EFFECT OF HEMORRHAGIC SHOCK ON LIVER AND MUSCLE GLYCOGEN CONTENT IN HIGH ALTITUDE ACCLIMATIZED RATS

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ชื่อวิทยานิพนธ์ ผลของช็อคจากการเสียเลือดต่อปริมาณกลัยโคเจนในตับและกล้ามเนื้อของหนูที่ปรับตัวต่อการอยู่ในที่สูง

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บทคัดย่อ

การวิจัยนี้มุ่งศึกษาผลของการเสียเลือดจนถึงภาวะช็อคต่อระดับกลัยโคเจนในตับและในกล้ามเนื้อ และระดับของสารบางชนิดในเลือดในหนู หลังจากปรับตัวอยู่ในที่สูงเหนือระดับน้ำทะเล 5,000 ม. โดยเปรียบเทียบกับหนูกลุ่มควบคุมที่อยู่ที่ระดับน้ำทะเล รวมทั้งศึกษาถึงความสัมพันธ์ระหว่างความต้านทานต่อการเสียเลือดกับการเปลี่ยนแปลงของปริมาณกลัยโคเจนในเนื้อเยื่อ และระดับสารเคมีในเลือดของหนูทั้งสองกลุ่ม ในการศึกษานี้ใช้หนูขาว (Wistar rats) เพศผู้ น้ำหนักเมื่อเริ่มการทดลอง 216-262 กรัม แบ่งออกเป็น 2 กลุ่มใหญ่ คือ กลุ่มควบคุมจำนวน 27 ตัว ถูกเลี้ยงที่ระดับน้ำทะเล และกลุ่มที่ปรับตัวต่อการอยู่ในที่สูง จำนวน 23 ตัว ซึ่งถูกเลี้ยงไว้ในถังที่สามารถปรับความดันบรรยากาศภายในถังให้อยู่ในระดับ 403 ม.ม.ปรอท (เทียบเท่าความสูง 5,000 ม. เหนือระดับน้ำทะเล) หลังจากนั้น ประมาณ 7 สัปดาห์ (5-10 สัปดาห์) จึงเริ่มทำการทดลอง หนูในแต่ละกลุ่มถูกแบ่งต่อไปเป็น 3 กลุ่มย่อย กลุ่มย่อยที่ 1 และ 2 (กลุ่มย่อยละ 5 ตัว) ไม่มีการเสียเลือด แต่มีการวัดระดับกลัยโคเจนที่เวลา 0 และ 150 นาที ของการทดลองตามลำดับ กลุ่มย่อยที่สาม จำนวน 17 ตัวในกลุ่มควบคุม และ 13 ตัว ในกลุ่มที่ปรับตัวต่อการอยู่ในที่สูง ถูกทำให้เสียเลือดทาง femoral artery จนกระทั่งความดันเฉลี่ยเลือดแดงลดลงอยู่ที่

ระดับ 35 ม.ม.ปรอท แล้วควบคุมความดันเลือดให้อยู่ที่ระดับนี้ตลอดการทดลอง จนถึงนาทีที่ 150 จึงให้เลือดบางส่วนที่เสียไปคืนกลับเข้าไปในตัวหนูในปริมาณ 35 เปอร์เซนต์ ของเลือดทั้งหมดที่เสียออกไป เก็บตัวอย่างเลือดในช่วงเวลาต่างๆ คือเริ่มต้นการเสียเลือด ช่วง 40-60 นาที ของการควบคุมความดันเลือดต่ำ และช่วงสุดท้าย ภายหลังให้เลือดคืนกลับไปแล้ว 10 นาทีหลังจากอยู่ในสภาวะความดันเลือดต่ำนาน 150 นาที นำเลือดไปวิเคราะห์หาระดับฮีมาโตคริท กลูโคส แลคเตท และตรัยกรีเซอไรด์ และทำการเก็บเนื้อเยื่อตับ หัวใจ และกล้ามเนื้อ ทันทีหลังจากเก็บตัวอย่างเลือดครั้งสุดท้าย เช่นเดียวกับหนูกลุ่มย่อยอื่นๆที่มิได้มีการเสียเลือด จากการทดลองพบว่าหนูที่อยู่บนที่สูงมีปริมาณกลัยโคเจนในตับ หัวใจ และกล้ามเนื้อ extensor digitorum longus ต่ำกว่าหนูในกลุ่มควบคุม และพบว่าปริมาณเลือดที่หนูเสียในครั้งแรก เพื่อให้ความดันเลือดลดลงสู่ระดับ 35 มม.ปรอท และปริมาณเลือดที่เสียได้มากที่สุดขณะทดลอง รวมทั้งระยะเวลาของการเสียเลือดมากที่สุด ในกลุ่มของหนูที่ปรับตัวต่อการอยู่ในที่สูงมีค่ามากกว่าหนูกลุ่มควบคุมอย่างมีนัยสำคัญ การเสียเลือดของหนูทั้งสองกลุ่ม มีผลให้ปริมาณกลัยโคเจนในตับลดลงจนเกือบหมด ส่วนในกล้ามเนื้อ extensor digitorum longus ลดลงน้อยกว่าในตับ ในขณะที่ในกล้ามเนื้อ soleus ไม่มีการเปลี่ยนแปลง ส่วนระดับกลัยโคเจนในหัวใจเพิ่มขึ้น การเปลี่ยนแปลงของระดับกลัยโคเจนนี้ ไม่แตกต่างกันระหว่างหนูทั้งสองกลุ่ม สำหรับระดับ lactate ในเลือดขณะเสียเลือดในหนูที่ปรับตัวต่อการอยู่ในที่สูง เพิ่มขึ้นน้อยกว่าในหนูกลุ่มควบคุมอย่างมีนัยสำคัญ ขณะที่กลูโคสในเลือดเพิ่มขึ้นเท่าๆ กัน ผลการศึกษานี้บ่งชี้ว่าปริมาณกลัยโคเจนในอวัยวะเหล่านี้ ทั้งก่อนและหลังเสียเลือด และระดับกลูโคสในเลือดไม่ได้บอกถึงภาวะความทนทาน ที่ต่างกันของหนูทั้งสองกลุ่ม แต่ระดับของ lactate ในเลือดที่ต่างกัน อาจบอกถึงภาวะการขาดพลังงานและระบบการสร้างพลังงานที่ไม่ต้องการออกซิเจนขณะช็อคจากการเสียเลือด และอาจบอกถึงการมีส่วนสัมพันธ์กับความรุนแรงจากการเสียเลือด ซึ่งมีผลให้ความต้านทานต่อการเสียเลือดจนถึงช็อคทั้งในระยะเริ่มต้น และระยะรุนแรงที่ไม่กลับคืนของหนูทั้งสองกลุ่มที่แตกต่างกัน แม้หนูทั้งสองกลุ่มถูกควบคุมให้ความดันเลือดแดงต่ำที่ระดับ 35 ม.ม.ปรอทเท่ากัน และเป็นเวลานานเท่ากัน

Thesis Title EFFECT OF HEMORRHAGIC SHOCK ON LIVER AND MUSCLE GLYCOGEN CONTENT IN HIGH ALTITUDE ACCLIMATIZED RATS

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ABSTRACT

Experiments were undertaken with 23 high-altitude acclimatized and 27 control sea-level rats to determine the effect of hemorrhagic shock at sea level on their liver, heart and skeletal muscle glycogen content and some blood parameters and to relate these hemorrhagic responses to their hemorrhagic tolerance. The rats in each group were divided into 3 subgroups. Animals in subgroups I and II were non-hemorrhaged and sacrificed under anesthesia at 0 and 150 min, respectively, while rats in subgroup III were bled into a reservoir which maintain mABP at 35 mmHg. At 150 min of hemorrhagic hypotension, an amount of the shed blood remaining in the reservoir was reinfused (approximately 35 %MBL). Hematocrit, plasma glucose and blood lactate were measured at the initial, 40-60 min of hypotension, and at 10 min after blood reinfusion at the

end of 150 min hypotension. The liver, heart and soleus (SOL) and extensor digitorum longus (EDL) muscles were immediately excised and fixed in liquid nitrogen for determination of their glycogen content. It was found that chronic exposure to high altitude caused lower liver, heart and EDL glycogen content compared to the controls. The initial blood withdrawal (IBW), the maximum blood loss (MBL) and also the time to maximum blood loss (TMBL) were significantly greater while the blood volume reinfused to maintain the level of hypotension was less in the altitude-acclimatized rats than those of the control rats. Glycogen content was found to be depleted in liver, reduced in EDL, increased in myocardium, and not changed in SOL by hemorrhagic shock and these post-hemorrhagic glycogen levels were similar in both animal groups. In contrast, blood lactate concentration was significantly higher in the control rats than that of the altitude rats. It is concluded that the higher tolerance to hemorrhagic hypotension in the high-altitude acclimatized rats than the control rats was not attributed to the levels of hyperglycemia induced by hemorrhage or to the initial and the post-hemorrhagic levels of glycogen(indication of the carbohydrate reserves) in the liver and skeletal muscles of the two animal groups. Such the difference in hemorrhagic tolerance is associated with the blood levels of lactate which reflect the development of acidosis and the extent of tissue ischemia during hemorrhagic shock.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ADP	=	adenosine diphosphate
ATP	=	adenosine triphosphate
BR	=	blood reinfused
BW	=	body weight
CO_2	=	carbon dioxide
cm	=	centimetre
CP	=	creatine phosphate
°C	=	degree celcius
EDL	=	extensor digitorum longus
FFA	=	free fatty acid
g	=	gram
G-6-P	=	glucose-6-phosphate
h	=	hour
H_2O_2	=	hydrogen peroxide
IBW	=	initial blood withdrawal
kg	=	kilogram
L	=	litre
M	=	molar
m	=	metre
mABP	=	mean arterial blood pressure
MBL	=	maximum blood loss
µ	=	micron
µl	=	microlitre
mg	=	milligram
mg%	=	milligram per 100 millilitres
min	=	minute

ml	=	millilitre
mmHg	=	millimetre of mercury
mmole	=	millimole
N	=	normal
No.	=	number
nm	=	nanometre
O_2	=	oxygen
%	=	percent
RBC	=	red blood cell
SEM	=	standard error of mean
SOL	=	soleus
TMBL	=	time to maximum blood loss
WBC	=	white blood cell
wks	=	weeks

CHAPTER I

INTRODUCTION

GENERAL ASPECTS OF HEMORRHAGIC SHOCK

1. DEFINITION OF SHOCK

Over the last century a number of attempts have been made to define adequately the term "shock". Although various definitions of shock have been proposed, most of them were accurate only to a certain extent and far from exact. According to MacLean (1), a more precise definition of shock has been proposed as "inadequate blood flow to vital organs or the inability of the body cell mass to utilize oxygen and other nutrients". However, more recently, Hardaway (2) stated that the best definition of shock should be "inadequate capillary perfusion" as he has defined previously since the year 1963.

Shock and all its ramifications, resulted from a sustained reduction in perfusion of capillaries and therefore of tissue and organs lead to generalized cellular hypoxia, ischemia, and, finally, organ damage. Major operations, trauma, myocardial diseases, and severe infection all may lead to circulatory failure or shock. As a result of prolonged reduction in tissue perfusion, various alterations in tissue metabolism, structure, and function occur at the systemic, cellular, and subcellular

levels. The metabolic disturbance is related to the delivery of oxygen and nutrients to the tissue. The oxygen deprivation, hence a decrease of oxidative-phosphor ylation, in almost any cell leads to a prompt induction of anaerobic glycolysis which in turn produces metabolic acidosis. Depending upon the time of exposure and the severity of the insult, this process proceeds to cell destruction, progressive multisystem failure, and death (2).

The commonest cause of shock is hemorrhage which is the interesting aspect of the present study. Its severity depends upon the amount and rate of blood volume loss. Whole blood loss occurs frequently as a result of traumatic injury with severance of blood vessels, gastro-intestinal ulcer or obstetic complications which lead to postpartum bleeding (3).

2. PATHOPHYSIOLOGICAL RESPONSES DURING HEMORRHAGIC SHOCK

In the face of a loss of circulating blood volume, the homeostatic compensatory adjustments (by which the extracellular and intracellular milieu are maintained) are necessary for vital biochemical processes to proceed. The physiologic, metabolic, hemostatic, and immunologic resposnes to the insult, if adequate, lead to survival and recovery. If not, shock and death ensure. There are essentially three major systems which play very important roles in homeostasis : the nervous system, the hormones,

and the immunologic mechanism (2). Only the first two will be addressed in appropriate places in the following items concerning the responses to hemorrhage.

2.1 Compensatory [Early] Phase

General Responses

The early shock phase is characterized by efficient compensatory mechanisms and if property treated it seldom causes organ failure or systemic complication. The homeostatic response of an animal to severe blood loss results in a shunting of blood from nonvital organs such as skeletal muscle, skin, kidney and splanchnic bed to preserve blood flow to the organs of most essential, the heart and the brain.

The initial response to massive blood loss is sympathoadrenal stimulation with negative-feedback system maintaining homeostasis. A decrease venous return to the heart and a subsequent decrease in cardiac output causes reduction in mean arterial pressure and pulse pressure. These incidents trigger a response via the baroreceptors in aortic arch and carotid sinus which relay the information to the cardiovascular center in the medulla oblongata (reduction in vagal tone). The response is the release of catecholamines (epinephrine and norepinephrine), which remain high throughout shock, from the adrenal medulla and the postganglionic fibers of the sympathetic

nervous system. The indications of sympathetic stimulation during hemorrhage are pale, cold and sweaty skin, a rapid and thready pulse, and elevation in blood glucose (2,3).

Epinephrine stimulation increases heart rate and myocardial contractility, while norepinephrine causes intense vaso- and venoconstriction in almost all vascular beds (except the cerebral and coronary beds) which results in an increase of total peripheral resistance and promotes venous return to keep up cardiac output. At the same time fluid loss are minimized by increased secretion of hormones such as antidiuretic hormone (a very potent vasoconstrictor) and aldosterone which its effects are body retention of sodium ion and water.

Histamine which has been reported to be secreted from mast cells adjacent to each capillary bed of ischemic tissue during hemorrhage can cause capillary opening but the effect is not potent because histamine is quickly destroyed in the blood stream (2).

Adrenocorticotropic hormone (ACTH) and cortisol are also released during hemorrhage. Their actions which involve specific metabolic responses to stress are hepatic gluconeogenesis, lipolysis and muscle glycogen breakdown which can be stimulated by catecholamines (2,3).

Transcapillary Refill

A powerful feedback system for maintenance of homeostasis exists in the mechanism called "transcapillary refill" which can increase blood volume during hypovolemia. The main factors governing the exchange of fluid between the capillaries and interstitial space which were first elucidated by Starling in 1896 are capillary hydrostatic pressure (filtration force) and plasma oncotic pressure (absorptive force) (2). The sympathoadrenal stimulation plays a role in transcapillary refill by more pronounced activity in precapillary arteriolar vasoconstriction than in postcapillary venoconstriction. A fall of capillary hydrostatic pressure occurs and allows fluid to move by osmotic force into the capillary from the interstitium.

In the early phase of the response to hemorrhage, the hyperglycemia, secondary to high catecholamines (alpha-adrenergic inhibition of insulin secretion) (4), may contribute significantly to the absorptive force, because of a pronounced hyperosmolality (5), fluid and proteins is thus mobilized to blood plasma along this force via the lymphatics or by the transcapillary route, causing hemodilution. This glucose osmotic defence of plasma volume seems of importance for the shock tolerance (6).

Microcirculatory Disturbances

A reduction of the hydrostatic pressure within the capillary bed does not seem to be critical for the passage of red blood cells (7). In spite of the fact that red blood cells have an average diameter (about 7.5 μm) larger than that of most capillaries (5-6 μm) they deform rapidly since their high surface area to volume ratio make them extremely flexible. White blood cells (WBCs), on the other hand, are spherical and they have an average diameter of 7-9 μm, i.e. they are larger than most capillaries. Therefore they have to deform considerably in order to pass through a capillary, but the coefficient of viscosity of the WBCs is almost 2000 times higher than that of red blood cells. Thus, the resistance to deformation is large and WBCs deform relatively slowly. One important factor for the deformation of WBCs for their passage through the capillary is the hydrostatic driving pressure, which is reduced during oligemic shock and therefore WBCs are seen to plug many capillaries not only in peripheral tissue but also in the pulmonary network. The nutritive blood flow through tissue will be heterogeneous since a high remaining flow is seen in some of the capillaries that remain patent while others are intermittently or constantly blocked by trapped WBCs. There is consequently a significant perfusion defect in shock, which is also evidenced by a heterogeneous distribution of hypoxia induced cellular metabolic disturbance within tissues (6).

Tissue Metabolic Disturbances

The cellular metabolic consequences of hemorrhagic shock will vary considerably between various tissues and are related to the redistribution of remaining blood volume. The cellular hypoperfusion resulted from a loss of circulating blood volume, occurs first in the nonvital tissues of the gastrointestinal tract, skeletal muscle, connective tissue, and skin. The ability of the animal to recover normal function depends on the duration and extent of the flow deprivation in the various affected organs.

Normal cell produces energy in the form of adenosine triphosphate (ATP) which is a primary energy source for maintenance of normal cellular functions such as energy transport systems, protein synthesis, and enzyme synthesis. The main source of ATP is the oxidative phosphorylation of glucose metabolites in the inner membrane of mitochondria. Smaller quantities of this energy substrate are synthesized during anaerobic glycolysis in the cytoplasm. Fatty acids and amino acids can also be metabolized for energy production. The decreased levels of ATP found in liver, kidney, and other tissues of animals in severe hemorrhagic shock have been assumed to be a significant factor in cellular dysfunction. Not only a decrease in ATP, but with dephosphorylation of adenosine diphosphate (ADP) and creatine phosphate (CP) were decreased as well.

Normally energy metabolism requires a constant supply of oxygen as electron acceptors and a constant supply of an appropriate substrate such as glucose. The delivery of oxygen to the tissues is cut off in anoxia and the supply of both oxygen and substrates is cut off by ischemia. The peripheral vasoconstriction of shock may partially or completely reduce the tissue's ability to produce energy aerobically. There is a rapid marked decrease in the production of energy by aerobic pathways. In case of inadequate oxygenation, anaerobic process is dominant for energy supply to the cells. In the glycolytic process, glucose is brokendown to pyruvate which combined with hydrogen ions released in the process to form lactic acid. This acid then diffuses out of the cell to the surrounding interstitial fluid, gains access to the circulatory system and produce metabolic acidosis. This could be partly neutralized by the buffer systems of the body which include extracellular and intracellular buffers, and the means of excreting the acids such as respiration. The respiratory center in the medulla is stimulated by the acid environment, causing increased respirations. Eventually, however, all the body buffer systems are exhausted and acidosis ensues (2,3).

With the exception of red blood cell (because of the lack of mitochondria) and the brain (because of blood brain barrier), all tissues utilize free fatty acid (FFA) as an important energy fuel (8). In skeletal muscle, FFA is normally the major source of energy for resting muscle

such as soleus muscle (9). Also in the heart, plasma free fatty acids normally serve as the predominant energy substrate. During hemorrhagic hypotension, lipolysis occurs in response to alpha-adrenergic stimulation by sympathoadrenal discharge which responds to the stress of hypoxia and ischemia. The triglycerides released from adipose tissues are promptly broken down to free fatty acids and glycerols but these cannot be utilized by tissues in anoxic or ischemic conditions since their passage in the circulation may be inhibited by the intense vasoconstriction and the reduce perfusion of the adipose tissue during shock (2,10,11). During oligemia, there is a decrease in whole body utilization of FFA as a metabolic substrate, hence a rise in the respiratory quotient (RQ) occurs, indicating a shift toward greater metabolic utilization of carbohydrate (12) (Figure 1). The tissues of the body vary considerably in their carbohydrate reserves such as glycogen and this accounts for their varying abilities to survive anaerobically under anoxic conditions.

Glycogen, the storage form of glucose, is present in most body tissues, but the major supplies are in the liver and skeletal muscle. During stress such as hemorrhage, glycogen especially in the liver are rapidly dissipated as a result of the effects of catecholamines. Glycogen synthesis and breakdown are shown in Figure 2. Thus, the glucose pool in the body increases above normal and hyperglycemia occurs not only by glycogenolysis but

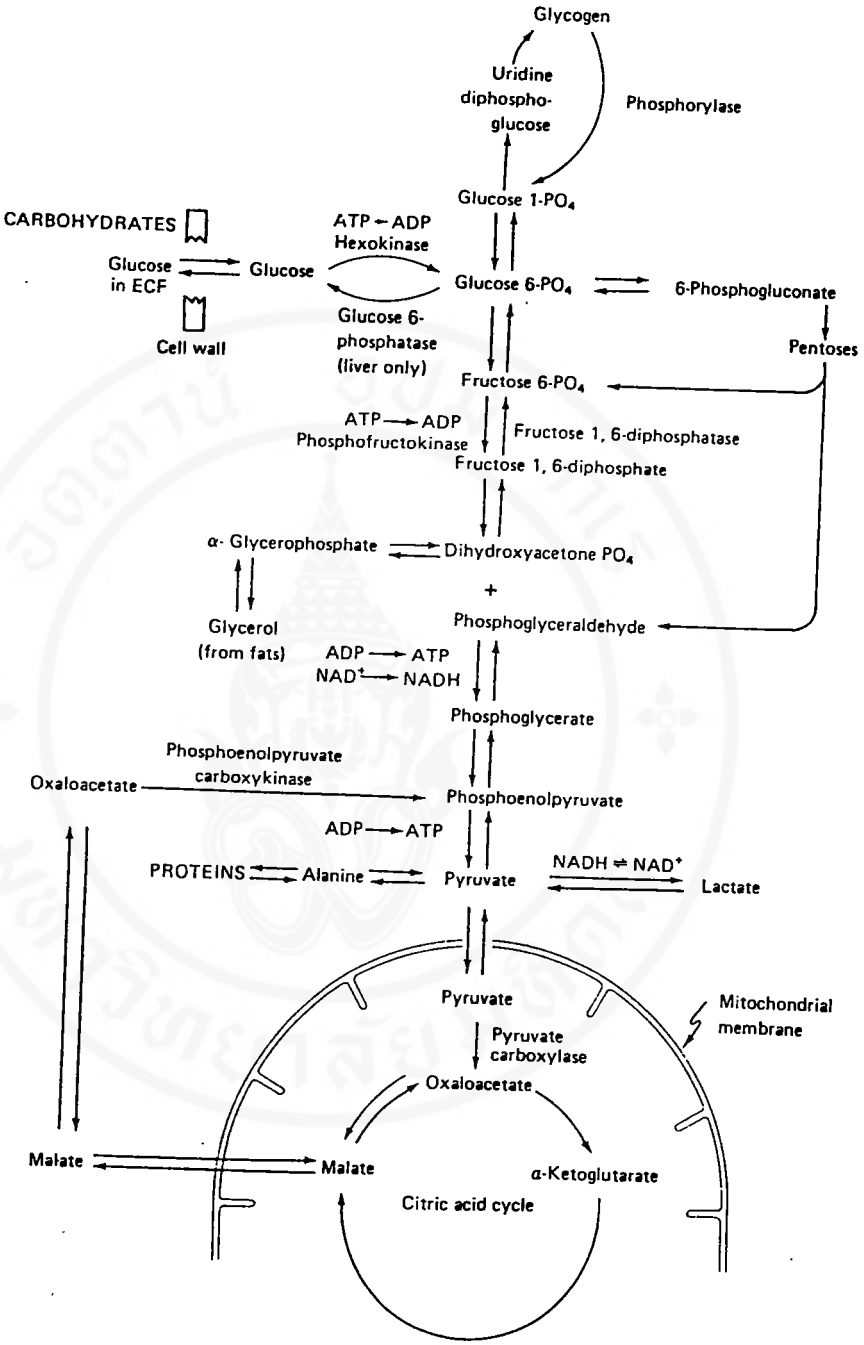


Figure 1. Outline of the metabolism of carbohydrate in cells (13).

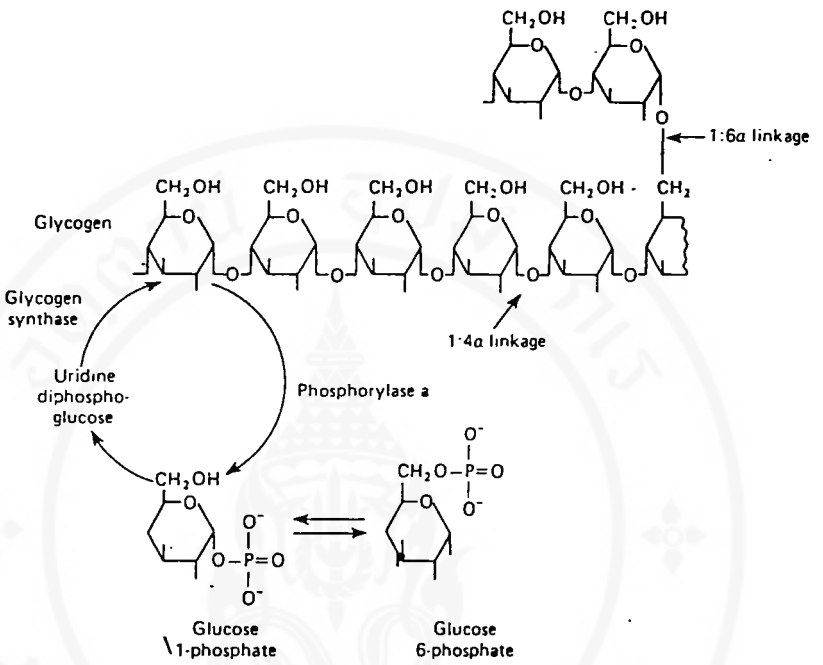


Figure 2. Glycogen synthesis and breakdown (13).

also by gluconeogenesis (from noncarbohydrate precursors) (2,14). The glycogenolytic and gluconeogenetic capacities of the liver are important determinants of shock tolerance and survival (6). The metabolic disturbance is markedly different in various organs and the status of energy metabolism is important in predicting viability of the ischemic organs (2).

Energy Metabolism in Skeletal Muscle

Skeletal muscle is a tissue specialized to transform chemical energy into mechanical energy of work and motion, and has the capacity to accelerate energy metabolism several hundred fold during transition from rest to full physical activity. Since skeletal muscle constitutes about 40 percent of the total body mass, studies in this tissue provide information on the energy metabolism of an important part of the total cellular compartment. At rest, the metabolic rate of skeletal muscle is low since the muscle being used only for the preservation of resting motion. Most of the energy used is directed to the work of transporting ions and nutrients across membranes, and to biosynthesis of structural elements and cell membranes (15).

The largest tissue mass sustaining hypoperfusion in hemorrhagic shock is the skeletal musculature. The changes in total and nutritive blood flow occuring in striated muscle in shock have been extensively described

in various experimental models (16,17). The ischemic tolerance of skeletal muscle itself is high and a complete recovery is seen even after several hours of complete ischemia (18).

Skeletal muscle fibers are not homogeneous with regard to their chemical and physiological characteristics. It consists of two main fibers types : slow twitch fiber (type I) and fast twitch fiber (type II). The fast twitch fibers are further subdivided into fast twitch oxidative fiber (type IIA) and fast twitch glycolytic fiber (type IIB). Type I fibers, with a predominantly oxidative metabolic profile and rely mainly on aerobic metabolism are slowly contracting and are relatively resistant to fatigue. On the other hand, type IIB fibers have a high activity of glycolytic enzymes and a metabolism based partly on anaerobic pathways. They also possess a shorter contraction time and show rapid fatigue. Type IIA fibers are endowed with both aerobic and anaerobic metabolic machinery; they contract rapidly but at the same time are fatigue resistant ("super fiber") (19).

Most muscles contain a randomly distributed mixture of the fiber types, but their relative proportions may vary considerably between different muscles or even between different regions of the same muscle. In certain muscle there is an overwhelming numerical predominance of one type fiber. For instance, in the soleus (SOL) muscle the vast majority (approximately 80 percent) of the fibers are type I and 20 percent of fast twitch oxidative fibers.

In extensor digitorum longus (EDL) muscle, there is a high percentage of fast twitch oxidative fibers (approximately 60 percent), the remaining fibers are fast twitch glycolytic fibers (20). Because of the differences in contractile and metabolic characteristics between these two types of muscles, their responses to exercise stress have been widely studied; but, relatively, few studies on their metabolic responses to hemorrhagic stress have been reported (21,22). Moreover, in the aspect of muscle glycogen metabolism during shock, it seems to be that only the SOL muscle has been conducted (22) although it is known that muscle glycogen serves as an important source of energy during anaerobic glycolysis in types IIA and IIB fiber (19). The SOL and the EDL have been used for study the effect of hemorrhagic hypotension on their glycogen contents. Glycogen, a large molecule containing thousands of D-glucose molecules in 1,4-and 1,6-glucosidic linkages, is situated in the sarcoplasm and appears as dense granules on electron microscopy. Muscle glycogen can only be employed locally since muscle does not contain the enzyme glucose-6-phosphatase essential for releasing glucose into the extracellular fluid.

In shock condition, there is a shift from aerobic to a more anaerobic metabolism in both slow and fast contracting muscles, as evidenced by the rise in lactate level in both tissues (21). It has been shown that lactate concentrations in skeletal muscle were higher than those of arterial blood and other organs (intestine, heart

and liver). Sahlin et al. (23) has found a good correlation between tissue lactate and tissue pH in skeletal muscle which means that energy production during hemorrhage comes from anaerobic glycolysis. However, Pearce et al. (23) observed the energy reserves in rat soleus during hemorrhagic shock, and found no change in either ATP, creatine phosphate, or glycogen content in any phase of hemorrhagic shock while a depletion of hepatic ATP and glycogen levels occurred. Lacticacidemia, which occurs during the oligemic time, possibly comes from the loss of hepatic clearance of lactate (23) and the release from the fast contracting muscle (24). The latter was supported by Jennische et al. who studied in cats and found that soleus muscle, although were metabolically more susceptible than gastrocnemius muscle (fast contracting muscle) to total ischemia, were metabolically affected to a lesser extent under conditions of hypovolemia.

An early response to hemorrhage is a pronounced vasoconstriction in skeletal muscle, due to an increased sympathetic outflow (25). The vascular beds of slow skeletal muscle have been shown to be less sensitive to sympathetic stimulation than those of fast skeletal muscle (26,27). It therefore assumed that during shock, a redistribution of the blood flow within the skeletal muscle tissue take place, favouring slow contracting muscle at the expense of fast contracting muscles, thereby initially maintaining a relative high oxygen supply to slow contracting muscles and entrance clearance of waste

products. The shorter diffusion distances in slow contracting muscles, due to a richer capillary supply and a smaller mean fiber diameter (28) compared to the fast contracting muscles, may be important factor in this context.

Energy Metabolism in Liver Cell

The liver, like other vital organs, shares the disturbance in circulation and anoxia brought about by hemorrhagic shock. The unusual blood supply of this organ as well as its essential role in energy metabolism and detoxification implicate the importance of the liver in this conditions. Because of its unique anatomic location between the heart and the other splanchnic organs coupled with its complex and essential functions, the liver is said to be involved in the detoxification of humoral substances produce from the splanchnic tissues during hypovolemia (e.g., lysosomal hydrolases, myocardial depressant factor (MDF), reticuloendothelial-depressant substance, endotoxin, fibrin, and serotonin) by various mechanisms (29). However, hemorrhagic shock with its harmful stimuli (i.e., hypoxia, ischemia, and acidosis) reduce liver efficiency in detoxification of humoral substances with resultant failure to prevent deleterious substances from depressing or inhibiting myocardial function.

The initial metabolic response and alterations were more rapid in hepatic glycogen and energy metabolism than in those muscle metabolism in hemorrhagic shock rats (30). The liver receives about 30% of the cardiac output, three quarters of which comes through the portal vein. Hepatic artery flow responds directly to hepatic oxygen requirements and metabolic needs, whereas portal flow is depended upon splanchnic arterial flow (31). Both the flow and oxygen saturation decrease in advanced hypovolemic shock. Immediately following blood loss, the release of catecholamines, glucagon, and glucocorticoids causes a significant increase in production of glucose by the liver (gluconeogenesis and glycogenolysis). Much of the glucose-6-phosphate released from liver is converted by glucose-6-phosphatase to free glucose for release into the blood stream, causing the brief hyperglycemia of early shock (2). The depletion of hepatic glycogen and ATP levels by the time the animal reach the maximal compensatory phase of shock occurs coincidentally with the most dramatic increase in plasma glucose and lactate (22). Lactate produced can be converted to glucose via Cori cycle principally in the liver and to some extent in the renal tissue. However, Pearce et al. (32) found that anoxia and hypovolemia impair gluconeogenesis which due to the decrease in ATP produced by oxidative mitochondrial reactions. The energy from anaerobic metabolism can sustain liver viability temperatorily, but it is not enough to carry out all liver functions. If the systemic

crisis is surmounted without endangering hepatocellular integrity, the liver can return to its normal role of assisting peripheral organs (33). Lost gluconeogenic potential is an early casualty in the shocked animal (34). The levels of lactate dehydrogenase (LDH), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and total bilirubin can determine the severity of liver damage (2).

Energy Metabolism in Myocardium

The heart muscle cell with its abundant mitochondria must produce high concentration of ATP. This cell extracts lactate, glucose, and FFA and converts these substrate to ATP. Hypovolemic shock causes inadequate tissue oxygenation. The heart is not one of the early target organs. Total body hypoxia is uneven during the compensatory phase of shock. This does not mean that the heart is entirely spared. The sensitivity of the heart cells to hypoxia depends on the work performed. Mild degrees of under perfusion can be torelated by the heart cells (35). Many physiological reactions occur in such a way to keep the degree of hypoxic low and favour coronary circulation. Hemorrhagic shock affects myocardial metabolism by limiting the supply of oxygen and substrate utilization. In normal condition, energy metabolism of the heart is largely dependent on ATP generation by mitochondria. FFA are the major substrate and lactate is

the second most important one that utilized by the myocardium. In phase of hemorrhage, FFA uptake is greatly reduced but lactate uptake is enhanced in the face of marked elevation in arterial concentration. Hence, a shift from FFA oxidation to an increased reliance on lactate utilization occurred (36). Lactate accumulation causing acidosis in circulation does not appear to be responsible for impaired cardiac function at a pH above 6.8 without associated hypoxia (37). Myocardial glucose uptake is also diminished following hemorrhage (38). The pyruvate is diverted into lactate formation rather than entering the citrate cycle. So, ATP is formed by anaerobic metabolism.

2.2 Decompensatory (Late) Phase

The development of shock due to hemorrhage is the result of failure of several interrelated organ systems. During late shock, decompensation starts due to local as well as central effects of substance released from ischemically injured cells. Functional disturbance of vital central organs are critical and multiple organ failure may occur. The cellular metabolic deterioration will of course increase with the duration of a hypovolemic condition and depending on tissues. Locally accumulating metabolites (lactate, hydrogen ion etc.) and other substances leaking out from hypoxically damaged cells (potassium, lysosomal factors, polypeptide etc.) will

interfere with the responsiveness of the vascular smooth muscle to sympathetic vasoconstrictor stimuli. Precapillary vessels seen more sensitive to the vasodilatory effect of local tissue factor than postcapillaries one (39). Consequently, arteriolar resistance falls and water escapes from capillaries.

An early decompensatory response is therefore an increase blood flow into the capillary bed as well as loss of fluid into the tissue leading to an additional reduction of venous return, cardiac output and blood pressure (6). A terminal phase of physiologic decompensation is then present when the blood plasma starts to leave the vascular system (vasomotor exhaustion) and histamine released from damaged cells further increases vasodilation and capillaries permeability (40). At this point, hematocrit may start to rise (hemoconcentration) (2).

If vascular tone is lost as a consequence of local accumulation of metabolites and cellular factors then a situation of survival is very critical and poor. The reasons for this are the combined effects of losses into hypoxic tissues and wash out to tissue factors which will interfere with the function of central vital organ. The complex disturbances that are thereby initiated are schematically summerized in Figure 3(6).

Prolonged hypotension also causes immunologic depression in the intestine. The hepatic reticuloendothel ial system fails, allowing bacteria absorbed from the

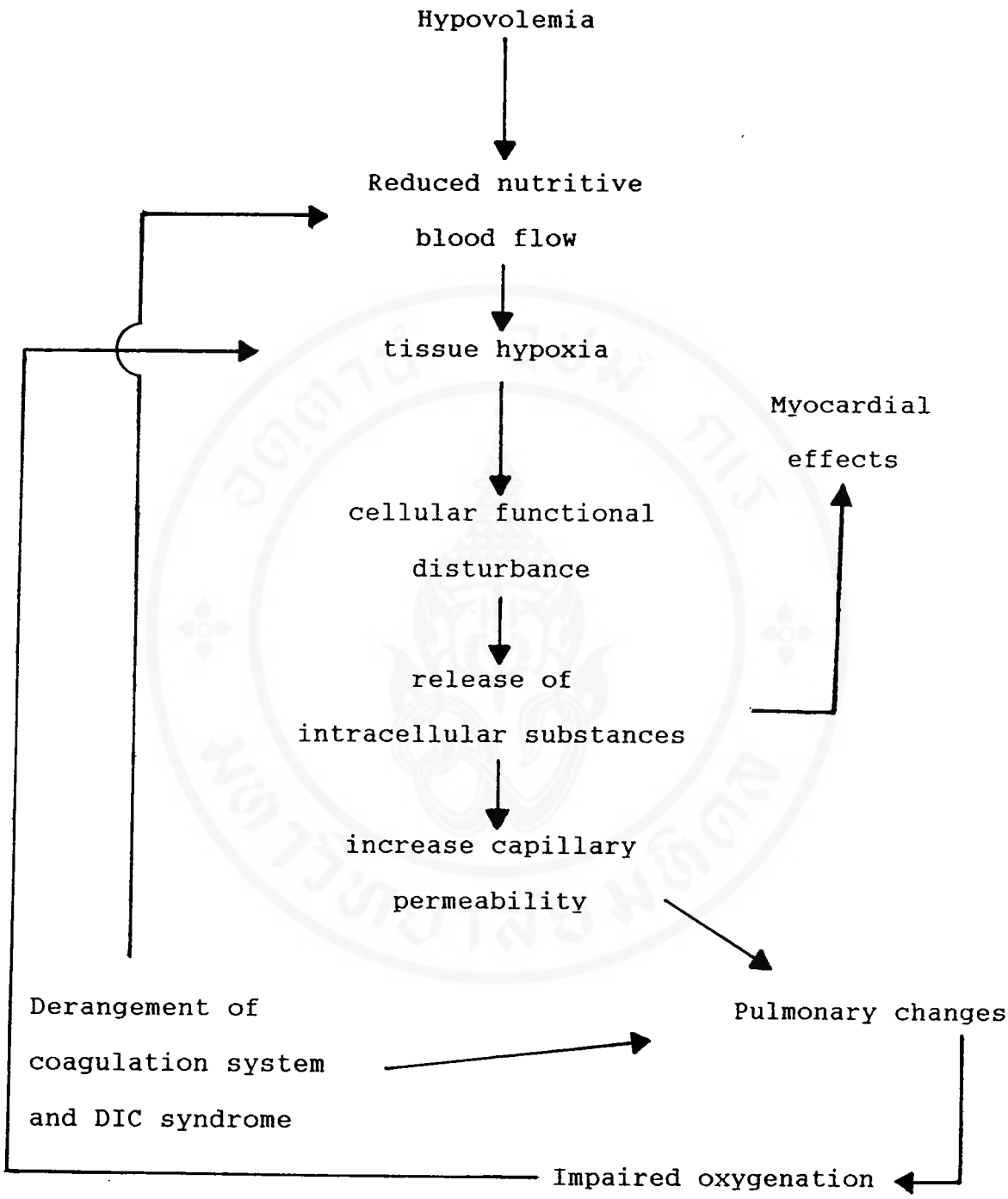


Figure 3. Deletorious vicious circles started by the release of intracellular substances following severe hypovolemia (6). DIC = Disseminated intravascular coagulation.

intestine access to the circulation. Bacterial toxins have adverse effects on cell function and vascular centrol, and activate the coagulation and complement cascades (40).

Substances of intracellular origin may have direct effects on myocardial function. Cardio-inhibitory toxins (e.g. Myocardial Depressant Factor-MDF) seem to origin from pancreas and gastrointestinal tract. MDF will aggravate the shock state by further impairment of nutritive blood flow, tissue oxygen availability and thereby of cellular function (Figure 3). Substances released from hypoxically injured cells may also affect the cascade systems and derange the coagulation system. Thereby respiratory distress syndrome (RDS) or disseminated intravascular coagulation (DIC) may develop. DIC is characterized by the inappropriate initiation of the clotting process in large segments of the capillary bed. If the DIC continues the shock may progress to multiple organ failure (MOF). MOF most commonly involves organs such as lungs, kidney, liver and stomach. Cardiovascular instability, depressed immune function, impaired coagulation, and abnormality in intermediary metabolism are frequent associated phenomena (2).

3. HEMORRHAGIC SHOCK PROTOCOL

There are two types of protocols which are in general use for the hemorrhagic shock study (41).

Type I is a constant-pressure protocol which is a modification of Wiggers original protocol. This protocol uses a variable stress (blood loss) to maintain a constant level of response (blood pressure).

Type II is a constant rate of hemorrhage which is thought to be a better analogue of the clinical events. This protocol uses a fixed stress (constant rate of blood loss) while the blood pressure response is uncontrolled.

A typical pattern of blood loss and reinfusion using the Type I protocol is illustrated in Figure 4. This curve can be subdivided into sections that correspond to the hemodynamic phases of the physiological response to hemorrhage. The early period, during which blood must be removed to maintain the target pressure, will be referred to as the compensatory phase. During this period peripheral resistance increase, and fluid from the extravascular space is absorbed. As a result, there is hemodilution and a fall in the hematocrit. There is then a period, near the point of maximal blood loss, during which very little blood needs to be added or removed. This is a transition period and will be referred to as the maximal compensatory phase. The time to reach maximal blood loss (total compensation time) and the volume of maximal blood loss (total compensatory volume) are well-defined values in this protocol. Finally blood must be reinfused in order to maintain the target blood pressure. This is the decompensatory phase. This phase is thought to result from prolonged tissue ischemic. It

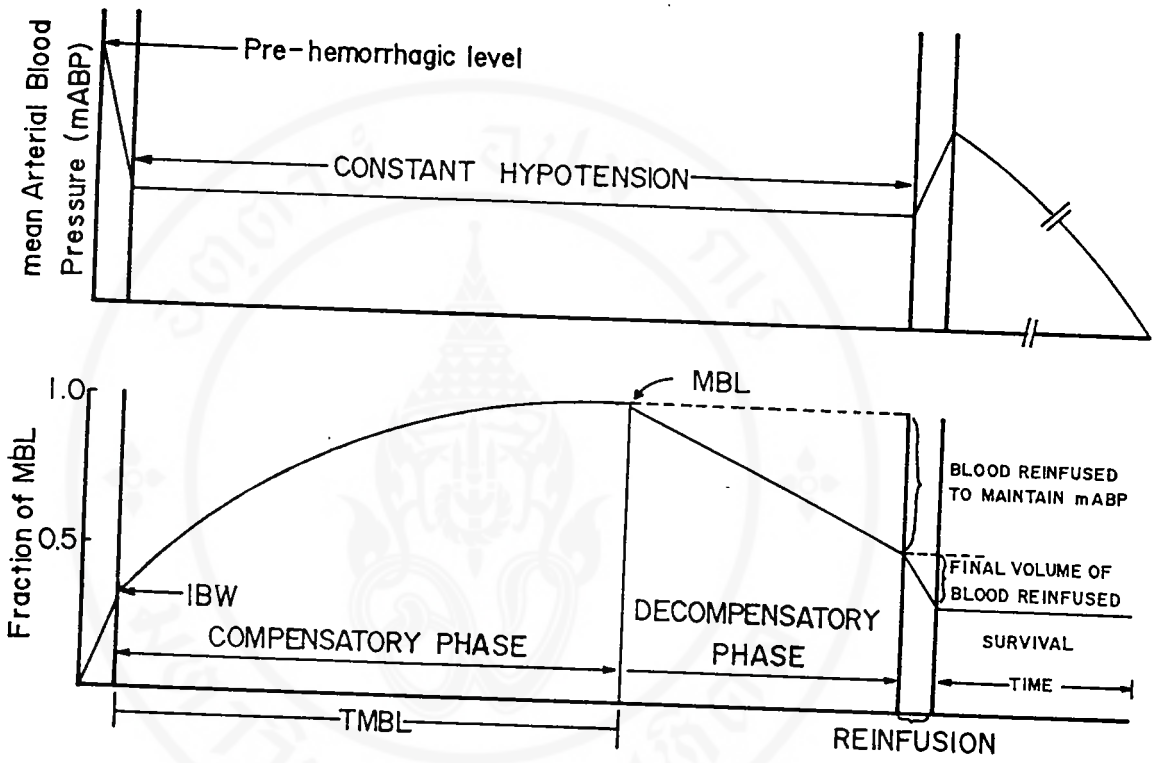


Figure 4. Blood loss curve for constant-pressure protocol. Modified from Connett JR (41).
Abbreviations :

IBW = initial blood withdrawal,

MBL = maximum blood loss

TMBL = time to maximum blood loss

is associated with a fall in total peripheral resistance which leads to an increasing loss of circulating volume even with the reinfusion of blood. This is indicated by a rising hematocrit. If the protocol is continued, death ensues, and a measure of survival time is obtained.

GENERAL ASPECTS OF HIGH ALTITUDE AND HYPOXIA

1. HIGH ALTITUDE CONCEPT

The definition of "high altitude" is not clear since no exact altitude level has been indicated as "high". The wide ranges of the height of altitude above sea level reported rely on the different elevations of the high-altitude research stations in various parts of the world. Heath and williams (42), however, defined "high altitude" an elevation at 3,000 m or more, because at this point, in the majority of subjects ascending to high mountains, unequivocal signs and symptoms associated with the ascent appear. Above this altitude level, biochemical, physiological, and anatomical features of acclimatization become progressively more pronounced. However, there is a critical altitude above which successful, permanent acclimatization cannot take place. This limit appears to be somewhere around an elevation of 5,500 m.

The term "acclimatization" to high altitude (or hypoxia at high altitude) means the changes that occurred

in a sea level resident who chronically exposed to high altitude or hypoxia. This term is different from the term "adaptation" which will be used to imply genetic selection of a strain suited to high altitude or hypoxic conditions.

2. HYPOXIA

The amount of oxygen in the atmosphere remains constant at 20.93 percent up to an altitude of 110,000 m as demonstrated by Frisancho (43). The percentage of oxygen in the ambient air is the same at high altitude as it is at sea level. However, gas is compressible and this means that the number of molecules a unit volume contains is greater at sea level than at high altitude. In other words, the barometric pressure which depends upon the molecular concentration of the air decreases with increase in altitude. This in turn means that the partial pressure of oxygen in the ambient air at high altitude, is reduced. The relation between altitude, barometric pressure and air (and oxygen) pressure as a percentage of that at sea level is shown in Figure 5.

3. BODY WEIGHT AT HIGH ALTITUDE

One of the most common observations at high altitude is an initial loss of body weight. The weight loss at moderate altitudes stabilized after several weeks of exposure (44,45). Only at extreme altitudes (above

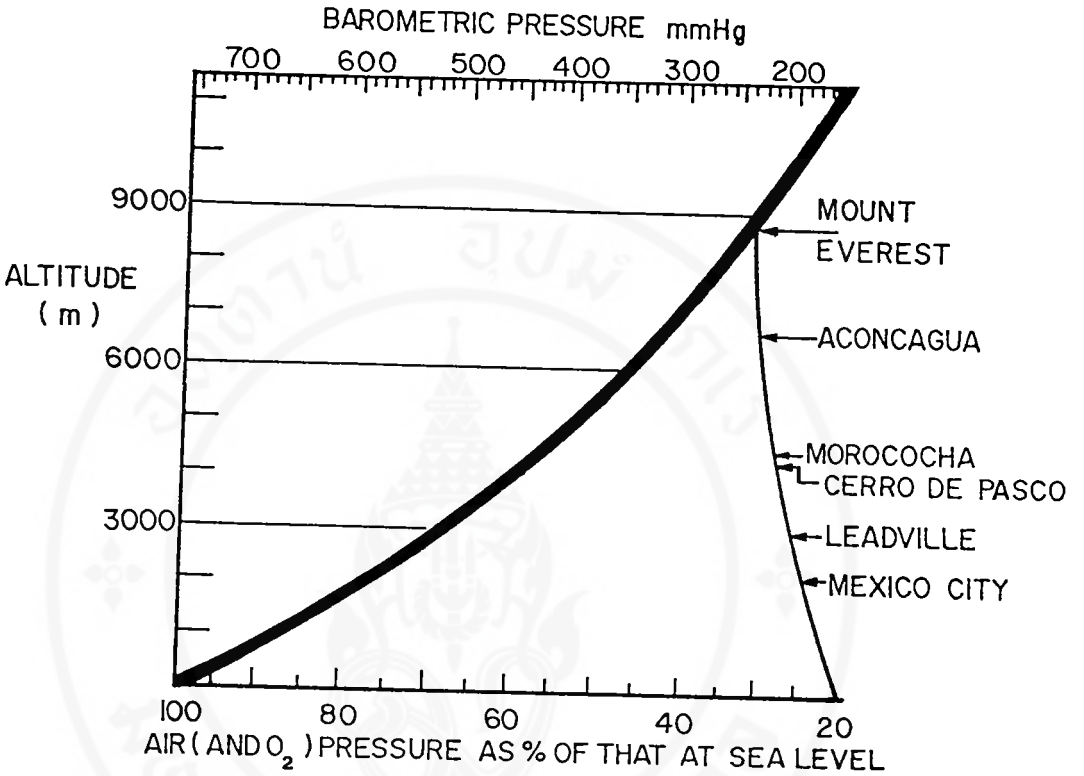


Figure 5. The relationship between altitude, barometric pressure, and air (and oxygen) pressure as a percentage of that at sea level. Modified from Frisancho RA (43).

5,000 m) does the weight loss appear to be progressively and continuous (46).

The initial weight loss has been attributed to a combination of effects such as reduced dietary intake (47), increased water loss mainly from the lungs due to hyperventilation (45), and the loss of stored body fat (44). The reduction in dietary intake resulting from anorexia and hypophagia limits the amount of exogenous energy available to the body, which forces the tissues to utilized stored energy materials especially in body fat and the development of a negative nitrogen balance (48).

4. SKELETAL MUSCLE IN HIGH ALTITUDE EXPOSED SUBJECTS

One possible microanatomical adjustment to the hypoxemia resulting from life at high altitude would be a reduction of the distance over which oxygen has to diffuse from blood capillaries to reach the mitochondria. In acclimatized animals there is an increased number of capillaries per unit of tissue in skeletal muscle (49). Banchero (50) studied in mongrel dog after exposure to a simulated altitude of 4,880 m for three weeks found that the mean capillary density in cross-sectioned muscle and the relative area of capillaries per unit volume of tissue are both risen. The shorter diffusion distances are effective in facilitating oxygenation of cells.

An increased amount of myoglobin are present in the muscular tissue of man and animals at high altitude

(42). The increased myoglobin content of skeletal muscle has also been reported in rats exposed to high altitude by Anthony et al. (51). Myoglobin has the property of combining loosely and reversibly with oxygen, it takes up oxygen rapidly at low oxygen tissue tensions and acts as a reserve store of oxygen which is available during periods of activity.

Young et al. (52) found that rats after chronic high altitude exposure (19 days, 4,300 m), substrate which increased mobilization and utilization during heavy exercise is mostly in FFA result in sparing of muscle glycogen, which detected by respiratory exchange ratio. Respiratory exchange ratio, and also blood lactate concentration following exercise, were lower in chronic high altitude rats.

5. LIVER METABOLISM AT HIGH ALTITUDE

The circulating level of glucose is often used as a nonspecific indicator of the state of carbohydrate metabolism. Lower fasting blood glucose level was found in high altitute (3,800 m) than in sea-level animals (53). In addition to low blood glucose levels, liver glycogen content decreased in rats during both acute and chronic exposure to 3,800 m high altitude hypoxia. It was suggested that the lower glycogen level could be resulted from the following factors :diminished food intake, decreased intestinal absorption of glucose, increased

glycogenolysis associated with an increased anaerobbic glycolysis, or a generalized increased in tissue utilization of glucose (54).

Blume and Pace (55) reported a significant decrease in glucose oxidation to carbon dioxide during the postpandial period in mice chronically exposed to an altitude of 3,800 m. This effect was not observed during the absorptive state. The results suggest an alteration of liver glycogen that manifests itself when the body depend on endogenous glucose sources. These authors, with the use of radioactive-labelled glucose (56), also found that the liver glycogen contained higher amounts of radioactive after labelled glucose injection in high altitude rats compared to their sea-level counterparts. This suggests that glycogen synthesis is enhanced at altitude despite the lower total liver glycogen content which implies that hepatic glycogen turnover is generally increased at altitude.

Carbohydrate metabolism is regulated by the actions between insulin and glucagon. During the normal diurnal fluctuations in exogenous glucose supply, these hormones, acting together, maintain the plasma glucose level within fairly norrow limits. The altitude-induced changes in exogenous energy supply, hepatic glycogen turnover, and tissue glucose utilization could be resulted from alteration in the circulating levels of these hormones (54).

The catecholamines, epinephrine and norepinephrine, have significant metabolic effects, including stimulation of glycogenolysis, glycogen turnover, and gluconeogenesis. These hormones act, similar to the glucocorticoids by inhibitinginsulin action and increasing glucagon release in response to hypoglycemia (54). Myles and Ducker (57) found that increased activity of the sympathoadrenal medullary system (norepinephrine and epinephrine) appears to be involved only in the initial stage of acclimatization to altitude.

6. CARDIAC MUSCLE AT HIGH ALTITUDE

The alterations in cardiac muscle following prolonged high-altitude exposure are similar to those in prolonged forced exercise (58). The changes which result in the limitation of oxygen diffusion to the cells include mitochondrial enlargement, dilation of the cisternae within the sarcoplasmic reticulum, seperation of myofilaments and a slight increase in the number of lipid droplets and glycogen, and also induce cardiac hypertrophy which observed in dogs and rabbits. In rats, they appear to be less susceptible to lower oxygen levels and are able to withstand prolonged periods under hypoxic conditions without producing cellular alterations. These rats showed only moderate changes in the myocardium. The mitochondria were minimally swallen as was the sarcoplasmic reticulum. There was no observable increase

in lipid droplets and no seperation within myofilament (59).

McGrath et al. (60) demonstrated that the cardiac muscle of animals acclimatized to high altitude have an increased ability in tolerance to anoxia by continuing its function under anaerobic conditions. The increased anoxic tolerance in tissues from altitude acclimatized rats involves an increase capacity for glycolysis.

In the studies of Moret (61) measurements were made on the arteriovenous differences of glucose, lactate, pyruvate and free fatty acids. The arterial concentration of glucose was slightly lower at high altitude and the myocardial uptake tended to decrease. The arterial concentration of lactate and pyruvate were increased at high altitude and the uptake of each tended to increase. The lactate pyruvate ratio of arterial and coronary sinus was not changed, suggesting that the redox potential of the myocardial cell was the same. The arterial concentration of FFA was similar at high and low altitudes while the myocardial uptake tended to decrease at high altitude.

QUESTION CONCERNED

During hemorrhagic shock there is a rapid, marked decrease in the production of energy by aerobic pathways and prompt to anaerobic glycolysis process (2). The main sources of energy substrate in anaerobiosis is the carbohydrate reserves such as glycogen which vary considerably on different tissues (2) and this accounts for their varying abilities to survive anaerobically under hemorrhagic shock. Glycogen is a storage form of carbohydrate in liver and muscle, being depolymerized to glucose when stress occurred. The level of blood glucose is also an important determinant of shock tolerance which related to the level of liver glycogen (62). A state of hyperglycemia which is present in hemorrhagic stress is due to glycogenolysis with depletion of hepatic glycogen while a concomitant increased insulin level failed to produce a stimulatory effect on glucose uptake in skeletal muscle (2,63).

The homeostatic response of an animal to hemorrhagic shock results in a shunting of blood from nonvital organs such as skeletal muscle, skin, kidney, and splanchnic bed to preserve blood flow to the vital organs, the heart and the brain. The impairment of tissue perfusion which results in tissue hypoxia, and hence a limitation in oxidative pathway by the oxygen deficiency, during hemorrhagic hypotension favors anaerobic glycolytic process and leads to lactic acidemia, which related to

massive glycogen breakdown in skeletal muscle for ATP production (21). In general, lactate can be recycled to glucose in Cori cycle at liver, however, the liver has been implicated as a source of lactate in severe shock (22).

Previous studies have shown that high-altitude acclimatized rats can tolerate hemorrhagic shock at sea level better than control sea-level rats (64,65). Such increased hemorrhagic tolerance has been suggested to be resulted, in part, from an increased initial blood volume (64), an increased ability for arterial blood pressure regulation (64), and more hemodilution during hemorrhagic hypotension (65). However, other possible contributing factors have not yet been elucidated. Since, in sea-level animals subjected to hemorrhage, it has been reported that endogenous substrate (glycogen) in tissues (especially in liver) prior to hemorrhage and the blood glucose level during hemorrhagic shock are important determinants of shock tolerance (2,62), it is therefore of interest to know whether the higher tolerance to hemorrhagic shock in the high-altitude rats than the controls is contributed by any differences in their carbohydrate reserves in tissues as well as the hyperglycemic responses to hemorrhagic hypotension. Accordingly, the present study was conducted to determine glycogen stores in the liver, heart, and skeletal muscles before and during hemorrhagic shock in rats after chronic exposure to high altitude. Changes in plasma levels of

glucose and lactate in response to hemorrhage and their relations with the tissue glycogen and hemorrhagic tolerance were also determined.

CHAPTER II

OBJECTIVES

1. To determine the glycogen content in the liver, the heart and skeletal muscles of the chronically high altitude exposed rats and their sea-level counterparts before and after a standardized hemorrhagic hypotension at mABP of 35 mmHg.

2. To correlate any changes in the organs glycogen content and some blood parameters induced by hemorrhage in both groups of rats to their hemorrhagic tolerance.

CHAPTER III

MATERIALS AND METHODS

1. ANIMALS

Adult male Wistar strain rats with initial body weight of 216-262 g supplied by the National Animal Center, Salaya Campus, Mahidol University, Nakornpathom were used in this study. The animals were housed in the cleaned-stainless steel cages and fed ad libitum with dry-regular rat chaw (Gold Coins Co, Ltd., Singapore, authorized by F.E. Zuellig Company, Thailand) and free excess of water. The animals were weighed at the beginning and every two days during the course of the experiment.

The animals were randomly divided into two major groups : control and altitude. The altitude animals were kept in a well ventilated decompression chamber where they were continuously exposed to a simulated altitude of 5,000 m (barometric pressure of 403 mmHg) for about 5 to 10 wks while animals of the control group were kept outside the low-pressure chamber in the experimental room where the barometric pressure was about 760 mmHg which is approximate to that at sea level, and ambient temperature and relative humidity were 30±2°C and 52-60%, respectively. Cage cleaning and replenishment of food and water were performed every two days at sea level. For altitude animals, this was done by bringing the altitude

chamber to sea level pressure; about an hour was spent on this purpose which included the return of the animals to the altitude.

The animals in each group were further randomly divided into three subgroups :

Subgroup I : Non-hemorrhaged, 0 min. The animals were sacrificed at the time the animal preparation was finished (i.e. at time 0).

Subgroup II : Non-hemorrhaged, 150 min. The animals were sacrificed at 150 min after the animal preparation had been finished.

Subgroup III : Hemorrhaged, 150 min. The animals under anesthesia following cannulation until their mABP decreased to 35 mmHg. The mABP had been maintained at this hypotensive level for 150 min before the animals were sacrificed.

2. INDUCTION OF HIGH ALTITUDE EXPOSURE

The decompression chamber in which the altitude group of rats were kept consisted of an iron cylindrical tank 40 cm in diameter and 62 cm long (approximately 78 L capacity) with one end permanently closed. The open end of the chamber could be tightly closed with a reinforced plexiglass plate 2 cm thick. The plexiglass plate fits tightly against a rubber gasket making and air-tight seal.

Air entered the chamber through two inlet valves via two calibrated flowmeters (Brooks, type 1355-O1F1AAA). These flow meters could simultaneously measure and control the rate of air flow at any designed altitudes. The chamber was evacuated via two seperate outlet valves which were combined as one with a Y tube and connected to a vacuum pump (Model S-35, Precision Scientific Co., U.S.A.). The position of the inlet and outlet valves were set apart allowing even distribution of air throughout the chamber. Barometric pressure inside the chamber was read from a mercury manometer which connected to the chamber and had been previously calibrated for various altitude levels. The relation of altitude with the corresponding barometric pressure was calibrated according to Gilbert (Gilbert, D.L. Handbook of physiology, Sec. 3 Respiration. Am Physiol Soc : Washington Vol. 1, pp. 153-176, 1964). The decrease of chamber pressure accomplished by reducing the rate of air flow into the chamber until the designed altitude level was reached. At the altitude equivalent of 5,000 m the steady rate of air flow through the chamber was 13 L/min. Oxygen gas tension inside the altitude chamber was calculated from the barometric pressure at the altitude by assuming the presence of 21% of O_2 in the air. Excess CO_2 absorbent (Sodasorp, W.R. Grace, Canada) was place inside the chamber to prevent accumulation of carbon dioxide. The rate of pressure increase and decrease was approximately equivalent to an altitudinal rate of change of 1,000 ft/min. Chamber temperature and relative

humidity measured by a thermometer and an electronic hygrometer (Api Instrument Co., Silver Spring, Maryland), were 30+2°C and 52-63%, respectively.

3. CHEMICALS

All chemicals and solvents used in the present study were analytical grade. Nembutal (Pentobarbital sodium) was purchased from Abbott Laboratories, North Chicago, Illinois, U.S.A.,; and heparin from Leo Pharmaceutical products, Bullerup, Denmark. Reagents for determination of plasma glucose and tissue glycogen were purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A. Reagents for determination of triglycerides and blood lactate were purchased from Wako Pure Chemical Industries, Osaka, Japan, and Analox Instruments Ltd., U.S.A., respectively.

4. ANIMALS'DIET

All animals received the same diet. The regular composition of the diet is shown in Table 1..

Table 1. Composition of rat's diet.

Composition	Imported Pellets (per 100 g)	
Food energy	335.9	Kcal.
Moisture	8.6	g
Fibers	3.0	g
Ash	7.3	g
Proteins	25.2	g
Carbohydrates	53.6	g
Vitamins		
Thiamine	4.62	mg
Riboflavin	1.95	mg
Niacin	7.8	mg
Folate	85.0	ug
Minerals		
Calcium	2108.5	mg
Phosphorus	541.1	mg
Iron	41.5	mg
Sodium	230.0	mg
Potassium	840.0	mg

Food analysis was done by Dr. Songsuk Srianuchart, Division of Nutrition, Research Center, Ramathibodi Hospital, Bangkok 10400.

5. ANIMAL PREPARATION FOR HEMORRHAGIC STUDY

At the end of the altitude exposure period, the altitude animals were brought to sea level and given the following experimental treatment along with the control group. Each rat was weighed and anesthetized intraperitoneally with Nembutal (Pentobarbital sodium) at 5 mg per 100 g body weight. The animal was restrained in a supine position on a surgical board under a surgical lamp where the temperature around the animal was maintained at 30+2°C. Tracheostomy was performed using a short piece of PE 240 polyethylene tube for aspiration of the secretion that may block the airway under anesthetic condition. The left common carotid artery was cannulated by a modified catheter placement unit (Angiocath 22G, Deseret Medical, Inc., U.S.A.) which connected to a strain gauge pressure transducer (Statham P23 AC) and Beckman type R Dynograph recorder for continuous monitoring of arterial blood pressure. The left femoral vein and artery were cannulated with modified catheter placement units (Angiocath 24G, Deseret Medical Inc., U.S.A.). Each rat received 500 IU.kg^{-1} rat weight of heparin via the left femoral vein (Fig. 6).

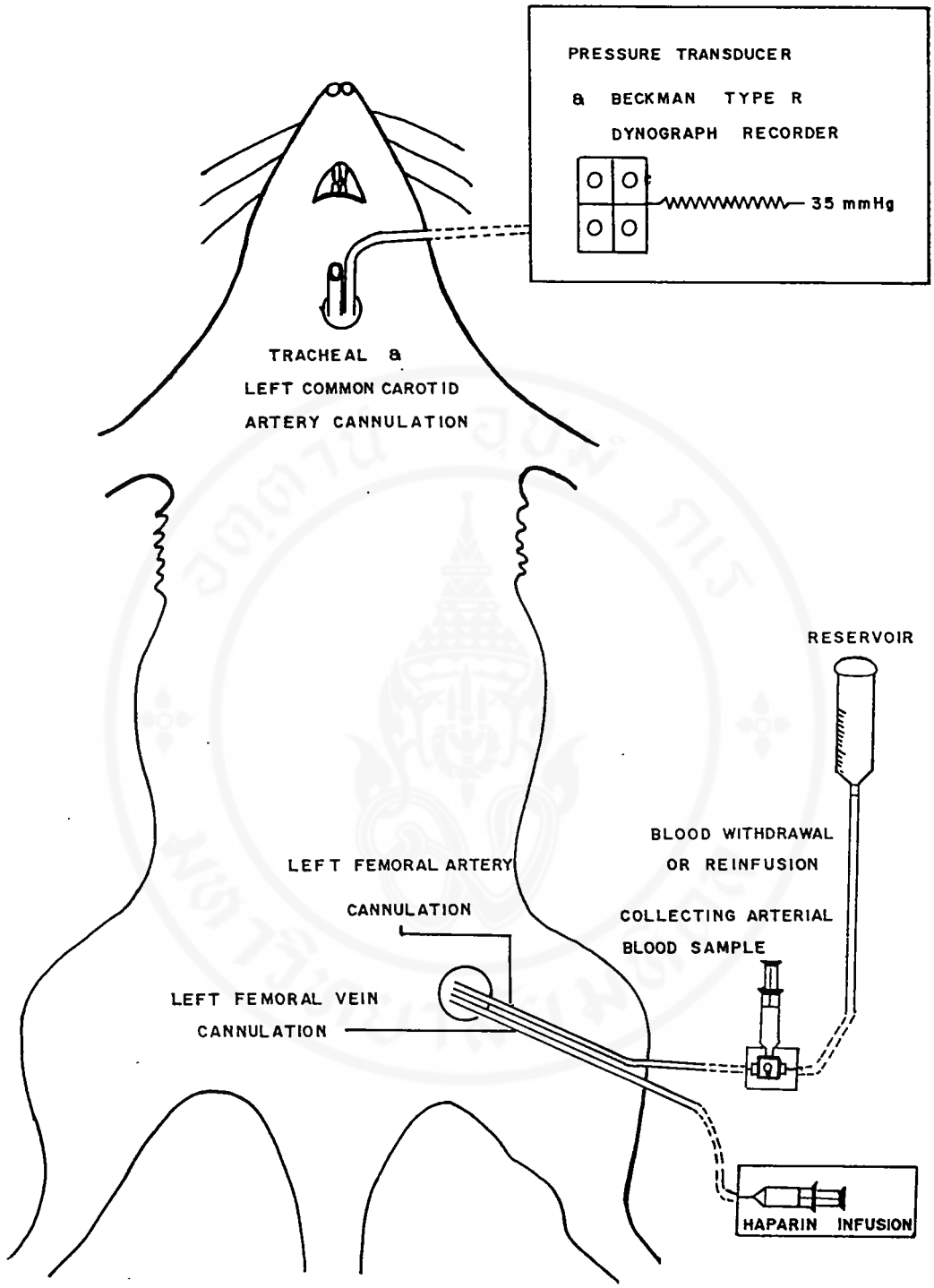


Figure 6. General animal preparation in the present study.

6. INDUCTION OF HEMORRHAGIC SHOCK AND BLOOD/TISSUES SAMPLING

At 10 min after heparin injection, hemorrhagic shock was induced in subgroup III rats by blood withdrawal at a rate of about 0.5 ml/min from the left femoral artery into a standard 10 ml glass syringe which serve as a reservoir until mean arterial blood pressure (mABP) recorded from the left common carotid artery had dropped to 35 mmHg. This degree of hypotension was usually reached after removal of 2-5 ml of blood in about 4-10 min. A 0.5 ml of heparinized saline, initially used to filled the dead space (i.e. the polyethylene tubing connecting the modified catheter placement unit to the reservoir) remained layered on top of the blood calcium in the reservoir and was allowed to remain in the system after reinfusion.

Mean arterial blood pressure was maintained at a constant level of 35 mmHg for 150 min. At the first part of hemorrhagic hypotension, additional volume of blood have to be drawn from the animals due to their compensation until a point of maximum blood loss was reached after which gradual reinfusion of the shed blood was performed due to their decompensation of arterial blood pressure. At 150 min of hypotension, a precalculated fraction of the shed blood remained in the reservoir was reinfused at a rate of about 1 ml/min until the animal received a relative blood volume of 35% maximum

blood loss. The volume of the initial blood withdrawal (IBW), the maximum blood loss (MBL), the time to the MBL (TMBL), the volume of blood reinfused during the control hypotensive period (Hypotensive BR), and the final volume of blood reinfused at the end of the hypotensive period (Final BR) were determined.

Arterial blood samples approximately 2 ml each were taken via the cannulated left femoral artery once prior to hemorrhagic shock, twice during hypotensive period (40-60 and 90 min), and once at 10 min after blood reinfusion for analysis of lactate, plasma glucose, triglycerides and also measurement of hematocrit.

The animals in subgroup I and II were treated identically to the subgroup III except bleeding. The rats in these first two subgroups were maintained normotensive under anesthesia for either 0 min (subgroup I) or 150 min (subgroup II).

At the end of the experiment, each rat in all animal groups was killed by saturated KCl infusion via the left femoral vein. Soleus and extensor digitorum longus muscles from the non-cannulated leg and a portion of the liver and the heart were immediately excised and frozen in liquid nitrogen for glycogen analysis.

7. BIOCHEMICAL ANALYSIS

7.1 Blood lactate

Blood lactate was measured by an enzymatic method (oxidoreductase method). See Appendix I.

7.2 Plasma glucose

Plasma glucose was measured by a colorimetric method (oxidase method). See Appendix II.

7.3 Plasma triglycerides

Plasma triglycerides were determined by an enzymatic colorimetric method which based on a glycerol-3-phosphate oxidase (GPO)-P-chlorophenol coloring reaction. The yield of the reaction, a red color compound, was then measured colorimetrically on a spectrophotometer at a wave length of 505 nm. See Appendix III.

7.4 Tissue glycogen

Tissue glycogen was determined by a colorimetric method in which the glycogen was hydrolysed by sulfuric acid to glucose which then reacted with anthrone in the presence of thiourea. See Appendix IV.

8. STATISTICAL METHODS

Statistical methods were used to calculated the mean and the standard error of mean (SEM). Student's unpaired t-test was used to determine the significant difference between the control (sea level) and the

altitude groups as well as among subgroups at the same period of time. Student's paried t-test was employed to determine any changes within subgroups. The statistical significant differences were considered at the probability level (P-value) of less than 0.05.

9. EXPERIMENTAL PROTOCOL

A. EXPOSURE TO HIGH ALTITUDE

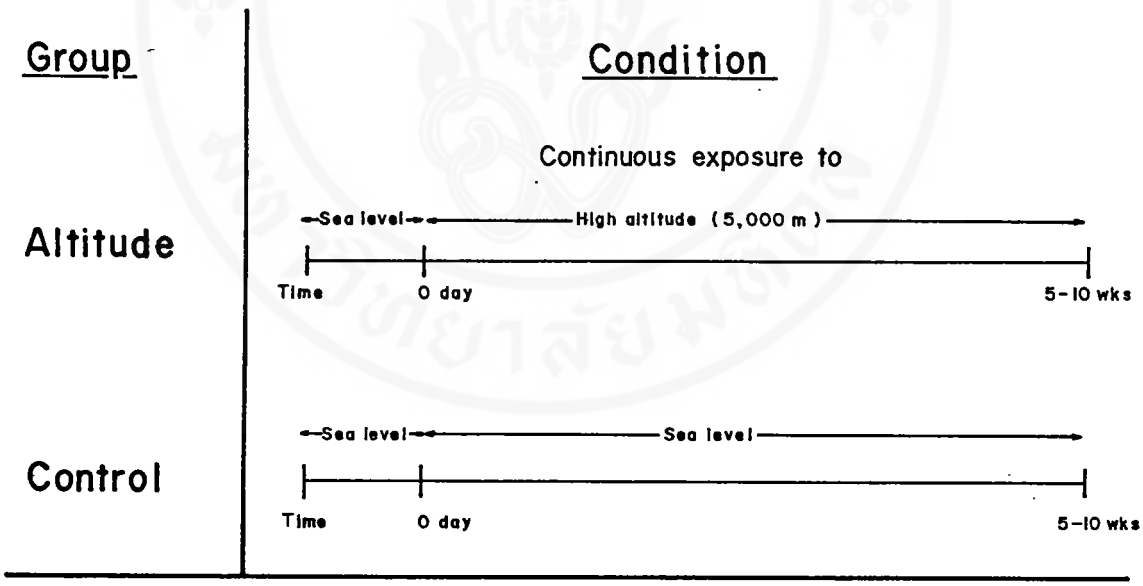
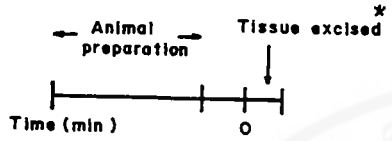


Figure 7a. Experimental protocol of exposure to high altitude

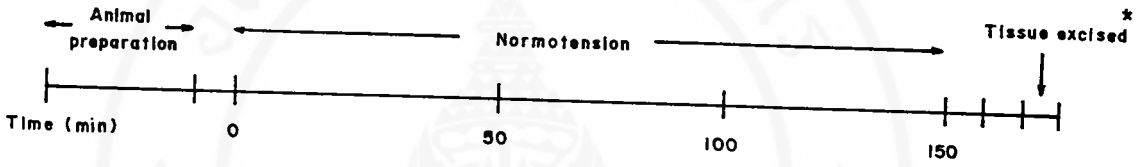
B. INDUCTION OF STANDARDIZED HEMORRHAGIC SHOCK AT SEA LEVEL

Group : Control and altitude

1. Subgroup I (Non-hemorrhaged , 0 min)



2. Subgroup II (Non-hemorrhaged , 150 min)



3 Subgroup III (Hemorrhaged , 150 min)

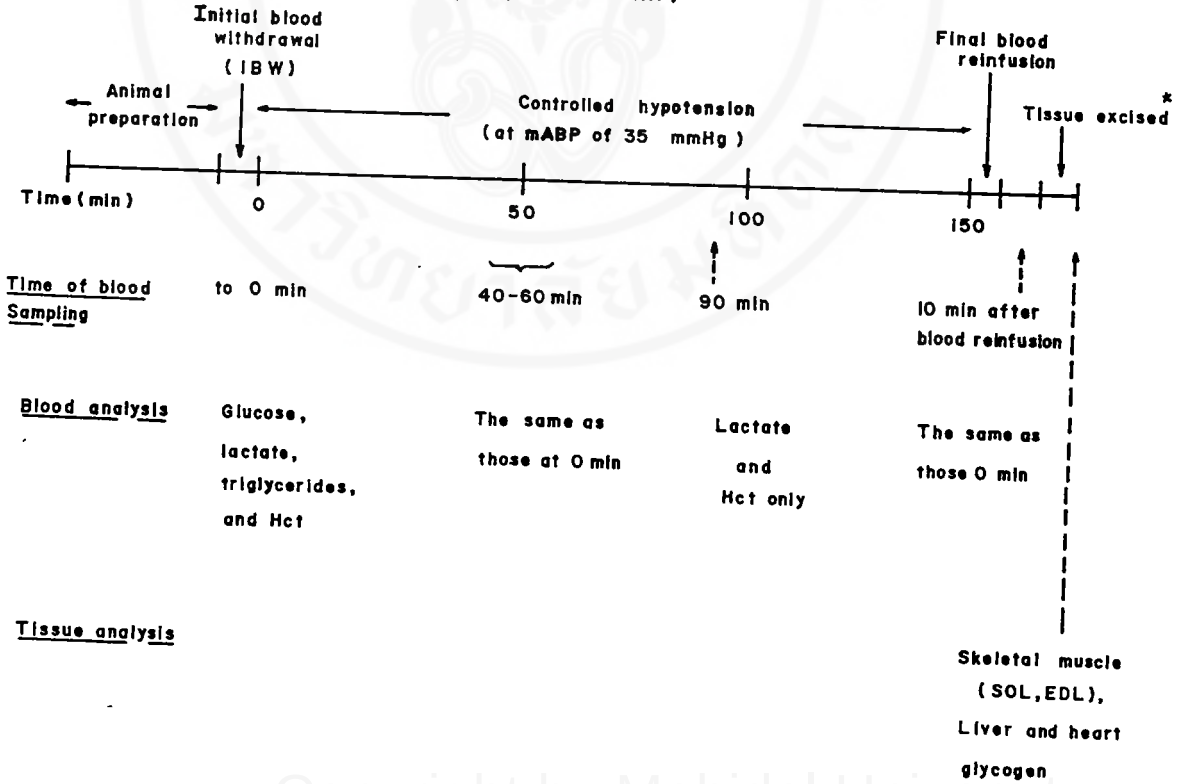


Figure 7b. Experimental protocol for induction of standardized hemorrhagic shock at sea level. *Tissues were excised within 5 min.
Animal preparation includes anesthetization, tracheostomy, arterial and venous cannulation, and heparin injection.
Initial blood withdrawal is the first volume of blood withdrawn at a rate of 0.5 ml/min until mABP dropped to 35 mmHg

CHAPTER IV

RESULTS

1. EFFECT OF CHRONIC EXPOSURE TO HIGH ALTITUDE ON PHYSIOLOGICAL PARAMETERS

The differences in mean body weight and various physiological parameter between the control (sea level) and the high altitude exposed rats are shown in Table 2. After 5-10 wks of altitude exposure, mean arterial blood pressure (mABP) of the high altitude acclimatized rats was higher while their mean body weight was lower than the control rat living at sea level. The mABP of the control and the altitude exposed rats were 125.9±3.55 and 137.9±2.93 mmHg, respectively ($P<0.05$), and their body weight were 371.8±5.93 and 303.4±6.00 g, respectively ($P<0.01$). The SEM values of these parameters in both groups of rats were relatively small when compared to their means (less than 3% of the means) although different exposure periods (range from 5 to 10 wks) were used.

2. EFFECT OF CHRONIC EXPOSURE TO HIGH ALTITUDE ON HEMORRHAGIC TOLERANCE

2.1 Initial blood withdrawal and maximum blood loss

The initial bleeding rate in both animal groups was manually controlled at 0.5 ml/min. The initial blood withdrawal required to produce hypotension at the mABP of

Table 2. Body weight (BW), mean arterial blood pressure (mABP), initial blood withdrawal (IBW), maximum blood loss (MBL), time to maximum blood loss (TMBL), volume of blood reinfused during hypotensive period (Hypotensive BR) and the final volume of blood reinfused at the end of hypotensive period (Final BR) of control rats and high-altitude acclimatized rats (subgroup III).

Group	BW† (g)	mABP (mmHg)	IBW (%BW)	MBL (%BW)	TMBL (min)	Hypotensive BR (%MBL)	Final BR (%MBL)
Control	371.8±5.93 (17)	125.9±3.55 (17)	0.79±0.04 (17)	2.79±0.09 (17)	101.5±3.0 (17)	19.7±3.0 (17)	35.8±0.9 (17)
Altitude acclimatized	303.4±6.00** (13)	137.9±2.93* (13)	1.92±0.12** (13)	3.55±0.12** (13)	127.3±6.4** (13)	8.4±1.6* (13)	34.6±1.4 (13)

Values are means±SEM. The numbers in parentheses indicate the number of rats tested.

*Values are significantly different from the control, $P<0.05$.

**Values are significantly different from the control, $P<0.01$.

†Prior to exposure to altitude equivalent pressure, both groups of animals had statistically the same average body weight.

35 mmHg was about 1.13 %BW greater in the altitude-acclima tized rats than their sea level counterparts (Table 2). The maximum volume of blood withdrawn was also higher in the attilude-exposed rats than the control rats, i.e., 3.55±0.12 and 2.39±0.09 %BW ($P<0.01$), respectively.

When the values of the initial blood withdrawal and maximum blood loss of all rats in both groups were plotted against each other. It was found that these two parameters were significantly correlated, the correlation coefficient (r) is 0.82 (Figure 8).

2.2 The time to maximal blood loss and the volume of blood reinfused

During the first part of oligemia,both groups of rats lost blood continuously into the reservoir. The time for blood loss reached its maximum (TMBL) in both animal groups are shown in Table 2. The average TMBL of the altitude acclimatized rat (127.3±6.4 min) was significantly greater than that of the control rats (101.5±3.0 min) ($P<0.01$). After reaching its maximum blood loss, the animal took blood back from the reservoir (by reinfusion) for maintenance of the mABP at 35 mmHg up to the time of 150 min of hemorrhagic hypotension. The average blood volume reinfused for this purpose, expressed in term of % MBL, was 19.7±3.0% for the control sea level group which was significantly higher than 8.4±1.6% for the altitude-exposed group ($P<0.01$).

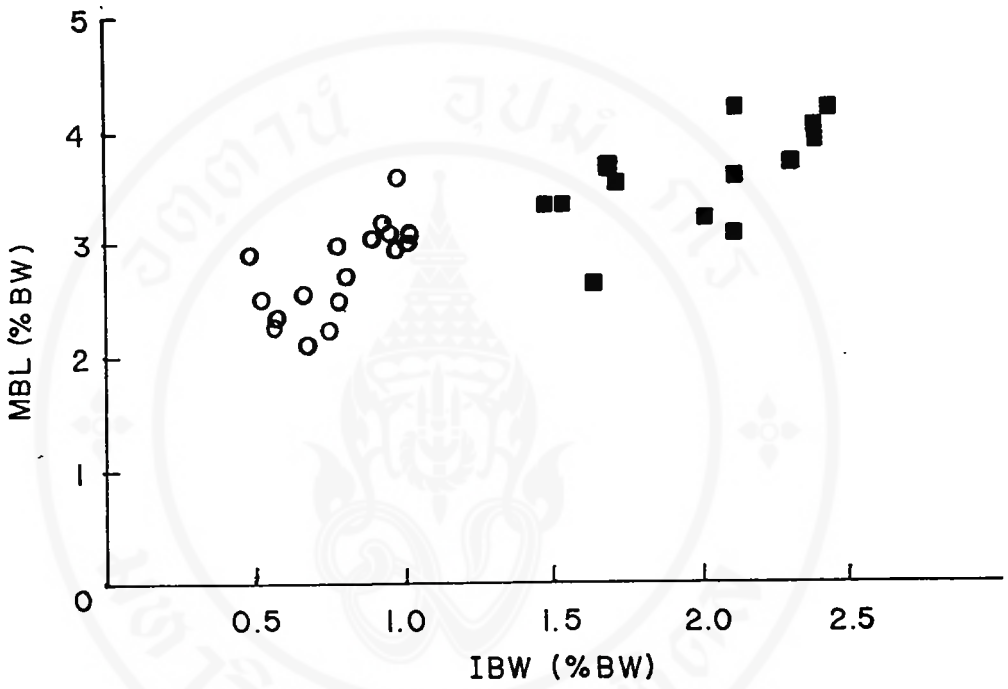


Figure 8. Correlation between initial blood withdrawal (IBW) and maximum blood loss (MBL) for all the rats studied; 17 control rats (O) and 13 high altitude acclimatized rats (■), r=0.82.

At the end of 150 min hypotensive period, the blood remaining in the reservoir was reinfused within 4 min to the animals. The volume of blood reinfused at this time was judged with calculation to have the rats in each group received the same fractional volume of maximum blood loss. This was about 35% for both animal groups (Table 2, Figure 9).

3. EFFECT OF HEMORRHAGIC SHOCK ON HEMATOCRIT, PLASMA GLUCOSE AND BLOOD LACTATE CONCENTRATIONS

The changes in hematocrit during hemorrhagic hypotension period are shown in Figure 10. The hematocrits of the control and the post-altitude rats at the initial phase of hypotension were 50.5±0.55 and 63.2±1.16%, respectively. At the time of 40-60 min of hemorrhagic hypotension, they were significantly decreased from the initial values to 40.8±1.5 and 49.6±1.5%, respectively ($P<0.01$) and further decreased to 36.4±1.0 and 46.6±1.4% respectively at 90 min of hypotension ($P<0.01$). The hematocrit values returned slightly upward and reached the levels of 43.5±1.4% for the control rats and 54.3±1.1% for the altitude-exposed rats when hypotensive time was extended to 150 min. These values, however, were remained lower significantly than their corresponding initial values ($P<0.01$). Figure 10 also shows that the hematocrit values of the altitude rats were always significantly

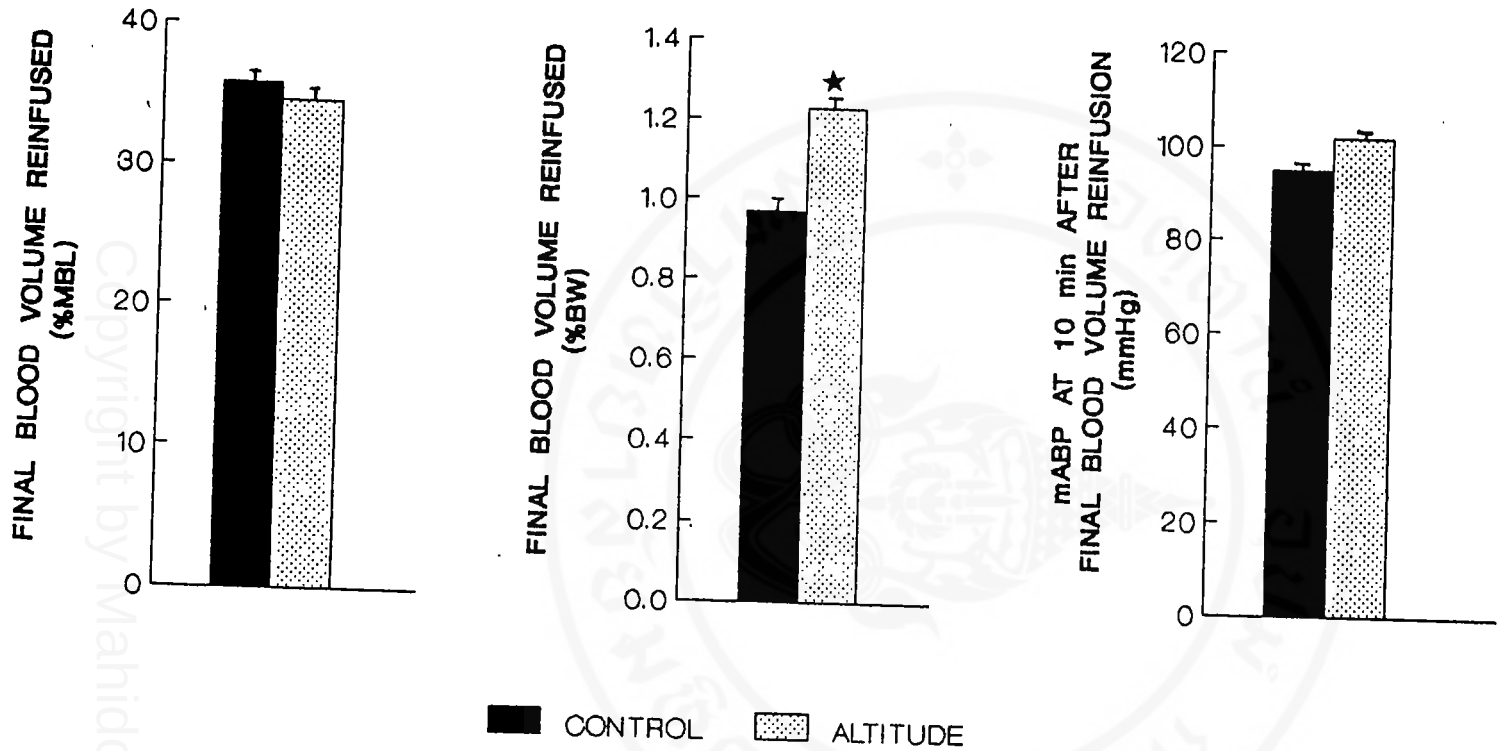


Figure 9. Volume of blood reinfused, expressd in percent of maximum blood loss (% MBL) and percent of body weight (% BW), at the 150 min of hemorrhagic hypotensive period and mean arterial blood pressure (mABP) at tenth minute after final blood reinfusion in the control rats and high altitude acclimatized rats. Values are mean±SEM.
★Significantly different from the control : $P<0.01$.

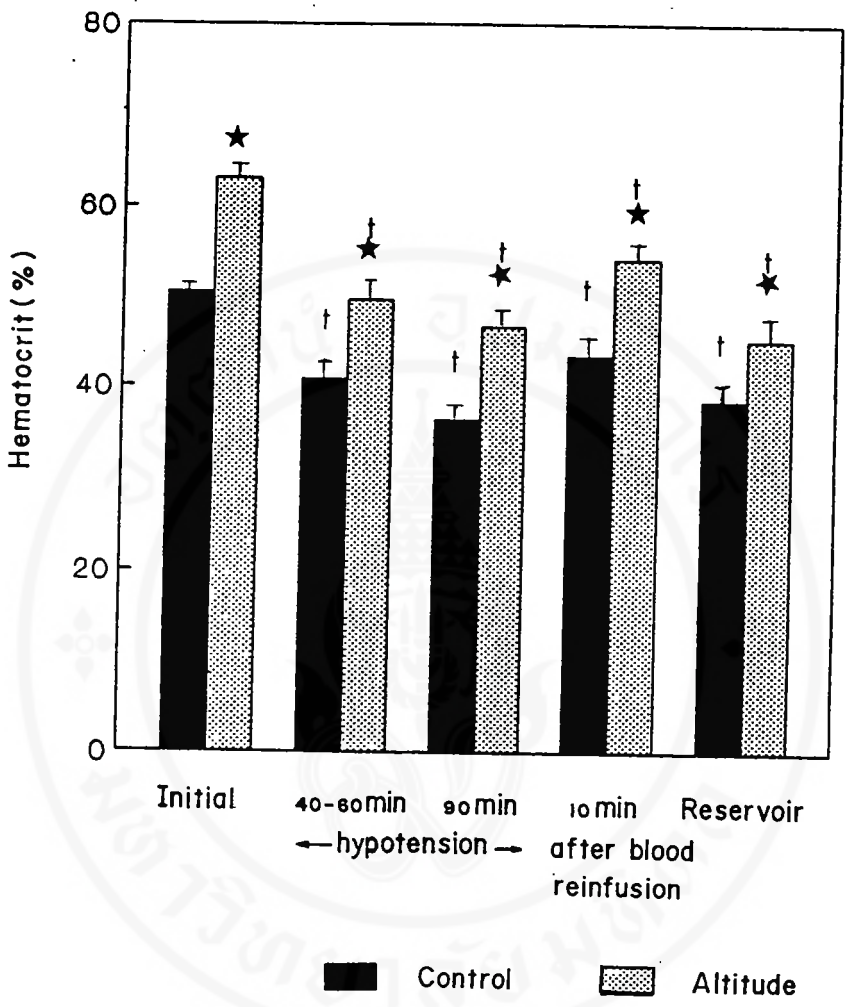


Figure 10. Percent hematocrit of the control rats and altitude-exposed rats at the initial, during 40-60 min and 90 min of standardized hemorrhagic hypotension, at 10 min after blood reinfusion, and of blood in the reservoir at 10 min after blood reinfusion.
Values are means±SEM.
★Significantly different from the control : $P<0.05$.
†Significantly different from the initial values : $P<0.05$.

higher than those of the control sea level animals ($P<0.01$).

The hematocrit in the reservoir at the end of hemorrhagic experiment was 38.6±1.4% for the control rats and 45.2±1.9% for the altitude rats. These values were significantly less than those in the animals reinfused with the blood from the reservoir ($P<0.01$).

During standardized hemorrhage at 35 mmHg of mABP, plasma glucose and blood lactate in animals were dramatically increased with time and remained high after reinfusion of blood (Figure 11,12). Plasma glucose concentrations at the initial phase, 40-60 min of hypotension and at 10 min after blood reinfusion in the control rats were 145.4±7.2, 390.3±23.9 and 419.2±38.7 mg%, respectively, and in the altitude rats were 134.0±6.5, 415.8±23.8 and 444.5±44.8 mg%, respectively. Blood lactate concentrations at the initial phase, 40-60, and 90 min of hypotension and at 10 min after blood reinfusion in the control group were 2.5±0.2, 6.1±0.6, 9.1±0.6, and 9.6±0.3 mmole/L, respectively, and in high altitude rats were 2.6±0.2, 4.2±0.3, 5.3±0.2, and 6.5±0.4 mmole/L, respectively.

Means±SEM of plasma glucose and blood lactate concentrations in the reservoir at the end of hemorrhagic experiment were 290.1±30.2 mg% and 6.9±0.4 mmole/L in control rats, respectively, and in high altitude rats they were 193±14.4 mg% and 5.3±0.2 mmol/L, respectively (Figure 11,12). These values in the reservoir were significantly

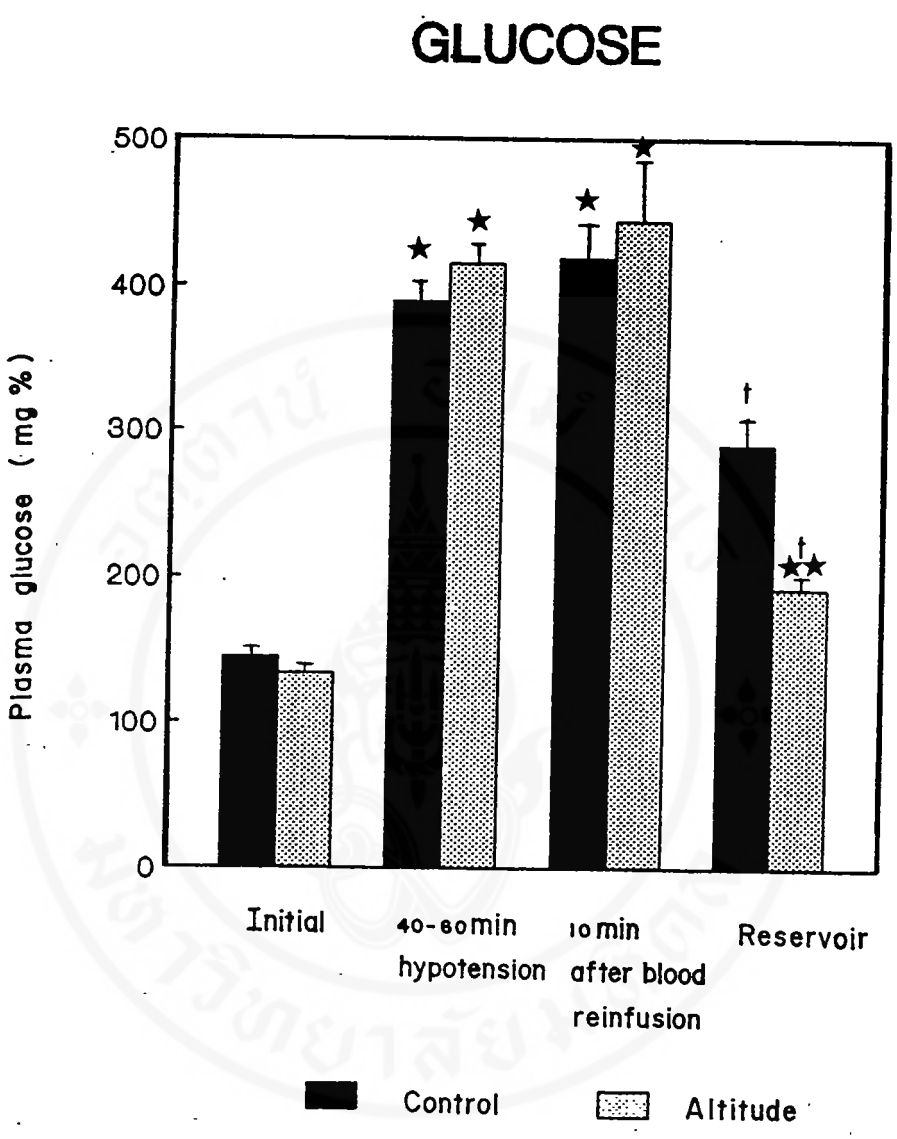


Figure 11. Plasma glucose in the control rats and altitude-exposed rats at the initial, during 40-60 min of standardized hemorrhagic hypotension, at 10 min after blood reinfusion, and in blood from the reservoir at the end of the experiment.
Values are means±SEM.
★Significantly different from the initial value : $P<0.01$.
★★Significantly different from the control : $P<0.05$.
†Significantly different from the 10 min after blood reinfusion : $P<0.05$.

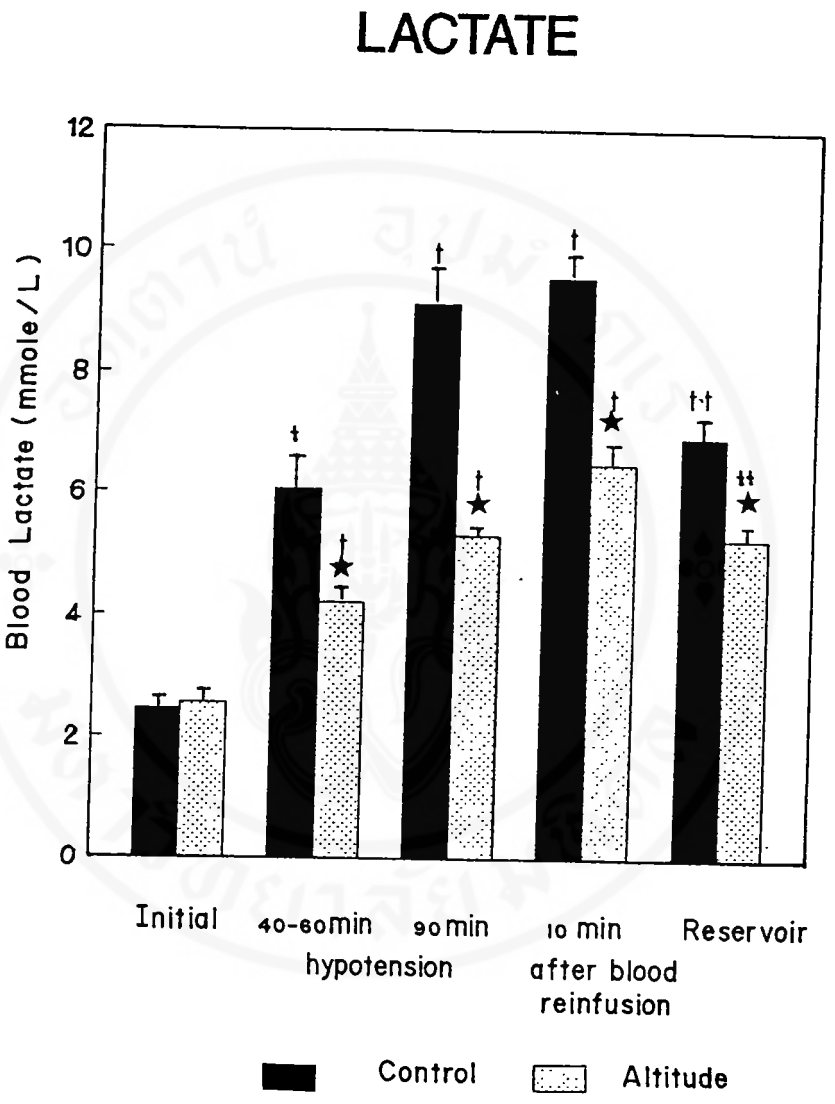


Figure 12. Blood lactate concentrations of the control rats and high-altitude acclimatized rats at the initial, during 40-60 min and 90 min of standardized hemorrhagic hypotension, at 10 min after blood reinfusion, and of blood in the reservoir at the end of the experiment. Values are means±SEM.
★Significantly different from the control : $P<0.01$.
†Significantly different from the initial value : $P<0.01$.
††Significantly different from the 10 min after blood reinfusions : $P<0.05$.

lower than their corresponding values in both animal groups at the 10 min after blood reinfusion ($P<0.05$).

The plasma glucose concentration was not significantly different between the control and the altitude rats at any time of the test. There was also no significant difference in blood lactate between the two animal groups at the initial phase, but during the subsequent phase of shock, the control sea level rats produced greater blood lactate levels than those of the altitude-exposed rats throughout the course of the experiment ($P<0.01$ at both 40-60 min and 90 min of hypotension, and also at 10 min post-reinfusion).

4. EFFECT OF STANDARDIZED HMORRHAGIC SHOCK ON LIVER, HEART AND SKELETAL MUSCLE GLYCOGEN CONTENTS

4.1 Liver glycogen content

Liver glycogen content expressed in term of g/100 g tissue, in control or non-hemorrhaged rats at time 0 (subgroup I) and 150 min (subgroup II), and in rats subjected to 150 min hemorrhagic hypotension (subgroup III) of both animal groups are shown in Figure 13. The average values in subgroups I, II and III of the control animals were 11.9±0.77, 9.03±0.75, and 0.17±0.04 g/100 g, respectively, while those of the altitude exposed animals were 7.39±1.20, 6.38±0.69 and 0.14±0.04 g/100 g, respectively. In each animal group, significant

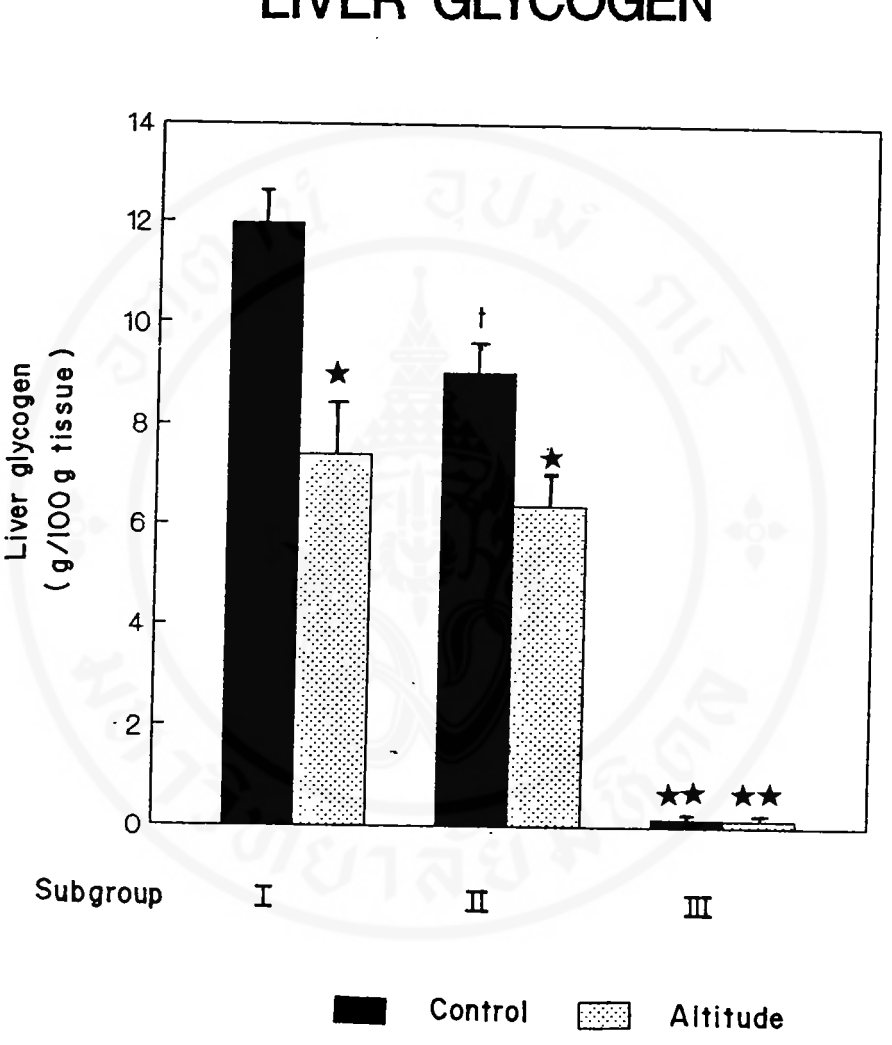


Figure 13. Liver glycogen content of the control rats and high-altitude acclimatized rats in subgroup I, II and III.
Values are means±SEM.
★Significantly different from the control : P<0.01.
★★Significantly different from subgroup I and II : P<0.001.
†Significantly different from subgroup I : P<0.05.

difference in liver glycogen level existed between subgroups I and III ($P<0.001$), and between subgroup II, III ($P<0.001$). There was also significant difference between subgroup I and II for the control rats ($P<0.05$) but not for the altitude-exposed animals. Hepatic glycogen content was similar for both groups which was almost completely depleted by hemorrhage, i.e., the lowest measured values showed no significant difference. In non-hemorrhaged animals, however, the hepatic glycogen levels at 0 and 150 min were significantly higher in the control rats than those in the altitude-exposed rats ($P<0.01$).

4.2 Myocardial glycogen content

Figure 14 illustrates the myocardial glycogen content in various subgroups of both control and altitude rats. The relative amount of glycogen in subgroups I, II and III of the control rats were 1.19±0.05, 0.91±0.11 and 1.53±0.09 g/100 g, respectively, and of the altitude rats were 1.00±0.05, 0.94±0.10, and 1.50±0.16 g/100 g, respectively. The glycogen contents in subgroups II and III of the altitude rats were not significantly different from those corresponding values of the control rats. Significant difference between groups existed only for subgroup I animals ($P<0.05$). In both the control and altitude groups, the myocardial glycogen content in subgroup III was significantly higher than those in

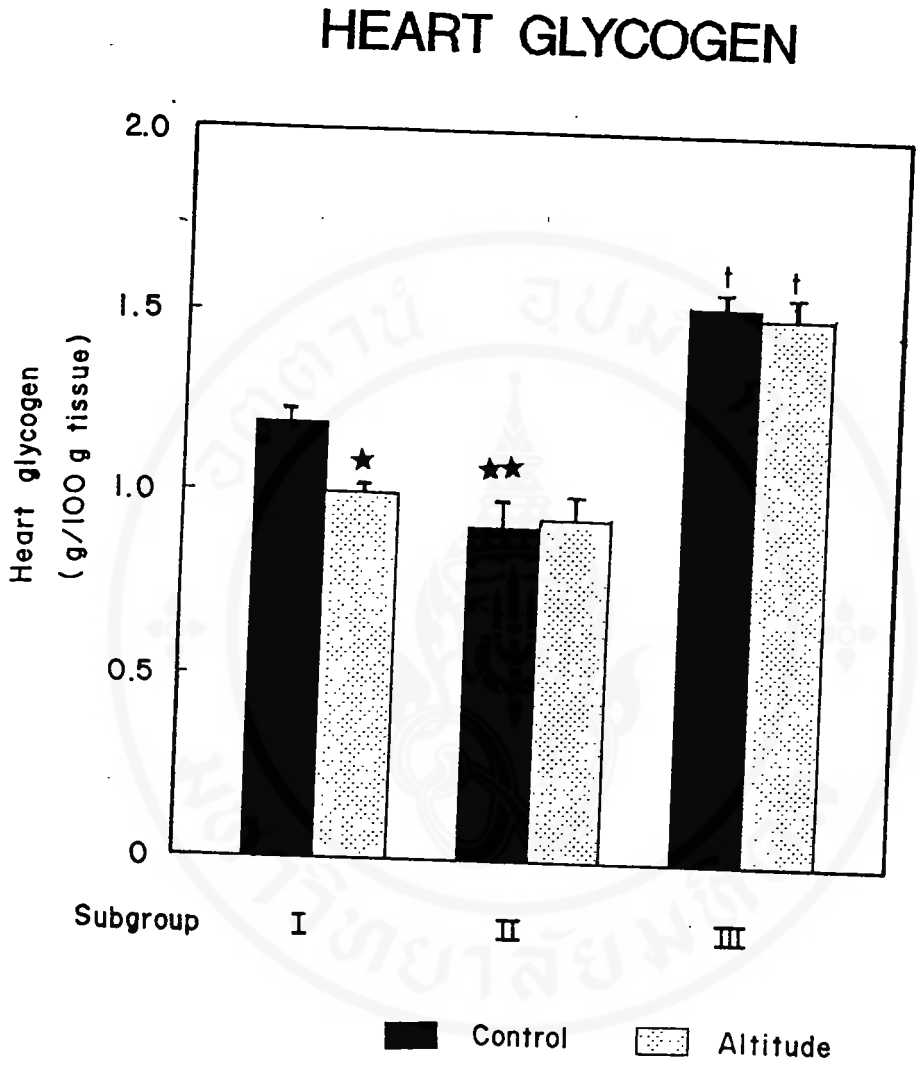


Figure 14. Myocardial glycogen content of the control rats and high-altitude acclimatized rats in subgroup I, II and III.
Values are means±SEM.
★Significantly different from the control : P<0.05.
★★Significantly different from subgroup I : P<0.05.
+Significantly different from subgroup I and II : P<0.05.

subgroups I and II ($P<0.05$).

4.3 Skeletal muscle glycogen content

The means+SEM of SOL and EDL glycogen content are shown in Figures 15 and 16. The average SOL glycogen content of the control rats was 0.97±0.11 g/100 g in subgroup I, 0.97±0.04 g/100 g in subgroup II, and 1.06±0.10 g/100 g in subgroup III. In altitude rats, the values in subgroups I, II and III were 0.87±0.13, 0.97±0.15 and 0.88±0.07 g/100 g, respectively. For the SOL glycogen content, there were no significant difference between groups and also among subgroups. In contrast, the glycogen content in the EDL of both animal groups was significantly lower ($P<0.05$) in subgroup III (0.49±0.05 and 0.51±0.07 g/100 g for control and altitude rats respectively) when compared to subgroup I (1.02±0.07 and 0.75±0.05 g/100 g for control and altitude rats, respectively) and subgroup II (0.90±0.06 and 0.92±0.1 g/100 g for control and altitude rats, respectively). However, comparison of the EDL glycogen content in each subgroup between the two groups of animals showed no significant differences, except in subgroup I, the value was significantly lower in the altitude-acclimatized rats when comapred to the control rats ($P<0.05$).

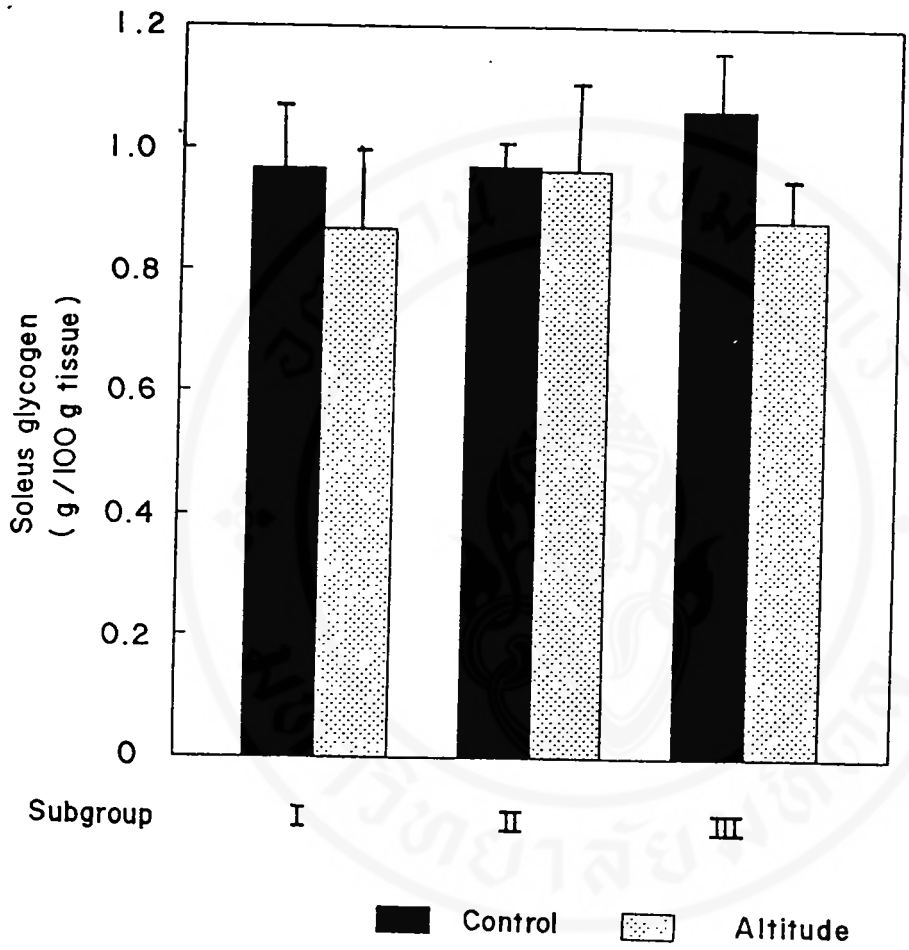


Figure 15. Soleus (SOL) glycogen content of the control rats and high-altitude acclimatized rats in subgroup I, II and III values are means±SEM.

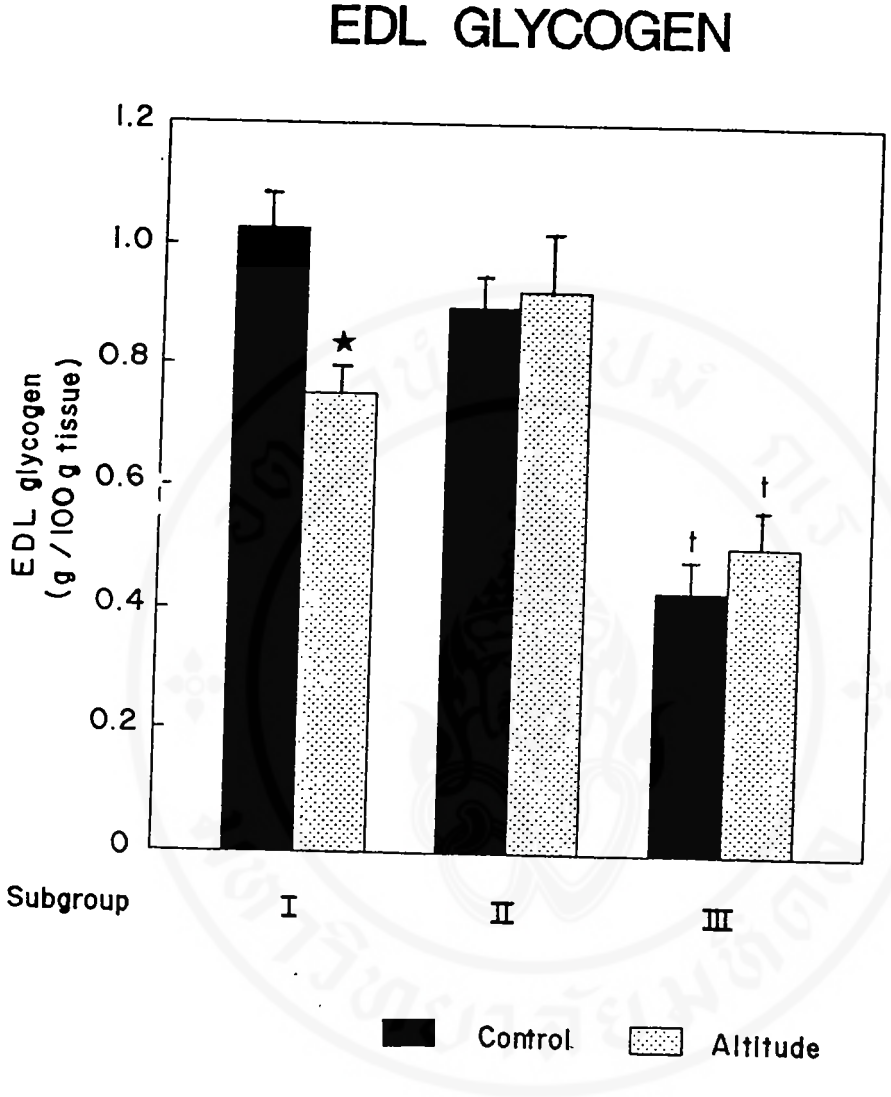


Figure 16. Extensor digiterum longus (EDL) glycogen content of the control rats and high-altitude acclimatized rats in subgroup I, II and III. Values are means±SEM.
★Significantly different from the control : $P<0.05$.
†Significantly different from subgroup I and II : $P<0.05$.

5. EFFECT OF HEMORRHAGIC SHOCK ON PLASMA TRIGLYCERIDE

The level of plasma triglyceride in subgroup III of each animal group is shown in Figure 17. The means±SEM at the initial of the experiment, during 40-60 min hypotension and 10 min after blood reinfusion in the control group were 52.6±5.66, 45.33±4.34, and 97.9±13.12 mg%, respectively, and in the altitude group were 39.4±4.04, 44.0±5.58, and 71.5±4.67 mg%, respectively. Plasma triglyceride was significantly increased at 10 min after blood reinfusion when compared to the values at initial and 40-60 min ($P<0.05$) in both groups. The values at the initial and 40-60 min of hemorrhagic hypotension in each animal group were not significantly different and there were also no significnt differences between the groups. However, the plasma triglyceride level at 10 min after blood reinfusion of the high-atltide rats was significantly lower than the control rats ($P<0.05$). The plasma triglycride level of the blood in the reservoir at the end of experiment in the control and altitude groups were 64.1±5.60 and 47.5±5.64 mg%, respectively, both of which were significantly lower than those corresponding levels in hemorrhaged animals infused with blood from the reservoirs ($P<0.05$).

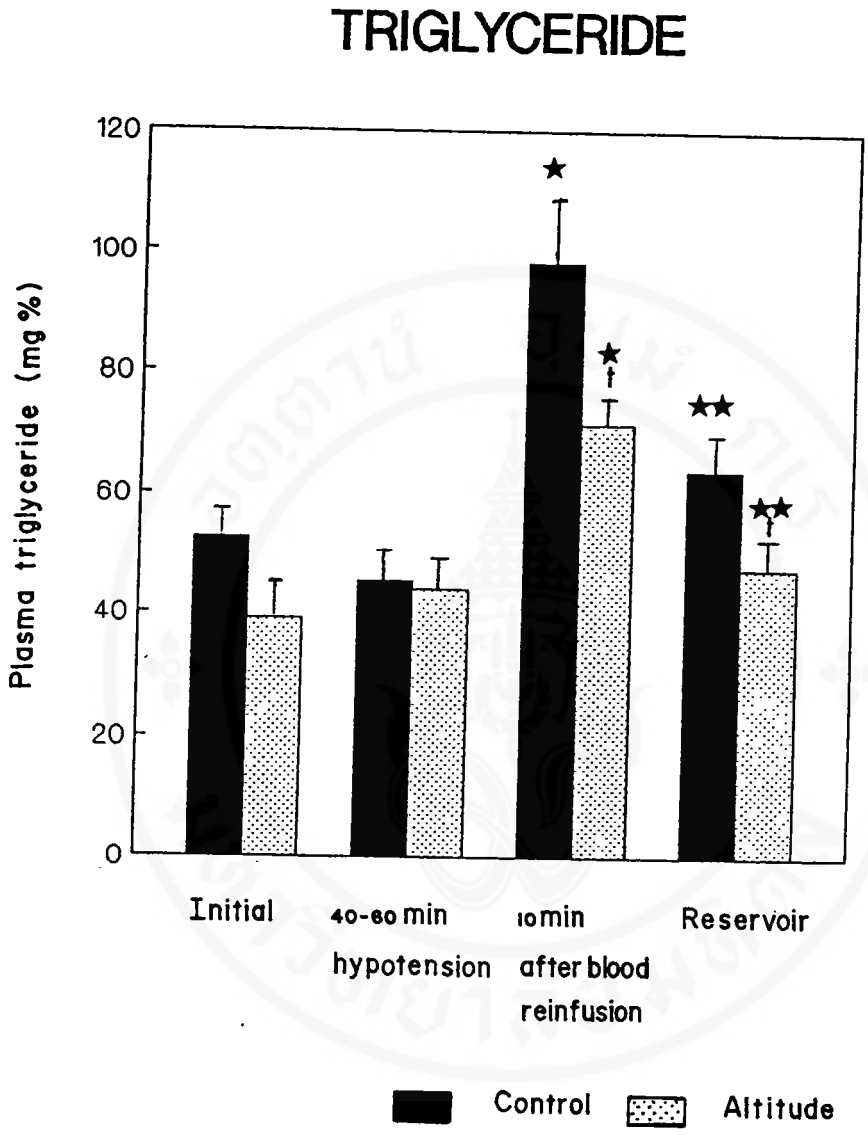


Figure 17. Plasma triglyceride of the control rats and high-altitude acclimatized rats at the initial, during 40-60 min of standardized hemorrhagic hypotension, at 10 min after blood reinfusion and of blood in the reservoir at the end of the experiment. Values are means±SEM.
†Significantly different from the control : $P<0.05$.
★Significantly different from the initial : $P<0.05$.
★★Significantly different from the 10 min after blood reinfusion : $P<0.05$.

CHAPTER V

DISCUSSION

Continuous exposure of rats to high altitude hypoxia at the level of 5,000 m in the present study caused body weight loss when compared with the control sea-level rats (Table 2). Many investigators have observed body weight loss in human and animals exposed to simulated altitudes of greater than 3,400 m (45,66). The extent of the loss in body weight varied considerably with the animal species and the level of altitudes (56,67). The data in this experiment confirmed various previous studies in rats (45,67). The body weight loss in rats at high altitude has been described to be attributed to dehydration, alterations in nutrient absorption and digestibility, disturbances in protein metabolism (negative nitrogen balance) and anorexia (53). Schnakenberg and coworkers (68) found in rats that body weight which was markedly decreased on the first day of altitude exposure increased sharply at the third day but still remained below the control level group throughout the period of altitude (14 days).

The increase in systemic arterial blood pressure in the high altitude acclimatized rat compared to the control rats found in this study confirmed previous investigations (65,69). Grover et al. (69) reported that such the rise in arterial blood pressure is due to an increase in systemic vascular resistance reflected from

the high levels of hematocrit which increased blood viscosity.

The greater initial blood withdrawal (IBW) and maximum blood loss (MBL) as well as the longer time to maximum blood loss (TMBL) found in the altitude rats compared to the control rats indicate a greater tolerance to hemorrhagic shock in the former group of animals. These results supported the previous reports of Cherdrungsi (64,65). The factors responsible for the increased hemorrhagic tolerance in altitude-acclimatized rats have been suggested to be due, in part, to a higher prehemorrhagic blood volume and a better arterial blood pressure regulation (64). Other factors responsible for this, however, are still unknown.

Prolonged exposure to high altitude hypoxia caused a large increase in hematocrit (53) which is believed to be the effect of a sustained increase in erythropoietin, the glycoprotein hormone that stimulates the formation of red blood cells. The rise in hematocrit at high altitude reflects a shrinkage of plasma volume and is accompanied by increased total blood volume (70). The development of polycythemia results in an increase in hemoglobin concentration which is potentially important in increasing the oxygen carrying capacity of blood.

Hemorrhagic shock is characterized by loss of red cell mass and intravascular volume which result in tissue ischemic, decreased cardiac output, and also reduced the mean arerial blood pressure. The stress imposed on the

animals by hemorrhage is met by a number of rapid or more gradual compensatory defense mechanisms aimed at restoring cardiovascular homeostasis by returning the mABP toward normal and keep up cardiac output. Recovery from hemorrhage by physiological compensatory mechanisms requires both red cell and plasma volume restoration. However, recruitment of red cells by increased erythropoesis is a slow process. Early compensation for hypovolemia is therefore mainly depending on expansion of the plasma volume (71) which results in hemodilution. Hemodilution occurs due to the transfer of fluid across cell and capillary membranes from intracellular and interstitial space into the vascular bed, following the sympathoadrenal discharge (increase peripheral vasoconstriction) and hyperosmolality, which associated with the drop in the hematocrit. Hemorrhagic hyperosmolality can play an important role in the cardiovascular compensatory during bleeding. One of the beneficial effects of hemorrhagic hyperosmolality is the transcapillary osmotic absorption of fluid from the extravascular space in different tissue. This increases the plasma volume and refills the circulatory system. In the present study, both groups of rats showed gradually increased hemodilution (Figure 10). The minimum hematocrit values were about 36 and 47% in the control and altitude-exposed rats, respectively. These values were about 71-74% of their initial levels.

With the lower hematocrit and accompanying decreased in oxygen carrying capacity, one might suspect that tissues would be suffering from oxygen deprivation. Fortune et al. (72) found that a hematocrit near 30% in patients following acute injury and hemorrhage is sufficient to maintain metabolic stability which means that the sufficient oxygen delivery can be provided at a lower hematocrit without impairing oxygen consumption or hemodynamic function. Jan and Chien (73) also showed that the oxygen consumptions in total body and in myocardium were essentially constant over a wide range of hematocrit (20-60%). Accordingly, the decreased hematocrit during hypotension in both groups of rats in this study should not account for their difference in hemorrhagic tolerance since the lowest hematocrit in both animal groups was remained greater than 30%.

It has been reported that the hyperglycemic response to hemorrhage is an important determinant of an animal's tolerance to hemorrhagic shock (74,75). It is known that loss of blood volume can lead to a fall in blood pressure via a reduction of the venous return. The sympathoadrenergic reaction in response to hypotension is initiated reflexly via baroreceptors in the carotid sinus and aortic arch. This regulatory mechanism is supported by the rise in blood sugar with the amount of blood withdrawn in hemorrhagic shock experiment (23,75). Glucose is released from the liver by glycogenolytic and gluconeogenesis via neural and hormonal reflexes

(catecholamines), leads to arterial plasma hyperosmolality and can evoke glucose-osmotic mechanism as described and indicated by Friedman and colleagues (62) that a rise of blood glucose is an important determinant of plasma refill. The mechanisms whereby glucose exerts these effects may involves its role as an osmotic agent and as substrate for energy metabolism. Holzrichter et al. (75) suggested that the rise in blood glucose in hemorrhagic shock increases with the amount of blood withdrawn per unit time and with the blood level of epinephrine which stimulates hepatic glycogenolytic and gluconeogenesis. Epinephrine is also tissue insulin resistance and suppressive effects on pancreatic islets (insulin insufficiency) (63), however the concentration gradient favoring transport of glucose into the cell is maintained. The altitude-exposed rats in the present study exhibited larger blood volume withdrawn and less blood volume reinfused during a 150 min period of constant hemorrhagic hypotension at 35 mmHg mABP when compared to the control while their plasma glucose level showed similar to that of the control (Figure 11). This might suggest that the larger blood volume loss in the altitude-exposed rats caused no more hemorrhagic stress in these animals than their control animals although the plasma levels of stress hormones such as epinephrine have not been measured in this study. The less reinfused blood volume and the longer time to maximal blood loss in the altitude rats with an equal plasma glucose level to that of the control

rats might support this suggestion of a lower stress of hemorrhagic shock in these altitude animals than their sea-level counterparts.

It has generally been assumed that lactic acidosis developed in shock is due to a decreased peripheral perfusion to tissues which leads to tissue hypoxia with an increased reliance on anaerobic metabolism. Glycolytic flux would therefore increase and pyruvate would be reduced to lactate rather than undergone to oxidation in tricarboxylic acid cycle. A direct relationship between blood lactate and mortality has been claimed in human patients by several clinical workers (76). In severe hemorrhagic patients the blood lactate levels rise from the normal values of about 1 mmol/L to 9 mmol/L or more (13). However, if at any time, excess lactate was above 4 mmol/L, the usually fatal outcome was not altered even by promptly reducing it (76). The lactate values in the present study showed that the blood lactate concentration in the control rats during hypotensive period until the end of the experiment was increased in higher rate than that of the altitude rats. This again suggests the more severity out come from hemorrhagic shock in the control animals when compared to the post-altitude animals.

The relationships between epinephrine, acidosis, and hemorrhagic resistance have also been reported. A previous study on standardized hemorrhagic shock has shown that adrenal gland is stimulated by acidosis to secrete epinephrine during periods of hypotension associated with

shock and that correction of the acidosis during the hemorrhagic hypotension could reduce the blood epinephrine levels, markedly improved cardiac function, reduced respiratory rate, increased arterial PO_2, and increased hemorrhage volume (77). Although, in the present study, arterial pH and blood epinephrine levels have not been determined during hemorrhage, the rise in blood glucose should reflect the increased sympatho-adrenal activity and the rise in blood lactate indicates the development of lactic acidosis in both animal groups. Since a greater volume of blood loss and a less requirement of blood volume reinfused to maintain mABP at 35 mmHg were found in the post-altitude rats which also exhibited less blood lactate levels compared to the control sea-level animals, this might suggest that less degree of acidosis was developed and contribute, in part, to the higher hemorrhagic tolerance in the post-altitude rats in the present study.

The resulting lactic acidemia could depress the myocardium and peripheral vascular responsiveness to catecholamines (13). The prolonged hyperlactatemia of shock reflects an imbalance between production and clearance. Erythrocytes and skeletal muscle are the chief sites of lactate production and that the liver, kidney and heart are involved in its removal from the blood. The liver has a large capacity for lactate uptake, if the oxygen delivery is adequate. Daniel et al. (21) showed an evidence that the major source of lactate in dog shock

came from muscle glycogen breakdown in anaerobiosis. In normal circumstances the glucose stored as glycogen in muscle is not released into the blood stream as free glucose and is available as fuel only to that muscle. However, the experimental in dog by Cucinell and coworkers (78) showed that if hemorrhagic shock is severe enough and with the decrease in hepatic venous PO_2 and pH (while arterial PO_2 was stable) liver has been implicated as a source of lactate production. They suggested that, in shock and acidosis, the liver changes from a lactate-using to a lactate-producing organ, hence, hepatic gluconeogenesis is impaired. In exercise at high altitude, Young et al.(79) found that blood lactate after exercise was lower in chronic hypoxic subjects when compared to the sea level group. Binn et al. (80) found that blood lactate after exercise in chronically hypoxia exposed subjects was not significantly changed from basal level and suggested that chronic exposure to hypoxia might include pH effects on rate-limiting enzymes of the glycolytic pathway such as phosphofructokinase, diminution in muscle glycogen and a greater use of free fatty acids. These evidences on exercise stress might go in parallel with the the results in this experiment on hemorrhagic stress which showed the lower blood lactate at any point of time of hemorrhagic shock in post-altitude group when comapred to the control group (Figure 12).

Liver and skeletal muscle metabolism are normally linked through a symbiotic metabolic relationship known as

the Cori cycle. By virtue of its mass, skeletal muscle is the major consumer of glucose in the body, oxidizing a portion to carbon dioxide and releasing a portion primarily as lactate, pyruvate, alanine, and glutamine into the blood stream. These products may then serve as gluconeogenic precursors for hepatic gluconeogenesis to replenish the glucose stores. However, the successful operation of this substrate cycle depends on maintenance of hepatic blood flow. and ATP supplies which are sufficient to support the metabolic demand. In the present study, the high blood lactate levels in hemorrhaged rats of both animal groups might result, at least in part, from the defect of hepatic gluconeogenesis, because of oxidative deprivation leads to mitochondrial dysfunction. From the lactate data in this study, however, it could not be suggested that the liver function of the altitude-exposed rats was less deteriorated by hemorrhage than that of the control rats eventhough they possessed a lower increased lactate levels when compared to the control rats since there might also be a possibility that lactate production from skeletal muscle and other tissues may be lower in the altitude-exposed group and this is still unknown.

The liver is one of the first organ systems to demonstrate loss of ATP in fasted animals during hemorrhagic shock (81). Pearce et al. (23) found that a depletion of hepatic levels of ATP, glcogen, and CP which occurred between the early compensatory and maximal

compensatory phase of shock, appeared at the time coincident with the most dramatic increase in plasma glucose and lactate and that the greatest increase in lactate, which occurs between these phases, appears at a time coincident with the loss of hepatic potential for lactate utilization. They also suggested that the very low ATP levels in the liver at the maximum compensatory phases and in decompensatory phases of shock would not allow significant lactate uptake or gluconeogenesis. Hift and Strawitz (82) in their studies of the dog showed that the onset of the irreversible stage of hemorrhagic shock was related to an elevation of plasma lactate which occurred coincident with depletion of hepatic glycogen and waning of the hyperglycemia implicating hepatic failure during hemorrhagic shock.

The former study done by Strawitz et al. (74) in fed and fasted rats suggested that by glucose reserves may influence the onset of irreversibility and that the ultimate death or survival of an animal in hemorrhagic shock is independent of its initial glucose reserves but seems some how to be related to its ability to maintain itself in a hyperglycemic state (i.e. animals which ultimately died the blood sugar levels were reduced; the rates of glucose loss were inversely proportional to survival time; the results were terminal hypoglycemia and near exhaustion of liver glycogen). This evidence was confirmed by a more recent study (Friedman et al., 1982) (62). In the present study, the pre-hemorrhagic hepatic

glycogen in high-altitude rats was lower significantly than the control rats (subgroups I and II, Figure 13) whereas, there was a higher resistance to hemorrhage (more IBW, MBL, and TMBL) in the altitude rats while the levels of blood glucose during hemorrhagic hypotension were not different (Figure 11,13). Thus, it may be assumed that the pre-hemorrhagic levels of hepatic glycogen in altitude-exposed rats did not determine their higher resistance to hemorrhage.

Observation that hepatic glycogen in subgroups I and II of the altitude-acclimatized rats in the present study was lower significantly than control rats confirms the previous report by Blume and Pace (56) who found that glycogen synthesis is enhanced at altitude despite the lower liver glycogen content which implies that hepatic glycogen turnover is generally increased at altitude.

The heart glycogen at the end of controlled hypotensive phase in the present study showed a significant increase from the zero times (subgroup I) in both altitude and control rats. If the coronary circulation is depressed the myocardium under ischemia and anoxia relies on anaerobic glycolysis of glucose and will utilize its energy stores of myocardial glycogen and may lead to heart failure. The prior to main festations of myocardial hypoxia, the early changes in myocardial metabolism are decreased uptake and oxidation of FFA and increased reliance on substrates of carbohydrate sources (36). This does appear to be confirmed by Wittnitch et

al. (83), who demonstrated clevated serum FFA after hypovolemia with significantly decreased glucose and insulin. But when they facilitated the glucose uptake by high insulin level, they found an increase in myocardial glycogen in hemorrhagic shock. However, in the present study the same high blood glucose was maintained throughout the experiment in both groups of rats, it might be assumed that high glucose level could enhance the level of myocardial glycogen. These data did not determine the different tolerance to hemorrhagic shock in these animals in the control and the altitude groups because there was no difference in heart glycogen in both groups at the end of the hemorrhagic experiment. In addition, it should be suggested that at 35 mmHg of hemorrhagic hypotension, the hearts of the rats in both groups can maintain normal function by FFA utilization for 150 min, and since hyperglycemia was presented, the heart could uptake glucose to increase storage of glycogen.

However, in subgroup I, the heart glycogen of altitude-exposed rats was lower significant than those of the control rats. This might be due to the action of epinephrine on glycogenolytic action which may induces carbohydrate metabolism by glycolysis.

The changes in glycogen content in SOL and EDL muscle in this study were in different patterns. Glycogen in SOL muscle in both groups subjected to hemorrhagic shock were not different from the non-hemorrhagic state. This is in agreement with a previous study of Pearce et al.

(84) who have observed that SOL did not mobilize glycogen anaerobically and also did not contribute to the observed lactic acidemia because of no changes in high energy phosphate and glycogen levels in this muscle. In addition, Jennische et al. (24) have found no changes in ATP and CP in SOL muscle while the decline of ATP and CP have been observed in gastrocnemius (fast contracting muscle) concomittant with the increases in G-6-P and lactate and suggested that during hypovolemia slow contracting muscle appear to be protected from early hypoxic damage, probably due to a redistribution of skeletal muscle blood flow. Their studies support the present experiment which showed that glycogen content in EDL muscle in both groups were declined in hypovolemic hypotension state reflecting the predominant of anaerobic glycolysis. The hyperglycemic levels are not susceptible for glucose uptake by muscle because of epinephrine mediated insulin resistance (6) and because, during hypovelemic shock, less blood supply occurs at skeletal muscle and metabolic profile of EDL favors the glycolytic process, these evidence might support the decrease in EDL glycogen.

The decrease in EDL glycogen was correlated to the increase in arterial blood lactate ($r=-0.5$) (figure 18) in all animals of both groups. This might suggest that the high blood lactate levels were reflected from fast twitch muscle glycogen breakdown. Since there were no correlations between blood lactate levels and the liver or

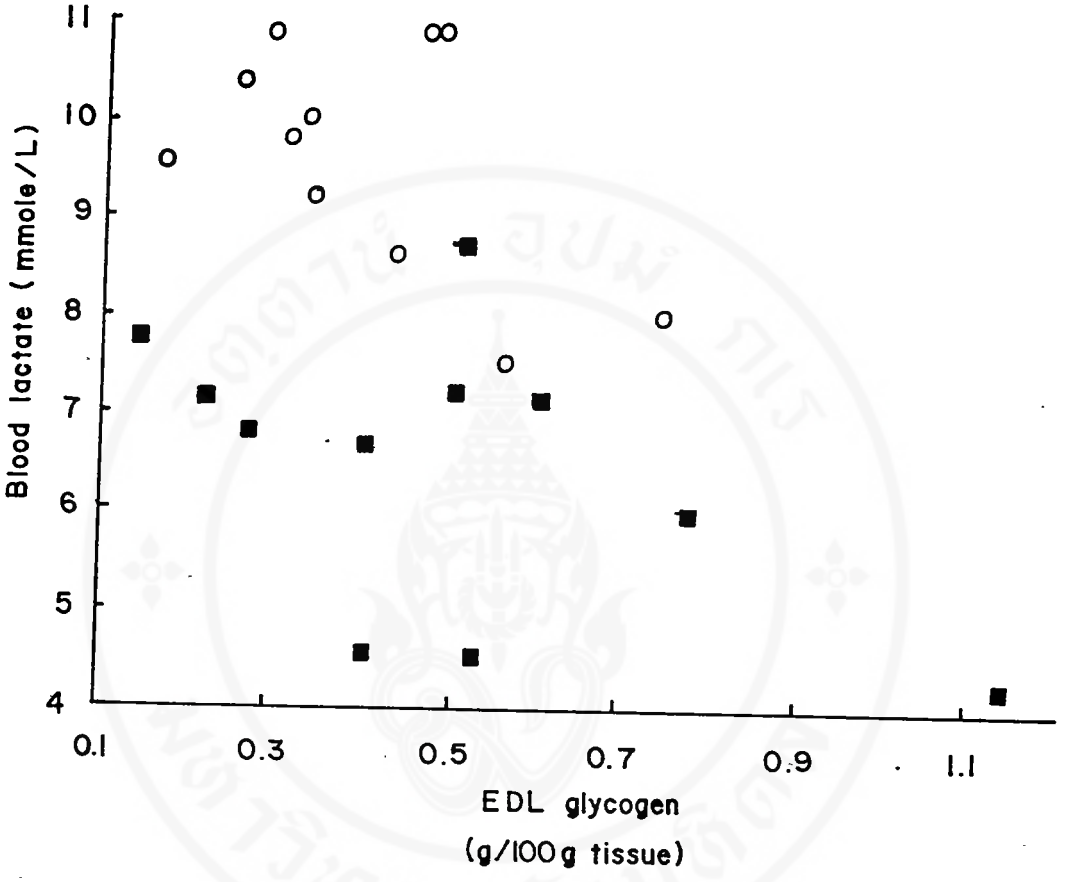


Figure 18. Correlation between blood lactate concentrations and the EDL glycogen content at the end of the hemorrhagic shock experiment for all rat studied; 11 control rats (o) and 11 high altitude acclimatized rats (■), r = -0.5.

myocardial glycogen content, it could be explained that the liver glycogen had been depleted before the increased blood lactate was found at the end of the experiment, and that the liver glycogen was almostly brokendown to blood glucose during compensatory phase of shock. The other parameters of liver such as the lactate level in hepatic vein should be measured, because during the decompensatory phase of shock, the liver can produce lactate, instead of consuming. In contrast, the heart, in this study, showed an increase in glycogen, not depletion, so the heart may uptake lactate during hypotension in this experiment.

High blood catecholamine levels following hemorrhagic stress (neurohormonal response) stimulate lipolysis with the release of triglycerides from adipose tissues. The released triglycerides are prompty brokendown to FFA and glycerol by lipase in compensatory phase (11). In the present study, the plasma levels of triglycerides in both groups were increased by hemorrhage and the plasma level of triglyceride after blood reinfusion in altitude rats was lower than that of the control rats. This might suggest that during hemorrhage the altitude-acclimatized rats relied greater on FFA metabolism when compared with the control rats. Kashyap et al. (85) reported that induced hemorrhagic hypotension in dogs results in a significant rise in arterial free fatty acids concentration reflecting a decrease in FFA utilization. However, if impaired glucose utilization is compounded with a fall in circulating FFA following

hemorrhage, the resulting fuel failure could have an adverse effect on organ or tissue function.

During hemorrhagic shock , the tissue of the body especially the peripheral tissues can survive by anaerobically depending on their carbohydrate reserves such as glycogen (2) and the survival time of the shock animal depend on the level of blood glucose (62). Therefore, the lower pre-hemorrhagic liver glycogen level found in altitude rats in this study cannot account for the higher resistance of these animals to hemorrhagic shock. In addition, the blood glocose level during shock and the glycogen content in the liver, the heart, and the skeletal muscles (SOL and EDL) were not different between animal groups, but only the blood lactate levels of the altitude rats showed lower significantly than those of the control rats. The hemorrhagic tolerance indices, theIBW, MBL, and TMBL, of the animals in both groups in this study were found to be inversely correlated significantly with the blood lactate levels after 150 min hypotension [r=-0.76for IBW (Figure 19), r=-0.57 for MBL (Figure 20) and r=-0.49 for TMBL (not shown), respectively]. Significant correlation between TMBL and the blood lactate level during 40-60 min hypotension (r=-0.57) was also observed.Accordingly , it is concluded that the higher tolerance to hemorrhagic hypotension in the high-altitude acclimatized rats than the control rats was not attributed to the levels of hyperglycemia induced by hemorrhage or to the initial and the post-hemorrhagic

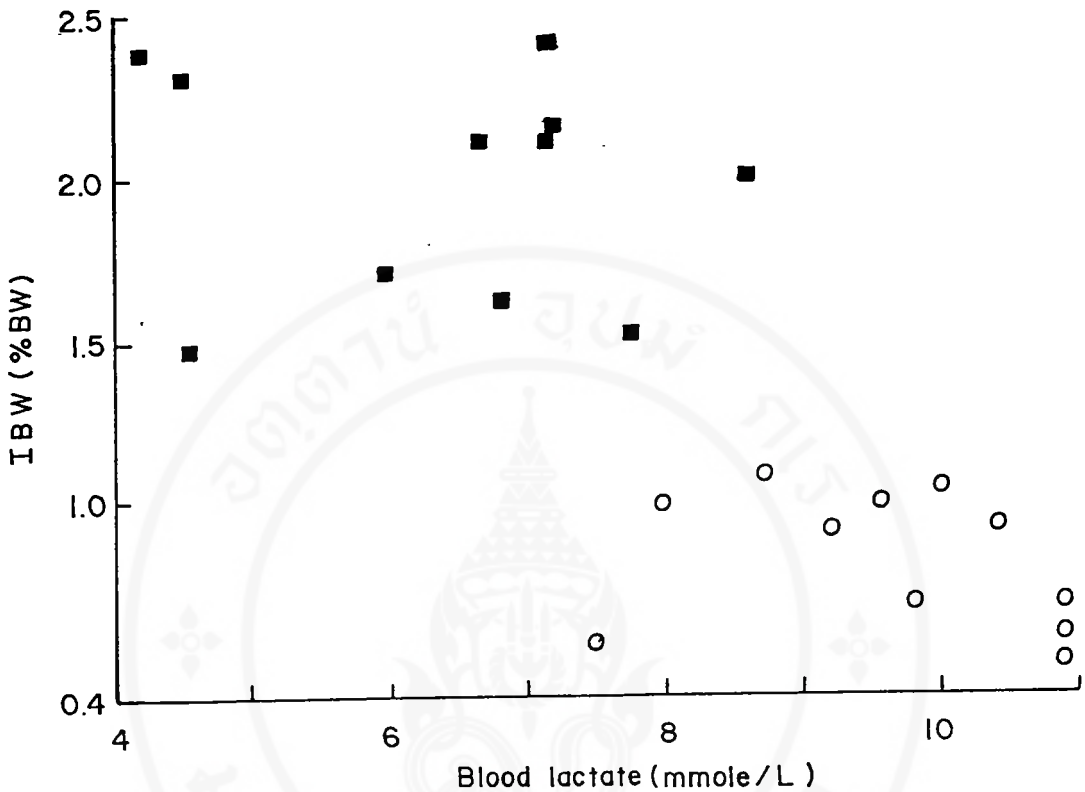


Figure 19 Correlation between initial blood withdrawal and blood lactate concentration at 10 min after blood reinfusion for all the rats studied ; 11 for controls (O) and altitude-acclimatized rats (■), r =-0.76.

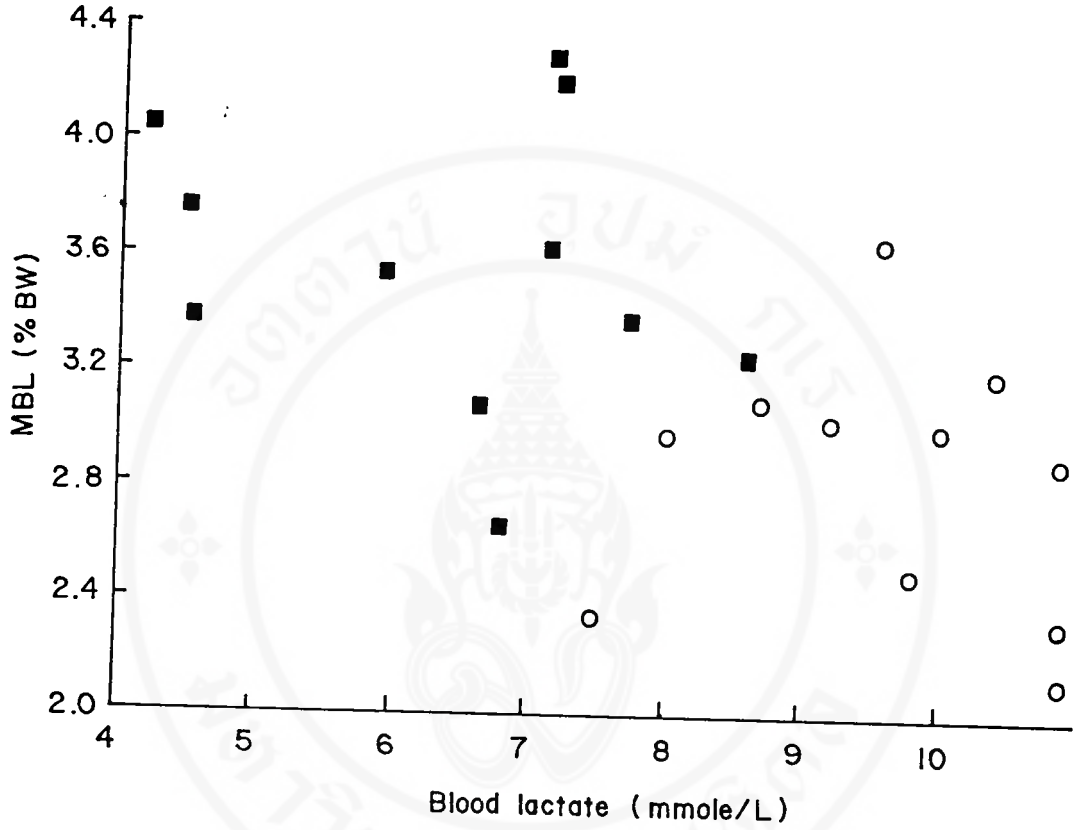


Figure 20 Correlation between maximum blood loss and blood lactate concentration at 10 min after blood reinfusion for all the rats studied ; 11 controls (o) and 11 high-altitude acclimatized rats (■), r =-0.57

levels of glycogen (indication of the carbohydrate reserves) in the liver and skeletal muscles of the two animal groups. Such the difference in hemorrhagic tolerance is associated with the blood levels of lactate which reflect the development of acidosis and the extent of tissue ischemia during hemorrhagic shock.

CHAPTER VI

CONCLUSION

1. Rats chronically exposed to high altitude possessed higher IBW, MBL, and longer TMBL, with a lower reinfused blood volume required to maintain mABP at 35 mmHg at sea level than the sea level control rats indicating that the high altitude-acclimatized rats could tolerate hemorrhagic shock better than the control rats.

2. The glycogen content was found to be depleted in liver , reduced in EDL , increased in myocardium , and not changed in SOL by hemorrhagic shock and these post-hemorrhagic glycogen levels were similar in both animal groups. In contrast, blood lactate concentration was significantly higher in the control rats than that of the altitude rats.

3. The higher tolerance to hemorrhagic hypotension in the high-altitude acclimatized rats than the control rats was not attributed to the levels of hyperglycemia induced by hemorrhage or the initial and the post-hemorrhagic levels of glycogen in the liver and skeletal muscle the two animal groups. Such a difference in hemorrhagic tolerance is associated with the blood levels of lactate which reflect the development of acidosis and the extent of tissue ischemic during hemorrhagic shock.

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APPENDIX I

BLOOD LACTATE DETERMINATION

(Analox Lactate II Analyzer)

Principle

The assay is a simple one-step procedure, which oxygen oxidoreductase (LOD) catalyses the oxidation of L-lactate to pyruvate.

$$\text{L-Lactate} + O_2 \xrightarrow[\text{pH 6.5}]{\text{LOD}} \text{Pyruvate} + H_2O_2$$

LOD is highly specific for L-lactate, reacting with neither D-lactate nor pyruvate and is catalase free. Buffered reagent is entrained into the analyser and the reaction is initiated by injection a 7.0 µl standard or sample. The maximum rate of oxygen consumption is directly related to lactate concentration.

Reagents : Lactate II Reagents No. GMRD-090 (Analox Instrument, U.S.A.).

a.	Lactate Reagent Buffer Solution	70 ml
b.	Lactate Oxidase Enzyme	1 vial
c.	Lactate Standard	3 ml

Reagent Preparation

1. Bring required reagents to room temperature.

2. Add buffer solution to oxidase enzyme using the plastic pipette provided and mix gently. When dissolved, transfer to buffer bulk and wash in residue. Where appropriate transfer to a clean 250 ml reagent reservoir and attach to analyser.
3. The reagents should be stored at 0-5°C. The working life of reagent stored is at least 2 months.

Equipment : Analox Analyser, Model GM-7, Analox Instruments Ltd., U.S.A.

Procedure :

1. Single point calibration at 8.0 mmole/L is recommended.
2. Check stability by analysing the standard as a sample. If satisfactory, proceed with analysis of the test samples.
3. After use, excess reagent should be stored at 0-5°C. The working life of reagent stored in this way is at least 2 months.

Reference :

Analox Instruments. No. GMRD-090, Revised October (1989).

APPENDIX II

DETERMINATION OF PLASMA GLUCOSE

Reagents :

1. Solution A

   Dissolve a capsule of PGO enzyme (Sigma, stock No. 510-6) in 100 ml distilled water. Stable for one week at 0-5° C

2. Solution B

   Reconstitute O-Dianisidine dihydrochloride vial (Sigma, stock No. 510-50) with 20 ml distilled water. stable at 0-5° C for 3 months.

3. Solution C

   Add 1.6 ml of solution A to 100 ml of solution B and mix (freshly prepared).

4. Glucose standard solution (Sigma, Stock No. 635-100)

   Contains a solution of β-D-Glucose 100 mg% in benzoic acid 0.01%. Store at 0-5° C.

Procedure :

1. Pipette 0.025 ml of plasma sample, and glucose standard solution (100 mg%) into the tubes.
2. Add 5.0 ml of solution C into each tube and mix thoroughly.
3. Incubate at 37° C for 30 min.

4. Read the optical density at 450 nm by spectronic-20 against the reagent blank within 30 min.

Calculation

Plasma glucose concentration (mg%)

$$= \frac{\text{O.D. unknown}}{\text{O.D. standard}} \times 100$$

References :

Raabo E, Terkilsen TC. On the enzymatic determination of blood glucose. Scan J Lab Invest. 1970; 12: 402.

Sigma Technical Bulletin. No. 510, Revised October, 1989.

APPENDIX III

DETERMINATION OF PLASMA TRIGLYCERIDES

(Glycerol-3-phosphate oxidase-P-chlorophenol coloring method)

Principle

Serum triglycerides are decomposed to glycerol and free fatty acids by lipoprotein lipase. The glycerol thus produced is converted to glycerol-3-phosphate by glycerol kinase in the presence of ATP. This glycerol-3-phosphate is then oxidized by glycerol-3-phosphate oxidase to yield hydrogen peroxide.

The hydrogen peroxide thus produced yields a red color compound upon oxidative condensation with p-chlorophenol and 4-aminoantipyrine in the presence of peroxidase.

Reagents :

1. Buffer solution, each contains 0.05 M Tris buffer solution, pH 7.5 and 0.07% p-chlorophenol (Wako Pure Chemical Industries).
2. Color reagent (Wako Pure Chemical Industries).
3. Color reagent solution.

   Dissolve the entire contents of one bottle of color reagent in 105 ml of Buffer solution. Stable for one week at 2-10° C.

4. Triglycerides standard 300 mg% (Wako Pure Chemical Industries) contains 0.0312% Glycerin, 0.05% Sodium azide.

Procedure :

1. Pipette 20 µl of sample, and standard into the appropriate test tube. Add nothing for blank.
2. Pipette 3.0 ml of color reagent solution to all tubes, including the blank.
3. Thoroughly mix tube contents.
4. Incubate at 37°C for 15 min.
5. Read optical density at 505 nm against the blank tube within one hour.

Calculation :

Triglycerides Concentration in Plasma (mg%)

$$= \frac{\text{O.D. unknown}}{\text{O.D. standard}} \times \text{Concentration of standard}$$

Reference :

Wako Pure Chemical Industries, Osaka, Japan.

APPENDIX IV

DETERMINATION OF GLYCOGEN

Reagents :

1. 1 N Sodium Hydroxide
2. 1 N Perchloric acid
3. 95% Ethanol containing 0.1% Lithium Chloride
4. 80% Ethanol containing 0.1% Lithium Chloride
5. Anthrone reagent
   a. 280 ml distilled water and 720 ml of highest purity concentrated H_2SO_4
   b. 500 mg Anthrone
   c. 30 g Thiourea

   Store the reagent in the dark under refrigeration
6. Glucose standard
   a. Stock solution

      Dissolve 100 mg of dry, highest purity glucose in 100 ml of saturated benzoic acid.
   b. Working standard

      Pipette 5 ml of stock solution and make up to 100 ml with saturated benzoic acid.

Procedure

1. Animal tissue samples were excised and fixed immediately in liquid Nitrogen.
2. The samples were then weighed on a precision torsion balance approximately 200 mg and

transferred into a 15 ml graduated centrifuge tube containing 3 ml 1 N NaOH.

3. Place the tubes in a boiling water bath for 45 min and shake occasionally to facilitate disintegration. Fit each tube with an air condenser.
4. Place the tubes in a cold water bath and cool to room temperature
5. Add 6 ml 1 N $HClO_4$ to each tube and adjust the final volume to 10 ml with distilled water.
6. Shake mixture vigorously, allow to stand for 15 min, and filter through acid-washed filter paper (Whatman No. 541).
7. Place 1 ml of each filtrate in a 15 ml centrifuge tube; add 2 ml 95% EtOH containing 0.1% LiCl.
8. Agitate the tubes to mix the content thoroughly, then stopper and incubate at 37°C for 6 h.
9. Centrifuge the tube at 4000 rpm for 10 min. Decant the supernatants carefully and allow the tubes to drain at a 45 angle for 10 min.
10. Add 5 ml of 80% EtOH containing 0.1% LiCl to each tube and centrifuge the mixture again for 5 min. Discard the supernatants and drain the tube as above for 10 min (9).
11. The glycogen is dissolved in 2.0 ml distilled water. For the blank, uses 2.0 ml distilled water and 0.1 mg glucose in 2.0 ml saturated benzoic acid for a standard.

12. Add 10 ml anthrone reagent and mixed the contents thoroughly.

13. Fit each tube with air condenser. Immerse the tubes in boiling water bath for 15 min and cool to room temperature.

14. Read the absorbance at 620 nm.

Calculation :

Glycogen content in the tissue (mg glycogen/100 gm tissue)

$$= \frac{\text{O.D. unknown}}{\text{O.D. standard}} \times 0.1 \times 10 \times \frac{100}{\text{gm tissue}} \times 0.9$$

Reference :

Roe JH, Dailey RE. Determination of glycogen with the anthrone reagent. Anal Biochem 1966; 15: 245-250.