ผลของพิษงูแมวเซาต่อการทำงานของไตในหนูแรทที่ให้ธาตุซีลีเนียมเสริม

ร้อยตำรวจเอกหญิง อังธนา จุลสุคนธ์

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สหสาขาวิชาสรีรวิทยา
บัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย
พ.ศ. 2538

ISBN 974-631-901-9

ลิขสิทธิ์ของบัณฑิตวิทยาลัย จุฬาลงกรณ์มหาวิทยาลัย

EFFECTS OF RUSSELL'S VIPER VENOM ON RENAL FUNCTIONS IN SELENIUM-SUPPLEMENTED RATS.

POLICE CAPTAIN ANGTANA JULSUKON

A Thesis Submitted in Partial Fulfillment of the Requirements
for the degree of Master of Science
Inter-Department of Physiology
Graduate School
Chulalongkorn University
1995

ISBN 974-631-901-9

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Thesis Title	Effects of Russell's viper venom on renal functions in selenium-supplemented rats.
By	Police Captain Angtana Julsukon
Inter-Department	Physiology
Thesis Advisor	Professor Narongsak Chaiyabutr, Ph.D.
Thesis Co-advisor	Professor Visith Sitprija, Ph.D.

Accepted by the Graduate School, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree.

Santi Thoongsuwan
.....Dean of Graduate School
(Associate Professor Santi Thoongsuwan, Ph.D.)

Thesis Committee

.....Chairman
(Associate Professor Bungorn Chomdej, Ph.D.)

.....Thesis Advisor
(Professor Narongsak Chaiyabutr, Ph.D.)

Visith Sitprija
.....Thesis Co-advisor
(Professor Visith Sitprija, Ph.D.)

Choogiart Sucanthapree
.....Member
(Assistant Professor Choogiart Sucanthapree, Ph.D.)

พิมพ์ต้นฉบับบทคัดย่อวิทยานิพนธ์ภายในกรอบสีเขียวนี้เพียงแผ่นเดียว

หอสมุดกลาง สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

อังธนา จุลสุคนธ์ : ผลของพิษงูแมวเซาต่อการทำงานของไตในหนูแรทที่ให้ธาตุซีลีเนียมเสริม (EFFECTS OF RUSSELL'S VIPER VENOM ON RENAL FUNCTIONS IN SELENIUM-SUPPLEMENTED RATS) .ที่ปรึกษา : ศ.น.สพ.ดร.ฉรงค์ศักดิ์ ชัยบุตร และ ศ.นพ.ดร.วิศิษฏ์ สิตปรีชา, 75 หน้า. ISBN 974-631-901-9

การศึกษาครั้งนี้มีจุดมุ่งหมายเพื่อศึกษาผลของการฉีดพิษงูแมวเซาในขนาด 0.9 มิลลิกรัม ต่อน้ำหนักตัว 1 กิโลกรัมต่อการเปลี่ยนแปลงของระบบไหลเวียนในไตและการทำงานของไต รวมถึงศึกษาผลการให้พิษงูต่อการทำงานของไมโตคอนเดรียซึ่งแยกจากส่วนคอร์เทกซ์ของไตหนูแรทที่ให้ธาตุซีลีเนียมเสริม การทดลองใช้หนูแรทตัวผู้ น้ำหนักระหว่าง 90-110 กรัม แบ่งออกเป็นกลุ่มไม่เสริมธาตุซีลีเนียม และกลุ่มเสริมธาตุซีลีเนียมในขนาด 8 ไมโครกรัมต่อวันเป็นเวลา 40 วันอย่างต่อเนื่อง แล้วจึงทำการแบ่งสัตว์ทดลองออกเป็น 4 กลุ่มคือ กลุ่ม 1) กลุ่มควบคุม กลุ่ม 2) กลุ่มเสริมธาตุซีลีเนียมอย่างเดียว กลุ่ม 3) กลุ่มได้รับพิษงูแมวเซา กลุ่ม 4) กลุ่มเสริมธาตุซีลีเนียมและได้รับพิษงูแมวเซา พบว่า 40 ชั่วโมงภายหลังได้รับพิษงูแมวเซา ค่าเม็ดเลือดแดงอัดแน่นลดลงในกลุ่ม 4 ($P<0.05$) และกลุ่ม 3 แต่กลุ่ม 2 ไม่เปลี่ยนแปลง อัตราการเต้นของหัวใจเพิ่มขึ้นในกลุ่ม 3 และกลุ่ม 4 ($P<0.05$) ค่าความดันเลือดแดงเฉลี่ยของกลุ่ม 3 ไม่เปลี่ยนแปลง แต่มีแนวโน้มลดลงในกลุ่ม 2 และ กลุ่ม 4 ค่าความแตกต่างระหว่างความดันซีสโตลิคและไดแอสโตลิคในกลุ่ม 4 เพิ่มขึ้น ($P<0.05$) ปริมาณเลือดที่ไหลเข้าสู่ไตและอัตราการกรองผ่านกลอเมอรูลัสมีแนวโน้มลดลงในกลุ่ม 3 แต่เพิ่มขึ้นในกลุ่ม 2 ปริมาณพลาสมาที่ไหลผ่านไตเพิ่มขึ้นในกลุ่ม 4 ($P<0.05$) แต่อัตราการกรองผ่านกลอเมอรูลัสกลับลดลงเมื่อเปรียบเทียบกับกลุ่ม 1 ความต้านทานของหลอดเลือดภายในไตเพิ่มขึ้นในกลุ่ม 3 แต่ลดลงในกลุ่ม 2 และกลุ่ม 4 อัตราการขับทิ้งของโซเดียมและคลอไรด์ทางปัสสาวะในกลุ่ม 3 และ 4 มีแนวโน้มลดลง ส่วนอัตราการขับทิ้งของโปแตสเซียมเพิ่มขึ้นในกลุ่ม 3 แต่ลดลงในกลุ่ม 4 ในกลุ่ม 2 อัตราการขับทิ้งของโซเดียมทางปัสสาวะมีแนวโน้มลดลงในขณะที่ความเข้มข้นของโซเดียมในพลาสมามีแนวโน้มเพิ่มขึ้น การขับทิ้งกรดและแอมโมเนียทางปัสสาวะไม่แตกต่างกันระหว่างกลุ่ม สำหรับการทำงานของไมโตคอนเดรียการใช้ออกซิเจนในการหายใจของไมโตคอนเดรียในระยะกระตุ้นด้วย ADP ลดลงในกลุ่ม 3 ส่วนในกลุ่ม 2 และ 4 การใช้ออกซิเจนของไมโตคอนเดรียทั้งระยะพักและระยะกระตุ้นด้วย ADP มีแนวโน้มเพิ่มขึ้น ส่วนค่าดัชนีบ่งชี้การหายใจของไมโตคอนเดรียของทุกกลุ่มไม่แตกต่างกันอย่างเด่นชัด

จากผลการทดลองการได้รับพิษงูแมวเซาเป็นเวลา 40 ชั่วโมง ทำให้เกิดการเปลี่ยนแปลงต่อระบบหัวใจและหลอดเลือดเพียงเล็กน้อยและการไหลเวียนเลือดสู่ไตยังคงลดลงอยู่ การให้ธาตุซีลีเนียมเสริมในขนาด 8 ไมโครกรัมต่อวันไม่มีผลต่อหัวใจและหลอดเลือดอย่างเด่นชัด แต่ส่งเสริมให้เกิดภาวะเสี่ยงต่อการเสียเลือดและมีแนวโน้มเพิ่มปริมาณเลือดเข้าสู่ไต พิษงูแมวเซาลดการทำงานของไมโตคอนเดรียในขณะที่ธาตุซีลีเนียมกระตุ้นการทำงานของไมโตคอนเดรียทั้งในระยะพักและระยะกระตุ้นด้วย ADP

ภาควิชา จุลชีววิทยา สัตวแพทย์ ลายมือชื่อนิสิต อังธนา จุลสุคนธ์

สาขาวิชา สัตวแพทย์ ลายมือชื่ออาจารย์ที่ปรึกษา

ปีการศึกษา 2539 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

พิมพ์ต้นฉบับบทคัดย่อวิทยานิพนธ์ภายในกรอบสีเขียวนี้เพียงแผ่นเดียว

C 445529 : MAJOR PHYSIOLOGY
KEY WORD : RUSSELL'S VIPER VENOM / RENAL FUNCTIONS / MITOCHONDRIAL ACTIVITY /SELENIUM
ANGTANA JULSUKON : EFFECTS OF RUSSELL'S VIPER VENOM ON RENAL FUNCTIONS IN SELENIUM -SUPPLEMENTED RATS.
ADVISOR : PROF. NARONGSAK CHAIYABUTR, D.V.M.,Ph.D. AND PROF. VISITH SITPRIJA, M.D.,Ph.D. 75 pp. ISBN 974-631-901-9

The present study was designed to study effects of intraperitoneal injection of 0.9 mg/kg.BW Russell's viper venom (RVV) on renal hemodynamic and renal function and effects of the venom on mitochondrial activity in selenium supplemented rats. The study was carried out on male Wistar rats, 90-110 gBW. The animals were divided into nonselenium-supplemented group and group of rats supplementation with 8 μg selenium daily for consecutive 40 days. After supplemental period, the animals were divided into 4 groups ; Group I : control group ; Group II : selenium supplemented group ; Group III : Russell's viper venom administration group and Group IV : selenium supplemented rats given Russell's viper venom group. After 40 hours of RVV envenomation, packed cell volume was decreased in group IV ($P<0.05$) and group III but did not change in group II. Mean arterial blood pressure in group III was not affected but there were trend to decrease in group II and IV. Pulse pressure was apparently increased in group IV ($P<0.05$). The heart rate was markly increased in both group III and IV ($P<0.05$). Renal blood flow and glomerular filtration rate were decreased in group III while there was trend to increase in group II. In group IV renal plasma flow was significantly increased ($P<0.05$), however, glomerular filtration rate showed trend to decrease when compared to group I. The renal vascular resistance was increased in group III but there were trend to decrease in group II and IV. Fractional excretion of sodium and chloride were slightly decreased in group III and IV while fractional excretion of potassium was slightly increased in group III but decreased in group II and IV. In group II fractional excretion of sodium was slightly decreased while plasma sodium concentration was slightly increased. The urinary titratable acid and ammonium excretion were not differently among groups. In view of mitochondrial activity, mitochondrial state 3 respiration was decreased in group III. The increment in both mitochondrial respiration in state 3 and state 4 have been observed in group II and IV. The mitochondrial respiratory control index value were not significantly different among groups.

These results indicated that after 40 hours of RVV envenomation would produce minor changes in the systemic circulation while decrease in renal blood flow was still observed. The supplementation of selenium did not affect to changes in systemic circulation. Selenium supplementation would promote bleeding tendency and would increase in renal blood flow. Russell's viper venom caused changes in mitochondria activity by decreasing mitochondrial state 3 respiration . The increase in mitochondrial activity induced by selenium supplementation has been noted.

ภาควิชา จุฬาลงกรณ์ สรีรวิทยา ลายมือชื่อนิสิต อังคนา จุลสุคนธ์

สาขาวิชา สรีรวิทยา ลายมือชื่ออาจารย์ที่ปรึกษา

ปีการศึกษา 2537 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

ACKNOWLEDGEMENT

I would like to express my deep gratitude to my advisor, Professor Dr. Narongsak Chaiyabutr for his kind advice, guidance, frank, keen interest and constant encouragement thoughout this study.

I am also deeply grateful to my co-advisor, Professor Dr.Visith Sitprija for his suggestion in this work. In addition, I would like to extend my thanks to Associate Professor Dr.Bungorn Chomdej and Assistant Professor Dr.Choogiart Sucanthapee, who served on my commitee.

My sincere thanks are expressed to Miss Siripen Komolvanich, Mr. Somchai Pondeenana, teachers and all the staffs of Department of Physiology, Faculty of Veterinary Science, Chulalongkorn University for their kindness and provision the facilities used in experimental work, laboratory technique and manuscript of this thesis.

I am also indebted to all experimental rats for their sacrifice which brought me to succed in my study and thanks to the Science Division of Thai Red Cross Society for the donation of Russell's viper venom and to Chulalongkorn University for the research grant to support this study.

Finally, I am extremely grateful to my parent, family and friends for their love, encouragement given to me.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย

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ABBREVIATION

ADP	=	Adenosine diphosphate
ATP	=	Adenosine triphosphate
Cl	=	Chloride
DBP	=	Diastolic blood pressure
ERBF	=	Effective renal blood flow
ERPF	=	Effective renal plasma flow
FE_e	=	Fractional excretion of electrolyte
FF	=	Filtration fraction
Fig	=	Figure
GFR	=	Glomerular filtration rate
F_e	=	Filtered load of electrolyte
GSH-px	=	Glutathione peroxidase
H_2O_2	=	Hydrogen peroxide
HR	=	Heart rate
In	=	Inulin
K	=	Potassium
kg.bw	=	kilogram of body weight
MAP	=	Mean arterial blood pressure
mEq	=	Milliequivalent
mg	=	Milligram
min	=	Minute
ml	=	Milliliter
mmHg	=	Millimeter mercury
Na	=	Sodium
NH_4	=	Ammonium
O_2	=	Oxygen
$O_2^{-\bullet}$	=	Superoxide
$OH^{\bullet}$	=	hydroxyl radical

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P	=	plasma
PAH	=	Para-aminohippuric acid
PCV	=	Packed cell volume
PGI_2	=	Prostacyclin
PG_S	=	Prostaglandins
PLA_2	=	Phospholipase A_2
PLOOH	=	Phospholipid
PP	=	Pulse pressure
RCI	=	Mitochondrial respiratory control index
ROS	=	Reactive oxygen species
RVR	=	Renal vascular resistance
RVV	=	Russell's viper venom
S_3	=	Mitochondrial respiration state 3
S_4	=	Mitochondrial respiration state 4
SBP	=	Systolic blood pressure
Se	=	Selenium
SOD	=	Superoxide dismutase
T_e	=	Tubular reabsorption of electrolyte
Ta	=	Titratable acid
TXA_2	=	Thromboxane A_2
TXB_2	=	Thromboxane B_2
U_eV	=	Urinary excretion of electrolyte
V	=	Urine flow rate
V/GFR	=	Ratio of urine flow rate to GFR
μEq	=	Microequivalent
μg	=	Microgram
μL	=	Microliter

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CHAPTER I

INTRODUCTION

Kidney is one of the important organ in the body, its functions are formation of urine, control homeostasis of the body fluid and electrolytes, excrete toxic substances and other functions. Many toxic substances can be cause of renal damage. Several papers have reported effects of Russell's viper venom on renal injury. The injury might be due to direct toxicity of the venom to renal tissue including tubules (Chung et al., 1975, Sitprija and Boonpucknavig, 1977), cortex (Chung et al., 1975) interstitium (Sitprija et al., 1982) and/or reduction in systemic and renal circulation from hypotension, vasodilatation (Chomdej and Pfaller, 1987) or rise in systemic and renal vascular resistance (Chaiyabutr et al., 1985). The decrease in renal blood supply might lead to renal ischemia, decreased glomerular filtration rate (GFR), decreased excretion of electrolytes and urine volume (Tungthanathanich, Chaiyabutr and Sitprija, 1986; Chomdej and Pfaller, 1987). The studies in ischemic cell injury have shown that mitochondrial dysfunction and reduction of mitochondrial respiratory rate might be important factors of renal impairment (Jung and Pergande, 1988; Wolfgang et al., 1990). Experimental ischemic acute renal failure also showed inverse correlation between calcium accumulation and mitochondrial respiration. Mitochondrial calcium content was increased, whereas mitochondrial respiratory control index as well as kidney functions were decreased during postischemic peroid (Wilson et al., 1984; Gronow, Skrezek and Kossmann, 1984). A number of reports have been demonstrated that in postischemic period, the generation of oxygen free radicals (ROS) occured (Freeman and Cropo, 1982; McCord, 1985; Marx, 1987) and play pivotal roles in ischemic cell injury. Many investigators have suggested that ROS could injure cell by altering cellular and subcellular membrane structure, increased membrane permeability and altered cellular

functions (Wilson et al., 1984; Matthys et al., 1984; Kako et al., 1988; Kaminishi et al., 1989; Malis and Bonventre, 1986; Nath and Paller, 1990). However, Shlafer and coworkers have suggested that ischemic cell injury was not due to increase oxygen free radicals production but might be due to decrease antioxidant ability (Shlafer, Myers and Adkins, 1987).

Biological antioxidants, compounds that protect biological systems against the potential harmful effects of processes or reactions that can cause excessive oxidations (Krinsky, 1992). One of the cell protective mechanisms is the development of glutathione peroxidase activity which be result from cooperation of two enzymes, selenium dependent glutathione peroxidase and selenium independent glutathione peroxidase. As selenium dependent glutathione peroxidase, the connection of selenium deficiency and decrease in glutathione peroxidase activity have been reported to development of oxidation injury (Olsson, 1985; Weitzel, Ursini and Wendel, 1990). The deficiency of selenium accompanied with impairment of ketone body metabolism, renal functions and ischemic kidney injury were also noted (Nath and Salahudeen,1990; Nath and Paller, 1990). They have concluded that oxygen free radicals (ROS) might play roles in these pathology. Supplementation of selenium in diet given to rats could decrease cardiac ischemic-reperfusion injury (Konz et al., 1991; Poltronieri, Cevese and Sbarbati, 1992). Selenium was also shown to protect against gastric ulceration caused by necrotizing agents or hypothermic stress (Parmar, Tario and Agreel, 1988). Long term selenium supplementation to rats which were exposed to high sucrose diet could change microvascular morphology in renal glomeruli by increasing Bowman's capsule and glomerular tuft diameter (Eckhert et al., 1992) and high dose supplementation of selenium to man could increase creatinine clearance which was suggested to be due to modification of this trace element to arachidonic acid metabolism. (Guidi et al.,1990)

This study attempted to study the effects of Russell's viper venom on renal hemodynamic, renal functions and whether these changes are related to cortical mitochondrial activity in selenium-supplemented rats.

หอสมุดกลาง สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER II

BACKGROUND INFORMATION

The Russell's viper and its venom

Russell's viper or Vipera Russellii, one of poisonous snakes which can be found throughout Thailand. These snakes are in Viperidae family which have hemotoxic effects.

The snake's venom is a colourless or pale-yellow aqueous which be a mixture of polymeric protein compounds. The protein compounds can be divided to two types.

1) Protein compounds which have protease property such as hyaluronidase, a protein which can release histamine and bradykinin,lead to increase in vasodilatation and increase in membrane permeability. Jayanthi and coworkers have purified RVVX, a glycoprotein with MW. of 7900, which exhibited caseinolytic and factor X activating properties (Jayanthi and Gowda 1990). Hence, victims who are bitten by Russell's viper appear localized swelling very quickly.

2) Protein compounds which have hemotoxic effects on red blood cells, coagulation system and fibrinolytic system. The venom is composed of phospholipase A_2, lecithinase, phosphodiesterase, etc which might be cause of change in RBC membrane structure and permeability or increased osmotic pressure which might lead to RBC hemolysis or irregular shape. It is known that the venom could retard coagulation system by its component, factor X activator, which might be cause of disseminated intravascular coagulation (DIC). These clotting blood would circulate in vessels and could block blood supply which be cause of ischemia and tissue hypoxia through organ

failure. Moreover, this venom could also activate fibrinolytic activity which would lead to hemorrhagic tendency (สุดิมา ทุตะจิตต์, 2529)

Effect of Russell's viper venom on general and renal circulation

It has been known that Russell's viper venom (RVV) contains a number of enzymes which can synergistic playing to produce pathophysiological actions. Huang and Lee have isolated the venom into five fractions, two of five fractions were phospholipase A_2 (PLA_2) which could be divided into many subfractions. By intravenous injection of most PLA_2 subfractions to rats showed hypotensive effect. They suggested that hypotensive action of this venom was indirect effect which might be due to release or form highly active autocoids (Huang and Lee, 1984). Huang has also identified slow reacting substances (SRS) which was released from lung after incubated with PLA_2 that was isolated from RVV. The SRS has been shown to be a mixture of Thromboxane A_2 (TXA_2), prostacyclin (PGI_2) and leukothrienes (LTs). The positive linear correlation between lung perfusion pressure increment and the ratio of TXB_2 to 6-Keto-$PGF_{1\alpha}$ has been observed (Huang, 1984b). The venom has been suggested to provide peripheral vessels contraction but those of the splanchnic area were widely relaxed which be the same way as shown in histamine shock (Chopra and Chowman, 1934). In later work, Huang has shown that hypotensive effect of Russell's viper venom in renal hypertensive rats might be due to increase plasma prostacyclin coincided with reduce plasma renin activity (Huang, 1984a).

Lee (1948) studied the circulatory action of Vipera Russelli Formosensis's venom, a subspecies of Russell's viper, in rabbits. The venom has been shown to produce intravascular clotting defect. Thus, he has concluded that intravascular clotting might be cause of sudden death. Li and coworkers have suggested that Vipera russelli siamensis snake venom has inhibitory effects on platelet functions. It could increase platelet cyclic AMP and changed cytoskeleton, therefore, the responses in adhesion,

aggregation and contractibility of platelet might be inhibited (Li, Liu and Wang, 1986). These defects would be causes of hemorrhagic disorders.

In addition to hypotensive effect and coagulopathy in systemic circulation, renal circulation effected by Russell's viper venom has been observed. In experimental animal, intravascular injection of the venom could decrease renal blood flow, glomerular filtration rate and urine flow, whereas renal vascular resistance was increased (Tungthanathanich, Chaiyabutr and Sitprija, 1986 ; Chomdej and Pfaller, 1987). The reduction in renal blood flow, GFR and urine flow have been suggested to be effect of renal vascular constriction which was induced by renin-angiotensin system because administration of converting enzyme inhibitor, enalapril, could decrease renal vascular resistance and reversed those defects (Chaiyabutr et al., 1985a). However, converting enzyme inhibitor could decrease only 23% of renal vascular resistance which was increased after envenomation. After the venom administration, the excretion of sodium, potassium and chloride was decreased which might be result from decrease in renal blood flow and GFR (Chaiyabutr, 1985 ; Tungthanathanich, Chaiyabutr and Sitprija, 1986). After two hours of the RVV administration to rat, fractional excretion of sodium was significantly increased which might possibly be due to directly damage renal tubular cells and interfered with renal functions by the venom during this period (Chomdej and Pfaller, 1987).

The pathophysiological effects of Russell's viper venom on renal functions might be due to changes in systemic and renal circulation. In addition, renal lesion including arteritis (Sitprija, 1974), tubular necrosis (Sitprija, 1974; Chung et al., 1975 ; Shastry et al., 1977) cortical necrosis (Chung et al, 1975) glomerulonephritis (Sitprija and Boonpucknavig, 1980) and interstitial nephritis (Sitprija et al, 1982) have also been observed after RVV administration.

Acute renal failure

Acute renal failure (ARF) is a pathophysiological state of reduce renal function which develops following acute damage to the kidney and, typically, is reversible. This abnormality is commonly referred to three major different pathophysiological origins a) prerenal acute renal failure, b) intrarenal acute renal failure and c) postrenal acute renal failure

In prerenal acute renal failure, there are renal vasoconstriction and decrease renal blood flow result in a reduction of glomerular filtration rate. These changes in renal hemodynamics are volume conserving in state of severe electrolyte and volume disturbance, however, cell functions and structure are not apparently changed. This type of renal failure will be improved with normalization of electrolyte and fluid volume. Intrarenal acute renal failure results from damage to intrarenal cellular components, preferentially, the tubular epithelial cells. Tubular function is reduced and structural damage may occurs. Characteristic of intrarenal acute renal failure is decrease in glomerular and tubular functions as shown by a low glomerular filttration rate and a high fractional sodium excretion, reduce tubular secretory activity and a diminish concentrating ability , finally, urine volume may be low or high. Renal functional changes and severity of damage are relatively uniform, range from simple ischemic damage to damage by nephrotoxic substances. In postrenal acute renal failure results from obstruction of urine outflow.

Acute renal failure and mitochondrial functions

Mitochondria is the major part of cellular energy generation in normal function organ. Mitochondrial activity reflects the physiological function of the tissue and determine its capacity to perform aerobic metabolic functions. Mitochondria is composed of two membranes, an outer and inner membrane. The outer membrane is thought to be

a rather simple membrane with relatively few enzymatic or transport functions, whereas, inner membrane is structurally and functionally much more complex. The inner membrane contains most of enzymes involved in electron transport and oxidative phosphorylation, various dehydrogenases and several transport systems, which are involved in transferring substrates, metabolic intermediates and adenine nucleotides between the cytosol and mitochondrial matrix. Inside mitochondrial inner membrane, the mitochondrial electron transport chain is arrenged in a sequential pattern. The electron transport chain is a sequence of linked oxidation-reduction reactions, typically, occur when there is a transfer of electron from a suitable electron donor (the reductant) to a suitable electron acceptor (the oxidant). The component of electron transport chain is composed of four complexs including complex I or NADH-Q dehydrogenase, complex II or succinate-Q dehydrogenase, complex III or Q-cytochrome C dehydrogenase and complex IV or cytochrome oxidase. The electron transferring is initiated by transduction of the reducing equivalent, $NADH+H^+$ or $FADH_2$, in the presence of oxygen into utilized energy by synthesizing ATP. It should be noted that the electron transfer reactions from $NADH+H^+$ through coenzyme Q transfer two electrons, whereas the reaction between coenzyme Q and oxygen involving the various cytochromes are one-electron transfer reactions. The formation of ATP occur at only three coupling sites including coupling site I occurs at complex I, coupling site II occurs at complex III and site III occurs at complex IV inside F_0-F_1 complex. The rate of mitochondrial respiration can be controlled by concentration of ADP because mitochondrial oxidation and phosphorylation are tightly coupled. In generally, most cells in resting state are in state 4 and the availability of ADP allowing more respiration to occur, which in turn replenish of the ATP storage. The mitochondrial state 3 respiration occur when all substrates and components are presented in saturating amount. Examination of intact respiring mitochondria reveals that when substrates are oxidized via NAD-linked dehydrogenase and the respiratory chain, 3 mol of ATP are formed per ½ mol of oxygen consumed, P:O

ratio is 3. On the other hand, when a substrate is oxidized via flavoprotein-linked dehydrogenase, only 2 mol of ATP are formed and P:O ratio is 2.

Several investigators have suggested that dysfunction of mitochondria might be an important factor in ischemic cell injury. Study in ischemic renal failure, mitochondrial respiratory control index (RCI) value in both renal cortex and medulla has been shown to decrease, however medullary mitochondria were less sensitive to ischemia than the other one (Jung and Pergande, 1988). One of many functions of mitochondria is to regulate intracellular ions concentration. After four hours of ischemia, a continuous decrease in the ability of mitochondria to accumulate calcium following a high initial stable phase of calcium accumulation have been noted. The decrement in the ability to regulate calcium concentration was suspected to depend on both ATP level and mitochondrial respiration. However, any form of calcium accumulation, even mildly or partially inhibited calcium accumulation, resulted in a marked swelling of mitochondria (Mergner et al, 1977). After a 45 minutes period of renal artery clamping, mitochondrial calcium content, blood urea nitrogen and serum creatinine have been shown increased at 1 to 24 hours of blood reflow. Fractonal excretion of sodium was increased which could confirm the presence of tubular function impairment at 24 hours of blood reflow. At the time of renal artery clamping, mitochondrial acceptor control value and respiratory rate was reduced, slightly improved, however it was lower than control value, and then was reduced again after 4 hours of blood reflow. Therefore, mitochondrial calcium accumulation might occur predominately during blood reflow peroid and closely related to the progressive deterioration of both mitochondria and total kidney functions (Wilson et al., 1984). The calcium blocker, verapamil, has been shown to prevent mitochondrial damage by renal ischemia. However, there were no difference in mitochondrial respiratory capacity in either animals with or without verapamil pretreatment after 30 minutes of renal ischemia. Verapamil pretreatment could only delay the impairment of mitochondrial functions (Widener and Mela-Riker, 1984).

In hypoxia or ischemic-reperfusion, oxygen free radicals (ROS) might be generated (Mccord, 1985). ROS which be formed in the ischemic-reperfusion kidney has been suspected to have effect on enzyme proteins as well as membrane lipids. In vitro rat kidney perfusion, reoxygenation peroid after severe hypoxia has been shown to decrease in mitochondrial functions and tubular sodium reabsorption. The correlation between a decrease in fractional sodium reabsorption , mitochondrial functions and the release of enzyme Acid phosphatase has been observed (Gronow, Skrezek and Kossmann, 1984). The synergistic action of calcium and oxygen free radicals on renal mitochondria has been demonstrated. By using pyruvate and malate as substrate, oxygen free radical alone induced a mild increase in membrane permeability which was reflected by an increase in mitochondrial respiration in substrate supporting respiration (S_4) and a 30% reduction in ADP-stimulated respiration (S_3) but uncoupled respiratory rate was unaffected. ATP synthetase activity was reduced to 68% of control. The marked decrease in state 3 respiration was due to a defect in ATP synthetase. After treatment with calcium and oxygen free redical induced 1) an increase in mitochondrial membrane permeability 2) a marked impairment of uncoupled respiratory rate 3) ATPase activity was reduced to 45% of control value 4) a decrease in ADP translocase activity to 35% of control value and 5) complete uncoupled respiration has been observed. These results could reflect an inability of mitochondria to synthesize ATP. It was noted that mitochondria which was exposed to calcium and oxygen free radicals remained partially couple and could, therefore, synthesize ATP if succinate was used as substrate (Malis and Bonventre, 1986). By incubation rat liver mitochondria with oxygen free radical generating system, HX/XO, and $CaCl_2$ resulted in enhancement of peroxidative decomposition of mitochondrial membrane lipids coincided with the swelling of organelle. Monoamine oxidase acitivity was increased, whereas glutathione peroxidase and cytochrome C oxidase activity were inhibited (Kakkar, Mehrotra and Viswanathan,1992).

Oxygen free radicals or Reactive oxygen species

A free radical is any molecule that has odd number of electrons which can be generated in vivo as by products of normal metabolism or be generated by organism which is exposed to ionizing radiation, to drugs capable of redox cycling, or to xenobiotics that can form free radical metabolites in situ. The free radicals can be generated in both extra and intracellular sources. Intracellular sources of free radicals are autooxidation of thiols, catecholamines, flavins, tetrahydroproteins or during catalytic cycling of numerous enzymes such as xanthine oxidase, aldehyde oxidase, dihydroorotate dehydrogenase, etc. Mitochondrial electron transport chain, endoplasmic recticulum, nuclear membrane electron transport chain, peroxisome are sources of free radicals generation also.

In all cases, first oxygen free radical formed by the reduction of dioxygen is superoxide ($O_2^{-\cdot}$). Superoxide radical is highly reactive in hydrophobic environment, but is poorly in aqueous solution. It can across membrane via transmembrane anion channels. After the step of oxygen reduction, the spontaneously or enzymatically catalyzed dismutation reaction of superoxide is presented, thus, hydrogen peroxide (H_2O_2) and singlet oxygen ($1\Delta g$) are formed. In aqueous solution, superoxide dismutation reaction rate is faster at acidic pH values.

$$\text{Enzyme-Flavin-}H_2 + O_2 \longrightarrow \text{Enzyme - Flavin - H} + O2^{-\cdot} + H^+ \qquad ①$$

$$O2^{-\cdot} + O2^{-\cdot} + 2H^+ \xrightarrow{\text{superoxide dismutase}} H_2O_2 + O_2 \qquad ②$$

Hydrogen peroxide is a poorly reactive oxygen free radical in aqueous solution at physiological concentration but it can across biological membrane freely as water can. Hydrogen peroxide, itself, is a weak oxidant but be harmful by participation in produce more noxious oxidant, hydroxyl radical ($OH^{\bullet}$), via two step reaction termed an "iron-catalyzed Haber-Wiss reaction"

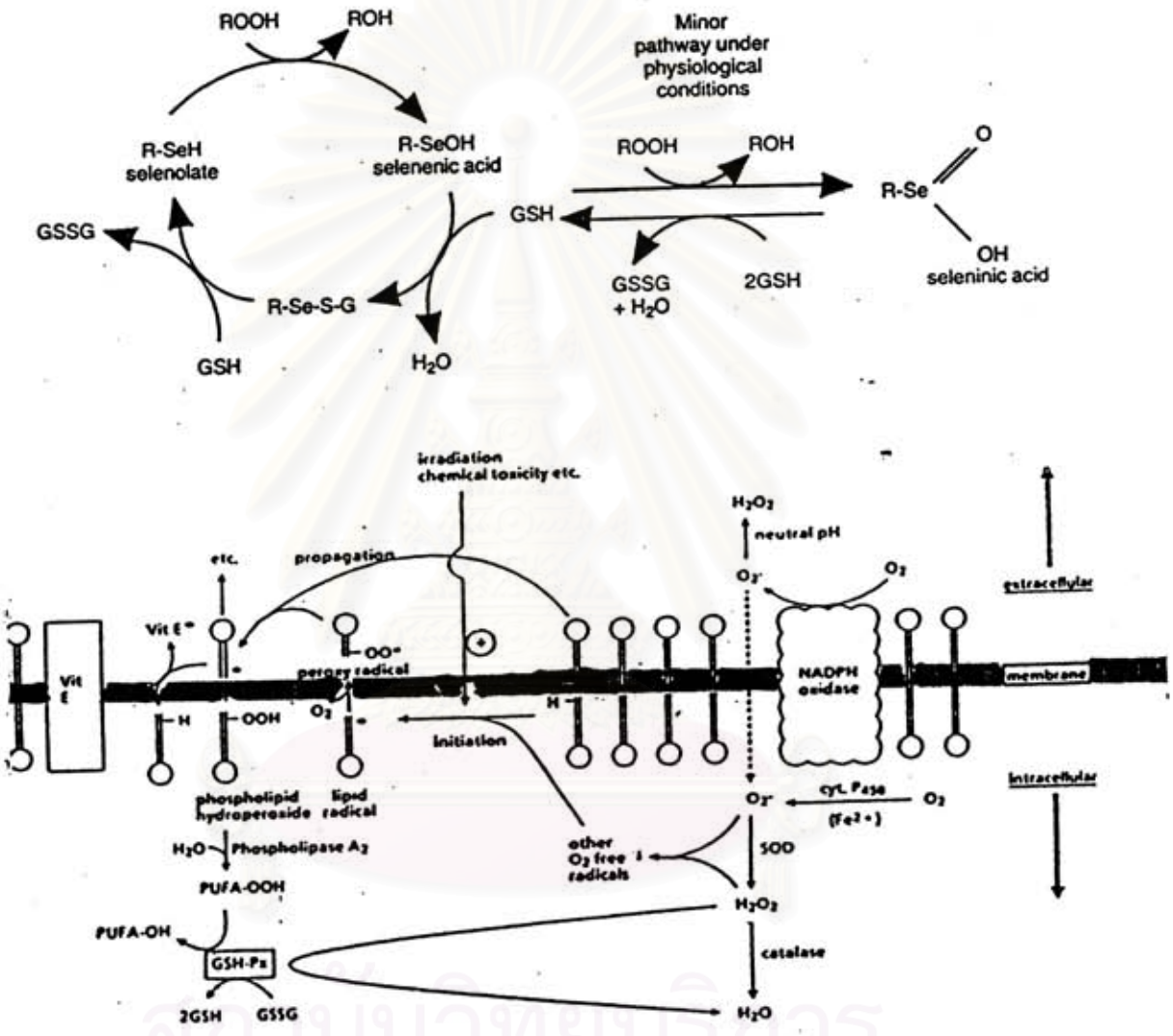

Fig 1. Scheme of the role of glutathione peroxidase (GSH-Px) in the body's defense system against oxidative stress.

$$Fe^{3+} + O2^{\cdot-} \longrightarrow Fe^{2+} + O_2 \quad ③$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH^\bullet \quad ④$$

$$\text{Net reaction : } O2^{\cdot-} + H_2O_2 \xrightarrow[\text{Catalyst}]{\text{Fe}} OH^\bullet + OH^- + O_2$$

Copper is an other cation that be capable of reacting with hydrogen peroxide to generate the hydroxyl radical. The hydroxyl radical is an extremely reactive radical which can react with almost every type of molecules formed in living cells including sugar, amino acids, phospholipids, DNA bases and organic acids at diffusion controlled rate It has a short half life but is capable of causing great damage within a small radius of site of production.

The other oxygen metabolites that can be generated by the reaction of various peroxidases with hydrogen peroxide in the presence of halides is hypohalous acids. The iodide derivatives are the most reactive hypohalous acid on a molar basis, however, in most biological systems, hypochlorous acid is the predominent species formed. Long-acting reactive oxygen metabolites can be generated when peroxidase-H_2O_2-chloride system interact with free amino groups (Freeman and Cropo, 1982 ; Halliwell and Gutteridge, 1986 ; Dimond, 1992 ; Cheeseman and Slater, 1993).

Biological antioxidants

Biological antioxidants are compounds that protect biological systems against the potentially harmful effects of processes or reactions that can cause excessive oxidation. They can be divided into two types, enzymatic and non-enzymatic processes.

Enzymatic processes

Several enzymes are capable of decrease the amount of oxidants or potential oxidants in the body, therefore, serve the protective function with respect to biological oxidants.

1) Superoxide dismutase (SOD). This enzyme catalyzes reaction of superoxide dismutation.

$$2O_2^{-\cdot} + 2H^+ \xrightarrow{SOD} O_2 + H_2O_2$$

2) Catalase. After dismutation of superoxide, one of the products is H_2O_2. This molecule can be detoxified by several enzymes such as catalase which catalyzes the following reaction.

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2$$

3) Selenium glutathione peroxidase (Se-GSH-Px). This enzyme was originally reported to reduce fatty acid hydroperoxide in cell membranes. Currently, Se-GSH-Px has been postulated to act together with Phospholipase A_2(PLA_2) in converting potential harmful phospholipid hydroperoxide (PLOOH) to free fatty acid hydroperoxides(LOOH) and lysophospholipids, and then to harmless fatty acid alcohols (LOH).

$$PLOOH \xrightarrow{PLA_2} Lysophospholipid + LOOH$$

$$LOOH + 2GSH \xrightarrow{Se\text{-}GSH\text{-}Px} LOH + GSSH + H_2O$$

4) Phospholipid hydroperoxide glutathione peroxidase (PLOOH-GSH-Px). A relatively new enzyme which has been described to act directly on phospholipid hydroperoxides without necessity of hydrolyzing the free fatty acid hydroperoxide from phospholipid.

$$PLOOH + 2GSH \xrightarrow{PLOOH\text{-}GSH\text{-}Px} Phospholipid\text{-}LOH + GSSH + H_2O$$

In this way, the membrane perturbing properties of phospholipid hydroperoxide are removed without the requirement of activation or mobilization of PLA_2 which might liberate excessive amount of substrates for prostanoid synthesis. In addition PLOOH-GSH-Px can also reduce membrane-associated cholesterol hydroperoxides, thereby further diminishing the amount of potentially harmful lipid hydroperoxide.

Non-enzymatic processes

A large number of compounds has been shown to posses antioxidant activity. This group of antioxidant was divided into two subgroups, lipid-soluble antioxidants and water-soluble antioxidants.

Lipid-soluble antioxidants

1) Tocopherols such as α-tocopherol or Vitamin E, have proven to be effective inhibitors of the propagation of lipid peroxidation. Each molecule can react with two peroxyl radicals.

α-tocopherol + LOO• ⟶ α-tocopherol• + LOOH

α-tocopherol• + LOO• ⟶ LOO + α-tocopherol

The α-tocopherol is low mobility in the membranes and greater probability of chain propagation in the more tightly structured environment of the membrane.

2) Carotenoids. A large family of conjugated polyenes which have biological antioxidant activity. The β-carotene (CAR) might react directly with peroxy radical (LOO•) to form a resonance-stabilized carbon centered radical.

CAR + LOO• ⟶ LOO - CAR•

LOO••LOO-CAR• ⟶ LOO-CAR-LOO

3) Quinones or Coenzyme Q (UQ). Coenzyme Q, in its reduced form (UQH_2) might function by reducing the α-tocopherol radical back to α–tocopherol. It

can also function in the absence of α-tocopherol, presumably by acting directly on either peroxyl or alkoxyl radical.

4) Bilirubin. It can effectively inhibit lipid peroxidation in both homogenous solution and liposomes.

Water-soluble antioxidants

1) Ascorbic acid. This compound has been reported to act synergistically with α-tocopherol to prevent lipid peroxidation in a micell or preperation of linoleic acid or methylinoleate.

2) Uric acid. It shows strong antioxidant activity with respect to water soluble free radicals at a physiological concentration.

3) Metal binding proteins. This group of proteins such as transferrin, lactoferrin, ceruloplasmin and albumin can reduce the effective concentration of transition metal that are capable of reacting with hydroperoxides.

$$LOOH + Fe^{2+} \longrightarrow LO\bullet + OH^{-} + Fe^{3+}$$

4) Binding proteins for heme and heme-containing proteins. Both free heme and heme proteins, hemoglobin or myoglobin are pro-oxidants which can react with H_2O_2, forming ferryl species that can initiate lipid oxidation. Haptoglobin can bind hemoglobin or myoglobin with high affinity, whereas, hemopectin binds free heme and then rapidly clear from the circulation. These binding proteins can prevent reaction of hemoglobin and heme to stimulate peroxidative reaction (Krinsky, 1992).

Oxygen free radicals and renal damage

Oxygen free radicals or reactive oxygen species (ROS) may contribute to the development or exerbation of many of mankind's most comman illness including cancer, heart atteck, stroke and emphysema (Marx, 1987). A number of studies have shown the

correlation between ROS and oxidative tissue damage in both in vitro and in vivo. An important role of ROS in initiate glomerular injury has been observed. In isolated glomeruli, ROS has been shown to alter the glomerular component, particularly, the glomerular basement membrane (GBM) (Shah, Baricos and Basci, 1987) and increased cyclic AMP content in isolated glomeruli (Shah, 1984). These changes were markly inhibited by catalase, therefore it has been suggested that hydrogen peroxide was responsible for these changes. ROS generated in ischemic-reperfusion kidney has been suspected to effect on the enzyme proteins as well as membrane lipids by depression membrane bound Na^+-K^+ATPase activity and decreased amount of sulhydryl groups in microsomal fraction. ROS was postulated to render the glomerular to be more susceptibility to enzymatic degradation (Kako et al., 1988). By direct injection of myeloperoxidase, a neutrophil cationic enzyme localized in glomeruli, following with a mixture of hydrogen peroxide in chloride-containing solution provided the development of proteinuria, endothelial cell swelling and spreading of the foot processes in glomerular basement membrane (Johnson et al, 1987). ROS could also degrade hyaluronic acid in glomerular basement membrane (Johnson, Rehan and Ward, 1988). Hydrogen peroxide has been demonstrated to change glutathione redox cycling during ischemic-reperfusion peroid in rat kidney (McCoy etal., 1988). By incubation isolated glomeruli with ROS generating system, xanthine/xanthine oxidase, could stimulate production of prostaglandins such as PGE_2, $PGF_{2\alpha}$, 6 keto $PGF_{1\alpha}$ and TXB_2 up to two folds. Moreover, ROS has been shown to stimulate the release of arachidonic acid before incorporated into isolated glomeruli. This occurance revealed that ROS might activate glomerular phospholipase A_2, then increased prostaglandins synthesis (Baud et al., 1981). However, the causation of oxidative damage was not only due to generation of ROS but the activity of enzymatic antioxidant was also decreased (Shlafer, Myers and Adkins, 1987; Konno and Kako, 1992).

Selenium

Selenium (Se) is an essential micronutrient for animals and man. It is considered a major breakthrough in nutrition, working synergistically with vitamin E, therefore its role as an antioxidant

Selenium was first discovered in 1817. It is a non metallic element which occurs ubiquitously in the earthcrust, mainly in association with sulfide minerals or as selenides of various metals. It is easily oxidized to selenite (SeO_3^{2-}) at pH 6.3-6.7 in the presence of iron or to selenate (SeO_4^{2-}) at pH 8. In these forms, selenium is taken up by plant, which occurs predominantly as selenomethionine or selenocystheine.

Selenium metabolism

By oral administration, selenite, selenate, selenocysteine or selenomethionine, can be absorped by duodenum (selenite on average by 55%). The absorption of selenium is inhibited by methionine, high fiber content diet, arsenic, cadmium and mercury. The absorption rate is depended on different oxidation state of selenium. This trace element may be absorpted by respiratory tract or skin , however there is no quantitative human data. Plasma selenium levels is immediately changed by its uptake, bound to plasma proteins and distribute to tissue. The half life of selenium is between 65-115 days. The distribution and concentration of selenium are markly depended on species and age. Under normal condition of dietary intake, selenium is highly concentrated in kidney, liver, testis, pineal gland, hypophysis, hair and finger nails. The high level of this trace element in kidney and liver than other tissues may account to involvement in its excretion. Total body selenium content is controlled by renal elimination (Diplock, 1987; Fox, 1992). The range between nutritional requirement and toxic dose is very narrow. Currently, there are insufficient data to recommend selenium supplementation as a prophylactic measure for any kind of diseases. Thus, at present , Se dietary supplementation cannot be

recommended as a routine basis (Fan and Kizer, 1990). However, the US National Research Council has calculated the basis for 1989 Recommended Dietary Allowance (RDA) for selenium. The RDA was calculated as 70 and 55 mg/day for adult man and woman,respectively, after correcting for differences in body size and individual variations in requirements (Lavender, 1991)

Biological function of selenium

Selenoenzymes

Selenium was considered for a long time to be toxic element for human and animals until 1970s. The first break through to understanding in its nutritional role as essential component of enzyme glutathione peroxidase came.

Glutathione peroxidase (GSH-px) is an enzyme that can metabolize many peroxides, and synergistially acting with vitamin E in protecting polyunsaturated membrane lipid from oxidative degradation that would result in membrane dysfunction. This enzyme has been shown to present in cytosol and mitochondrial matrix of cells as well as in body fluid (Sunde and Hoekstra, 1980) and has been shown to be the most abundunt rat kidney selenoprotein (Viljoen and Tappel, 1988). Glutathione peroxidase has four identical subunits with one atom of selenium as selenocysteine at each active site. Selenocysteine is directly incorporated into GSH-px by phosphorylation a serinyl t-RNA with specific kinase, giving rise to selenocysteine t-RNA, which is specific incorporated into the peptide backbone of GSH-px (Sunde and Hoekstra, 1980 ; Combs, 1990 ; Neve, 1991). Glutathione peroxidase show specificity for any hydroperoxide substrates including hydrogen peroxide (H_2O_2), lipid peroxides, steroid hydroperoxides and peroxidized DNA, however, peroxidized phospholipids are not substrates for this enzyme. It has been suggested that GSH-px which located within mitochondrial matrix may be responsible for protection mitochondrial inner membrane, while cytosolic enzyme may protect the outer membrane (Combs, 1984).

The activity of glutathione peroxidase is entirely depended on intake of dietary selenium. A low selenium status is frequently associated with an impairment of GSH-px functions. Normal activity can be restored by selenium supplementation, however, if selenium is consumed above certain level that can be considered as optimal dose for the enzyme function, the enzyme activity does not further increase (Neve, 1988). Enzyme activity of GSH-px is markly reduced in selenium deficient status such as Keshan's disease, Kashin back's disease. Plasma GSH-px is also low in myocardial infarction, cancer, psoriasis, rhumatoid arthritis and chronic renal failure (Parnham et al., 1987). At twenty four hours after one hour of renal ischemia, the selenium deficient rats showed a marked decrease in glomerular filtration rate, renal blood flow and increased renal vascular resistance than those occurred in selenium sufficient rats (Nath and Paller, 1990). The enhancement of renal growth, urinary protein excretion and ammoniagenesis without metabolic acidosis, glucose intolerance, potassium depletion or elevation of plasma amino acid concentration have been observed in selenium deficient rats. These impairment might be due to decrease in GSH-px activity (Nath and Salahudeen, 1990). The enzyme activity in leukocyte was decreased during early phase of inflammation, which was suggested that the loss of GSH-px activity might be a general response to inflammation and injury (Parnham et al., 1987).

The discovery that selenium is an integral part of GSH-px, an enzyme which act by removing H_2O_2 and hydroperoxides, could provide explanation for the protective role of selenium in amelioration or protection tissue damage. Further interesting in dietary selenium supplementation was become popular. Selenium was suggested to reduce cancer risk or to have some health benefit. The isolated heart from rats which were fed with diet supplemented with 0.5 mg Se per kg diet for 10 weeks showed a lesser degree of change in cardiac function after recieved stress condition which was induced by infusion of 375 nmolH_2O_2/min while nonsupplemented rats showed an impairment of left ventricular relaxation and the elevation of left ventricular end diastolic pressure after recieved the same stress condition. This changes was suspect to be the selective

impairment of calcium uptake by sarcoplasmic recticulum caused by H_2O_2 (Konz et al., 1991). Protective effect of selenium supplementation by action of GSH-px was comfirmed by Poltronieri and coworkers. They have demonstrated that supplementation 6 mg/day of selenium as sodium selenite for 4 weeks to rats produced a wide spread increase in Se-dependent glutathione peroxidase activity in their all tissues. Isolated heart from these rats showed the improvement of recovery of ventricular contraction, which was presented as left ventricular pressure (LVP) and left ventricular end diastolic pressure (LVEDP), during reperfusion period. The changes of LVP and LVEDP between before ischemic peroid and in reperfusion period in Se supplemented rats were less than those occured in nonselenium supplemented rats. The increment in creatine kinase activity was also less in selenium supplemented rats (supplemented rats, from 185 ± 36 to 351 ± 76, nonsupplemented rats, from 273 ± 34 to 3401 ± 914 mU/min/g of wet tissue). Supplementation of selenium might increase GSH-px activity,therefore, exerted protective action against functional and biochemical cardiac impairment which was induced by ischemia and reperfusion (Poltronieri, Cevese and Sbarbati, 1992).

The other selenoenzyme was discovered during pursuing the GSH-px activity in liver cytosol by Ursini and coworkers (1982). They have isolated a selenoenzyme which showed the different glutathione peroxidase activity from the classic GSH-px in several respects including 1) relatively high membrane affinity (hydrophobic character) 2) molecular weight on average 20 KDa for monomeric of this enzyme versus 85 KDa for tetrameric of classic GSH-px 3) lack of absolute specificity for GSH as the reducing substrate 4)abroad specificity for hydroperoxides including phospholipid hydroperoxides, cholesterol hydroperoxide etc. (Thomas et al., 1990). This enzyme was designated to ' phospholipid hydroperoxide glutathione peroxidase' (PHGPX) because of its capacity that could metabolize fatty acid hydroperoxides which were esterified in phospholipids (Ursini et al., 1982). This enzyme was identified in rat liver, brain, heart, kidney, lung, in dog heart, liver, kidney and brain and could be extracted by high ionic strenght treatment from mitochondria, nuclei and microsome of cell (Ursini, Maiorino and Gregolin, 1983).

The requirement of selenium for PHGPX is different from GSH-px. Its activity was more slowly depleted by selenium deficiency than GSH-px (Weitzel, Ursini and Wendel, 1990). By immunoaffinity chromatography and radioimmunoassay study, the other selenoprotein in plasma which has a distinct character from GSH-px has also been purified. It is called 'Selenoprotein P', a glycoprotein contained seven or more selenium atoms per molecule as selenocysteine. It is a selenoprotein with molecular weight 80 KDa and was postulated to be transport protein for selenium because of rapid incorporation of ^{75}Se into this selenoprotein and the apperance of selenoprotein P in plasma before ^{75}Se was accumulated into other tissues (Burk, 1990). However, this selenoprotein might, somehow, be involved in selenium transport but this role was not direct function. Selenoprotein P has been shown to have selenium-rich regions, and it might possibly be breakdown to an intermediate form that was true transport form of this element (Burk et al., 1991).

Recent study has demonstrated the other selenoprotein which was membrane bound protein and has an enzymatic activity likely to type I iodothyronine 5' deiodenase (5'DI). It has been shown to catalyze the reductive deiodination of L-thyroxine (T_4) to the biological active thyroid hormone (T_3). It was shown that liver homogenate from Se deficient rats decreased the production of T_3 whereas animals which were fed with diet containing selenium 0.5-1.0 ppm, showed an increase in 5'DI activity, however, the change was relatively small in correlation to the increase in dietary Se (Zachara, 1992). The discovery of several mammalian selenoproteins has been reported. Up to thirteen selenoproteins has been shown to present in different organs but only two of them namely GSH-px and 5'DI, were well characterized.

Physiological roles of selenium

The roles of selenium in some metabolic pathways or physiological functions have also been identified. This trace element was suggested to be involved in metabolism

of arachidonic acid . In the cyclooxygenase pathway, selenium has been shown to regulate the balance between the proaggregatory and vasoconstrictory agent,thromboxane A_2, and antiaggregatory and vasodilatory agent, prostacyclin. The biosynthesis of these eicosanoid derivatives has been shown to need the activation of hydroperoxides. Prostacyclin synthetase has been suggested to be more sensitive to inhibitory action of lipid hydroperoxide at concentrations that could stimulate thromboxane synthase. By supplementation of selenium as sodium selenite in dose of 10 mg/kg bw.for 8 weeks to human could induce progressive increase in platelet GSH-px activity and enhanced platelet aggregation threashold for arachidonic acid stimulation. These changes were concurrent with inhibition of platelet biosynthesis of thromboxane B_2. The progressive rise in bleeding time was unmodified by aspirin (Perona et al., 1990). By direct supplementation of selenium to cultured human mesangial cell caused increase in production of three major prostaglandins including PGE_2, $PGF_{2\alpha}$ and 6 keto-$PGF_{1\alpha}$, while TXB_2 production was decreased. In this case, phospholipase A_2 activity was not suppressed (Hampel, Rinke and Hren, 1991). Prostaglandins are thought to play roles in control renal blood flow and glomerular filtration. PGE_2 and PGI_2 are vasodilator in the kidney while TXB_2 is a potent vasoconstrictor and TXB_2 has been shown to contract glomeruli. By oral selenium supplementation to man could increase creatinine clearance coincide with decreased serum creatinine. Because of high level of selenium in kidney cortex, selenium has been suspected to involve in renal vascular regulatory mechanism (Guidi et al., 1990). Selenium supplementation could increase glutathione peroxidase activity and increased cellular seleno-tRNAs to raise the protein synthesis efficiency in cells (Jia et al., 1989). A year long of 0.21 mg Se/kg diet as sodium selenite supplementation to rats which were exposed to high sucrose diet resulted in higher GSH-px activity and changes in glomerular microvessel morphology. The selenium supplemented rats showed an increase in diameter of Bowman's capsule and capillary tuft. The more openning of capillary lumen but smaller in extracellular and cytoplasmic material than those in nonselenium supplemented rats have been noted. By radioisotope

study has demonstrated that almost ^{75}Se (90%) were associated with nuclei from glomerular cells, the remaining about 10% was distributed between the cytosol and mitochondria. The protective effect of selenium against renal damage which was produced by exposure to high sucrose diet might be due to directly utilize this trace element by renal microvessels (Eckhert et al., 1992). Selenium supplementation was shown to increase the response to acetylcholine administration. By intraperitoneal injection of 4.33 mM/g bw.per day for 3 consecutive days caused increase in endothelial dependent relaxation in rat aortic rings. The enhancement of aortic relaxation has been suspected to be due to increase in the release of nitric oxide. Selenium supplementation might possible be effected on endothelium dependent relaxation by involvement in cellular changes (Lu, Liu and Man, 1994). By oral supplementation of 50 mg Se/Kg bw. for 15 consecutive days produced rise in contractile response of rabbit ileum to acythycholine in dose dependent manner. This response might possible be due to change in the cell membranes openning voltage-gate calcium channel which could lead to increase calcium uptake and might possibly stimulate calcium release from mitochondria as well (Dalay et al., 1993). However, Guo and hiscoworkers have suggested that sodium selenite have inhibitory action on slow calcium channel in simmilarly to action of verapamil. This trace element has been shown to have hypotensive effect and could decrease myocardial tension, left ventricular pressure, tension time index of ventricular ejection, declined myocardial oxygen consumption, therefore, cardiac load was relieved at low dose (<1 mg/kg bw) intravenous infusion . In high dose (over 2 mg/kg bw) intravenous infusion caused decrease in blood flow, total peripheral resistance, heart rate and cardiac contractile force and could depress left ventricular work (Guo et al., 1988). Selenium supplementation has been suggested to effect on plasma glucocorticoid. By intraperitoneal injection of 1.6 mgSe/Kg bw.to fasted rats cold increase in hepatic glucose output (Bell, Soliman and Early II, 1990) and plasma cortisone level but plasma insulin was not affected (Rasekh et al., 1991). The change in plasma corticosterone, ACTH and β-endorphin in dose dependent of selenium injection have also been noted (Potmis et al.,

1992). By oral supplementation with selenium 2 to 4 PPM to autoimmune mices could improve servival rate from lupus-like disease and increased neural killer cell activity (O'Dall et al., 1988). Selenite at micromolar concentration has also been shown to produce irreversible inhibition on glucocorticoid receptor in vitro, whereas receptor-hormone complex was insensitive to selenite inhibition (Tashima, 1989). By direct incubation 0.1-0.3 PPM Se with erythrocyte ghost could increase membrane bound Na-K ATPase acitivity and plasma membrane lipid fluidity (Fuyu and Weihan, 1986).

Effect of selenium on mitochondrial activity

Selenium as sodium selenite in suprapharmacological dose, 30 to 100 mM incubated to isolated hepatocytes made changes in the glutathione redox cycling. Selenite at 50 mM could rise oxygen consumption rapidly, depleted thiols and NADPH level, whereas oxidized glutathione was increased. At concentration of 100 mM selenite produced more pronounced effects and could lyse cell within 3 hours because of an insufficient regeneration of reducing equivalents such as NADPH, thiols (Anundi, Stahl and Hogberg, 1984). Selenite has also been shown to stimulate glutamate, succinate and ascorbate oxidation, increased ATPase activity without any effect on phosphorylation efficiency. Selenite has been demonstrated to increase mitochondrial membrane swelling in dose dependent manner. It has been suggested that an increase in oxygen uptake might be cause of mitochondrial swelling (Shuyu et al., 1986). However, 5 mM selenite showed any inhibition on respiration or function of mitochondria (Morrison et al., 1988).

CHAPTER III

MATERIALS AND METHODS

The experiment was performed on male Wistar rats, weighing between 90 and 110 g.

Animal preparation

Rats were divided into two groups, selenium-supplemented and nonselenium-supplemented group.

1) In nonselenium-supplemented group, rats were fed with standard lab chow and free access tap water until renal clearance study. During this period, they recieved gastric feeding with tap water 0.8 ml daily, 40 days continuously.

2) In selenium-supplemented group, rats also been fed with standard lab chow and free access tap water like the other one but different treatment was carried out by feeding with 1.0 mg% sodium selenite dissolved in tap water in volume 0.8 ml daily, 40 days continuously.

During preparation period, both groups of rats were weighed every 4 days. Body weight were recorded and observed for abnormal weight gain or development.

Experimental period

After preparation period, animals were divided into four groups of treatment

Control group

The rats from nonselenium-supplemented group were injected with 0.9% normal saline in volume 1 ml per kg.bw intraperitoneally at 40 hours before renal clearance study.

Selenium-supplemental group (Se group)

Selenium-supplemental group (Se group)

The rats from selenium-supplemented group were injected with 0.9% normal saline in volume 1 ml per kg.bw intraperitoneally at 40 hours before renal clearance study.

Russell's viper venom administration group (RVV group)

The rats from nonselenium-supplemented group were injected intraperitoneally with Russell's viper venom dissolved in 0.9% normal saline in dose 0.9 mg/ml/kg.bw at 40 hours before renal clearance study.

Selenium-supplemental rats given Russell's viper venom group (SeRVV group)

The rats from selenium-supplemented group were injected intraperitoneally with Russell's viper venom dissolved in 0.9% normal saline in dose 0.9 mg/ml/kg.bw at 40 hours before renal clearance study.

Operative procedure for renal clearance study

After 40 hours of treatment, the rats were anesthetized by intraperitoneal injection with sodium pentobarbital (nembutal) 60 mg/kg.bw. A small supplemented doses could be given in order to control anesthetic condition. After animal was anesthesia, the surgical procedure was started. Tracheostomy was carried out and inserted a short piece of PE 240 tube into trachea for aspirated secretion and used as artificial airway. Right femeral artery, vein and left femeral artery were cannulated with PE 50 tubes. The right femeral artery was used for monitored arterial blood pressure (ABP) by connection to a pressure transducer (Statham PE23A) and ABP was recorded on a Grass polygraph (Model 7) recorder. Polyethylene tube which be cannulated into right femeral vein was used for Inulin and PAH solution infusion. Left femeral artery was used for blood sampling. Abdomen was explored in midline incision, carefully found out both ureter, then cannulated with PE 10 tubes for urine collection. Urine was collected in the pre-weighed cups.

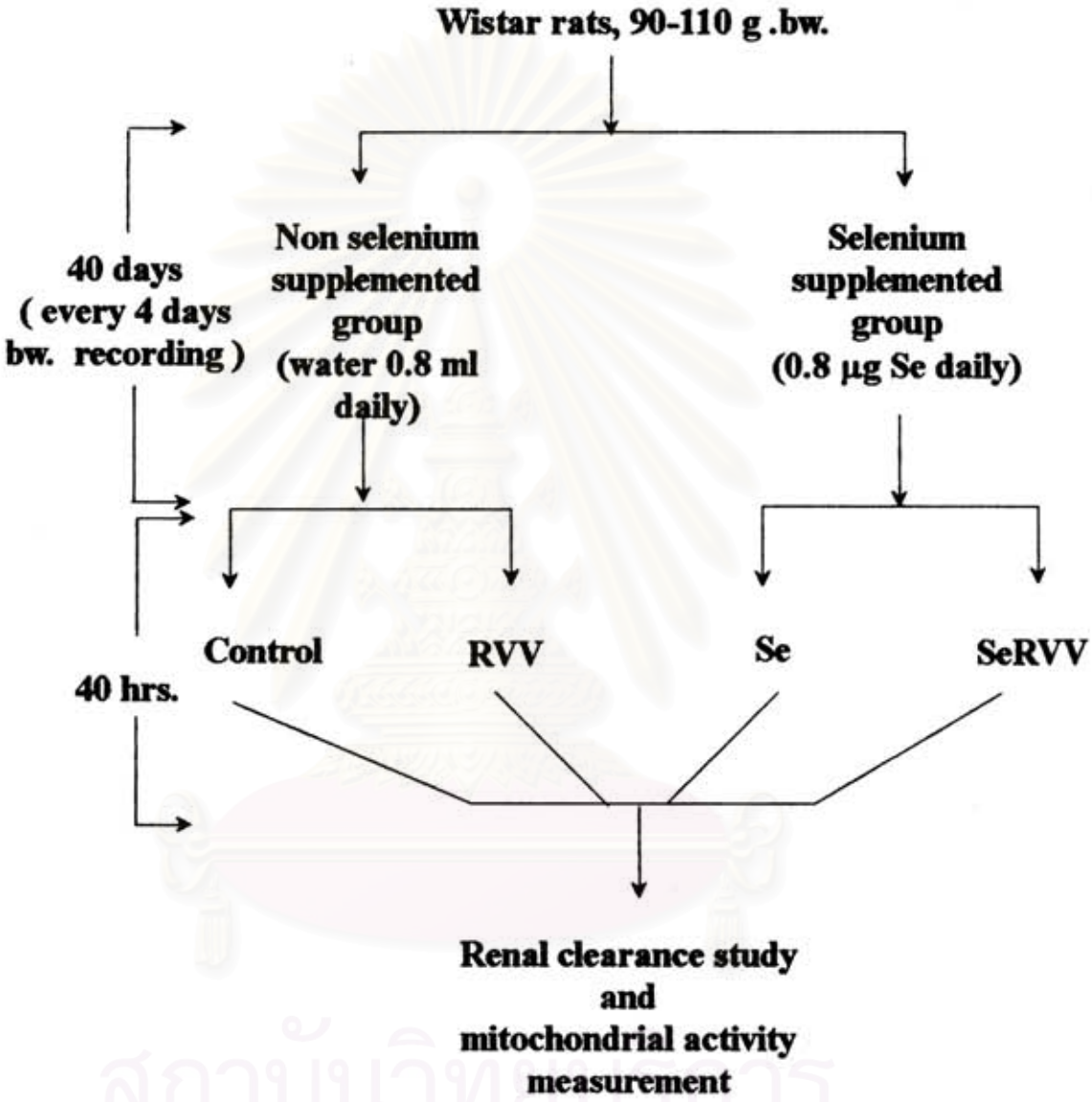

Fig. 2 Diagram of experimental protocol

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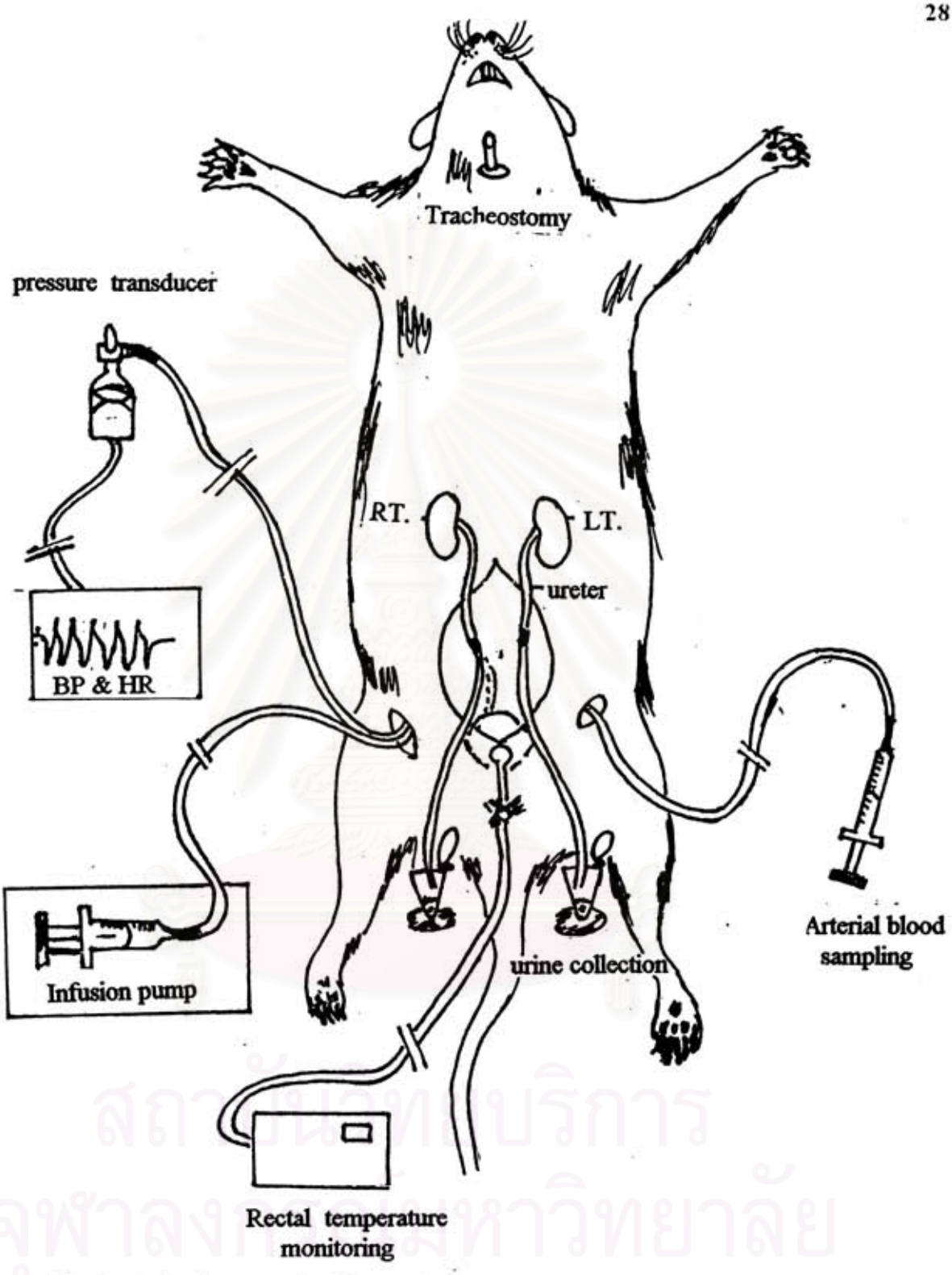

Fig. 3 Animal preperation for renal clearance study.

Renal clearance study

Before clearance study, fluid replacement was done with 0.9% normal saline in volume 1% per kg.bw, in 30 minutes. Clearance study was started by infusion of 1% Inulin and 0.1% PAH solution at rate 1% per kg.bw per hour for 45 minutes continuously to stabilize plasma Inulin and PAH concentration and general conditions.

After equilibrium period, three times of urine collection along with arterial blood sampling at midpoint of urine collection were done. Duration of a urine collection period was 30 minutes, blood sample was taken for 0.8 ml for each. Blood collection might be replaced with packed cell volume from each sample before added with 6% BSA and made volume equal to blood sample in each period. Urine volume was estimated from weight changes of urine cup. Blood samples were determined for packed cell volume by centrifugation to seperate packed cell. Serum and urine were kept for further analysis. Both kidneys were rapidly excised, weighed and stored in ice-cold homogenizing medium.

Cortical mitochondrial preparation

All steps of mitochondrial preparation has been done at temperature 0-4°C (Fig 4). Renal capsules were removed, longitudinal dissected and seperated inner medullary part out. Remaining part was minced and washed two times in ice-cold homoginizing medium; 210 mM mannitol, 70 mM sucrose, 100 mM EGTA and 0.5% BSA buffered at pH 7.4 with 2 mM K-Hepes. The tissue was hemogenated with Teflon motorized Potter Elvejham hemoginizer by using four up and down stroke of pastle rotating at 1000 rpm. The homogenate was centrifuged at 700 g for 10 minutes with automatic high refrigurated centrifuge (model IEC B22M applied with Roter model 877 and adaptor #7234 for roter #877) at 0-4°C to remove cell debris. The supernatant was centrifuged again at 13,000 g for 5 minutes to obtain mitochondrial fraction. Washing mitochondrial pellet with ice-cold homoginizing medium 2 times.

The resultant mitochondria formed a soft brown pink pellet, then resuspended mitochondria in ice-cold homoginizing medium 2.0-2.5 ml.

Determination of Mitochondrial Respiratory Control Index (RCI) and P/O ratio

Mitochondrial respiratory control index was measured in incubation chamber (Gilson chamber with Clark-typed oxygen electrode) which be controlled temperature at 25°C by circulating water supply surround the chamber and incubation mixture was stirred with magnetic stirrer continuously. The incubation mixture in chamber contained 1530 μL of incubation medium (120 mM KCl, 2 mM phosphate, 4 μM rotenone, 1 mM EGTA, 1 mM $MgCl_2$, adjusted pH 7.2 with 5 mM Tris-HCl), 130 μL of mitochondrial suspension and 10 mM succinate. Mitochondrial state 4 respiration (substrate supporting respiration) was monitored with oxygen monitor (YSI model 5300) and recorded with Kipp & Zonen type BD112 recorder for 1-2 minute, then added 300 nM ADP to start state 3 respiration (ADP stimulated respiration). The mitochondrial respiratory control index (RCI) was calculated from the ratio of oxygen that be consumed by mitochondria in state 3 and in state 4. Oxygen uptake value was expressed in ng-atom O_2/min/mg protein mitochondria and P/O ratio value was calculated from ratio of ADP content added and oxygen uptake in state 3. RCI value was used as an indicator of the integrity of mitochondrial preparation.

Determination of blood and urine samples

Inulin concentration in plasma and urine was determined by Antrone method as described by Davidson et al. (1963), PAH concentration was determined by the method of Bratton and Marshall which be modified by Smith (1962). Determination of plasma and urinary sodium, potassium was done by using Beckman flame photometer (model K, Li, Na), chloride by using Corning Chloride Analyzer (model 925).Packed cell volume was determined by preparation blood in international

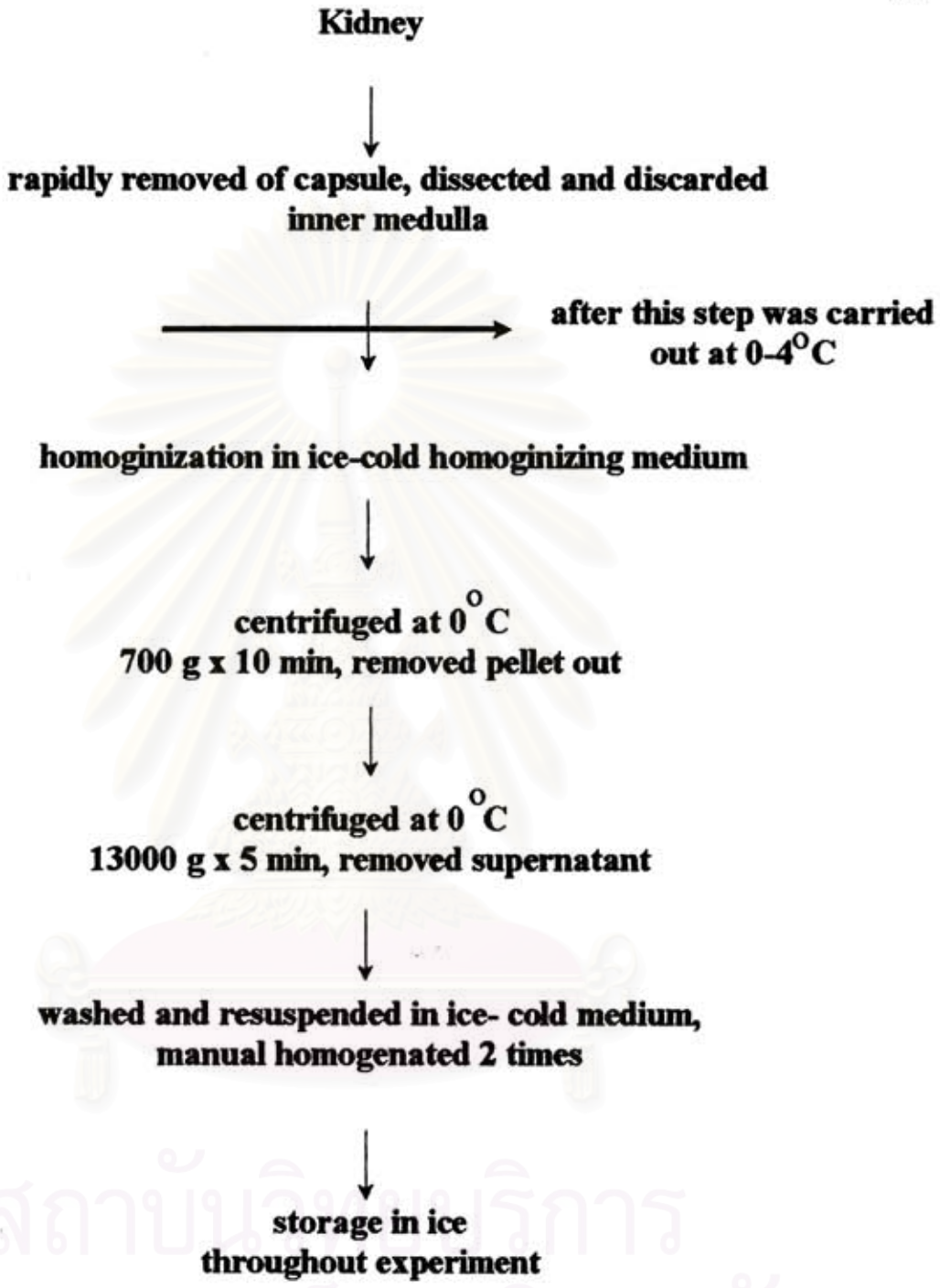

Fig. 4 Diagram of mitochondrial preperation

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microcapillary tube, centrifuged by using Adam microhematocrit centrifuge (model 850) and measured by using Hawksley micro hematocrit reader. Titratable acid (Ta) in urine was determined by titration with 0.001 N NaOH and ammonium content by formalin as described by Cunarro and Weiner (1974). Determination of plasma and urine urea nitrogen were carried out by a diacetyl monoxine method as described by Reed et al., (1972) and Wybenya (1971). Urinary pH was measured by using pH meter (Hanna instrument 8520 and pH electrode HI 1330). Protein content in mitochondrial suspension was measured by using Lowry method.

Chemical substances

Sodium selenite (Na_2SeO_3), Para-aminohippurate (PAH), Inulin (In), Russell's viper venom (RVV), Succinic acid, Bovine serum albumin (BSA), mannitol, sucrose, and Adenosine diphosphate

Calculation :

Mean arterial blood pressure (MAP) = $Pd + 1/3 (Ps-Pd)$

Pulse pressure (PP) = $Ps-Pd$

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

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

Effective renal blood flow (ERBF) = $\frac{ERPF \times 100}{(100-PCV)}$

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

Renal vascular resistance (RVR) = $\frac{MAP}{ERBF}$

Urinary electrolyte excretion = U_eV

Filtered load of electrolyte (F_e) = $GFR \times P_e$

Fractional electrolyte excretion (FE_e) = $\frac{U_e V/P_e \times 100}{GFR}$

Normality of Ta or NH_4^+ = $\frac{0.001\ NaOH \times volume\ of\ NaOH}{volume\ of\ urine}$

Urinary Titratable acid (UT_a) = $\frac{Normality\ of\ Ta \times V}{time\ of\ urine\ collection}$

Ammonium excretion (UNH_4^+) = $\frac{Normality\ of\ NH_4 \times V}{time\ of\ urine\ collecti\ n}$

Mitochondrial respiratory control index (RCI)

= $\frac{O_2\ consumption\ in\ mitochondrial\ state\ 3\ respiration}{O_2\ consumption\ in\ mitochondrial\ state\ 4\ respiration}$

Mitochondrial state 3 respiration (State 3)

= $\frac{O_2\ consumption\ in\ state\ 3\ (added\ ADP)}{mg\ protein\ of\ mitochondria}$

Mitochondrial state 4 respiration (State 4)

= $\frac{O_2\ consumption\ in\ state\ 4\ (added\ succinate)}{mg\ protein\ of\ mitochondria}$

P/O ratio (P/O ratio) = $\frac{nmole\ of\ ADP}{total\ O_2\ consumption\ in\ state\ 3}$

Data analysis

Data analysis was presented as mean ± SEM. These data were statistically compared by unpaired T-test among the groups. Results considered statistical difference had p-value less than 0.05.

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CHAPTER IV

RESULTS

Effect of selenium-supplementation on body weight gain

During supplementation with 8 μg selenium (Se) per day for consecutive 40 days to rats, the body weight gain of animals supplemented with Se were not different from nonselenium-supplemented animals at the same time of growth (table 2).

Effect of Russell's viper venom (RVV) on systemic circulation and packed cell volume in nonselenium and selenium-supplemented rats

After given RVV about 1.5 to 3 hours, some of the rats showed symtom of drowsiness, cold , pale , hyperpnea and following with hematuria. Some of them died before renal clearance study because of massive hemorrhage. The changes in systemic circulation of the servival animals in both nonselenium-supplemented group and selenium-supplemented group were observed.

The changes are shown in table 3 and figure 5. After 40 hours of intraperitoneal injection of Russell's viper venom, packed cell volume (PCV) was decreased in either rats given RVV alone (RVV group) by approximately 5% or selenium-supplemented rats given RVV (SeRVV group) by approximately 21% ($P<0.05$) when compared to the control animals. In selenium-supplemented animals (Se group), packed cell volume was not significantly different from the control ($43.1 \pm 1.1\%$ VS $44.0 \pm 0.06\%$ respectively). The heart rate (HR) in Se group was not much different from the control whereas a marked increase in heart rate was observed in both

RVV and SeRVV group ($p<0.05$). The increment in heart rate in RVV group and SeRVV group were 8.39% and 12.34% higher than the control. The systolic blood pressure (SBP) were not significantly different among groups. The diastolic blood pressure (DBP) of RVV group was not much different from the control group (106.8 ± 2.5 VS 105.9 ± 2.1 mmHg) but it was decreased in both Se group (100.1 ± 3.1mmHg) and SeRVV group (96.2 ± 3.8 mmHg, $P<0.05$) in comparison with the control (table 3). The pulse pressure (PP) of SeRVV group was significantly higher than the control (39.1 ± 3.4 VS 28.2 ± 2.1 mmHg, $P<0.05$) whereas those in Se group and RVV group were not different from the control. Mean arterial blood pressure (MAP) among groups were not statistically different, although it seemed to be lower in both Se and SeRVV group (table 3).

The effect of the venom on plasma electrolytes concentration are shown in table 4. There were not much different among groups after 40 hours of either envenomation or normal saline injection.

Effect of Russell's viper venom (RVV) on renal functions in nonselenium and selenium-supplemented rats

The rate of urine flow (V) in both RVV and SeRVV groups weere lower than those in the control and Se group. The glomerular filtration rate (GFR) was higher in animals fed with Se alone (494 ± 38 µl/min/100 g.bw) and was lower in the RVV group (435 ± 27 µl/min/100 g.bw)in comparison with the control (table 5). The ratio of urine flow rate to GFR (V/GFR) was decreased in both groups of RVV and SeRVV animals which were lower than the control group by approximately 9% and 23% respectively. As for animals who were fed with Se, the ratio of V/GFR was not much different (3.66% higher) from the control group (figure 7). Effective renal plasma flow (ERPF) and renal blood flow (ERBF) of the SeRVV group were higher by approximately 24% ($P<.05$) and 5% respectively than the control group. The ERPF in

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Se group was about 6% higher, whereas ERPF in RVV group was about 9% lower than those in the control group. The pattern of changes of ERBF in both Se group and RVV group were similarly to the changes of ERPF (figure 6). The SeRVV animals showed the lowest in filtration fraction (FF) and renal vascular resistance (RVR) in comparison with the control group.

The filtered load of sodium (F_{Na}), potassium (F_K) and chloride (F_{Cl}) were not significantly different among groups. However, the filtered load of sodium, potassium and chloride in Se group were higher than another groups. The urinary excretion (UV) of sodium and chloride in the Se group were also higher than the other groups whereas urinary excretion of potassium was lower than the control group by approximately 19% (figure 8). In SeRVV group, the urinary excretion of sodium, potassium and chloride were 18.4%, 13.8% and 24.6% respectively, lower than the control group. In view of fractional excretion of sodium (FE_{Na}), potassium (FE_K) and chloride (FE_{Cl}), there were not significantly different among groups. The tubular reabsorption of sodium (T_{Na}) and chloride (T_{Cl}) were not different among groups, although percentage tubular reabsorption of potussium (T_K) in both Se and SeRVV group were higher than in either the control or RVV group (table 7). There were not significantly different among groups for urinary excretion of titratable acid ($U_{Ta}V$) and ammonium excretion ($U_{NH4}V$) after envenomation or normal saline injection (table 8 and figure 11).

Effect of Russell's viper venom on kidney mitochondrial activity in nonselenium and selenium-supplemented rats

Mitochondrial activity is presented by the value of mitochondrial respiratory control index (RCI), oxygen uptake in mitochondrial state 3 respiration (S_3, ADP stimulated respiration), state 4 (S_4, substrate supportive respiration) and P/O ratio. The RCI value among four groups did not show any significant differences. Oxygen consumption in mitochondrial state 3 respiration in both Se and SeRVV group were

194.36 ± 11.31 and 223.85 ± 14.19 ng-atom O_2/min/mg protein of mitochondria, which were higher than those in the control, for Se was 9.4% and for SeRVV was 26% respectively. In RVV group, mitochondrial oxygen consumption in state 3 respiration was 169.44 ± 5.25 ng-atom O_2/min/mg protein of mitochondria which was lower than the control group by approximately 5%. Mitochondrial state 4 respiration did not show any significant differences among groups though a slightly increase were noted for Se and SeRVV group. The P/O ratio of RVV group was higher than the control group by approximately 11% whereas alteration of P/O ratio in either Se or SeRVV group were not observed (figure 12).

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Table 1 Composition of diet

Ingredient		Minerals		Vitamins	
Protein (%)	24	Calcium (%)	1.5	A (IU/Kg)	20,000
		Phosphate (%)	1.0	D (IU/Kg)	250
Fat (%)	3	Sodium (%)	0.25	E (mg/Kg)	10
		Manganese (PPM)	10	B1 (mg/Kg)	10
Fiber (%)	4	Cupper (PPM)	10	B2 (mg/Kg)	8
		Zinc (PPM)	80	B6 (mg/Kg)	8
Metabolic energy (Kcal/Kg)	2850	Cobolt (PPM)	1	B12 (mg/Kg)	0.02
		Iodine (PPM)	2	Thyamine (mg/Kg)	20
		Selenium (PPM)	0.1	Folic acid (mg/Kg)	0.05
				Pantacinic (mg/Kg)	20
				Choline chloride (mg/Kg)	1,500

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Table 2 Effect of selenium (sodium selenite) 8 μg supplementation daily for 40 days on body weight gain

selenium supplementation (days)	Body weight gain (g)	
	nonselenium-supplemented group (n=47)	selenium-supplemented group (n=53)
8 days	37.98 ± 11.40	39.43 ± 13.50
16 days	80.64 ± 18.14	82.64 ± 18.57
24 days	115.74 ± 20.38	118.96 ± 19.10
32 days	148.40 ± 21.65	151.69 ± 18.81
40 days	176.17 ± 22.0	179.91 ± 17.99

Data expressed as mean ± SD

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Table 3 Effect of Russell ' s viper venom on general circulation in nonselenium and selenium supplemented rats

	Control (n=11)	Se (n=11)	RVV (n=11)	SeRVV (n=11)
HR (BPM)	375.8 ± 9.5 [a]	386.5 ± 9.0 [a]	407.3 ± 7.7 [bc]	422.2 ± 8.8 [c]
SBP (mmHg)	134.1 ± 1.9	129.9 ± 2.8	137.7 ± 3.5	135.2 ± 3.7
DBP (mmHg)	105.9 ± 2.1 [a]	100.1 ± 3.1 [ab]	106.8 ± 2.5 [a]	96.2 ± 3.8 [b]
MAP (mmHg)	115.3 ± 1.7	110.0 ± 3.0	117.2 ± 2.7	109.2 ± 3.4
PP (mmHg)	28.2 ± 2.1 [a]	29.5 ± 1.1 [a]	31.1 ± 2.3 [a]	39.1 ± 3.4 [b]
PCV (%)	44.0 ± 0.6 [a]	43.1 ± 1.1 [a]	41.7 ± 1.8 [a]	34.9 ± 2.4 [b]

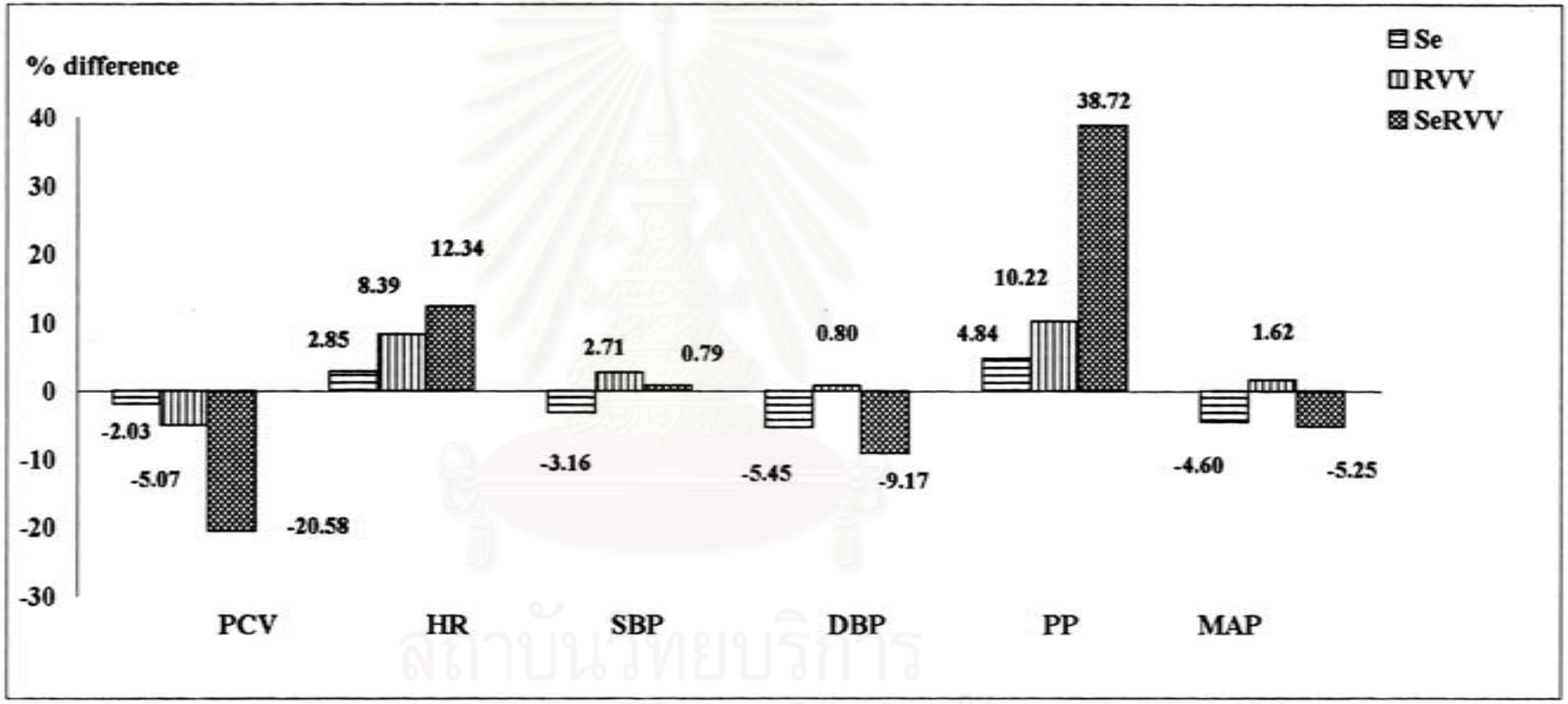
Data expressed as mean ± SEM

Control : Control group ; Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group ; SeRVV : selenium-supplemental rats given Russell ' s Viper venom group .

HR : heart rate ; SBP : systolic blood pressure ; DBP : diastolic blood pressure ; PP : pulse pressure ; MAP : mean arterial blood pressure ; PCV : packed cell volume

a,b,c : Means within a row with no common letters are significantly differented at $p<0.05$

Figure 5 Percentage difference in packed cell volume , heart rate , systolic blood pressure , diastolic blood pressure , mean arterial blood pressure and pulse pressure in each group of treatment compared to the control group



Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group and SeRVV : Selenium supplental rats given Russell ' sViper venom gruop

Data expressed as percentage difference in mean value

Table 4 Effect of Russell's viper venom on plasma concentration of sodium (P_{Na}), potassium (P_K) and chloride (P_{Cl}) in nonselenium and selenium supplemented rats

	Control (n=11)	Se (n=11)	RVV (n=11)	SeRVV (n=11)
P_{Na} (mEq/L)	143.2 ± 2.6	146.6 ± 1.8	144.6 ± 3.1	144.5 ± 3.0
P_K (mEq/L)	4.2 ± 0.1	4.1 ± 0.2	4.3 ± 0.2	4.1 ± 0.1
P_{Cl} (mEq/L)	104.9 ± 2.8	107.8 ± 2.1	107.5 ± 3.1	105.6 ± 2.7

Data expressed as mean ± SEM

Control : Control group ; Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group ; SeRVV : selenium-supplemental rats given Russell's Viper venom group .

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Table 5 Effect of Russell's viper venom on renal hemodynamic in nonselenium and selenium supplemented rats

	Control (n=11)	Se (n=11)	RVV (n=11)	SeRVV (n=11)
V (μl/min/100gBW)	16 ± 2	16 ± 3	14 ± 3	12 ± 4
GFR (μl/min/100g BW)	465 ± 36	494 ± 38	435 ± 27	448 ± 35
ERPF(ml/min/100gBW)	1.655 ± 0.154 [a]	1.754 ± 0.156 [a]	1.511 ± 0.112 [a]	2.049 ± 0. 202 [b]
ERBF(ml/min/100gBW)	2.950 ± 0.281	3.083 ± 0.275	2.594 ± 0.177	3.105 ± 0.262
V/GFR (%)	3.54 ± 0.58	3.66 ± 1.0	3.23 ± 0.65	2.72 ± 0.83
FF (%)	29.5 ± 2.44	29.6 ± 1.84	30.5 ± 2.03	24.5 ± 2.94
RVR (mmHg/ml/min/ 100gBW)	45.73 ± 4.43	42.72 ± 3.99	51.43 ± 4.60	39.84 ± 3.37

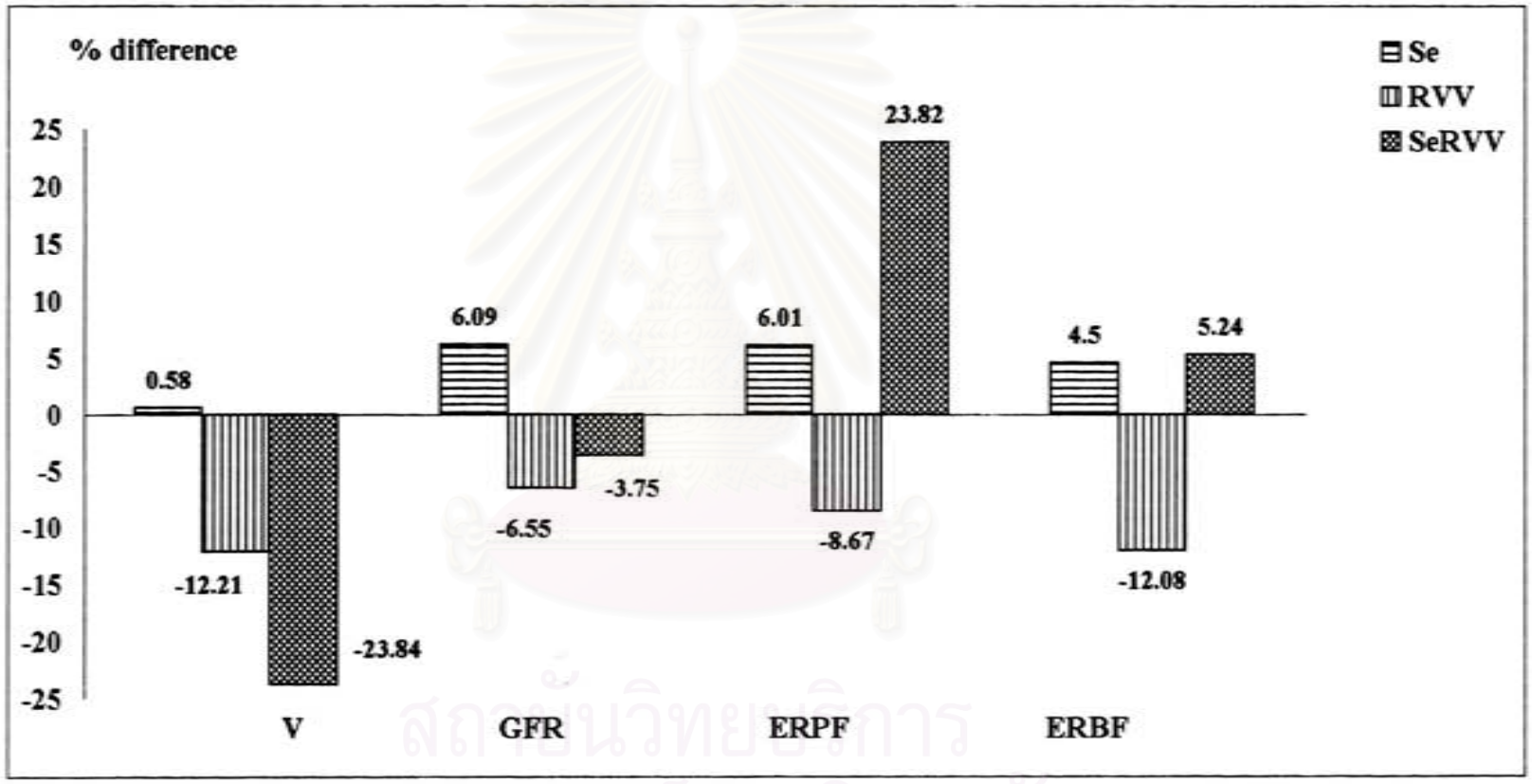
Data expressed as mean ± SEM

Control : Control group ; Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group ; SeRVV : selenium-supplemental rats given Russell's Viper venom group .

V: urine flow rate ; GFR : glomerular filtration rate ; ERPF : effective renal plasma flow ; ERBF : effective renal blood flow ; V/GFR : ratio of urine flow rate to GFR ; FF : filtration fraction ; RVR : renal vascular resistance .

a,b,c : Means within a row with no common letters are significantly differented at $p<0.05$

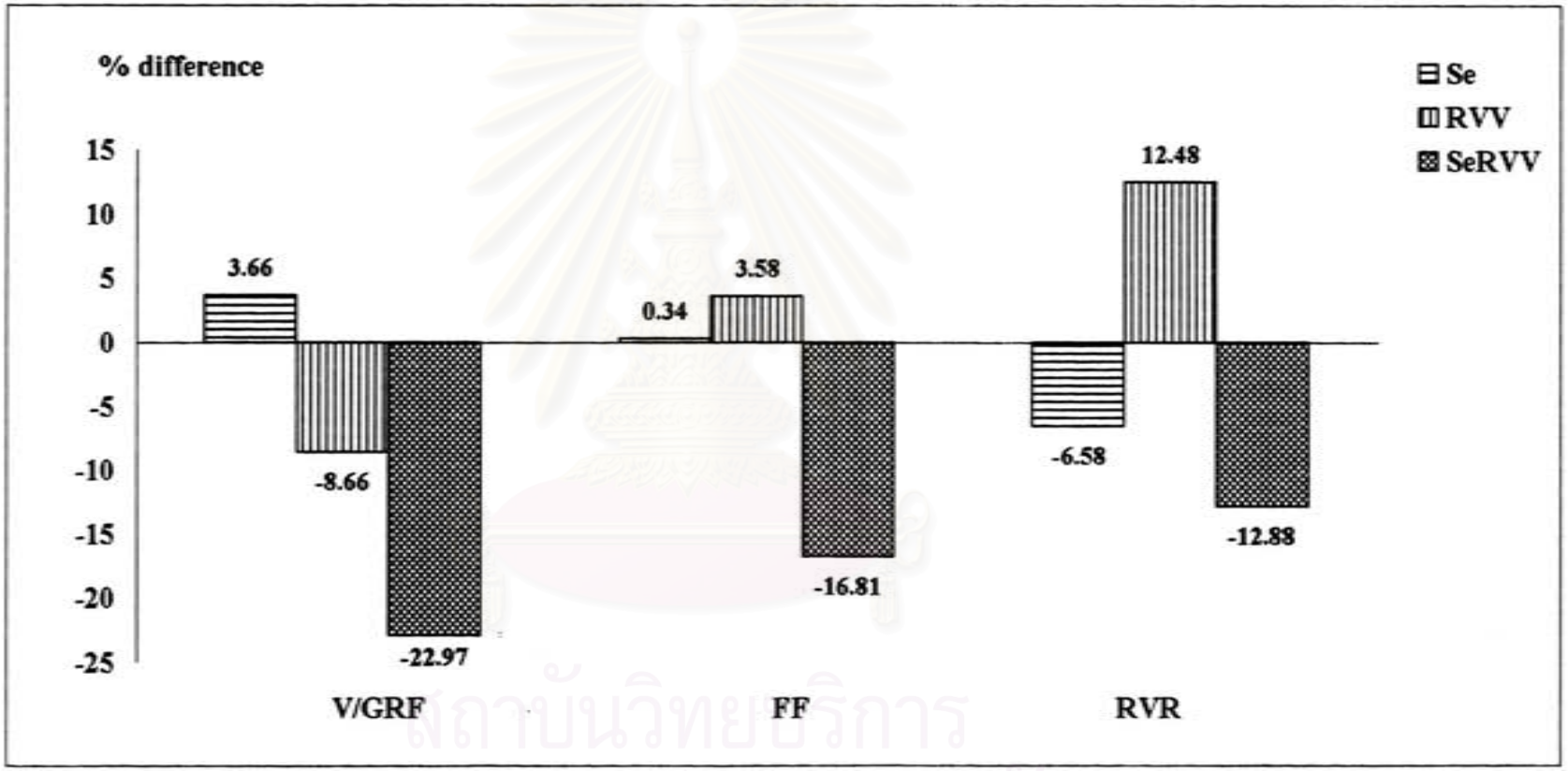
Figure 6 Percentage difference in urine flow ratte , GFR , effective renal plasma flow and effective renal blood flow in each group of treatment compared to the control group



Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group and SeRVV : Selenium supplental rats given Russell ' sViper venom gruop

Data expressed as percentage difference in mean value

Figure 7 Percentage difference in ratio of urine flow rate to GFR , filtration fraction and renal vascular resistance in each group of treatment compared to the control group



Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group and SeRVV : Selenium supplental rats given Russell ' sViper venom gruop

Data expressed as percentage difference in mean value

Table 6 Effect of Russell ' s viper venom on filtered load , urinary excretion and tubular reabsorption of electrolyte in nonselenium and selenium supplemented rats

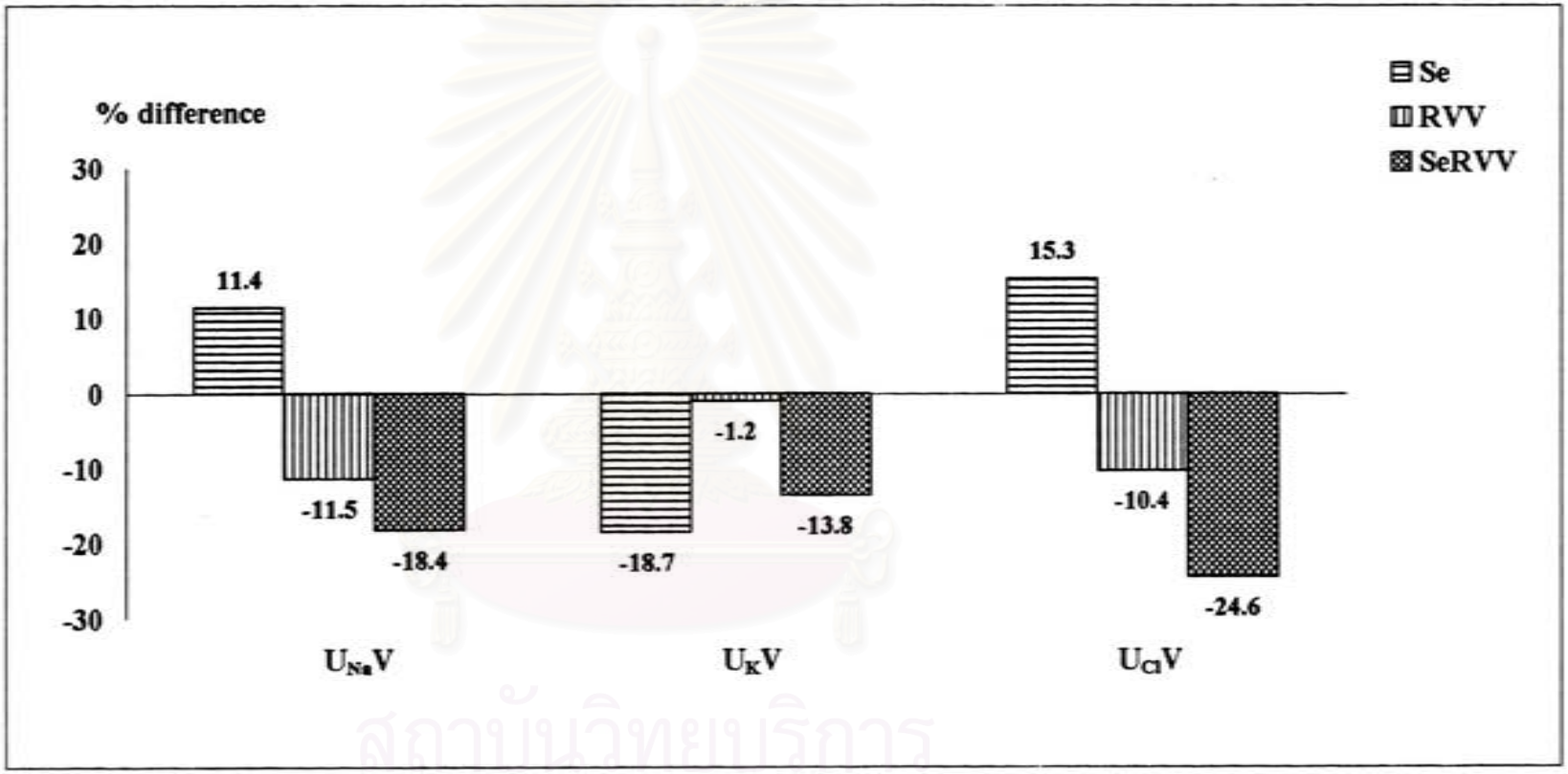
	Control (n=11)	Se (n=11)	RVV (n=11)	SeRVV (n=11)
F_{Na} (μEq/min/100gBW)	66.50 ± 5.17	72.25 ± 5.62	63.12 ± 4.30	64.78 ± 5.22
F_K (μEq/min/100gBW)	1.97 ± 0.16	2.04 ± 0.20	1.87 ± 0.14	1.84 ± 0.18
F_{Cl} (μEq/min/100gBW)	48.59 ± 3.65	53.42 ± 4.64	46.89 ± 3.27	47.32 ± 4.22
$U_{Na}V$ (μEq/min/100gBW)	2.42 ± 0.36	2.70 ± 0.44	2.14 ± 0.31	1.98 ± 0.41
$U_K V$ (μEq/min/100gBW)	0.74 ± 0.06	0.60 ± 0.05	0.73 ± 0.08	0.63 ± 0.05
$U_{Cl}V$ (μEq/min/100gBW)	2.12 ± 0.26	2.44 ± 0.46	1.90 ± 0.32	1.60 ± 0.36
T_{Na} (μEq/min/100gBW)	64.08 ± 5.14	69.55 ± 5.56	60.97 ± 4.22	62.81 ± 5.12
T_K (μEq/min/100gBW)	1.24 ± 0.14	1.46 ± 0.18	1.14 ± 0.16	1.20 ± 0.16
T_{Cl} (μEq/min/100gBW)	46.47 ± 3.61	50.97 ± 4.69	44.99 ± 3.29	45.72 ± 3.79

Data expressed as mean ± SEM

Control : Control group ; Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group ; SeRVV : selenium-supplemental rats given Russell ' s Viper venom group .

$F_{Na, K, Cl}$: filtered load of sodium , potassium and chloride ; $UV_{Na, K, Cl}$: urinary excretion of sodium , potassium , chloride ; $T_{Na, K, Cl}$: tubular reabsorption of sodium , potassium and chloride

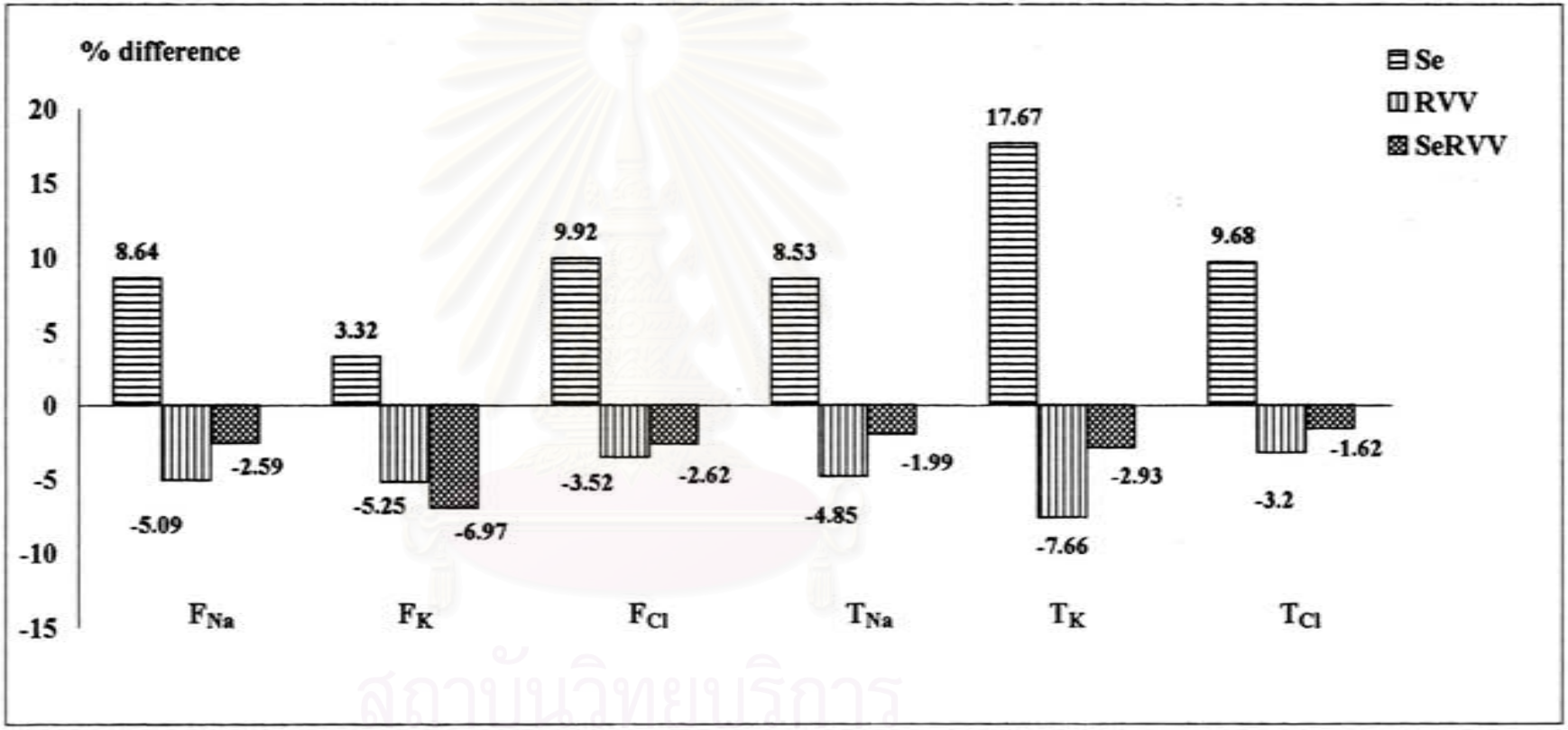
Figure 8 Percentage difference in urinary excretion of electrolyte in each group of treatment compared to the control group



Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group and SeRVV : Selenium supplental rats given Russell ' sViper venom gruop

Data expressed as percentage difference in mean value

Figure 9 Percentage difference in filtered load and tubular reabsorption of electrolyte in each group of treatment compared to the control group



Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group and SeRVV : Selenium supplental rats given Russell ' sViper venom gruop

Data expressed as percentage difference in mean value

Table 7 Effect of Russell's viper venom on fractional excretion and percentage tubular reabsorption of electrolyte in nonselenium and selenium-supplemented rats

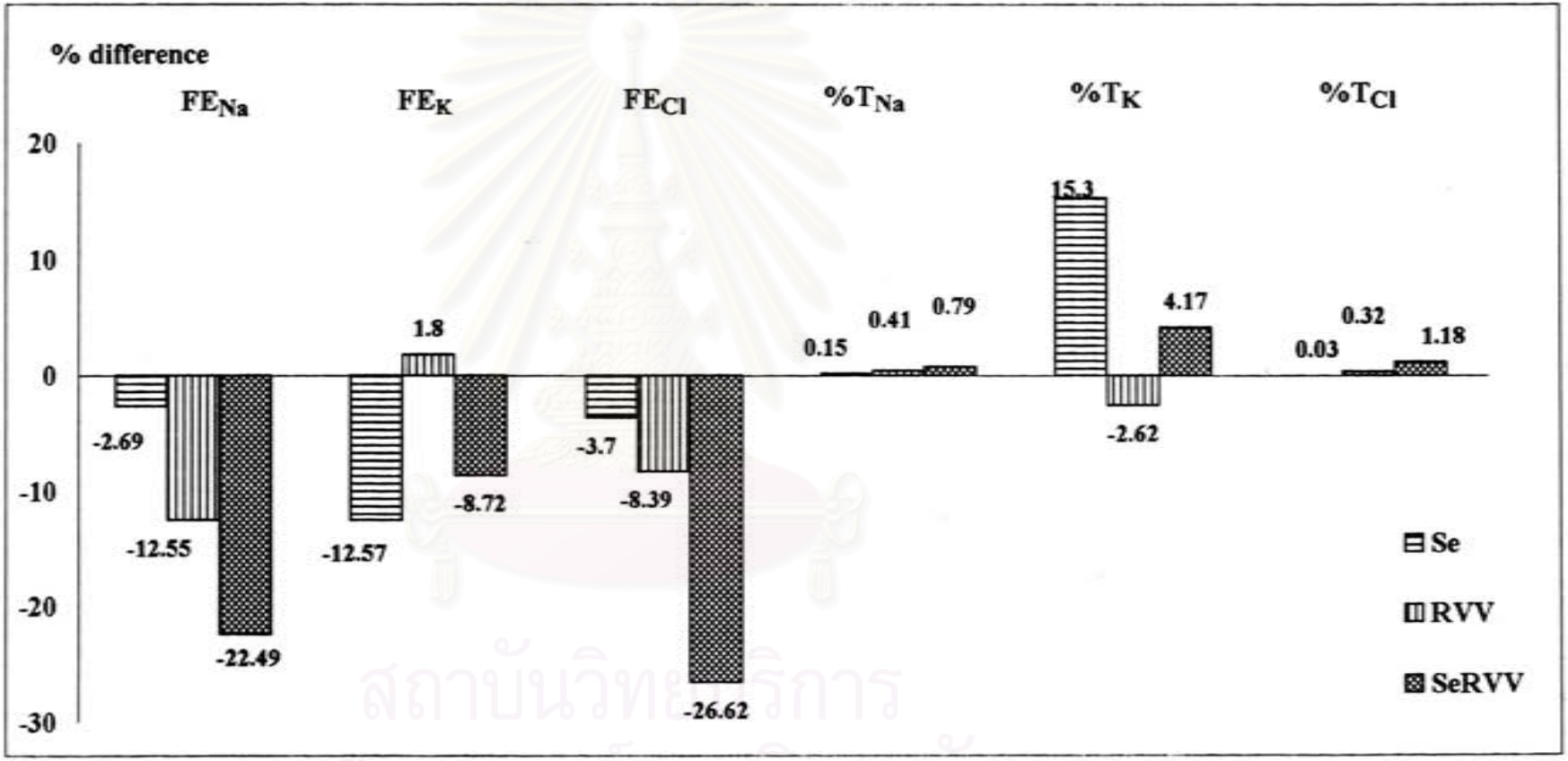
	Control (n=11)	Se (n=11)	RVV (n=11)	SeRVV (n=11)
FE_{Na} (%)	4.10 ± 0.84	4.00 ± 0.80	3.60 ± 0.55	3.20 ± 0.73
FE_{K} (%)	41.40 ± 4.39	36.20 ± 4.88	42.20 ± 5.02	37.80 ± 4.03
FE_{Cl} (%)	4.90 ± 0.72	4.70 ± 0.98	4.50 ± 0.86	3.60 ± 0.91
T_{Na} (%)	96.15 ± 0.69	96.0 ± 0.89	96.54 ± 0.50	96.61 ± 0.69
T_{K} (%)	60.95 ± 3.58	70.27 ± 3.78	59.35 ± 4.86	63.49 ± 3.80
T_{Cl} (%)	95.40 ± 0.63	95.44 ± 0.92	95.71 ± 0.80	96.53 ± 0.88

Data expressed as mean ± SEM

Control : Control group ; Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group ; SeRVV : selenium-supplemental rats given Russell ' s Viper venom group .

$FE_{Na, K, Cl}$: fractional excretion of sodium , potassium and chloride ; $T_{Na, K, Cl}$: percentage tubular reabsorption of sodium , potassium and chloride

Figure10 Percentage difference in fractional excretion and percentage tubular reabsorption of electrolyte in each group of treatment compared to the control group



Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group and SeRVV : Selenium supplental rats given Russell ' sViper venom gruop

Data expressed as percentage difference in mean value

Table 8 Effect of Russell's viper venom on urinary titratable acid and urinary ammonium excretion in nonselenium and selenium supplemented rats

	Control (n=11)	Se (n=11)	RVV (n=11)	SeRVV (n=11)
$U_{Ta}V$ (μEq/min)	2.6 ± 0.3	2.6 ± 0.8	2.9 ± 0.4	2.7 ± 0.4
$U_{NH4}V$ (μEq/min)	1.3 ± 0.2	1.1 ± 0.3	1.7 ± 0.4	1.5 ± 1.6

Data expressed as mean ± SEM

Control : Control group ; Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group ; SeRVV : selenium-supplemental rats given Russell's Viper venom group .

$U_{Ta}V$: urinary titratable acid ; $U_{NH4}V$: urinary ammonium excretion

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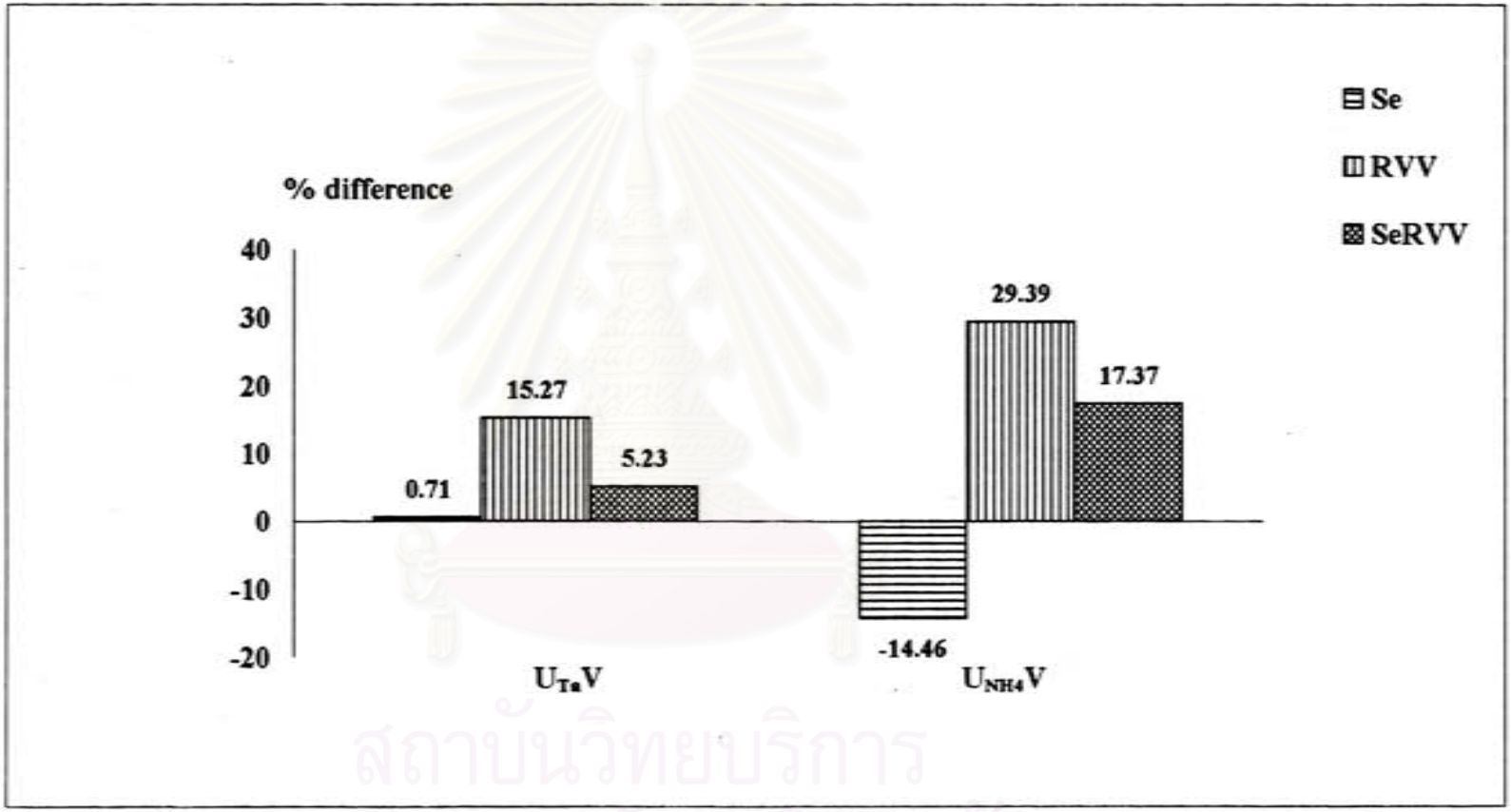
Figure 11 Percentage difference in urinary titratable acid and ammonium excretion in each group of treatment compared to the control group



Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group and SeRVV : Selenium supplental rats given Russell ' sViper venom gruop

Data expressed as percentage difference in mean value

Table 9 Effect of Russell's viper venom on mitochondrial respiratory control index , oxygen uptake in state respiration (S3) , state 4 respiration (S4) and P/O ratio in nonselenium and selenium-supplemented rats

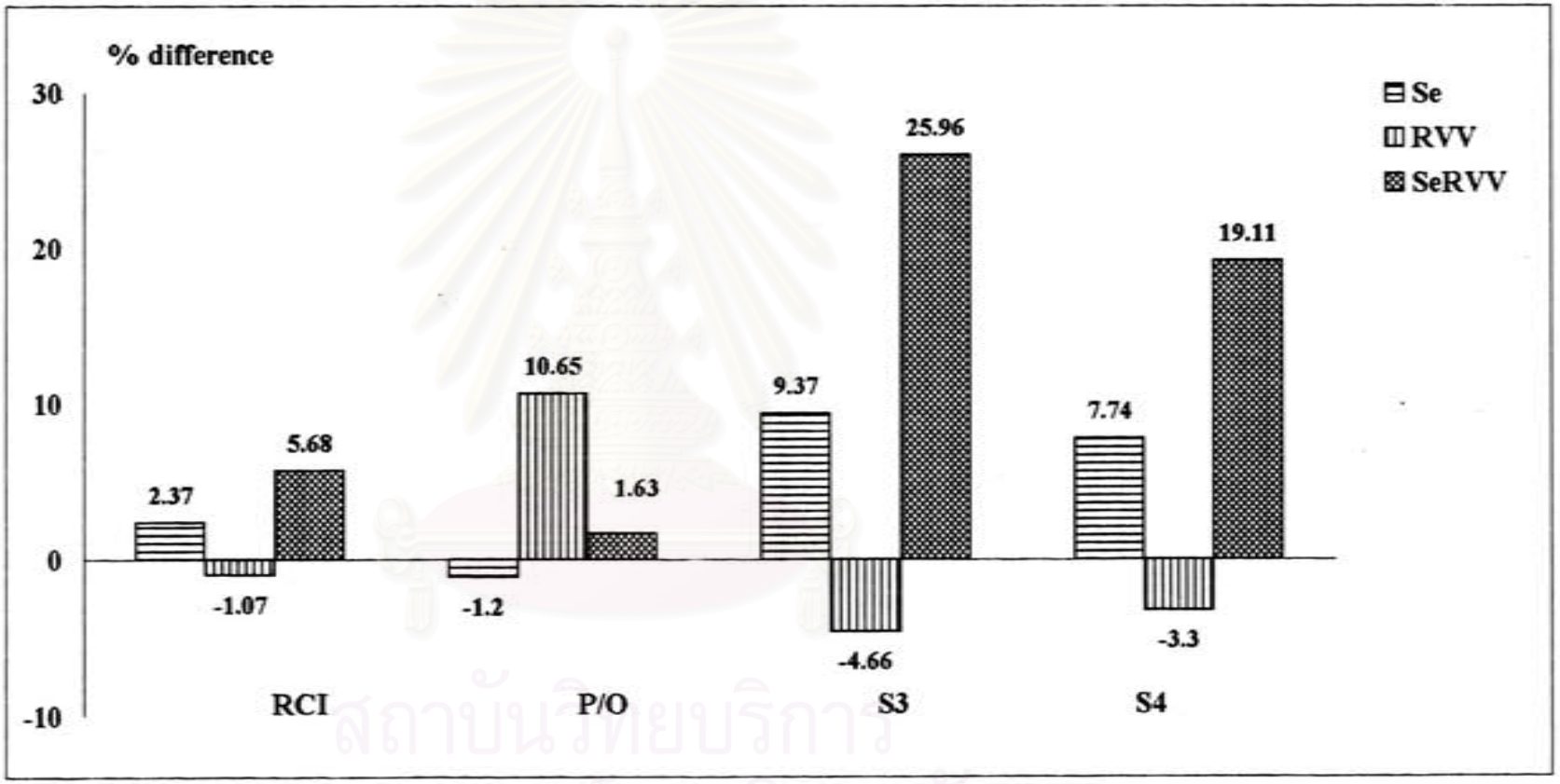
	Control (n=11)	Se (n=11)	RVV (n=11)	SeRVV (n=11)
RCI	4.45 ± 0.08	4.56 ± 0.07	4.40 + 0.37	4.70 ± 0.07
S3 (ng-atomO2/min/mgprot)	177.71 ± 8.55	194.36 ± 11.31	169.44 + 5.25	223.85 ± 14.19
S4 (ng-atomO2/min/mgprot)	39.77 ± 1.29	42.84 ± 2.53	38.45 +0.91	47.37 ± 2.99
O (ng-atomO2/min/mgprot)	196.16 ± 6.61	196.89 ± 3.02	179.02 + 8.68	194.14 ± 8.37
P/O ratio	1.55 ± 0.05	1.53 ± 0.02	1.71 + 0.08	1.57 ± 0.07

Data expressed as mean ± SEM

Control : Control group ; Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group ; SeRVV : selenium-supplemental rats given Russell's Viper venom group .

RCI : mitochondrial respiratory control index ; S3 and S4 : oxygen uptake in mitochondrial state 3 and state 4 respiration ; P/O ratio : ratio of ADP added to total oxygen uptake in mitochondrial state 3 respiration

Figure12 Percentage difference in mitochondrial respiratory control index , oxygen uptake in mitochondrial state 3 , state 4 respiration and P/O ratio in each group of treatment compared to the control group



Se : selenium-supplemental group ; RVV : Russell's Viper venom administration group and SeRVV : Selenium supplental rats given Russell ' sViper venom gruop

Data expressed as percentage difference in mean value

CHAPTER V

DISCUSSION

The present study was designed to find out whether pathophysiological changes in renal function after Russell's viper venom (RVV) administration was related to changes in activity of isolated rat renal mitochondria and whether selenium (Se) supplementation could protect injurious effects of the venom. Several investigators have shown that safety dose supplemental selenium could protect rat renal proximal tubules and glomerular injury from gentamycin toxicity (Ngaha et al., 1984), gastric ulceration induced by necrotizing agents and cold stress (Parmar et al., 1988). The protective effect of selenium on cardiac ischemic-reperfusion injury (Konz et al., 1991; Polronieri et al., 1992) and antiinflammatory action (O'Doll et al., 1988) have also been proposed.

After RVV administration, animals showed signs of shock such as drowsiness, cold, pale and hyperpnea following with hematuria in both nonselenium (RVV group) and selenium supplemental rats (SeRVV group). Hematuria and hemoglobinuria were apparently after RVV administration. These changes may reveal the actions of the venom, which contained many proteolytic and hematoxic enzymes (Huang et al., 1983; Huang, 1984 ; Li et al., 1986 and Jayanthi et al., 1989) The venom might be cause of intravascular hemolysis by changing red blood cell morphology (Chaiyabutr et al., 1987). The changes in glomerular basement membrane and variety region of renal lesion after envenomation has been reported in victims who were bitten by Russell's viper snakes (Sitprija, 1974 ; Chung et al., 1975 ; Shasty et al., 1977 ; Sitprija et al., 1980 and Sitprija et al., 1982) may be another posibility for massive hematuria and hemoglobinuria in the present study. In the present study, kidney swelling with

generalized spot, browncoal cortex and some cases of browncoal medulla were observed after 40 hours of envenomation at dose 0.9 mg/kg body weight. These appearences may indicate that renal damage occured.

The cardiovascular effects of RVV administration have been suggested to be attribute to two phases, initial and second phase (Chaiyabutr, 1985). In initial phase, mean arterial blood pressure, total peripheral resistance were decreased, sustained for 30 minutes and were restored to normal within 2 hours. In second period, 2 to 48 hours after envenomation, the cardiac output was increased while total peripheral resistance and mean arterial blood pressure were restored. However, blood flow through kidney, GFR and renal fraction were decreased (Tunthanathanich et al., 1986). The present results are compatible to those occured in the second phase. After 40 hours of envenomation, rat given RVV alone (RVV group) showed slightly decrease in packed cell volume (5%). Systemic blood pressure including systolic, diastolic and mean arterial blood pressure were not significantly different from the control group. However, heart rate was significantly increased ($P<0.05$) which was unlikely to those which were reported in dog (Tungthanathanich et al., 1986). An increment in heart rate in RVV group might be due to dehydration from hemorrhage stimulated baroreceptor reflexs (Guyton, 1991). The compensation mechanisms that return blood volume back toward normal has been suspected to occur because the decrease in ratio of urine to GFR (V/GFR)was observed (table 5).

Selenium supplemental rats given RVV (SeRVV group) showed a marked decrease in packed cell volume by approximately 20% ($P<0.05$) in comparison with the control group after 40 hours of envenomation . It indicated that more hemorrhage was induced in this group. This result may support the fact that selenium can inhibit thromboxane B_2 synthesis, rise platelet aggregation threshold and induce increase in bleeding time (Perona et al., 1990 ; Hampel et al., 1991) Severe hemorrhage in SeRVV group may be related to the action of selenium in promotion bleeding

tendency. The decrease in diastolic blood pressure ($P<0.05$) while systolic blood pressure was not changed ,therefore, result in lower mean arterial blood pressure and higher pulse pressure ($P<0.05$) in SeRVV group may be attributed to the decrease in total peripheral resistance (Guyton, 1991). Selenium has been shown to increase rat aortic ring relaxation which was induced by acetycholine ,in vitro, and this increment has been suggested to be due to increase in nitric oxide output from endothelial cell (Lu et al., 1994) The involvement of selenium in prostaniod metabolism has also been noted (Perona et al., 1990 and Hampel et al., 1991). These lines of evidences will support the decrement in total peripheral resistance in SeRVV group. The marked increment in heart rate in SeRVV animals were apparently which might be result from severe dehydration because a marked decrease in the ratio of V/GFR was observed (table 5).

Of interesting that animals supplemental with selenium alone (Se group) showed a decrease in diastolic blood pressure and mean arterial blood pressure while packed cell volume, heart rate and systolic blood pressure were not significantly different from the control group. These results may be due to a decrease in total peripheral resistance. Since, selenium has been shown to have negative inotropic and antiarrythmic effects in dose dependent manner (Guo et al., 1988). To the present study, supplementation of selenium could promote vasodilatation, however, the relative dose used in the present study was about 150 times lesser than those used in previous report in dogs (Guo et al., 1988).

As the effects of RVV administration on renal hemodynamics, in RVV group has demonstrated that the venom could increase in renal vascular resistance resulted in decrease in renal blood flow and GFR in RVV group. These changes might be due to renal vasoconstriction which was consistented with other observation in dogs (Tungthanathanich et al., 1986). These responses have been demonstrated to be mediated by renin-angiotensin system (Chaiyabutr et al., 1985) and/or norepinephrine

(Kidmungtangdee, 1989). In view of tubular function, the decrase in urine volume and fractional excretion of sodium and chloride in RVV group were noted while filtered load of these electrolytes were not significantly different from the control group. The ratio of urine to GFR (V/GFR) was decreased. These results might be due to more tubular reabsorption in compensation to dehydration which be resulted from hemorrhagic effect of Russell's viper venom. However, an increase in potassium excretion was observed which was similarly to the observation in previous study in dogs. It might be due to more tubular potassium excretion (Tungthanathanich et al., 1986).

In SeRVV animals, the changes in renal hemodynamics and tubular functions were observed . Renal blood flow was increased while GFR, filtration fraction and renal vascular resistance were decreased. These changes may indicate the present of vasodilatation in systemic throughout renal vascular bed. The vasodilatation seemed to be produced in both afferent and efferent arteriole, which was similarly to the changes during intrarenal infusion of vasodilators including acetylcholine (Thomas et al., 1983) prostaglandin E_1 and bradykinin (Baylis et al., 1976). The decrement in renal vascular resistance has also been observed in animal that supplemental with selenium alone (Se group) but these changes showed a lesser degree than those which were observed in SeRVV group. The difference in packed cell volume has been noted between Se group and SeRVV group (table 3). The decrease in packed cell volume may be caused of hypoxia. The effect of hypoxia has been demonstrated to stimulate endothelial derived relaxing factor (EDRF) output. (King and Brenner, 1991 and Park et al., 1994). Selenium supplementation has been shown to increase the release of EDRF and enhanced rat aortic ring relaxation which was induced by acetycholine, in vitro (Lu et al., 1994). A marked increase in renal vasodilatation in SeRVV animals might be potentiated by synergistic action of hypoxia and selenium. In Se group, animal also

showed an increase in renal blood flow coincided with increased in GFR which were consistent with the observation in man (Guidi et al., 1990).

The urinary excretion and fractional excretion of sodium and chloride in SeRVV group were decreased which might be due to decrease in filtered load (table 6). The urinary potassium excretion and fractional excretion of potassium were also decreased in the SeRVV animals. However, tubular reabsorption of potassium in this group showed trend to be higher than those in RVV group and the control group (table 7). The rise in tubular reabsorption of potussium coincided with a slightly decrease in plasma potassium has also been observed in animals supplemental with selenium (Se group). In Se group, slightly increase in plasma sodium concentration and decrease in fractional sodium excretion has also been noted (table 4 and 7). The sodium retention by the effect of Se might be due to an increase in plasma corticosterone level, since by intraperitoneal injection of Se 1.6 mg/kg to fasted rats caused increase in hepatic glucose output (Bell et al., 1990), plasma corticosterone level (Rasekh et al., 1991), plasma ACTH and β-endorphin (Potmis et al., 1992). The inhibitory action of selenium on glucocorticoid receptor has also been demonstrated (Tashima, 1989). However, the concentration of Se used in the present study was less than the dose used in previous study by approximately 200 times (Bell et al., 1990 ; Rasekh et al., 1991 and Potmis et al., 1992). Fuyu and coworker (1986) have demonstrated that selenium could increase erythrocyte ghost membrane-bound Na-K ATPase activity and lipid fluidity. The decrease in plasma potassium concentration in both Se group and SeRVV group may possible be mediated by effect of Se on Na-K ATPase activity, then it may lead to increase in tubular reabsorption of potassium. The decrease in plasma potassium might possible be due to an increase in another way of potassium loss such as chronic vomiting, diarrhea (Seldin, 1989). However, these signs were not observed in any group of rats.

In the study of mitochondrial activity which was demonstrated by mitochondrial respiratory control index (RCI), mitochondrial state 3 and state 4 respiration and P/O ratio. The animal given RVV (RVV group) showed a slightly decrease in mitochondrial respiratory control index coincided with a slightly decrease in oxygen consumption in mitochondrial state 3 respiration (table 9). The reduction in mitochondrial state 3 respiration may reflect defects in either the electron transport chain, ADP-ATP translocator, ATP synthetase or some combinations of the above (Malis et al., 1986). Russell's viper venom has been shown to have direct effect on proximal tubular cell of Triturus kidney by inducing depolarization in a similar manner to uncoupling agent, 2-4 dinitrophenol. The venom might possible directly intereact with composition of cell membrane or specific site that control ion transport across cell membrane (Chaiyabutr et al., 1985). Mitochondria is one of intracellular calcium pools and have been known to accumulate rather large quantities of calcium at the expense of ATP hydrolysis, mitochondrial respiration or an electrochemical gradient (Devlin, 1986). The reduction in mitochondrial state 3 respiration in RVV group may be suspect to be action of the venom on mitochondrial electron transport chain or sequential systems. However, in ischemic rat kidney, mitochondrial structure and membrane potential alteration has been noted . It has been demonstrated that ischemia could decrease oxygen consumption in mitochondrial state 3 respiration (Wilson et al., 1984), decreased adenine nucleotide translocator activity and impaired mitochondrial functions (Henke et al., 1990). Therefore, in the present study, the venom may possible either directly damage mitochondrial electron transport chain or the change in state 3 respiration was secondary changes in respect to ischemia.

In SeRVV animals, mitochondrial activity showed an increase in both mitochondrial state 3 and state 4 respiration (table 9). The similar changes have also been observed in selenium supplemental animals alone, but it was in lesser degree. An

increase in mitochondrial state 4 respiration when succinate was used as substrate reflected an increase in mitochondrial inner membrane permeability (Malis et al., 1986). The previous study has shown that selenium as sodium selenite could increase oxidation activity in redox cycling and increased glutathione oxidation(Anundi et al., 1984). Selenium has also been shown to stimulate oxygen consumption (Anundi et al., 1984 and Shuyu et al., 1986) and increased mitochondrial membrane swelling in dose dependent manner in vitro. Selenium as sodium selenite could increase mitochondrial ATPase activity which made available more ADP and then increased oxygen uptake to regenerate ATP. An increment in oxygen uptake might be cause of mitochondrial swelling (Shuyu et al., 1986). The increment in oxygen uptake by mitochondria state 4 respiration in either Se or SeRVV group in the present study may be suspect to be increase in membrane permeabiltiy or changes in membrane integrity. The increase in oxygen uptake in mitochondrial state 3 respiration may possible be the effect of selenium on mitochondrial ATPase activity and oxygen consumption as previous suggestion(Shuyu et al., 1986). However, the apparently increase in mitochondrial oxygen consumption has been observed in SeRVV group (table 7,fig 12). Hypoxia or ischemia was suspected to generate oxygen free radicals (ROS) and play critical roles in the damage from organ to cell membrane and functions (Freeman et al., 1982 ; Sedor et al., 1987 ; Shah et al., 1987 ; Konno et al., 1990 and Konno et al., 1992). Hydrogen peroxide (H_2O_2) has been demonstrated to change glutathione redox cycling during ischemic-reperfusion period in rat kidney (Mccoy et al., 1988). Enzyme glutathione peroxidase (GSH-px) has been suggested to catalyze many peroxides degradation (Sunde et al., 1980 and Krinski, 1990). The concentration of selenium used in the present study was shown to increase GSH-px activity (Jia et al., 1989 ;

Erkhert et al., 1992 and Poltronieri et al., 1991) and could protect cardiac functions from ischemic-reperfusion injury (Konz et al., 1991 and Poltronieri et al., 1991). The enzyme GSH-px has been suggested to be the most abundunt kidney selenoprotein (Viljoen et al., 1988) and resided in cytosol and mitochondrial matrix space (Sunde et al., 1980 ; Urini et al., 1987 and Eckhert et al., 1992). The increment in mitochondrial state 3 respiration in SeRVV animals may be suspected to be increase in enzyme glutathione peroxidase activity against metabolites of oxygen free radicals.

In conclusion, Russell's viper venom at dose of 0.9 mg/kg body weight injected to rats produce minor changes in systemic circulation at 40 hours after envenomation. The changes in renal hemodynamics including decrease in renal blood flow, GFR, urine volume and fractional excretion of sodium and chloride was persisted, however, fractional excretion of potussium still be increased. In addition, the venom produced minor decrease in mitochondrial state 3 respiration which may be suspect to be defects in mitochondrial elelctron transport chain or sequential systems. By supplementation with 8 μgSe/day for consecutive 40 days produce slightly changes in systemic circulation and plasma concentration of sodium and potassium. Renal circulation of the selenium supplemental animal (Se group) is also changed, increase renal blood flow and GFR while tubular functions are not much changed, however, increase in tubular reabsorption of potussium has been observed. The increase in mitochondrial activity has been observed in either selenium supplementation alone (Se group)or selenium supplemental animals given RVV (SeRVV group). The SeRVV animals have shown an increase in bleeding tendency, heart rate and vasodilatation which were observed in both systemic and renal circulation, however, GFR in this group was not increased

though renal blood flow was increased. The vasodilatation might occur in both afferent and efferent arteriole. A marked decrease in urine volume and fractional excretion of electrolyte has been noted in SeRVV group,moreover, decrease in fractional potassium excretion and an increase in potassium reabsorption has been observed.

The present study may expand the insight in effect of selenium supplementation and some benificial effects of this trace element, however, more available information are needed for demonstrating the benificial efficiency.

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จุฬาลงกรณ์มหาวิทยาลัย

BIOGRAPHY

Police Captain Angtana Julsukon was born on September 22, 1963 in Bangkok, Thailand. She graduated with B.S. in Nursing and Midwifery from Police Nursing College in 1985. At the present, she is a nurse in the surgical intensive care unit, Police general Hospital. She admissed with the Degree of Master of Science, Inter-Department of Physiology, Chulalongkorn University in 1991.

สถาบันวิทยบริการ
จุฬาลงกรณ์มหาวิทยาลัย