operating, Beckman instrument, U.S.A.), chloride by chloridometer (Buchler digital, U.S.A.), 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 (Advance osmometer model 3, U.S.A.). Urine pH was measured by pH meter (EIL 7050, Electric Instrument Ltd. U.K.)

Calculations of renal function

Clearances (C) of endogenous creatinine and PAH were used to measured GFR and ERPF respectively, based on the Fick Principle. Renal blood flow (RBF) was obtained by dividing ERPF by 1-packed cell volume. Filtration fraction (FF) was obtained by dividing GFR by ERPF. Fractional excretion (%FE) was obtained by dividing clearance of either electrolyte or urea by GFR. The tubular reabsorbed urea was obtained by the difference between the glomerular filtered and the renal excreted urea. Tubular solute-free water excretion (C_{H_2O}) was calculated by substraction urine flow rate (V) from osmolar clearance (C_{Osm}).

Packed cell volume was determined by microcapillary method. Heart rate was measured 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 ambient 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).

Plasma volume determination

Plasma volume was measured by dye dilution technique using Evans blue T-1824 as an indicator. After drawing blood as a control sample, the animal was injected with the bolus of 20 ml. of T-1824 (0.5% in normal saline). Sequence of blood samples were taken at 30, 45 and 60 min to determine the concentration of dye in the plasma. Plasma volume was calculated from the following equation.

$$PV = \frac{Id}{cd}$$

Where Id is total concentration of dye T-1824 bolus injection,

cd is the concentration of dye in the plasma at zero time which extrapolated from the concentration plotted at 30, 45 and 60 min. against semilogarithmic scale.

Blood volume was estimated from plasma volume and packed cell volume as follow :

$$\text{Blood volume} = \frac{\text{Plasma volume} \times 100}{100 - \text{Hct}}$$

Isotopic glucose determination

Blood samples used to measure glucose specific activity was collected in dried heparinized tubes. After centrifugation, plasma was seperated

and freezed at the temperature about -4°C until the day of determination. On the day of analysis, plasma was thawed and 2 ml. of plasma was deproteinized by equal volume of 5.5% Zinc sulfate and 4.73% barium hydroxide, four ml of supernatant was passed through IRA anion exchange resin (Amberlite, IRA-400 c.p, Mallinckrodt, St. Louis, MO., U.S.A.) that filled in a glass column (20 cm long c 4 mm. diameter) to eliminate contamination of the glucose radioactivity by other organic acids such as lactate and pyruvate (Radziuk et al, 1978). The resin bed volume was 2 ml. and the rate of flow of supernatant across the column was adjusted to 0.5 ml/min. The eluate was collected at the begining after it exited from the column. Three ml. of distilled water was added to the column to wash out the radioactive glucose. Total 7 ml. of eluate was evaporated at 80°C to reduced the volume to approximate 3 ml. The solution was divided into two portions. The first portion solution was used for measure of glucose concentration using enzymatic glucose oxidase method. Two ml. of the solution in the second portion was transferred to scintillating vial and evaporated to dryness at 80°C to remove all tritiated water. The residue was redissolved by 0.5 ml. of distilled water and counted in 10 ml. of the scintillant using liquid scintillation spectrometer and dual-isotope counting procedures. Crossover of ^{14}C counted into the channel for counting tritium was calculated from counting efficiency determined by using an external standard ratio. The scientillant contained toluene-triton X-100 (3:1 v/v), 5 g of PPO (2,5-diphenyloxazole) and 250 mg of POPOP (1,4-bis-(5-phenyl-oxazole-2 yl) benzene)/litre.

The glucose specific activity, in μCi/mg glucose, at zero time extrapolated from the specific activity plotted on semilogarithmic scale against time every 15 mins interval from 30 minute to 150 minute was used to calculate the glucose pool size as the following equation:

$$\text{glucose pool size (G)} = \frac{\text{Injection dose}}{\text{Specific activity at Zero time}}$$

Glucose turnover rate was calculated as follow:

$$\text{glucose turnover rate (T)} = \frac{\text{glucose pool size} \times 0.693}{t1/2}$$

Glucose carbon recycling (R) expressed in % was calculated using turnover rate of both U-^{14}C glucose and 3-^{3}H glucose.

$$R = \frac{\text{turnover rate of 3-}^{3}\text{H glucose - turnover rate of U-}^{14}\text{C glucose} \times 100}{\text{turnover rate of 3-}^{3}\text{H glucose}}$$

Statistical analysis

The results of experiments were evaluated using either paired or unpaired t-test.

Results

Changes of heart rate, respiratory rate, rectal temperature, packed cell volume, plasma volume and blood volume (Table 1)

No significant changes in rectal temperature, heart rate, respiratory rate and packed cell volume were observed in buffaloes kept in normal ambient temperature when exogenous urea was infused at 50 mg/min for 4 h. When animal exposed to heat for 3 h, the rectal temperature, heart rate and respiratory rate markedly increased. The progressive rises of heart rate and respiratory rate were detected in heat-exposed animals given exogenous urea by approximately 14% and 120% respectively. The packed cell volume slightly decrease during heat exposure. There were no significant differences in plasma volume and blood volume in animal given exogenous urea either during normal or high ambient temperature. The temperature humidity index (THI) was higher during heat exposure compared with preexposed value ($P < 0.01$).

Changes of glucose metabolism in buffaloes given exogenous urea during heat exposure. (Table 2)

Table 2 provided the data of changes of glucose metabolism. During exogenous urea infusion in buffaloes kept in normal ambient temperature, no significant changes of total body turnover rate of either U^{14}-C glucose or 3^{3}-H glucose, glucose recycling, glucose pool size, glucose clearance and plasma glucose concentration were observed. However animals given exogenous urea during heat exposure showed the marked reduction of the turnover rate of both U^{14}-C glucose and 3^{3}-H glucose by approximately 25.2% and 23.2% respectively. The glucose clearance rate significantly decreased during urea infusion while the significant

increase in plasma glucose concentration were determined ($P < 0.05$).

It has been noted that when animal exposed to heat, $U^{14}C$ glucose turnover rate significantly increased by 68% ($P < 0.05$). The rate of increase was higher than that obtained during urea infusion (38.5%). The similar pattern has also been found on the change of $3^{3}H$-glucose turnover rate. During heat exposure, the calculated glucose carbon recycling markedly reduced without any alteration in glucose pool size. There were significant increases in plasma glucose concentration in buffaloes exposed to heat in either before or after urea infusion.

Changes of renal functions (Table 3)

In buffaloes kept in normal ambient temperature, an intravenous infusion of urea for 4 h resulted in a significant decrease in the rate of urine flow by approximately 37% in comparison to the pre-infusion value. The marked reduction of urine flow rate was also detected in animals exposed to heat for 3 h and it showed a progressive decline after urea infusion which decreased by approximately 33% from the preexposed value. However, the positive correlation between the rate of urine flow and fractional urea excretion was only detected in animals kept in normal ambient temperature ($FE_{urea} = 47.19 + 1.8\ V$, $r = 0.665$, $P < 0.001$). During intravenous infusion of urea, no change in GFR was observed in animals kept in normal ambient temperature while a slight decrease in GFR was

apparent in heat exposed animals. RPF and 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 in heat exposed animals given exogenous urea. 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 exogenous urea and kept in normal ambient temperature. The calculated renal urea reabsorption showed significant increase by approximately 46% during the infusion of urea in animals kept in normal ambient temperature ($P < 0.05$) while a slight decrease was apparent during heat exposure. The osmolar clearance of animals kept in either normal ambient temperature or exposed to heat showed marked decrease after 4 h of urea infusion. However, no remarkably changes of free water clearance by the effect of urea infusion were apparent.

Changes of electrolyte excretion (Table 4)

No significant changes in the urinary and fractional excretions of sodium ion, calcium ion and inorganic phosphorus were apparent during the last 4 h of urea infusion. The urinary and fractional potassium excretion markedly decreased by approximately 43% during the infusion of urea for animals kept in normal ambient temperature ($P < 0.05$), whereas a slight decrease was detected for animals during heat exposure. On both series of experiments a positive correlation between the rate of urine flow and fractional excretion of potassium were observed ($FE_K = 49.53 + 7.97\ V$; $r = 0.684$; $P < 0.001$). The pattern of excretion of chloride ion was similar to that of potassium ion. Urine pH in animals kept in normal ambient temperature decreased after urea infusion whereas there were no changes in animals exposed to heat.

The concentrations of plasma constituents (Table 5)

No significant changes of the concentrations of plasma constituents were apparent in animals kept in normal ambient temperature. During heat exposure, the concentration of plasma sodium significantly decreased after 3 h. of heat exposure ($P < 0.05$) and it returned to preexposed value after urea infusion. Plasma inorganic phosphorus concentration decreased gradually by approximately 10% during heat exposure and a progressive decline was detected during the infusion of urea ($P < 0.05$). During heat exposure, there was a rise of plasma creatinine concentration with the concomitant increase in total plasma protein concentration ($P < 0.001$). The progressive rises of these plasma nitrogenous substances were obviously coincided with the time course of heat exposure. 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 1 Effects of intravenous urea infusion on rectal temperature, heart rate, respiratory rate, packed cell volume, plasma volume and blood volume of normaland acute heat exposed buffaloes. (Mean$\pm$S.E.M.)

	Normal temperature		Heat exposure		
	Pre-infusion	4 h-Urea infusion	Pre-heat exposure	3 h-Heat exposure	Heat exposure + 4 h-Urea infusion
Ambient temp (Dry bulb : Wet bulb) (^{o}C)	30:24	30:24	32:27	41:31	41:31
THI (%)	78.8$\pm$1.4	79.4$\pm$1.5	84.2$\pm$1.2	92.8$\pm$0.3**	92.2$\pm$0.8**
Rectal temperature (^{o}C)	38.05$\pm$0.38	38.21$\pm$0.22	38.40$\pm$0.22	38.90$\pm$0.13**	39.30$\pm$0.13**
Heart rate (beats/min)	43$\pm$3	43$\pm$3	43$\pm$3	47$\pm$4	49$\pm$2**
Respiratory rate (breaths/min)	23$\pm$3	21$\pm$3	25$\pm$4	58$\pm$6*	55$\pm$9*
Packed cell volume (%)	26.9$\pm$0.9	27.3$\pm$0.8	28.2$\pm$1.4	27.1$\pm$1.1	27.3$\pm$1.5
Plasma volume (L)	17.30$\pm$5.92	16.70$\pm$4.81	15.65$\pm$3.19	15.67$\pm$2.94	16.34$\pm$3.25
Blood volume (L)	20.63$\pm$4.53	22.93$\pm$6.18	21.86$\pm$5.03	21.76$\pm$5.04	22.60$\pm$4.84

P-values with respect to mean pre-infusion values and pre-heat exposure values for the normal temperature and heat exposure experiments respectively : * $P < 0.05$, ** $P < 0.01$.

Table 2 Effects of intravenous urea infusion on glucose metabolism of normal and acute heat exposed buffaloes.

	Normal temperature		Heat exposure	
	Pre-infusion	4 h-urea infusion	3 h-Heat exposure	Heat exposure + 4 h-Urea infusion
Glucose turnover rate (mg/min)				
U-^{14}C glucose	609.9±70.8	555.3±72.9^{NS}	1062.8±167.7†	800.3±148.1
	(7.21±0.93)	(6.54±0.95)^{NS}	(12.12±1.54)†	(9.06±1.40)
3-^{3}H glucose	840.4±108.7	873.1±141.5^{NS}	1173.6±196.8	904.6±162.4*
	(9.90±1.34)	(10.24±1.61)^{NS}	(13.37±1.86)	(10.27±1.59)*
Glucose carbon recycling (%)	25.99±5.17	31.85±9.65^{NS}	8.67±1.71†	11.30±3.96
3-^{3}H glucose pool size (gm)	132.9±20.9	92.5±11.6^{NS}	108.8±13.3	111.8±14.2
	(1.572±0.267)	(1.102±0.165)^{NS}	(1.249±0.134)	(1.279±0.131)
Plasma glucose concentration (mg%)	52.5±4.2	55.2±4.6^{NS}	69.5±2.0††	82.8±2.8*
Glucose clearance (ml/min)				
U-^{14}C glucose	1190.3±153.8	1024.4±137.2^{NS}	1548.8±275.3	951.9±159.8*
	(14.13±2.07)	(12.11±1.86)^{NS}	(17.65±2.62)	(10.79±1.44)
3-^{3}H glucose	1680.8±325.9	1671.6±368.1^{NS}	1171.4±321.3	1075.4±172.5)*
	(19.90±4.00)	(19.69±4.40)^{NS}	(19.49±3.12)	(12.23±1.61)

Figures in parentheses are values of glucose turnover rate and glucose clearance per metabolic rate

P-values with respect to mean pre-infusion values and pre-heat exposure values for the normal temperature and heat exposure experiments respectively : * $P < 0.05$, NS = Not significant

The significant difference from pre-infusion values of the normal temperature is indicated : † $P < 0.05$, †† $P < 0.01$.

Table 3 Effects of intravenous urea infusion on renal hemodynamic and urea excretion of normal and acute heat exposed buffaloes.

	Normal temperature		Heat exposure		
	Pre-infusion	4 h-Urea infusion	Pre-heat exposure	3 h-Heat exposure	Heat exposure + 4 h-Urea infusion
Urine flow rate (ml/min)	9.37±3.21	5.87±2.13*	7.08±1.24	5.87±1.00	4.73±0.90
Glomerular filtration rate (ml/min)	280.4±28.9	278.3±39.9	302.2±25.4	308.9±13.3	233.5±23.2
Effective renal plasma flow (ml/min)	1237.8±144.5	1091.0±179.7	1255.8±137.6	1321.9±98.9	1165.4±144.1
Renal blood flow (ml/min)	1732.0±202.2	1501.6±244.0	1751.1±194.4	1812.7±124.4	1609.6±208.7
Filtration fraction (%)	23.0±1.5	26.9±3.9	25.1±3.0	23.7±1.6	20.3±1.7*
Osmolar clearance (ml/min)	21.6±4.9	16.3±4.2*	17.1±2.5	17.4±2.7	13.4±2.1
Free water clearance (ml/min)	-12.2±1.8	-10.4±2.1	-9.9±1.3	-11.5±1.8	-8.6±1.2
Renal urea clearance (ml/min)	197.1±36.1	166.1±43.8	194.6±23.7	190.4±11.7	156.9±19.5
Renal urea excretion (mg/min)	89.4±18.3	84.3±22.5	91.0±12.1	88.9±7.6	81.1±7.8
Fractional urea excretion (%)	76.9±6.3	56.2±6.8**	63.8±4.3	62.1±4.6	68.2±6.9
Urea reabsorption (mg/min)	41.8±8.9	61.1±10.1*	52.7±8.9	57.3±11.3	42.2±11.5

P-values with respect to mean pre-infusion values and pre-heat exposure values for the normal temperature and heat exposure experiments respectively : * $P < 0.05$, ** $P < 0.01$.

Table 4 Effects of intravenous urea infusion on renal electrolyte excretion and urine pH of normal and acute heat exposed buffaloes. (Mean±S.E.M.)

	Normal temperature		Heat exposure		
	Pre-infusion	4 h-Urea infusion	Pre-heat exposure	3 h-Heat exposure	Heat exposure + 4 h-Urea infusion
Urinary Na^+ excretion (μEq/min)	186.3±87.3	212.9±70.9	320.2±134.9	217.2±60.9	280.2±119.6
† (μEq/min)	*(0.49±0.21)*	*(0.62±0.21)*	*(0.92±0.45)*	*(0.53±0.14)*	*(0.91±0.39)*
Urinary K^+ excretion (μEq/min)	1898.1±447.4	1093.8±315.1*	1181.3±289.7	1172.7±203.5	738.9±187.3
† (μEq/min)	*(162.3±37.2)*	*(90.9±11.0)**	*(87.5±12.0)*	*(93.4±9.2)*	*(80.5±15.2)*
Urinary Cl^- excretion (μEq/min)	1337.9±415.4	973.0±325.3*	1207.8±178.3	1051.7±274.7	699.2±192.3
† (μEq/min)	*(4.5±1.0)*	*(3.4±0.6)*	*(4.1±0.6)*	*(3.4±0.8)*	*(2.8±0.7)*
Urinary Ca^{++} excretion (mg/min)	0.41±0.19	0.73±0.25	0.92±0.17	0.73±0.20	0.55±0.22
† (mg/min)	*(1.6±0.7)*	*(3.1±0.8)*	*(4.1±0.9)*	*(2.9±0.8)*	*(2.7±1.0)*
Urinary $PO_4^=$ excretion (mg/min)	0.032±0.016	0.028±0.017	0.046±0.013	0.048±0.015	0.040±0.014
† (mg/min)	*(0.24±0.03)*	*(0.25±0.03)*	*(0.31±0.06)*	*(0.34±0.06)*	*(0.43±0.12)*
Urine pH	9.52±0.09	9.14±0.18	8.83±0.35	8.86±0.40	8.79±0.40

P-values with respect to mean pre-infusion values and pre-heat exposure values for the normal temperature and heat exposure experiments respectively : * $P < 0.05$,

† Italicized figures in parentheses are percentages of fractional electrolyte excretion.

Table 5 Effects of intravenous urea infusion on the concentrations of plasma electrolytes and plasma constituents of normal and acute heat exposed buffaloes.

	Normal temperature		Heat exposure		
	Pre-infusion	4 h-Urea infusion	Pre-heat exposure	3 h-Heat exposure	Heat exposure + 4 h-Urea infusion
Plasma urea (mg%)	46.3±5.6	53.2±6.7**	47.4±3.8	47.1±3.8	53.0±4.1***
Plasma sodium (mEq/L)	133.4±2.9	134.3±3.7	138.2±1.8	136.2±1.7*	137.1±1.3
Plasma potassium (mEq/L)	4.44±0.17	4.32±0.10	4.62±0.15	4.27±0.16	4.14±0.15
Plasma chloride (mEq/L)	95.8±2.2	99.1±3.1	99.6±2.5	98.4±2.2	100.1±1.3
Plasma calcium (mg%)	7.90±0.22	7.87±0.16	8.63±0.20	8.36±0.12	8.58±0.13
Plasma inorg. phosphorus (mg%)	4.34±0.47	4.10±0.54	4.68±0.37	4.19±0.30	3.59±0.20*
Plasma Protein (g%)	9.77±0.40	9.90±0.41	9.12±0.10	9.57±0.10**	10.27±0.11***
Plasma creatinine (mg%)	1.39±0.11	1.43±0.13	1.53±0.13	1.63±0.21	1.82±0.13*
Plasma osmolality (mOsm/kg H_2O)	264±3	263±4	274±2	263±3*	275±1

P-values with respect to mean pre-infusion values and pre-heat exposure values for the normal temperature and heat exposure experiments respectively : * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

Discussion

The previous study showed that during heat exposure, a marked increase in plasma glucose concentration was apparent (Chaiyabutr et al, 1987). However, there is little information on the turnover and oxidation of glucose in buffaloes. The measurement of glucose kinetic in heat exposed buffaloes showed that an increase in glucose turnover rate was related to the glucose concentration in the plasma. It has been known that only little glucose is absorbed from the digestive tract of the ruminating animal (Bergman et al, 1974). If availability of exogenous glucose absorption from digestive tract is assumed to remain constant throughout the present experiment, it would be suggested that the increase in the total glucose turnover rate in heat stressed animal could be related to an increase in the rate of gluconeogenesis particularly from amino acids since an increase in plasma protein concentration has also been noted. Amino acids would be mobilized and thus as precursors for gluconeogenesis. However, during short term exposure to heat, an increase plasma level of catecholamine in the ungulate has been reported before (Yousef, 1979; Barrand et al, 1981). The plasma level of biogenic amines were also used as indicator of thermal stress in cattle. (David, 1978). The changes of these hormone levels would be responsible for increase glucose production particularly the high rate of glycogenolysis from the liver and muscle. In the present study, the turnover rate of 3-^{3}H glucose may be considered to represent the total glucose turnover rate as the ^{3}H is not recycle from product of partial glucose degradation (Katz et al, 1965). Thus, one way of estimating carbon atom recycling is by simultaneously injecting 3-^{3}H glucose and U-^{14}C glucose as in the present experiment. The results of glucose carbon recycling suggest that a

constant level of tricarbon units originally derived from glucose is again reincorporated into glucose and that this was alleviated in heat stressed buffalo. The reduction of glucose carbon recycling is probably partly due to tricarbon atom of glucose more enter the tricarboxylic acid cycle which oxidized to CO_2. However, during urea infusion period, the renal urea reabsorption kept constant in comparison to the control normal ambient temperature. These results show that during heat exposrue the other nitrogen compounds were not synthesized from the urea nitrogen given into the blood which might be related to an increase of plasma protein concentration (Macfarlane, 1964). The extra exogenous urea may affect the biochemical reaction of intermediates in TCA cycle which have been demonstrated to inhibit isocitrate and glutamate dehydrogenase (Katunuma et al, 1966), and impairs the decarboxylation of α-ketoglutarate and pyruvate (McKhann and Tower, 1961). Energy yielding processes including the Krebs cycle and oxidative phosphorylation might be inhibited. Therefore the accumulation of glucose with a result of reduced utilization could account for hyperglycemia associated with the decrease in glucose turnover rate during urea infusion in heat stressed animal.

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 (Schmidt-Nielsen et al, 1957) Gans, 1966. However, in the present experiment, only 56% of 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 et al, 1958). In the present study no increase in GFR was observed, despite a marked increase in plasma urea concentration which was similar to the experiment in sheep fed high protein diet after short term of urea infusion (Ergene and Pickering, 1978). The decrease in the rate of urine flow during urea infusion seems to be responsible for an increase in urea reabsorption since there were significant positive correlations between the rate of urine flow and fractional urea excretion ($FE_{urea} = 47.19 + 1.81V$, $r = 0.665$, $P < 0.001$). This phenomenon has been noted before (Smith, 1962; Gans, 1966; Cocimano & Leng, 1967).

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. The similar decrease in urinary potassium excretion was also reported in sheep after increase urea level in the blood (Juhasz & Szegedi, 1969). Therefore, effect of urea alone had limited diuretic ability (Godwin & Williams, 1984). In the present study, the clinical signs of urea poisoning were not apparent. However, an intravenous urea infusion would contribute to a decrease in blood pH which has been previously demonstrated in sheep given ammonium compound (Singer & McCarty, 1971). This evidence could account for the decrease of urine pH concomitant with the decrease of potassium excretion. Such changes can be explained by the well known fact that kidney play a significant role in acid-base regulation by an attempt to ensure hydrogen ion with a reciprocal secretion of potassium ions (Johnson & Selkurt, 1966).

During acute heat exposure, buffaloes showed signs of distress and increase in respiratory rate which usually produce alkalosis (Hales & Findlay, 1968). The decrease of the concentration of plasma potassium and inorganic phosphorus were apparent. It could have been due to the shift of ions moving intracellularly during alkalotic state (Knochel & Caskey, 1977). However,both urinary and fractional potassium excretion of the heat stressed buffalo given exogenous urea showed no significant change. The effect of exogenous urea infusion might be superimposed by respiratory alkalosis during heat stress since urine pH kept constant.

In the present study, total plasma protein increased significantly by the time of heat exposure. This result has also been reported in both man (Senay, 1970) and buffalo exposed to severe heat (Chaiyabutr et el, 1983; 1987). An increase in the concentration of plasma protein would be due to the breakdown of muscle protein. It was also indicated by a marked increase in the concentration of plasma creatinine which has also been reported in steers exposed to high environmental temperature (Terui, Ishino, Matsuda, Shoji, Ambo & Tsuda, 1979). An exogenous urea infusion during heat exposure could not induce a greater extent of renal urea reabsorption in comparison to the buffalo kept in normal ambient temperature eventhough the decrease of the rate of urine flow has been observed. These findings suggest that the other control systems are responsible for the renal urea reabsorption. During heat exposure, one might expect an increase in plasma pool size of nitrogenous substances which can be attributed to an elevation of both plasma protein and creatinine concentrations coincided with the elevation of plasma urea level during urea infusion. The question then arise whether an increase in plasma pool size of nitrogenous substances in heat stressed animals

given exogenous urea resemble to the state of high dietary protein intake. Then such changes might inhibit renal urea reabsorption, since Cocimano & Leng (1967) showed that in sheep given a higher protein nitrogen intake led to a marked decrease in the rate of the urea transport from the tissue into the blood.

The previous study in acute heat stressed buffalo showed a marked increase in water turnover (Chaiyabutr et al, 1987). This change would therefore closely relate to an increase in energy metabolism (Macfarlane & Howard, 1970). This evidence supports another possibility that the limitation of renal urea reabsorption in heat stressed animal given exogenous urea would attribute to an increase metabolism within the kidney cells, especially since Rabinowitz and Co-workers demonstrated in sheep that an increase in the collecting duct permeability to urea of the low protein animal was a manifestation of reduced cellular volume consequence to reduce cellular metabolic activity (Rabinowitz et al, 1973).

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References

ANDERSON, R.S. & PICKERING, E.C. (1961). A technique for the accurate measurement of the rate of urine flow of the cow for renal clearance studies. British Veterinary Journal 117, 502-506.

BARRAND, M.A., DAUNCEY, M.J. & INGRAM, D.L. (1981). Changes in plasma noradrenaline and adrenaline associated with central and peripheral thermal stimuli in the pig. Journal of Physiology (London) 316, 139.

BERGMAN, E.N. (1973). Glucose metabolism in ruminants as related to hypoglycemia and ketosis. Cornell Veterinarian 63, 341-382.

BERGMAN, E.N., BROCKMAN, R.P. & KAUFMAN, C.F. (1974). Glucose metabolism in ruminants : comparison of whole-body turnover with production by gut, liver, and kidneys. Federation Proceedings 33, 1849-1854.

BIANCA, W. & FINDLAY, J.D. (1962). The effect of thermally-induced hyperpnea on the acid-base status of the blood of calves. Research in Veterinary Science 3, 38-49.

CHAIYABUTR, N., BURANAKARL, C., MUANGCHAROEN, V., LOYPETJRA, P. & PICHAICHARNARON, A. (1987). Effects of acute heat stress on changes in the rate of liquid flow from the rumen and turnover of body water of swamp buffalo. Journal of Agricultural Science (Cambridge) 108, 549-553.

CHAIYABUTR, N., CHANPONGSANG, S., LOYPETJRA, P. & PICHAICHARNARONG, A. (1983). Effect of heat stress on renal urea excretion of swamp buffalo. In "Current development and problems in swamp buffalo production" Ed. Shimizu, H. pp. 184-191. Tsukuba : University of Tsukuba.

CHAIYABUTR, N., FAULKNER, ANNE & PEAKER, M. (1982). Glucose metabolism in vivo in fed and 48 h. starved goats during pregnancy and lactation. British Journal of Nutrition 47, 87-94.

COCIMANO, M.R. & LENG, R.A. (1967). Metabolism of urea in sheep. British Journal of Nutrition 21, 353.

DAVID, T.P. (1978). HPLC of Biogenic Amines in Cattle : Effect of Heat, Cold and Dehydration, Ph.D. thesis, University of Missouri, Columbia, 1978.

ERGENE, N. & PICKERING, E.C. (1978). The effects of urea infusion on glomerular filtration rate and renal plasma flow in sheep fed low and high protein diets. Quarterly Journal of Experimental Physiology 63, 77-81.

GANS, J.H. (1966). Renal excretion of urea in sheep. American Journal of Veterinary Research 27, 1279-1283.

GODWIN, I.R. & WILLIAMS, V.J. (1984). Renal control of plasma urea level in sheep : The diuretic effect of urea, potassium and sodium chloride. Quarterly Journal of Experimental Physiology 69, 49-59.

HALES, J.R.S. & FINDLAY, J.D. (1968). The oxygen cost of thermally-induced and CO_2-induced hyperventilation in the ox. Respiration Physiology 4, 353-362.

JOHNSON, P.C. & SELKURT, E.E. (1966). Respiratory and renal regulation of acid-base balance. In "Physiology", Ed. Selkurt, E.E., pp. 537-552. Boston : Little Brown & Company.

JUHASZ, B. & SZEGEDI, B. (1969). Regulation of the acid-base equilibrium in sheep. Acta Veterinaria Academiae Scientiarum Hungaricae 19, 61-80.

KATUNUMA, N., OKADA, M. & NISHII, Y. (1966). Regulation of the urea cycle and TCA by ammonia. In "Advances in Enzymes Regulation" Vol.4, Ed. Weber, G., pp. 317-335. New York : Pergamon Press.

KATZ, J., ROGNSTAD, R. & KEMP, R.G. (1965). Isotope discrimination effects in the metabolism of ^{3}H glucose. Journal of Biological Chemistry 240, 1484-1486.

KNOCHEL, J.P. & CASKEY, J.H. (1977). The mechanism of hypophosphatemia in acute heat stroke. Journal of American Medical Association 238, 425-426.

MACFARLANE, W.V. (1964). Terrestrial animals in dry heat : ungulates. In "Handbook of Physiology (sec.4) : Adaptation to the Environment" Eds. Dill, D.B., Adolph, E.F. & Wilber, C.G., pp. 533. Washington, D.C. : American Physiological Society.

MACFARLANE, W.V. & HOWARD, B. (1970). Water in the physiological ecology of ruminants. In 'Physiology of Digestion and Metabolism in the Ruminant' Ed. Phillipson, A.T., pp. 362. Newcastle upon Tyne : Oriel Press.

MAUST, L.E., McDOWELL, R.E. & HOOVEN, N.W. (1972). Effect of summer weather on performance of Holstein cows in three stages of lactation. Journal of Dairy Science 55, 1133.

McKHANN, G.M. & TOWER, D.B. (1961). Ammonia toxicity and cerebral oxidative metabolism. American Journal of Physiology 200, 420.

RABINOWITZ, L., GUNTHER, R.A., SHOJI, E.S., FREEDLAND, R.A. & AVERY, E.H. (1973). Effects of high and low protein diets on sheep renal function and metabolism. Kidney International 4, 188-207.

RADZIUK, J., NORWICK, K.H. & VRANIC, M. (1978). Experimental validation of measurements of glucose turnover in nonsteady state. American Journal of Physiology 234, E84-E93.

RITCHER, H.J. & LAPOINTE, Y.S. (1962). Urea in blood serum or urine (diacetly mono-oxime procedure). Clinical Chemistry 8, 335.

SANO, H., TAKAHASHI, K., FUJITA, M., AMBO, K. & TSUDA, T. (1979). Effect of environmental heat exposure on physiological response, blood constituents and parameters of blood glucose metabolism in sheep. Tohoku Journal of Agricultural Research 30, 76-85.

SCHMIDT-NIELSEN, B., OSAKI, H., MURDAUGH, H.V. & O'DELL, R. (1958). Renal regulation of urea excretion in sheep. American Journal of Physiology 194, 221-228.

SCHMIDT-NIELSEN, B., SCHMIDT-NIELSEN, K., HOUPT, T.R. & JARNUM, S.A. (1957). Urea excretion in the camel. American Journal of Physiology 188, 477-484.

SENAY, L.C. Jr. (1970). Movement of water, protein and crystalloids between vascular and extravascular compartments in heat-exposed men during dehydration and following limited relief of dehydration. Journal of Physiology 210, 617-635.

SINGER, R.H. & McCARTY, R.T. (1971). Acute ammonium salt poisoning in sheep. American Journal of Veterinary Research 32, 1229-1238.

SMITH, H.W. (1962). Principle of Renal Physiology, p. 209. London : Oxford University Press.

TERUI, S., ISHINO, S., MATSUDA, K., SHOJI, Y., AMBO, K, & TSUDA, T. (1979). Effect of experimental high environmental temperature and humidity on steers. Tohoku Journal of Agricultural Research 30, 95-108.

VARLAY, H., GOWENLOCK, A.H. & BELL, M. (1980). Practical Clinical Biochemistry. 5th Ed. London : William Heinemann Medical Books.

YOUSEF, M.K. (1979). Adaptive responses of Ungulates to the environment. In "Biometeorological Survey" Vol.1, Eds. Tromp, S.W. and Bouma, J.J., pp. 90. London : Heyden & Son.