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The Effects of Exercise Induced Blood Acidity on The Albuminuria Responses of Trained Male Athletes

by

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Bachelor of Physical and Health Education
For Partial Fulfillment of the Requirements
For the Degree of Master of Science (KIN)

OTTAWA, Ontario, 1984

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ABSTRACT

Albuminuria, which has been defined as an abnormal increase in the urinary output of the plasma protein albumin, has become a common observation in the urine of subjects participating in severe physical activity. Identifying the processes which have been altered by the stimulus of exercise may be of extreme importance in attempting to explain the observance of post-exercise outputs of albumin.

In order for the albumin molecule to cross the glomerular capillary wall (GCW) both the size- and charge-selective features of the filter must be overcome. Theoretically, exercise stress can increase the concentration of circulating cations within the blood. These cations may then bind to the negatively-charged, carboxyl terminus of the GCW's glycoproteins. This consequently eliminates the charge-restrictive features of the GCW and allows the albumin molecule to enter the endothelial fenestrae.

Explanations regarding the further movement of albumin across the size-restrictive GBM are unclear. However, it has been suggested that the capillary lumen to Bowman's space hydrostatic pressure gradients, and albumin molecule reptation will be predominant influences in the transglomerular flow of albumin.

Presently, no single factor has been isolated as the most important precursor to the post-exercise increase in albuminuria.

The purpose of this study was to compare the albuminuria responses of trained male athletes to blood acidity alterations induced by two separate bicycle ergometer tests.

Fourteen healthy male subjects completed a continuous bicycle ergometer test (workload=75% VO2 max for 42 min) and an intermittent bicycle ergometer test (workload=100% VO2 max for 16 x 2 min work periods).

Blood and urine samples were collected pre-, post- and one hour post-exercise, and analyzed for changes in blood pH, blood lactate, urine albumin and urine total protein.

The results of this study indicate that the intermittent work test did not induce a significantly different physiological stress than the continuous work test as indicated by post-hoc evaluation of the dependent variables blood pH, blood lactate, urine albumin and urine total protein.

The significant relationships between post-exercise urine albumin and exercise-induced blood acidity which have been reported in previous similar studies was not observed as a general trend in the results of this study. However, relationships between blood acidity and total proteinuria were observed when specific regression equations were used to isolate and evaluate expected physiological effects.

The results of this study suggest that further research of a more comprehensive nature may be required in order to clearly illustrate the mechanisms which are responsible for the post-exercise urinary outputs of proteins.

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ACKNOWLEDGEMENTS

The author wishes to express his sincere appreciation to the following persons for their guidance and assistance in the completion of this paper.

Dr. M. Booth, Ph.D., Department of Kinanthropology
Dr. L. Peterson, Ph.D., School of Nephrology
Dr. F. Reardon, Ph.D., Department of Kinanthropology
Dr. A. Reed, Ph.D., Department of Kinanthropology
Dr. J. Thoden, Ph.D., Department of Kinanthropology
Mrs. P. Lok, Department of Kinanthropology
Mrs. J. Landriault-Goudreau, Department of Kinanthropology

Chapter I

THE PROBLEM

1.1 INTRODUCTION

The observation of increased concentrations of albumin in urine has been referred to as albuminuria (Boylan 1979, Hardwicke 1975). The occurrence of this anomaly in post-exercise urine samples has been commonly reported for subjects participating in strenuous physical activity (Alyea and Parish 1958, Castenfors 1977, Gardner 1956, Poortmans and Haralambie 1979). Some authors have suggested that the albuminuria condition is the result of exercise-induced changes in the physical structure of the glomerular filtration apparatus, while other researchers have proposed the theory that the condition is caused by pathologic changes in the capillary wall (Poortmans,1972). Still others have reported post-exercise albuminuria to be simply the result of a transient, exercise-induced alteration in the relationship between blood acidity and the normal processes of glomerular filtration (Cantone and Cerrettelli, 1960, Todorvic et al 1972). According to Zambraski et al (1981), the exact mechanisms which cause post-exercise albuminuria have not yet been determined. However, most studies which have pursued

the cause of albuminuria from an exercise point of view suggest that: a) the condition of post-exercise albuminuria is a transient phenomenon, b) mechanical trauma and pathologic degradation is not an apparent consequence of exercise, c) exercise has been observed to alter both blood acidity levels and albuminuria. Therefore, based on these suggestions the primary interests of this research study were to compare the effects of two different work protocols on blood acidity and proteinuria, and to measure the suggested relationship between exercise-induced blood acidity changes and the post-exercise increases in albuminuria within each work test used.

1.2 RATIONALE FOR THE STUDY

Identifying the processes which have been altered by the stimulus of exercise may be of extreme importance in attempting to explain the observance of post-exercise urinary outputs of albumin. Venkatachalam and Rennke (1978) have suggested that, within the glomerulus of the kidney there exist both molecular size- and charge-restrictive features which impede the transglomerular passage of the plasma protein albumin. Theoretically, exercise stress can increase the concentration of circulating cations within the blood (Refsum and Stromme, 1975). These cations may then bind to the negatively-charged, carboxyl terminus of the glycoproteins. This would consequently eliminate the charge-re-

strictive feature of the capillary wall and allow the albumin molecule to enter the endothelial fenestrae. Explanations regarding the movement of the albumin molecule beyond the size-restrictive glomerular basement membrane are unclear. Castenfors (1977) has suggested the "stretched pore phenomenon", whereby molecules are forced through the elastic-like pores of the glomerular basement membrane by a hydrostatic pressure gradient. This pressure gradient is located between the low-pressure, epithelial Bowman's space and the high-pressure, endothelial, capillary lumen. A major discrepancy in this theory exists in that there are no fixed pores located within the glomerular basement membrane (Kefalides 1969).

Ryan's discussion (1981) helps to clarify the underlying principles of Castenfors' theory. Although the hydrostatic pressure gradient may be effective in influencing the transglomerular passage of the albumin molecule, one must consider that the molecule is not a rigid sphere travelling through fixed pore structures as suggested by Deen (1979). According to Ryan, some molecules have the ability to undergo a process of "reptation", which refers to the structural change of a molecule attempting to cross an uncharged, pseudocollagenous membrane. Although this process has not yet been identified with regard to the transglomerular passage of albumin, its importance as a potential precursor to post-exercise proteinuria should be acknowledged.

The stimulus of exercise in creating a change in blood cation concentration has been determined through blood lactate and blood pH measurements. Although previous research by Todorvic (1972) has supported the theory that an apparent relationship exists between blood cation concentrations and albuminuria, no significant relationship between exercise-induced increases in blood cation concentrations and albuminuria has yet been identified. As such, this study had attempted to measure the apparent relationship between blood acidity changes and albuminuria, through physiologically relevant correlation and multiple regression equation techniques.

Measurements of the hydrostatic pressure gradient and its relationship to the transglomerular passage of the albumin molecule were not performed in this study, based on the observations and recommendations of Tietz (1970), who has stated that tests of renal clearance (a measurement of intraglomerular activity) are cumbersome and physically uncomfortable for human subjects. However, it was assumed that the effects of renal blood flow and the hydrostatic pressure gradient would be controlled by the duration and intensity of the exercise tests, as suggested by Delgado et al 1975, Brenner and Rector 1978, Kachadorian and Johnston 1970.

1.3 STATEMENT OF THE PROBLEM

The purpose of this research study was to compare the relative exercise-induced alterations in blood pH (BpH) and blood lactate (BL) with the urinary output of total proteins (TP) and albumin (UA) in trained male athletes participating in separate intermittent and continuous work tests.

The study had three objectives: to compare the changes in the four dependent variables (BpH, BL, TP, and UA) in response to the two separate bicycle ergometer work tests; to compare the differences between each dependent variable at the three collection times in each work test; to measure the possible relationships between exercise-induced blood acidity and albuminuria.

1.4 LIMITATIONS OF THE STUDY

The subject population was made up of club rugby players from the Bytown Blues Rugby Football Club, Ottawa, and fourth year Kinanthropology and Physical Education students attending the University of Ottawa.

Each of the subjects was required to report to the human performance laboratory at the University of Ottawa no fewer than three times during the course of the study.

The design of the study allowed for the subjects to act as their own controls through the measurements of pre- versus post-exercise results within the group.

All of the subjects tested had been participating in some type of fitness training program on a regular basis, either related to rugby or dry land training for hockey. The mean VO2 maximum value for the rugby players was 54 ml/kg.min +/- 4.

While the renal blood flow and the glomerular filtration rate were not measured, it was expected that they would be controlled at relatively the same level in all subjects because the duration and the intensity of exercise was chosen in relation to each subject's VO2 maximum value.

The recommended analytical techniques of radioimmunoassay for urine albumin and total proteins were not used in this study because of the inaccessibility of the necessary materials and equipment to the researcher. Therefore the spectrophotometric analysis of freeze dried urine samples, as suggested by Pierre Pilot of The University of Ottawa, Department of Biochemistry, was used.

1.5 DEFINITION OF TERMINOLOGY AND ABBREVIATIONS

BCG dye — Bromcresol Green dye

Clearance — Referring to the quantitative evaluation of a molecule being filtered or reabsorbed by the kidney. The clearance of a substance (Cx) is equal to the concentration of the substance in urine multiplied by the urine volume in ml/min and then divided by the concentration of the substance in the plasma.

Colloidal Osmotic Pressure
: Refers to the osmotic pressure gradient of the plasma in the capillary bed. This pressure is created by the plasma proteins which are restricted from crossing the glomerular capillary wall. The effect of maintaining a large concentration of plasma proteins within the capillary lumen is magnified by the principle of Donnan's equilibrium.

conditional relationship
: The terms conditional relationship and conditional significance have been used in the Results and Discussion chapter of this thesis. These refer to the specific conditions upon which the statistical models were used test. For example, regression equations were used to isolate the effects of specific exercise on a specific physiological parameter.

Donnan's Equilibrium
: Refers to the anionic to cationic electrical balance occurring within a capillary bed. The anionically natured plasma proteins which are repelled by the capillary wall are neutralized by cationically natured molecules (primarily sodium) to maintain an electrical neutrality within the capillary lumen.

GBM — Glomerular basement membrane

GCW — Glomerular capillary wall

GF — Glomerular filtrate

GFA — Glomerular filtration apparatus

GFR — Glomerular filtration rate

Glycoproteins
: low molecular weight amino-carbohydrate molecules.

Macromolecules
: substances which have a molecular weight

greater than sixty thousand daltons, and a molecular radius of 3.5 nanometers or larger.

N-acetylneuraminic Acid (Sialic Acid) is a glycoprotein which forms the foundation of larger proteoglycan structures.

RBF Renal blood flow

Chapter II

REVIEW OF LITERATURE

2.1 PART A: THE RENAL FILTRATION SYSTEM

2.1.1 Function of The System

The renal filtration system is made up of a complex network of arteries, filtering units (nephrons) and veins. According to Brenner and Rector (1976), the renal filtration system handles approximately 500 ml of blood per minute or ten per cent of the total cardiac output. Therefore, one of over two and a half million similarly functioning filtering units or nephrons handles approximately 0.0002 milliliters of blood per minute. Blood is transported to each of the nephrons according to the following scheme; oxygenated blood flows from the abdominal aorta to the renal arteries. The renal arteries divide into a ventral branch and a dorsal branch as they form the interlobar arteries. The interlobar arteries in turn feed the arcuate arteries. The interlobular arteries which originate from the arcuate arteries supply the afferent arterioles of the renal corpuscle. The renal corpuscle is more commonly referred to as the glomerulus of the nephron.

The cellular composition of the macula densa gives rise to an important feature in the anatomical structure of the afferent arteriole as it enters the glomerulus of the nephron. Bing and Kazimierczak (1960) observed that the cells of the afferent arteriole, which lie adjacent to the juxtaglomerular apparatus, contain the vasoregulatory hormone renin. The release of renin by the macula densa, as discussed by Brenner and Rector (1978), initiates the formation of the inactive molecule angiotensin I. However, within the structure described by Bing and Kazimierczak as the periglomerular region of the afferent arteriole or the macula densa, the molecule angiotensin I is converted to the active vasoconstricting chemical angiotensin II. Brenner and Rector have suggested that one potential initiator for the release of renin by the cells of the periglomerular region is through an increase in renal blood flow, which apparently stimulates the baroreceptors located in the afferent arterioles. This release of renin and the formation of the vasoconstricting chemical angiotensin II supports the autoregulatory function of angiotensin II to maintain a normal renal blood pressure and a normal GFR, even though cardiac output has increased.

Meanwhile, the fluid which is filtered through the glomerular capillary wall is referred to as the glomerular filtrate. The glomerular filtrate leaves the Bowman's space and enters first the proximal convoluted tubule and is then

passed on through the three segments of the loop of Henle before entering the distal tubule. Within the proximal tubule, loop of Henle and distal tubule system the glomerular filtrate is transformed into urine. This occurs through the processes of ionic exchange and fluid reabsorption. The fluid, which has become urine, leaves the distal tubule and travels via the collecting tubule to the ureter en route to the urinary bladder. The blood which entered the glomerular capillary via the afferent arteriole flows out of the capillary through the efferent arteriole after it has been filtered. This freshly filtered blood is then routed to a second set of microcapillaries, the peritubular capillaries (Crouch, 1978). The purpose of the peritubular capillaries is to allow the blood to reabsorb some of the necessary fluid which was given up to the glomerular filtrate.

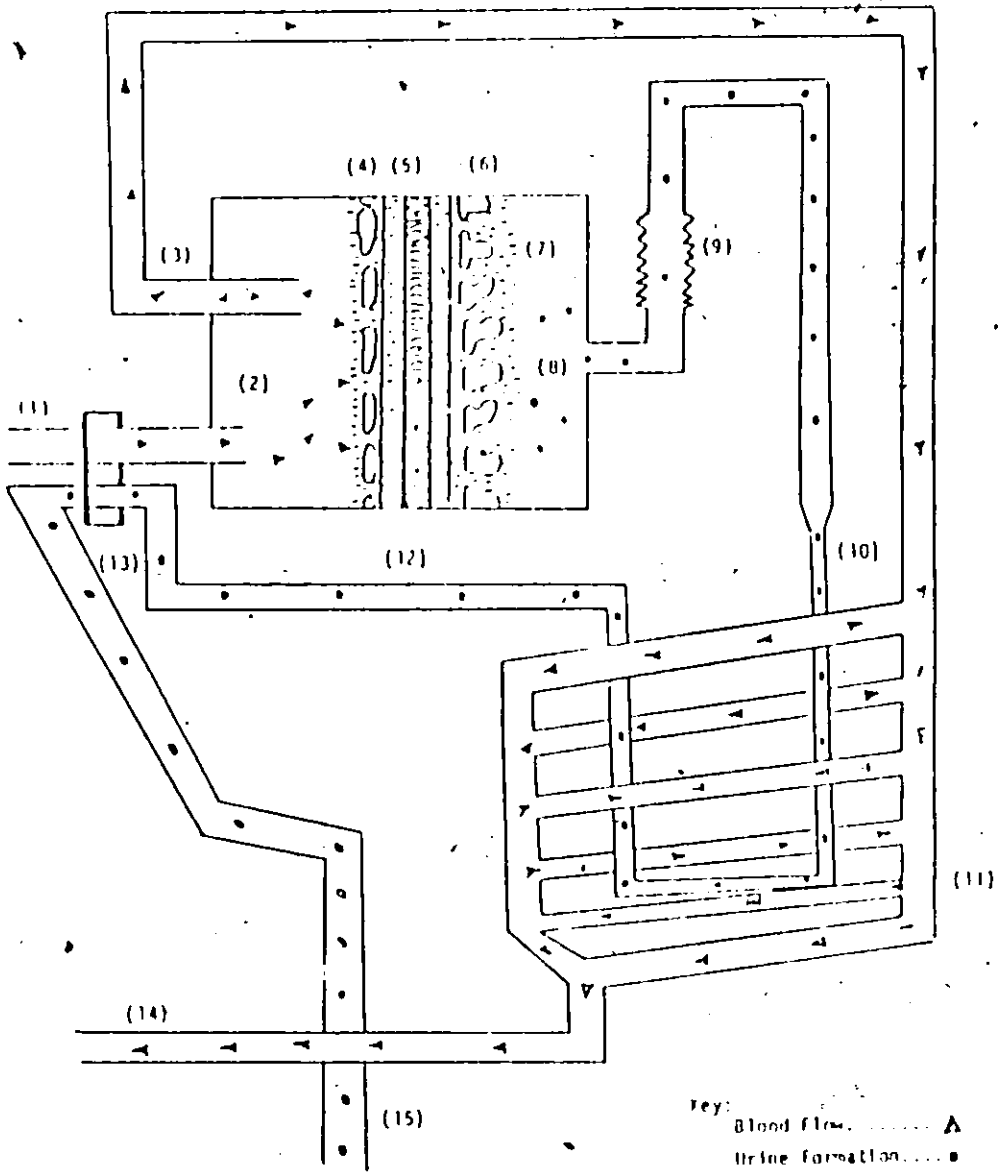
Also included in the filtration of blood and the formation of urine are the vasa recta. The vasa recta are appendages of the peritubular capillary system and are situated around the loops of Henle. The vasa recta are important to the further reabsorption of fluid and the concentrating of urine. The arcuate vein exports as much as ninety percent of the blood which entered the renal filtration system originally (Bing and Kazimierczak 1960, Brenner and Rector 1976, Ganong 1979, Crouch 1978, Guyton 1976). Diagram 1 on page 14 presents a simplified scheme of the function of a nephron and the formation of urine.

2.1.2 The Structure of The Glomerular Capillary Wall

The glomerular capillary wall (GCW) is composed of three different layers. The endothelium is the innermost layer, while the glomerular basement membrane (GBM) is centrally located and the epithelium protrudes outward into the Bowman's space. The middle layer, or the GBM, should be considered as a continuous structure, while the epithelial and endothelial layers lie intermittently along the respective inner and outer walls of the GBM. The separations between the endothelial cells are called fenestrae. The fenestrae are rather large openings with respect to filtration pore size (approximately 70 nanometers apart, Brenner and Rector 1978) and thereby create the potential for macromolecules equivalent in size to albumin to flow easily across the endothelial layer. However, an electrostatic barrier which has been located throughout the GCW functions to repel any anionically natured molecules away from the endothelial fenestrae. The anionic nature of the glomerular capillary wall results from the presence of the sialoprotein constituent N-acetylneuraminic acid, which appears to be located on both the endothelial and epithelial sides of the wall.

As was mentioned, the cells of the epithelium protrude outward into the space of the Bowman's capsule. These protrusions are referred to as "foot processes" and the spaces which exist between these protruding cells are referred to

as "slit pores" (Crouch, 1978). The slit pores are also coated with the glycoprotein, sialic acid. The size of the slit pore is smaller than the size of the fenestrae and as such may present both a physical- and an electrical-restriction to the macromolecule's transglomerular movement (Kanwar and Farquhar 1980, Ganong 1979, Crouch 1978, Venkatachalam and Rennke 1978, Guyton 1976, Jones 1969, Osawa et al 1966). The filtration layers of the GCW wall have been presented in Diagram 2 on page 15.



Simplified Scheme of a Functioning Nephron

(Venkatachalam and Rennke, 1978; Guyton, 1976)

Index Of Features In Diagram 1:
"Simplified Scheme of A Functioning Nephron"

(1) Afferent Arteriole
(2) Glomerular Capillary Space
(3) Efferent Arteriole
(4) Fenestrated Endothelium of Glomerular Capillary Wall
(5) Pseudo-Collagenous Glomerular Basement Membrane
(6) Podocyte Cells of the Epithelial Layer of the GCW
(7) Negative Charges, or Anionically Natured Glycoproteins
(8) The Bowman's Space
(9) Proximal Convoluted Tubule, Carries Glomerular Filtrate
(10) Beginning of Loop of Henle as it enters Peri-glomerular Network of renal concentrating, and dilution activities
(11) The Capillary Plexus as it surrounds the Loop of Henle
(12) Portion of the Distal Tubule
(13) Location of the Juxtaglomerular Apparatus
(14) Arcuate Vein
(15) Glomerular Filtrate transferred to Urine Collecting Duct

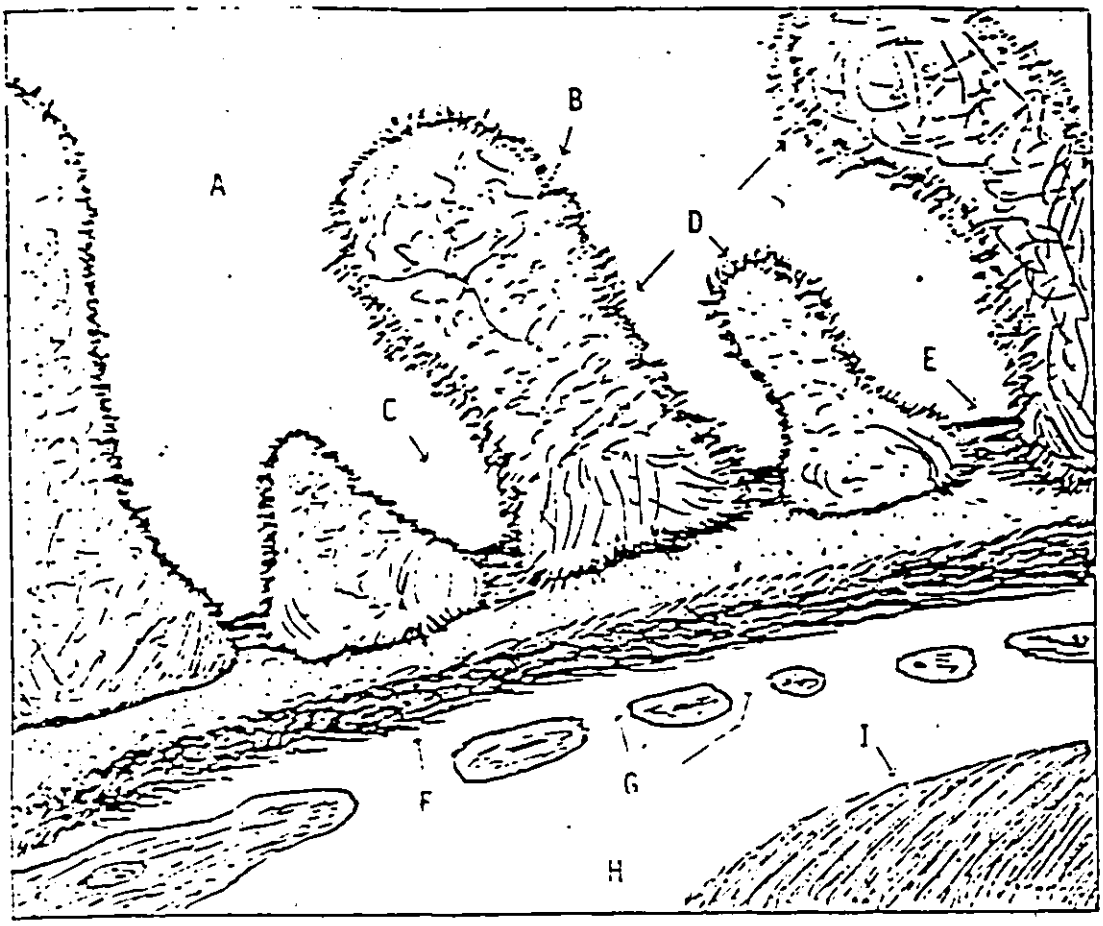


Diagram 2: Tracing of "An Electron Micrograph of A Rat Glomerulus", presented by Brenner and Rector, (page 17, 1976); (the original photo has been magnified 120,000 times normal).

Legend:

(A) Bowman's Space
(B) Podocyte Cells of the Epithelium
(C) Slit Pores of the Podocyte Cells
(D) Glycoproteins of the membrane, easily stained
(E) Lamina Rara Externa of the Glomerular Basement Membrane
(F) Pseudo-Collagenous Glomerular Basement Membrane
(G) Fenestra of the Endothelial Cells
(H) Capillary Space
(I) Portion of a macromolecular Erythrocyte

2.2 PART B: SUGGESTION FOR THE PERMSELECTIVE NATURE OF THE GCW

Various researchers have attempted to attribute an assortment of maladies of the GCW to being the cause of proteinuria. Bennett et al (1976) have abbreviated the possibilities for the transcapillary movement of albumin to either of the following categories: sieving properties with respect to molecular size; filtration as a function of hemodynamic principles; or macromolecular charge as compared to the charge of the glomerular capillary wall.

2.2.1 The Charge Selective Feature

Two of the more important inhibitors to the passive movement of albumin across the GCW are pore-size and electrical charge. The charge-selective feature of the GCW arises from the presence of the glycoprotein sialic acid which has been located on both the endothelial and epithelial layers of the GCW (Zambraski et al 1981, Andrews 1979, Venkatachalam and Rennke 1978, Mohos and Skoza 1970, Jones 1969, Kefalides 1969).

Venkatachalam and Rennke (1978) performed in depth analyses of the GCW using cationic staining. Their results have revealed that the carboxyl residues of the sialic acid molecule create the polyanionic nature of the GCW. In an attempt to identify and classify the glomerular filtering

mechanisms, the authors have developed the following model of their observations of electrical charges throughout the GCW. The endothelium was found to contain a large concentration of polyanionic substances located in the cell coat which accounted for a strong negative charge. The GBM was subdivided into the lamina rara interna, the lamina densa and the lamina rara externa. The lamina rara interna and externa were both found to contain a large polyanionic substrate concentration leading to a strong negative charge. However, the lamina densa which is made up of non-polar, collagenous type fibers provided a weak negative charge. Finally, the outer layer or the epithelial layer was observed to contain a large concentration of polyanionic substances in both the cell coat of the epithelium and the coating of the epithelial foot processes, thereby displaying a strong negative charge. Earlier research by Jones (1969), reported the existence of a differentiation between the layers of the lamina rara externa and interna versus the lamina densa in their ability to be stained by cationic substances. As well, Jones discussed the presence of the glycoprotein sialic acid within the slit pores of the podocyte cells, which enhanced the anionic nature of the outer layer of the GCW. Jones also mentioned that the anionic nature of the GCW was a result of the carboxyl components of the sialic acid layer.

The most convincing evidence of the glycoprotein component's ability to inhibit the transport of molecules across the GCW has been through the studies involving clearance rates of neutral versus charged particles. Studies of fractional clearance rates involving negatively-charged dextran sulfate by Bennett et al (1976) and Chang et al (1975) support the effectiveness of the GCW's charge-selective barrier. Both studies found there to be greater GCW fractional clearance values for the neutral dextran molecules versus the anionic dextran sulfate molecules. The authors preferred the use of nonprotein dextran molecules because of their similarity to albumin in size as well as their ability to be converted to anionically-natured substances. Chang et al, stated that the plasma protein albumin becomes polyanionic when it occurs in physiological solutions. Likewise, Chang reported to have observed cation binding activity in the area of the GCW'S epithelial, podocyte cell slit pores. The authors explained that the sialoglycoprotein coating may be neutralized through the binding with cationically-natured substances, which would led to a reduction of the GCW's charge-selective barrier. In the experiments of Brenner et al, the authors found the amount of cationic substances which crossed the GCW to be of a greater volume than both the neutral- and negatively-charged substances. The conclusions of Brenner were similar to those of Chang, in that these researchers also felt that the direct interaction be-

tween the sialoglycoprotein layer and the cationic test molecules created a neutralization of the carboxyl groups of the sialic acid.

The importance of the sialoglycoprotein layer has been discussed in studies involving the diseased GCW where there has been a reduction in the concentration of this charge-selective barrier through pathological circumstances. The Bennett et al study observed an increase in the clearance of anionically-natured dextran sulfate through the diseased GCW. Blau and Haas (1973) also found that an inverse relationship existed between GCW sialic acid concentrations and proteinuria. Urine, which was relatively free of albumin, was associated with subjects whose renal biopsy results confirmed the presence of a large concentration of GCW sialic acid, as measured through cationic staining. Likewise, those subjects who were considered severely proteinuric displayed poorly stained renal biopsies, implying that the absence of sialic acid led to a poor reaction between the GCW and the cationically-natured, colloidal iron stain. The experiments of Brenner et al (1978) also used cationic stains, which provided similar results. In the discussions by both Blau and Haas, and Brenner et al the authors mentioned the potential morphologic changes associated with a loss of GCW sialic acid. The primary area of concern for such changes was the slit pore filter of the epithelial podocyte layer. Blau and Haas were unable to distinguish whether the loss of

sialic acid created the changes in this area or whether the changes in the ultrastructure of the slit pores caused a loss of the sialoglycoprotein coating. Brenner et al referred to this morphologic change as "foot process fusion". The development of such a condition was determined to be the result of a decrease in sialic acid concentration, the latter was replaced by a continuous layer of uncharged epithelial cells. The loss of this charge-selective feature was reflected by an increase in albuminuria. These results provide convincing evidence that pathologic conditions which result in the loss of the negativity of the GCW through the reduction of its anionic glycoprotein coating enhance the transcapillary passage of albumin (Blau and Haas 1973, Chang et al 1975, Bennett et al 1976, Brenner et al 1978).

2.2.2 The Size Selective Feature

The GCW's impermeability to macromolecules based on filtration through selective pore size has been well documented (Maack et al 1979, Deen et al 1979, Venkatachalam and Rennke 1978, Kefalides 1969). Interestingly, observations of the GCW using microscopic techniques have reported the existence of spaces such as the endothelial fenestrae and the epithelial slit pores, yet researchers have been unable to locate fixed pathways or pores across the glomerular basement membrane. Kefalides (1969) described the GBM as a continuous layer of glycoproteins bound to a collagenous component. He

mentioned that the collagenous layer should not be considered synonymous with mature collagen fibers, as the uniqueness of this pseudocollagen layer allows for uninhibited interaction with the glycoprotein component. The author stated that one of the functions of the GBM was to provide elasticity to the filtration wall. This elasticity would not only create continuous support for the expanding Bowman's capsule but it would also be active in the specific selection of molecules to be filtered. If mature, rigid collagen fibers were to develop, then the size-permselectivity of the GBM would be eliminated.

Venkatachalam and Rennke (1978), and Deen, Bohrer, Robertson and Brenner (1977) observed an inverse relationship between the glomerular filtration rate and molecular radii. An attempt to explain this inverse relationship was made by Deen, Bohrer and Brenner (1979) using the principles of a pore theory model. However, in order to maintain the underlying mathematical concepts on which the theory was designed, the model allowed for few modifications in its adaptation to true physiological conditions. At best the model should be considered strictly theoretical. Two major requirements for the model were to assume that all pores were rigid cylinders situated at right angles to the flow of the liquid, and that all molecules were fixed uniform spheres, each displaying neutral electrical properties. In histochemical analyses of the GCW such conditions have not been

reported, nor are they consistent with the previously mentioned properties of the pseudocollagenous GBM or the anionically-natured GCW.

2.3 PART C: EXERCISE AND PROTEINURIA

According to Alyea and Parish (1958), two of the most important variables to be considered in the development of a post-exercise increase in albuminuria are the duration and the intensity of the exercise bout. Using within group comparisons of swimmers competing in events of varying distances, the authors concluded that, "the greater the intensity and the duration of the work bout, the larger will be the concentration of albumin in the post-exercise urine sample". The work intensities for the subject's examined by Alyea and Parish were not reported as a work/time relationship; instead the authors classed swimmers and runners into two groups: those who competed in events of fifteen hundred meters or longer, and those who competed in events of "short distances" ranging from fifty meters to four hundred meters. Alyea and Parish also compared the observations of red blood cell volume, hyaline casts and protein in the urine of contact and noncontact athletes. Their results showed that post-exercise urine albumin concentrations had increased in greater than seventy percent of all athletes tested. The two important features of the Alyea and Parish study, which should be recognized, are: that all measurements for albumi-

nuria were based on a 0 to 4+ qualitative scale; and that proteinuria may be a transient phenomenon, since normal proteinuria values had been observed in the twenty-four hour post-exercise sample. The results of the blood and urine analyses showed that the group of subjects having the highest proteinuria values was the noncontact long-distance runners. Thus, the authors had suggested that the effect may have been a consequence of posture and/or mechanical trauma. The possibility that mechanical trauma was the major cause of proteinuria, although very likely, remains difficult to measure in the asymptomatic human subject. Therefore, any diagnosis of mechanical trauma-induced exercise proteinuria should be considered at the very least strictly subjective. However, the duration and the intensity of the activity are extremely important to the development of the transient condition of exercise-induced proteinuria. Differences in the exercise duration and intensity may initiate specific changes in renal blood flow, hydrogen ion production and mechanical trauma to the renal system.

The three causes of exercise-induced proteinuria as suggested by Javitt and Miller (1952) were renal anoxia, increased blood and urine acidity, and a decreased reabsorption of proteins by the post-glomerular tubules. The experimental methodology involved subjects performing either a long treadmill run of 12-22 min at 9.0 to 10.0 mph at 0% grade or a short treadmill run of 3-5 min at 9.0-10.0 mph at

a 17.5% grade. The authors observed that the proteinuria values were the highest during the first 5 min post-exercise for all subjects tested. Most importantly, the authors reported that the blood and urine pH values remained low during the same post-exercise time period. This observation indicated to the authors that an apparent relationship between blood and urine acidity and proteinuria may exist. Interestingly, the values collected for the long runs did not differ significantly from those collected for the short runs. As well, Javitt and Miller reported a direct correlation between a decrease in blood pH and an increase in the concentration of urinary proteins. However, the authors were unsure of the effects which renal ischemia had on the development of proteinuria and suggested that the transglomerular passage of macromolecules was related to changes in blood acidity. Although Javitt and Miller mentioned that the increases in proteinuria were directly related to the changes in blood acidity, they were unable to correctly explain the effect which increases in blood acidity had on the biochemical properties of the GCW. The authors had suggested that there was a washing out of the intercellular cement between the cells of the GCW, which had created an increased permeability in the nature of the GFA. Later studies, which identified specific glycoproteins bound to the glomerular capillary wall, support the Javitt and Miller theory that blood acidity may be a precursor to the transglomerular pas-

sage of macromolecules. However, the ability for exercise-induced blood acidity to create a morphological change would be unlikely, since exercise-induced proteinuria should be considered as a transient-phenomenon and not a pathological condition.

Taylor (1960) reported that no increase in the urinary output of albumin or plasma proteins was observed in those subjects analyzed while performing a 15 min walk on a treadmill at 5 mph, yet the post-exercise urine samples were found to contain increased levels of albumin. Taylor suggested that such results did not indicate that circulating metabolites affected the glomerular filtration process. He felt that the post-exercise urine samples should have displayed the same characteristics as the urine samples taken during exercise. However, it would appear that Taylor neglected to consider the important function of the renal buffering systems, which would be effective in reducing the hydrogen ion concentration overload associated with exercise. The author concluded that the increased post-exercise albuminuria resulted directly from renal blood flow changes, most specifically a reduction in renal blood volume, and an increased vasoconstriction which had occurred following exercise. Taylor's review of exercise-induced proteinuria concluded that the most important aspect of albuminuria was the ability of the macromolecule albumin to overcome the size restrictive features of the GCW. Taylor attributed the suc-

cess of this activity to post-exercise renal vasoconstriction based on the observation that vasodilating drugs such as caffeine did not induce post-exercise proteinuria.

According to Poortmans (1972), the type of protein found in the urine may be specific to the anomalie occurring within the kidney. The author mentioned that the observation of increased levels of low-molecular weight proteins in urine do not reflect the extent of the GCW's filtration capability, based on the principle that proteins such as Lysozome, which has molecular weight of 15,000 daltons, are small enough to pass freely through the glomerular filtration apparatus. Therefore the presence of low-molecular weight proteinuria will not suggest that any problems exist in normal GCW activity. However, large volumes of low-molecular weight proteins in urine may indicate that problems exist in the tubular reabsorption mechanisms (Ravsknov, 1975). In view of this principle, investigations of low-molecular weight proteinuria were not within the scope of this review. This research study was much more concerned with the effects of exercise on the GCW's ability to filter macromolecules such as the plasma protein albumin.

2.3.1 Exercise and Changes in the GCW

Early research by Chesley et al (1939) found that vasoconstriction of the renal blood vessels initiated plasma

protein leakage across the GCW and into the Bowman's space. The authors used the cold pressor test of Hines and Brown to create the vasoconstriction response. The procedure of the test was to place the subject's hand in ice cold water and monitor the changes in pulse pressure. Chesley observed no relationship between the changes in blood pressure and the resulting changes in concentrations of protein measured in urine. However, there was a definite decrease in urinary fluid volume following the vasoconstriction test, primarily as a result of reduced renal blood fow through the constricted capillaries. More important though, was the observation that no proteinuria was measured until after the vasoconstriction had been eliminated. This prompted the authors to conclude that protein leakage only occurred after normal renal blood flow was restored.

The discussion of Chesley et al supports the hypothesis that the two restrictive factors of electrical charge and molecular size were partially reduced during renal vasoconstriction. Cationically-natured substances, such as sodium, potassium or magnesium, circulating in the area of the endothelial glycoproteins may have caused an electrical neutralization of the outer portion of the GCW, thereby allowing albumin to collect in the endothelial fenestra. The increased pressure created by the vasoconstriction of the glomerular capillaries would then force the albumin into the elastic GBM. The observation of post-experimental proteinu-

ria has been explained by Poortmans to be a washing-out of the trapped macromolecules as a result of the return to normal filtration flow (Castenfors and Piscator, 1967).

The GBM has been classified as a non-uniform structure which varies greatly in width (Osawa et al, 1966), therefore the time-course for this transient, proteinuria phenomenon is dependent upon the time taken for the macromolecules to cross the widest portion of the GBM. Castenfors and Piscator (1967) refer to the proteinuric condition observed following the cold pressor test as being catecholamine-induced, the active mechanisms being similar to exercise-induced proteinuria in that both are initiated by renal vasoconstriction.

Movement of macromolecules across the GCW becomes physically easier, and has been reported to occur more often, in persons who displayed damaged glomerular basement membranes as measured by renal biopsy techniques. Hardwicke (1975), and West (1966), concluded that a degeneration of the GBM through either glomerulosclerosis or glomerulonephritis caused marked increases in the transglomerular passage of plasma proteins. Morgenesen and Vittinghus (1975) reported that the GBM of diabetic patients underwent developmental changes early in their juvenile years. The authors compared post-exercise albuminuria measurements in normal versus diabetic subjects and found a statistically significant differ-

ence between the two subject populations. The exercise bouts consisted of riding a bicycle ergometer at the below-maximum workloads of 450 and 600 kpm/min. These work load intensities which may be classified as relatively low, caused proteinuria in the diabetic subject. Such results indicated that the time required for the movement of albumin across the GBM was reduced through the reduction in the width of the GBM. Morgensen and Vittinghus concluded that the need to maintain a constant glomerular filtration rate induced a rise in the single nephron filtration fraction, which consequently led to a rise in the movement of proteins across the damaged GCW. According to Bennett et al (1975), pathological conditions which may affect the GCW by affecting the pore size, the electrical charge or the normal renal filtration flow, will also be responsible for determining the movement of albumin across the GCW. The authors stated that consideration must be given to the type of maladie which is active when estimating the proteinuria values. As an example, they discussed the variances in neutral dextran clearance measurements for rats experiencing different types of glomerulonephritis. Interestingly, the values reported suggest that certain restrictive features attain varied levels of importance depending on the type of pathological condition.

Kleiman (1960) introduced the term "athlete's kidney" to classify the condition of renal trauma caused by prolonged

exposure to exercise. The condition was defined as the development of renal lesions leading to a pathogenic deformity of the kidney, thus producing measurements of post-exercise proteinuria in those subjects affected. Kleiman observed the incidence of athlete's-kidney to be greatest in professional boxers, followed by professional basketball players, professional hockey players and professional wrestlers. There are two important questions which result from the research of Kleiman: was renal trauma an effective causal agent in reducing the protein restrictive filtration features through the development of a deformed kidney? and do the sports observed provide measurements of orthostatically induced proteinuria, in which case the principles of renal vasoconstriction would become the predominant factors for instigating the proteinuria condition?

2.3.2 Renal Blood Flow Responses During Exercise

Kachadorian and Johnston (1970) used three 'standard treadmill exercise' bouts at rates of 5.6, 8.0 and 10.5 km/h to compare creatine clearance values, urine acidity measurements, and proteinuria. They noticed that only under the severe exercise stress of 10.5 km/h (or 6.5 mph which was considered to be a maximal workload) was there a marked increase in proteinuria and urine acidity. The authors reported that the glomerular filtration rate, as measured with a standardized technique, did not appear to be affected by

the steady state or submaximal workloads of 5.6 and 8.0 km/h respectively. These results imply that by exercising at a low intensity, normal renal blood flow during the activity is maintained and both the proteinuria and urine acidity responses are considerably lower. Delgado et al (1975) concluded from their research on dogs, which ran on a treadmill for between 20 and 25 min at the varied workload of between 3.0 and 8.0 mph with grades of between 8.0% and 15.0%, that no change in renal blood flow had occurred. The reduced cardiac output to the kidneys was compensated for by the overall increase in cardiac output associated with these exercise intensities. Therefore, although some renal vasoconstriction must have occurred, the authors suggested that it was not enough to affect either the GFR or the total RBF. Delgado stated that alterations in exercising renal blood flow were species specific and that, although they did not notice any exercise-induced modifications to the normal distribution of RBF in the canine kidney, changes in human RBF may be more apparent. Cadnapaphornchai et al (1981) supported this statement, as they observed that rats and dogs displayed inverse vascular responses to the administration of prostaglandins. This inability to transfer the results of RBF measurements from one species to another has lead to discrepancies between the animal model and the actual human response. The findings of Delgado did, however, suggest that changes in renal plasma fluid volume may occur during severe

exercise and in turn may affect the colloidal osmotic pressure within the filtering units, thereby potentiating a reduction in GFR.

The results of Kachadorian and Johnston, and Delgado et al, contradict the observations of many authors who have described considerable decreases in renal plasma flow during varied exercise intensities. Chapman et al (1948) reported an inverse relationship between renal plasma flow and workload intensity, suggesting that higher workloads may reduce the normal GFR. The exercise protocol for the Chapman study involved three runs of approximately 15 min on a motorized treadmill at the steady state intensities of 3.0 mph at 0.0% grade, 3.0 mph with 5.0% grade, and 3.5 mph with 10.0% grade. The authors noticed a slower recovery rate in the RBF values as compared to heart rate and blood pressure recovery. According to Refsum and Stromme (1975), who tested twenty one males involved in a 70 km ski race (completion time c. 5.47 hours, work rate c. 12-13 km/h), the reduced RBF which acompanied exercise also diminished the GFR and inhibited the urine concentrating processes. Further, the authors stated that although filtration of fluids was reduced, the tubular reabsorption of certain metabolites such as sodium was either held contant or increased, thereby reducing the secretion of these metabolites in the urine and negating their potential effect on urine acidity. Castenfors (1977), Castenfors (1967), Castenfors and Piscator

(1967) and Castenfors, Mossfeldt and Piscator (1967) all provided evidence that RBF decreased during exercise. According to Castenfors (1977), RBF as measured by evaluating GFR decreased approximately 30% during continuous, exhaustive exercise. Likewise, urine secretion of sodium was reduced as the result of increased tubular reabsorption, while hydrogen ion excretion was found to increase. Castenfors remarked on the inability to precisely define the cause of increased proteinuria during heavy exercise. He suggested that the increased glomerular permeability could be attributed to increases in body temperature, renal vasoconstriction and changes in the single nephron filtration fraction.

Castenfors (1977) used the principles of the "stretched pore phenomenon" to describe the resulting proteinuria from the increased pressure of renal vasoconstriction. The author explained that, since albumin has a molecular weight of 69,000 daltons and a molecular size of approximately 36 Angstroms (3.6 nanometers), "it is just slightly larger than the pore size of the GCW" (Castenfors, 1977). Under the condition of renal vasoconstriction, the albumin molecule is forced into the endothelial fenestra. The increasing pressure of the constricted renal capillaries forces the oversized macromolecule into the elastic-like GBM. Although this theory may explain the mechanisms for overcoming the size-restrictive feature of the GCW, it does not explain how the charge-selective barrier was overcome. This theory as-

sumes that all albumin molecules are similarily shaped, neutral spheres, of comparable weight and size, and that all pores are cylindrical, elastic tubules with the characteristic flexibility to allow macromolecular passage of the GCW.

As previously stated, researchers have as yet been unable to isolate an exact cause for the post-exercise increase in albuminuria. However, this review of the literature suggests that the causal mechanisms will involve the apparent relationship between the decrease in pH, as a result of an increase in circulating cations, and the neutralization of the glycoprotein constituent of the GCW. As well, some consideration should be given to the exercise-induced changes in RBF and the (capillary lumen to Bowman's space) pressure relationship which is essential to the transglomerular movement of molecules.

2.3.3 Reviewing the Activity of Lactate During Exercise

Investigations into some of the biochemical adaptations to exercise by Holloszy et al (1977), have suggested that the duration and intensity of the activity were important in affecting such parameters as blood flow, muscle glycogen depletion, fatty acid oxidation and blood lactic acid concentrations. The authors stated that the duration of time for which a subject can perform at a given intensity is dependent upon the individual's physiological energy supply. As

such, an inverse relationship between work intensity and work duration has been accepted. Therefore, to increase an individual's ability to perform at a greater work intensity for longer periods of time, the subjects must increase their physiological energy production capacity. This may be accomplished through exercise-programs which involve endurance training protocols.

Christensen et al (1960) investigated the responses of heart rate, pulmonary ventilation and blood lactate concentrations to the schedules of intermittent and continuous work. In the Christensen study the work intensity was similar for both situations, the subjects ran on a treadmill at 20 km/h. The authors mentioned that in establishing the schedules for the intermittent and continuous work experiments the most important consideration was the work duration, in that the ability to maintain a specific duration may be inhibited by the continuous depletion of the physiological energy stores and the progressive increase in circulating metabolic waste products.

Results of the measurements of blood lactate for the intermittent work bouts were directly affected by the ratio of work to rest time. The blood lactate concentrations were higher when the work to rest ratios were 3 to 1 as compared to the work to rest ratios of 1 to 1. In the case of the continuous exercise schedule the activity period was de-

scribed as "continuous at a high intensity". As such, the subjects could only work for a short period of time before reaching exhaustion. The post-exercise concentrations of blood lactic acid in these subjects was reported to have reached maximal values of 151 mg per 100 ml of blood. This was estimated to be an increase of greater than 10 times the normal resting values. Such an increase in lactate concentration was indicative of the subjects dependence on the anaerobic energy system to maintain the work effort. Finally, the beneficial effect of working intermittently was obvious from the comparison of maximum blood lactate values between the two work schedules. The highest blood lactate concentrations observed from the intermittent work test were as much as 50% below the values obtained from the continuous work test.

The ability to alter the concentration of blood lactate through the adjustment of the exercise duration and intensity is apparent in the research of Astrand et al (1963), Karlsson (1970) and Karlsson and Saltin (1971). Initially Astrand found that ski races with a high intensity short duration profile contributed to post activity blood lactate concentrations of 140 mg/100 ml. Likewise, the blood lactate concentrations of those subjects competing in ski races lasting longer than 3 hours were in the order of 40 mg/100 ml. Karlsson (1970) found that reductions in blood lactate concentrations had occurred in subjects following prolonged

exhaustive exercise. The author suggested that the expected increases in blood lactate concentrations were most likely inhibited by the process of lactate reconversion to pyruvate, which in turn would be oxidized by the Kreb's Cycle. Karlsson and Saltin (1971) referred to the oxidation of lactate as a possible explanation for the low blood lactate values observed following a heavy intermittent work schedule. Their exercise protocol consisted of 5 x 1 min rides on a bicycle ergometer at a work load of 2800 kpm, each work bout being separated by a 5 min rest period. Such a broad work to rest ratio would not only allow the subject to oxidize the post-exercise lactate accumulation but it would also enhance the ability to rejuvenate the oxymyoglobin stores in the muscles, as previously described by Christensen et al (1960).

Depocas et al (1969) had observed a minimum lactate oxidation rate of 43% per min in dogs involved in continuous treadmill running at 30% of their maximal oxygen uptake. The authors' methodology involved the infusion of radio-active labelled lactate into the exercising dogs while simultaneously measuring the amount of radio-active carbon dioxide produced against the amount of blood lactate which had accumulated. The results indicated that the infused lactate had combined with the naturally occurring lactate to provide a fuel source for the dogs during the moderate activity session through the reconversion of the lactate to pyruvate.

Previous studies by Huckabee (1958) reported that the efficiency of the lactate dehydrogenase enzyme to reconvert lactate to pyruvate was extremely important to the post-exercise recovery processes, especially the repayment of the oxygen debt. The oxygen debt has been characterized as the time which a subject takes to return to a resting state following exercise. The amount of time required to pay back the oxygen debt is essential to the restoration of the ATP-CP stores to their resting levels. The reconversion of pooled lactate to pyruvate and then on to the Kreb's Cycle produces 18 moles of ATP per mole of lactate. This represents the amount of energy which is gained through the elimination of "excess lactate" as it is utilized in the repayment of the oxygen debt. Huckabee also explained that the use of stored oxygen at the onset of exercise has been observed to create an increased dependence on the anaerobic energy system to maintain the specific workloads. Likewise such demands lead to increases in the normal production of lactic acid. The mechanisms of this process have been referred to as creating an oxygen deficit. The value of the oxygen deficit is usually equivalent to the oxygen debt, only in low-intensity exercise. The importance of Huckabee's research has been evident in the attempt to map the formation and oxidation of lactate during exercise.

2.4 PART D: REVIEW OF METHODOLOGICAL CONSIDERATIONS

2.4.1 Albumin and Sialic Acid

This research study was interested in the relationship between exercise duration and intensity on the activity of albumin and the expected alteration of the GCW'S filtration capabilities. The plasma protein albumin has been described as a lower weight macromolecule, ranging in weight between 66,000 daltons (Andersson,1979) and 69,000 daltons (Chang et al 1975, Castenfors 1977). Andersson (1979) has described the structure of albumin as being one polypeptide chain consisting of 585 amino acid residues strategically cross-linked by disulphide bridges. Although the molecule has a number of binding sites it has been suggested that albumin displays an overall negative-charge (Bennett et al, 1976). This characteristic is clearly seen in the molecule's low-binding affinity with the anionically-natured chloride ions, as opposed to its very strong attraction to certain positively-charged metallic ions (Andersson, 1979). The isoelectric point of albumin is 4.9, therefore all of the electrical charges of the albumin molecule display a net negative-charge in the relatively alkaline blood plasma. According to Birke et al (1979), the two most important functions of the albumin molecule are to act as a carrier substance and to maintain colloidal osmotic pressure. The seriousness of diseases which inhibit the hepatic synthesis of albumin become most pronounced during conditions in which

there has been an abnormal loss of the albumin concentration from the blood. Such imbalances in the concentration of serum albumin tend to initiate responses of acute edema through the reduction in the normal osmotic equilibrium between the blood and the tissues.

Sialic acid is the glycoprotein N-acetylneuraminic acid. As described by Gottschalk (1972), glycoproteins are conglomerates of proteins and sugars. Although variations exist within the glycoprotein classification, all glycoproteins may be found to contain a low number of sugar residues, which make up branched saccharide chains covalently bound to a polypeptide. A very important characteristic of the glycoprotein is that no repeating units are found, thus glycoproteins are considered to be non-linear as opposed to their proteoglycan counterparts, which may possess glycoproteins within their linear structure (Gottschalk, 1972). Most importantly, the glycoprotein sialic acid has been located as glycosidically linked units of larger polysaccharide chains. As an example, Spiro (1972) reported sialic acid to be one of the components in his isolation experiments using glycoproteins of the GBM. Sialic acid also has additional importance in analyzing macromolecular transport across the capillary membranes, in that this molecule displays a negative-charge on its free, carboxyl terminus, a characteristic which consitutes the anionic-nature of the GCW. Although no studies are as yet available which report

a free dissociation of the glycoprotein sialic acid from its position in proteoglycan structures, Warren (1972) has mentioned that certain microorganisms possess the enzyme N-acetylneuraminic acid-aldolase, which potentiates the ability to cleave the N-acetylneuraminic acid molecule into a molecule of pyruvate and a molecule of N-acetylmannosamine. The importance of such events is obvious in attempting to clarify whether capillary membrane structures are either neutralized or physically altered during experimentally and pathologically induced situations.

2.4.2 Theoretical Considerations in The Analysis of Urine

Prior to analyzing urine samples for specific plasma proteins with either a spectrophotometric technique, a radioimmunoassay technique or an electrophoretic technique, the urine samples should first be measured for their total protein content. The total protein content refers to all of the proteins present within the sample regardless of their size, weight or specific binding characteristics. There exist a number of methods which may be used in such an assessment. Three of these are the biuret method, the Lowry method and the trichloracetic acid method (Tietz, 1970). However, the technique which appears to be both the most sensitive and the most rapid is the 'Protein-Dye Binding' method described by Bradford (1976). This technique is based on the activity of coomassie brilliant blue G-250 dye,

which may be found in either a red or blue form. When a solution containing proteins is added to the red form of the dye the solution turns blue within two minutes. Further, the dye bound protein may hold this colour for up to one hour. Briefly explaining the mechanics of the dye bound protein method, Bradford has found that when the solution of proteins binds to the red form of the coomassie brilliant blue G-250 dye there occurs a change in the solution's light absorbancy characteristics. This alters the normal extinction coefficient of the solution. Since the extinction coefficient is a measureable variable, which is used in the equation to calculate the dye concentration, any change in the extinction coefficient will change the concentration measurement of the dye. The sensitivity of this dye bound protein method has been found to be better than both the commonly used biuret and Lowry methods, with the latter technique corresponding to only 25% of the sensitivity of the dye-bound protein method. One minor technical problem which has been found in the use of this dye-bound protein technique is that the blue dye tends to coat the inside of the cuvettes. This problem may be eliminated if proper clean-up instructions are followed (See Appendix G for complete technique).

Following a significant result in the Bradford assessment of total proteins, the researcher may wish to analyze for specific plasma proteins. As previously mentioned, there

are at least three reliable techniques which may be used: a) a spectrophotometric technique, b) a radioimmunoassay technique, and c) an electrophoretic technique. The technique which appears to be least expensive while maintaining a very high level of accuracy and efficiency is the modified spectrophotometric technique for the analysis of albumin, as outlined in Appendix H. The underlying theory in the development of this technique has been presented by Doumas and Biggs (1972), and Rodkey (1965). The Rodkey technique proposed that when the pH of a solution containing albumin is neutral there exists a strong binding affinity between the alkaline side of the albumin molecule and the anionically natured BCG dye. Although this method was successful, Doumas and Biggs (1972) modified the procedure and eliminated some of the potentially misleading features. These authors stated that the measurement of the binding activity between BCG dye to albumin may be increased if the pH of the solution is approximately 4.2 and the spectrophotometer is set at 28 nm. Both of these early experiments were based on the principle that the reactions involved in the binding of anionic dyes to albumin are equilibrium reactions. Therefore, as long as the volume of anionic dye is greater than the amount of BCG free albumin molecules then the measurement will be reliable.

A third technique, which may be utilized in the post-exercise assessment of proteinuria, is the electrophoretic

separation of colloidal substances. Although electrophoresis is very time consuming and is not superior in accuracy to the more widely used radioimmunological and spectrophotometric techniques (Doumas and Biggs, 1972), this method should also be considered by persons who do not have access to the radioimmunoassay or spectrophotometric equipment. However, this technique is not required if the researcher prefers to use the faster and more acurate spectrophotometric techniques previously discussed. Cann (1969), has described electrophoresis as an optimal method to separate macromolecular substances based on the principles of electrical charge migration. The basic theory of electrophoretic analysis is that oppositely charged particles attract each other. Therefore, given the proper supporting medium, electrically charged biological colloids will separate according to group classification and move towards oppositely charged poles of an electrical current. However, in electrophoretic analysis, certain considerations such as the pH of the electrolyte solution, overall strength of the colloid's electrical charge and the frictional coefficient of the transport medium must be acknowledged (Cann 1969, Moreland 1962).

Cann has also mentioned that electrophoretic techniques follow either the principles of moving-boundary electrophoresis or zone electrophoresis. Although both methodologies will provide measurements of the concentrations of col-

loids in biological solutions, the techniques of zone electrophoresis appear to be most effective in complete separation of the specific protein groups. Wybenga (1974), has suggested the use of cellulose acetate strips as opposed to paper filters in providing the support medium in the technique of zone electrophoresis. The author stated that in using cellulose acetate strips the researcher may eliminate the cumbersome preparation of the medium required when using paper. As well when using cellulose acetate strips the bio-colloids take less time to migrate to their destinations, and their final pattern displays a denser and clearer resolution. This is important to the densitometric reading of migrated proteins. The densitometer is the apparatus which is used to provide the quantitative value in the relative protein concentration analysis. The purpose of the machine is to read the amount of light which passes through the migrated proteins. An inverse relationship exists between the density of the migrated protein and the amount of light which is measured. The values for relative concentrations of proteins may then be read directly from the apparatus.

Chapter III

METHODOLOGY

3.1 THE SUBJECTS

The subject population was made up of first division club rugby players from the Bytown Blues Rugby Football Club, Ottawa, and fourth year Physical Education students attending the University of Ottawa. The ages of the subject population ranged from 19 to 36. The total number of subjects involved in this study was 14 (refer to Appendix A for physical profiles of subjects who were also training for rugby).

Although not having specifically trained on a bicycle ergometer, all subjects had previously been, and were at the time of testing, involved in high intensity endurance type training. All subjects were considered asymptomatic of illness or injury during the testing and were required to sign an informed consent letter which stated that the testing protocol had been explained completely prior to the onset of testing (see Appendix J).

3.2 PROCEDURE FOR EXERCISE

3.2.1 Testing Protocol

Each of the subjects made three visits to the human performance laboratory at The University of Ottawa. The first visit was necessary to determine the subject's maximal oxygen uptake (VO2 maximum), the information from which was used to establish the workload intensities for the subjects following visits: the continuous and intermittent exercise tests. It should be noted that the order in which the continuous and intermittent work tests were administered was completely randomized. However, no subject performed either test with less than 24 hours rest between the test sessions.

3.2.2 Measuring VO2 maximum

The procedure to determine each subject's VO2 maximum followed the protocol of a progressive bicycle ergometer test. Each subject was requested to arrive at the laboratory in a post absorptive state. He was immediately requested to lie down for 20 min so that an approximated basal heart rate reading could be established. The subject then rode intermittently on a Monark bicycle ergometer at progressively increasing workloads of 5 min duration until reaching VO2 maximum. The VO2 maximum was indicated by a plateauing of the successive VO2 measurements taken during the subject's ride. A complete description of the exercise protocol used in determinations of VO2 maximum is presented in Appendix E.

3.2.3 The Intermittent Work Test

Each subject was requested to arrive at the laboratory in a post-absorptive state. He was then instructed to empty his bladder and relax in a seated position in the laboratory at which time he was requested to drink water at a volume of 20 ml/kg of body weight. This was done to ensure that the subject would not become hypohydrated and as such be unable to provide the two post-exercise urine samples (Kachadorian and Johnston, 1970). The pre-exercise seated rest period lasted for 20 min. Immediately following the pre-exercise seated rest period the subject was asked to give a blood and urine sample. The subject was then given a 3 min warm-up ride on the bicycle ergometer at a workload of 300 kpm followed by a 3 min rest while seated on the bicycle. Immediately after the warm-up rest period the subject rode the bicycle at a workload equivalent to 100 percent of his predetermined VO2 maximum according to the schedule of 16 x 2 min work bouts each separated by a 2 min rest. Upon completion of the intermittent work test, the subject was asked to give a single post-exercise blood and urine sample. These samples were taken within the first 5 min after exercise and were designated as the post-exercise samples. After giving the 5 min post exercise urine sample, the subject was required to sit in a comfortable position for 1 hour so that the renal functioning mechanisms could be normalized prior to collecting the final blood and urine samples (Castenfors,

1977). The final blood and urine samples were taken as close to 60 min as possible after the subject completed the work test. The total intermittent work test protocol took approximately 150 min.

3.2.4 The Continuous Work Test

The continuous work test was conducted with a protocol similar to the intermittent work test. All warm up, and rest periods, and collection times for blood and urine were conducted according to a similar time schedule, with the following modifications in the exercise schedule: the subjects were asked to ride the bicycle ergometer continuously for 42 min at a workload equivalent to 75% of their predetermined VO2 maximum.

3.2.5 Blood and Urine Analyses

On the day of the respective intermittent and continuous work tests, each subject provided a pre-exercise blood and urine sample after the 20 min rest period. The urine sample was provided voluntarily by the subject and analyzed according to the procedure outlined in Appendix F. The urine samples were analyzed for total protein concentration by using the coomassie G-250 dye binding method of Bradford (1976). Refer to Appendix G for a complete description of the Bradford technique. The urinary output of albumin was measured

with the modified version of a spectrophotometric technique involving the anionic binding of BCG dye to the albumin molecule (Gustafsson, 1978). Refer to Appendix H for a complete description of this modified technique. The blood samples were taken from the subject's right antecubital vein by a qualified laboratory technician using a 10 ml normal vacutainer fitted with a 21gl1/2 size needle. Blood samples were analyzed for pH concentrations using the Corning pH blood-gas analyzer located in the biochemical laboratory of the Department of Kinanthropology. Lactate concentrations were measured using the Boehringer-Mannheim U-V method designed by Gutmann and Wahlefeld (1974) Refer to Appendix B for a complete description of the lactate analysis technique. The 5 min and 1 hour post-exercise blood and urine samples were analyzed following the same procedures.

3.2.6 Equipment

All blood, urine and exercise testing procedures were performed in the Department of Kinanthropology at the University of Ottawa. The bicycle ergometer tests were performed using a GIH Stockholm Monark bicycle ergometer. Heart rate responses were monitored using a Cambridge VS4 electrocardiogram. The subject's expired gas was collected with a Collins Chain Compensated Gasometer and analyzed for CO2 concentrations with a Roxon Meditech Capnograph. The expired O2 was measured using a Roxon Meditech Oxygen Analyzer and

recordings were taken with a Narco Physiograph Four-B. The urine samples were collected in standard 150 ml graduated laboratory flasks. The urine samples were analyzed for total protein and albumin concentrations using the techniques and chemicals presented in Appendices G and H respectively. The blood was collected using Beckton and Dickenson Normal Vacutainers which were equipped with a size 21G11/2 vacutainer needle. The blood samples were analyzed for pH concentrations using the Corning Blood Gas Analyzer, model number 165pH. Lactate concentrations were measured using the Boehringer-Mannheim method. The quantitative spectrophotometric analyses were performed with a Bausch and Lomb Spectronic 88.

3.3 DATA ANALYSES

3.3.1 Statistical Treatment of Data

All of the statistical results were calculated with the Bio-Medical Data Processing (BMDP) statistical packages available through the University of Ottawa's OS/VMS-1 computing facilities. The file definition utility programs (F Utility) were used to organize all of the data prior to the analyses. The 2 way analysis of variance with repeated measures design was used to measure the overall differences between the continuous and intermittent work tests of this study, as well as the differences between the pre-, post-, and one hour post-exercise collection times, of the depen-

dent variables blood pH (BpH), blood lactate (BL), urine albumin (UA) and urine total protein (TP). A probability level of $P < 0.05$ was established as the level of significance for the 2 way ANOVA designs used in this study.

Simple (pairwise) correlations, organized as a matrix for all possible within test pairwise comparisons, were also used in the statisitical analyses of this study. This procedure was used to support the premise that specific, physiologically relevant, relationships existed within the design of this study. The level of significance for the pairwise correlations, of this study, was based on the significant difference comparison of the correlation coefficient to zero.

Multiple linear regression equations were administered to the data following the ANOVA, and correlative relationship tests. The multiple linear regression equations (refer to Tables 8 to 11) were used to measure the relative contribution of BpH and BL to the urinary outputs of UA and TP, as a method of determining the potential relationships between the exercise-induced changes in blood acidity and the post- and one hour post-exercise outputs of UA and TP.

Chapter IV

RESULTS AND DISCUSSION

In this study, fourteen trained male athletes were requested to complete a continuous work test and an intermittent work test on a bicycle ergometer. (Refer to Appendix A for descriptive profiles of subjects involved in rugby training). Albuminuria and blood acidity responses of the subjects were measured pre-, post- and one hour post-exercise, within each work test. The resulting measurements were used to test for significant differences between the continuous and intermittent work tests of this study, as well as to test for significant differences between the three collection times within each work test. Further, the potential relationships between the exercise-induced albuminuria and blood acidity responses within each work test were also measured. All measurements of the potential relationships between the four dependent variables- blood pH (BpH), blood lactate (BL), urine albumin (UA), and urine total protein (TP) were performed independently for each work test.

The exercise-induced albuminuria responses were determined through the pre-, post-, and one hour post-exercise measurements of albuminuria and total proteinuria. The exercise-induced blood acidity responses were determined

through the pre-, post-, and one hour post-exercise measurements of venous blood lactate and venous blood pH. The mean scores for the blood pH, blood lactate, urine albumin and urine total protein measurements, within each work test, have been presented in Tables 1-2 and Figures 3-6. Raw data are tabulated in Appendix A.

TABLE 1

CONTINUOUS WORK TEST MEAN VALUES (±S.D.) FOR
BLOOD pH, BLOOD LACTATE, URINE TOTAL PROTEIN AND URINE ALBUMIN

VARIABLE NAME	SAMPLE SIZE	PRE EXERCISE	POST EXERCISE	ONE HOUR POST EXERCISE
BLOOD pH	13	7.35 (0.03)	7.31 (0.05)	7.35 (0.04)
BLOOD LACTATE (MMOLES/LITER)	13	0.76 (0.25)	4.89 (4.06)	1.32 (0.55)
URINE ALBUMIN (MILLIGRAMS/100 ML)	13	0.77 (0.40)	0.61 (0.28)	1.12 (0.75)
URINE TOTAL PROTEIN (MILLIGRAMS/100 ML)	13	1.14 (0.80)	1.19 (0.66)	1.45 (0.77)

TABLE 2

INTERMITTENT WORK TEST MEAN VALUES (±S.D.) FOR
BLOOD pH, BLOOD LACTATE, URINE TOTAL PROTEIN AND URINE ALBUMIN

VARIABLE NAME	SAMPLE SIZE	PRE EXERCISE	POST EXERCISE	ONE HOUR POST EXERCISE
BLOOD pH	12	7.34 (0.04)	7.28 (0.07)	7.36 (0.04)
BLOOD LACTATE (MMOLES/LITER)	12	1.12 (0.31)	8.29 (2.22)	1.75 (0.93)
URINE ALBUMIN (MILLIGRAMS/100 ML)	12	0.92 (0.61)	1.40 (0.34)	1.51 (0.80)
URINE TOTAL PROTEIN (MILLIGRAMS/100 ML)	12	1.35 (1.01)	1.84 (1.12)	2.28 (1.29)

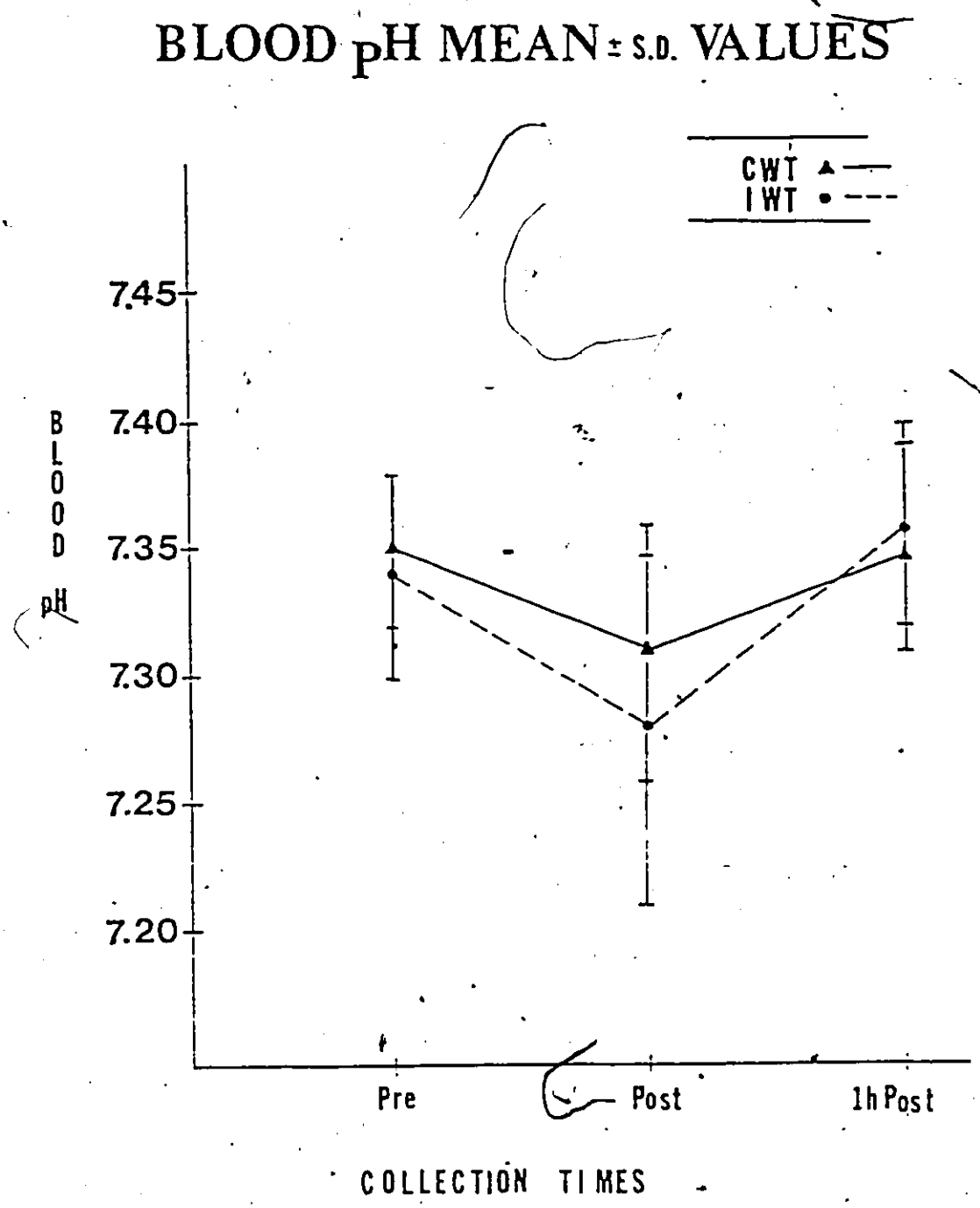


FIGURE 3; Blood pH Mean Values for The Continuous Work Test (CWT) versus The Intermittent Work Test (IWT)

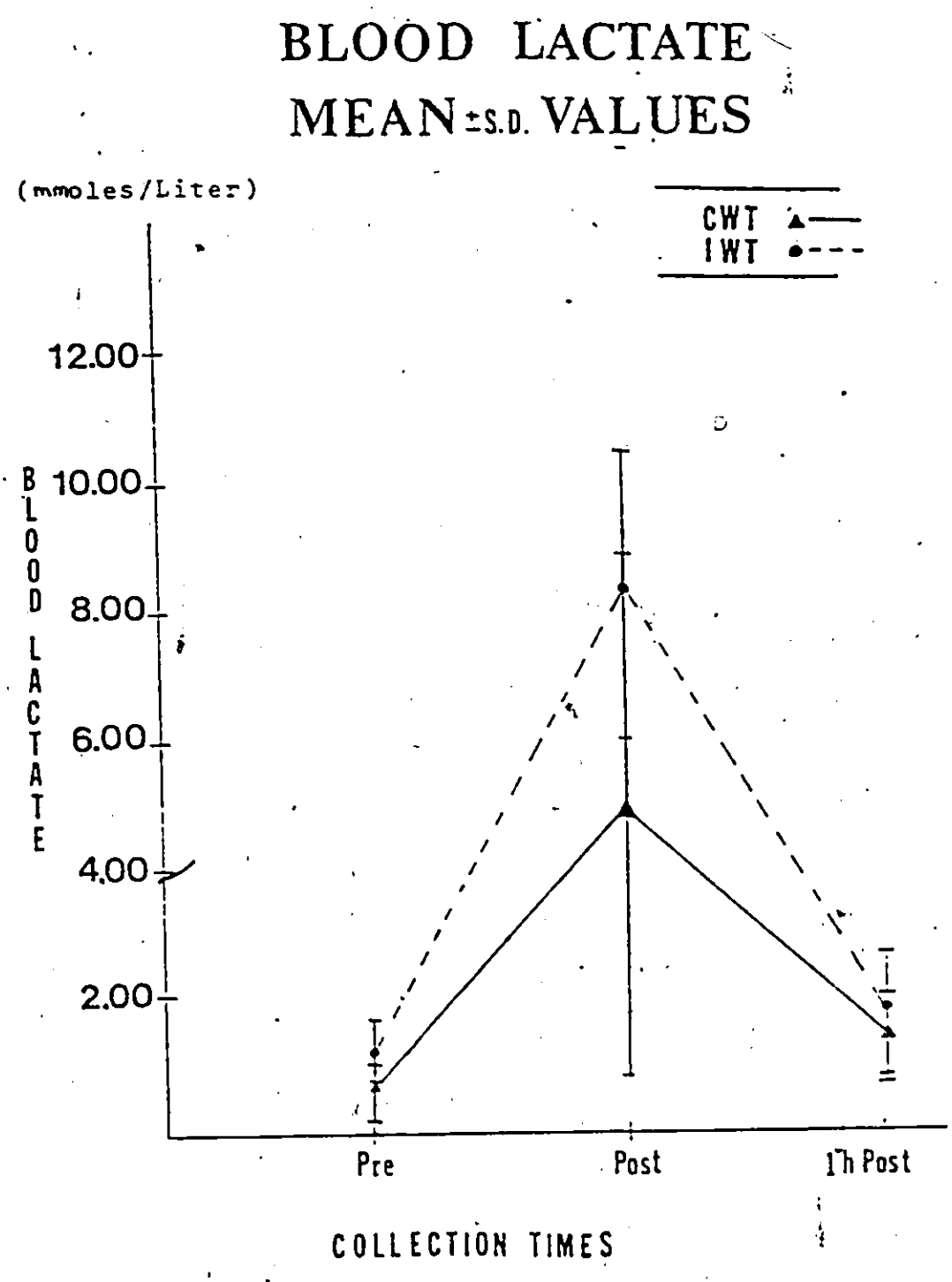


FIGURE 4: Blood Lactate Mean Values for The Continuous Work Test (CWT) versus The Intermittent Work Test (IWT)

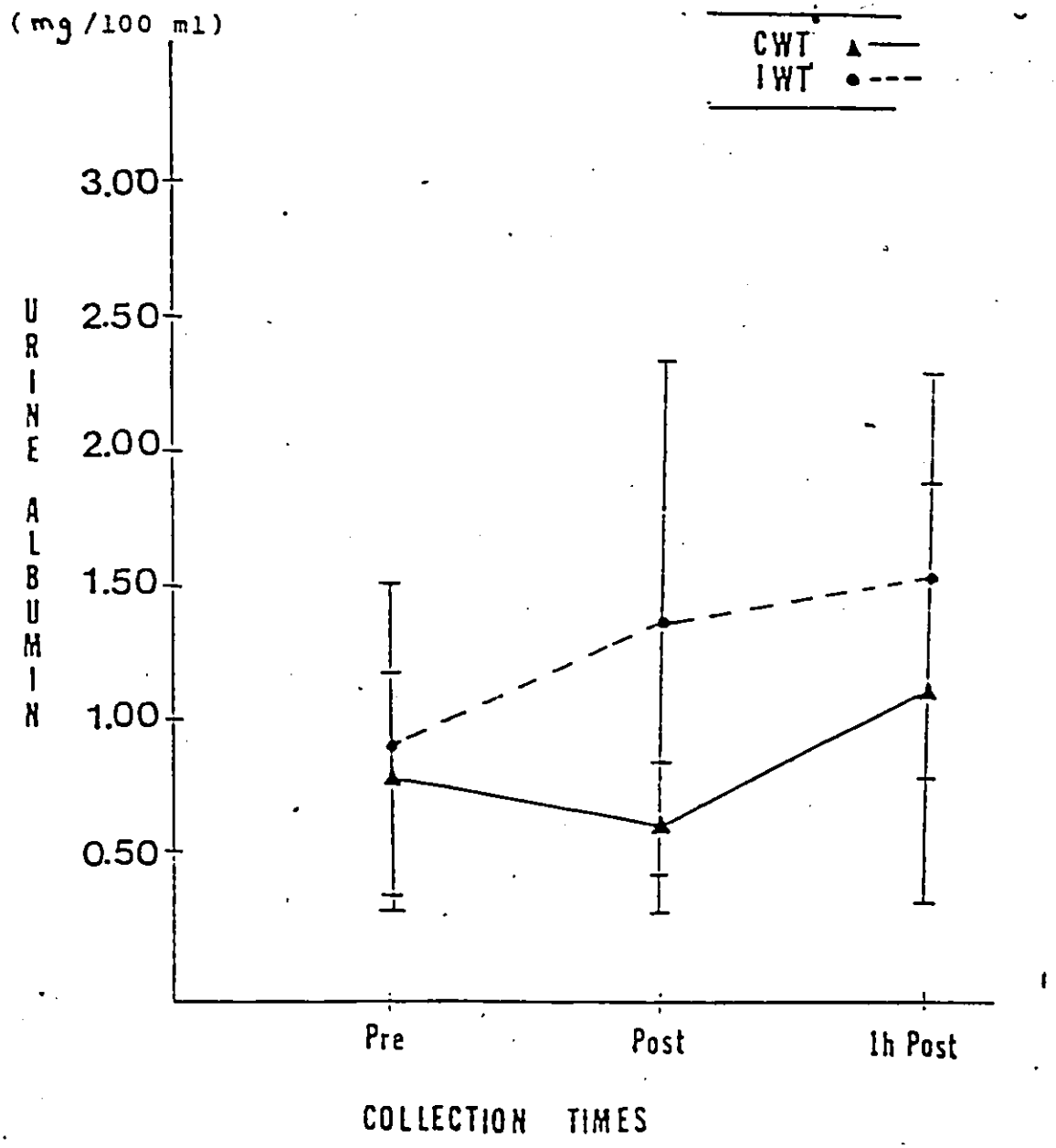


FIGURE 5 Urine Albumin Mean Values for The Continuous Work Test (CWT) versus The Intermittent Work Test (IWT)

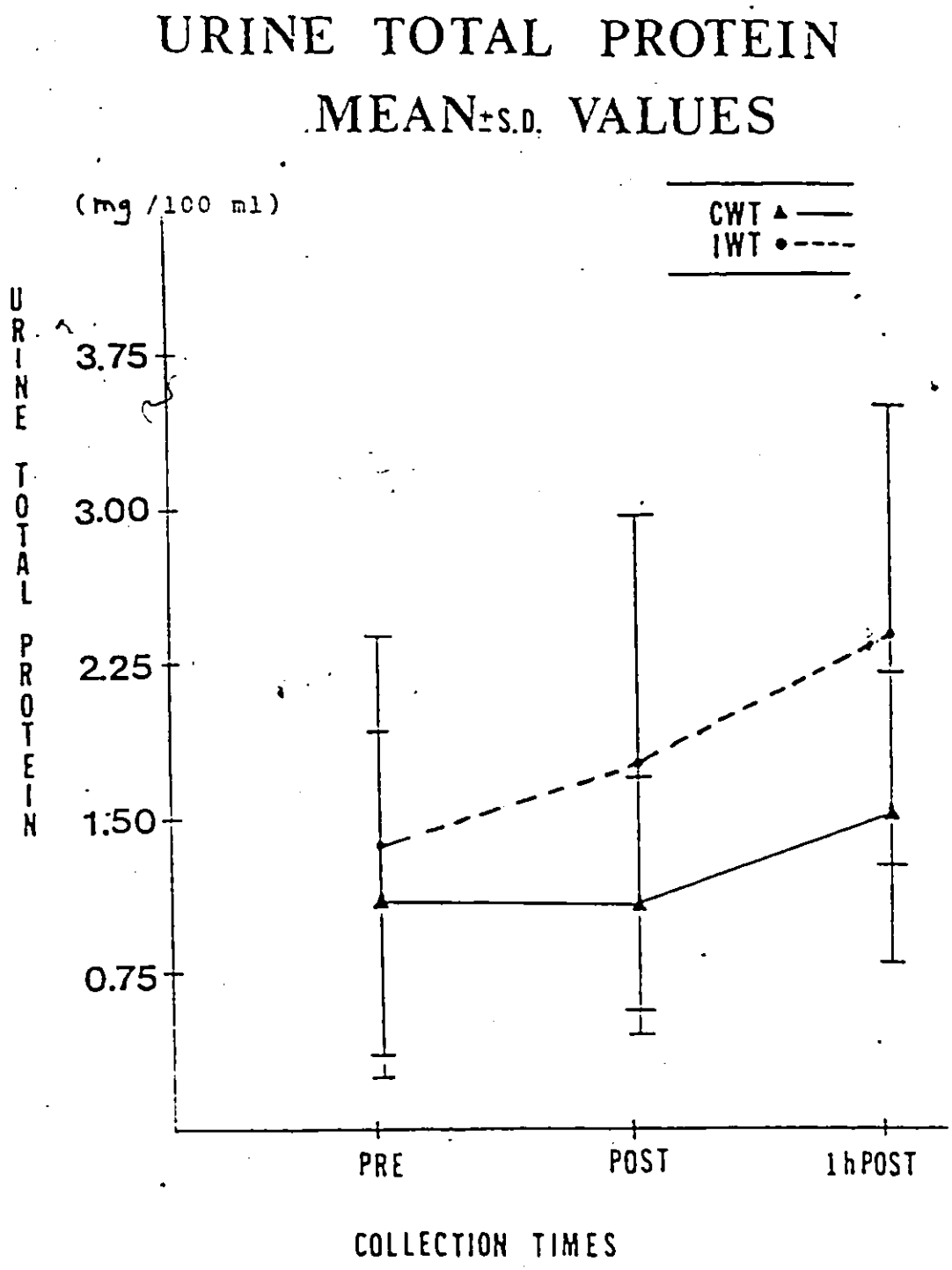


FIGURE 6; Urine Total Protein Mean Values for The Continuous Work Test (CWT) versus The Intermittent Work Test (IWT)

4.0.2 The Analysis of Variance Procedure

As previously stated, the two way analysis of variance procedure with repeated measures design was utilized in this study. This statistical procedure calculated comparisons on three levels: Level A, the main effects between the two work tests; Level B, the main effects between the three collection times for the two work tests combined (pre=Cont + Int, post=Cont + Int, 1h post=Cont + Int); and Level C, the interaction component, which is defined as Level A x Level B. A probability level of $P < 0.05$ had been previously established as the level of significance for the F values. The calculations within the analysis of variance procedures were performed using balanced groups, therefore in all ANOVA procedures n=11.

The results of the analyses of variance procedures, presented in Tables 3-6, have shown that the continuous and intermittent work tests, which were used in this study, produced significantly different effects on both the production of blood lactate, and the urinary outputs of albumin and total proteins. No significant difference was observed between the two work tests for the variable blood pH. Further, Level B of the ANOVA, which measures the main effects of the three collection times for the two work tests combined, has shown that a significant difference occurred between the pre- versus post- versus 1h post-exercise collec-

tion times for the variables BpH, BL and TP. Finally, the interaction component (Level A x Level B) of the ANOVA procedure, showed statistical significance only for the variable BL.

All significant F values, which resulted from the ANOVA procedures, were further analyzed with the Scheffe post-hoc technique. The Scheffe post-hoc procedure was also used to compare between test means for each level of collection time, as an attempt to determine the level of between test significance (eg. Int pre versus Cont pre). The post-hoc Scheffe test was not used to analyze comparisons which could be considered irrelevant, such as continuous exercise pre-test scores versus intermittent exercise post-test scores. The results of the Scheffe post-hoc procedures have been presented in Table 7.

The results of the blood pH measurements indicate that neither the continuous nor the intermittent exercise protocols had created a more strenuous effect on the ability of the subjects to buffer the increased acidity which may have been produced in the two work tests. Yet the significant pre- versus post-exercise results which were reported for Level B of the ANOVA suggest that each work test was independently effective in creating a stress on the subject's ability to buffer the increase in acidity. Further, the return of the subject's acidity measure to normal, as reported

in the one hour post- exercise values, indicates that the effect was short-lived.

TABLE 3

ANALYSIS OF VARIANCE RESULTS FOR THE DEPENDENT VARIABLE

BLOOD pH

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	F PROBABILITY	
Main Effects Between Tests	(1,10)	0.0006	0.0006	0.15	0.70	
Error		0.0429	0.0043			
Main Effects Between Times	(2,20)	0.0502	0.0251	14.02	0.01	*
Error		0.0358	0.0018			
Interaction Tests X Time	(2,20)	0.0058	0.0029	1.69	0.20	
Error		0.0343	0.0017			

* Denotes significance at the $p < 0.05$ level

TABLE 4

ANALYSIS OF VARIANCE RESULTS FOR THE DEPENDENT VARIABLE

BLOOD LACTATE

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	F PROBABILITY
Main Effects Between Tests	(1,10)	23.088	23.088	8.55	0.01 *
Error		26.988	2.698		
Main Effects Between Times	(2,20)	452.656	226.328	45.47	0.01 *
Error		99.557	4.978		
Interaction Tests X Times	(2,20)	22.240	11.12	5.54	0.01 *
Error		40.132	2.00		

* Denotes significance at the $p < 0.05$ level

TABLE 5

ANALYSIS OF VARIANCE RESULTS FOR THE DEPENDENT VARIABLE

URINE ALBUMIN

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	F PROBABILITY
Main Effects Between Tests	(1,10)	3.263	3.263	7.12	0.02 *
Error		4.584	0.458		
Main Effects Between Times	(2,20)	2.510	1.255	3.15	0.06
Error		7.979	0.398		
Interaction Tests X Times	(2,20)	1.128	0.563	1.52	0.24
Error		7.443	0.372		

* Denotes significance at the $p < 0.05$ level

TABLE 6

ANALYSIS OF VARIANCE RESULTS FOR THE DEPENDENT VARIABLE

URINE TOTAL PROTEIN

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	F VALUE	F PROBABILITY
Main Effects Between Tests	(1,10)	5.654	5.654	7.93	0.01 *
Error		7.131	0.713		
Main Effects Between Times	(2,20)	4.418	2.209	3.53	0.04 *
Error		12.508	0.625		
Interaction Tests X Times	(2,20)	1.241	0.621	0.75	0.48
Error		16.471	0.823		

* Denotes significance at the $p < 0.05$ level

Table 7: SCHEFFE POST-HOC RESULTS

PLANNED COMPARISONS		Degrees of Freedom	f VALUE
Variable name: blood pH			
(cont + int)	Pre vs Post	(2,20)	0.49
	Post vs 1hr Post	(2,20)	0.85
	Pre vs 1hr Post	(2,20)	0.05
Variable name: blood lactate			
(within test)	Pre(c) vs Post(c)	(2,20)	5.14 *
	Post(c) vs 1hr Post(c)	(2,20)	4.04 *
	Pre(c) vs 1hr Post(c)	(2,20)	0.07
	Pre(i) vs Post(i)	(2,20)	12.36 *
	Post(i) vs 1hr Post(i)	(2,20)	10.31 *
	Pre(i) vs 1hr Post(i)	(2,20)	0.09
(cont vs int)	Pre(c) vs Pre(i)	(2,20)	0.03
	Post(c) vs Post(i)	(2,20)	2.50
	1hr Post(c) vs 1hr Post(i)	(2,20)	0.04
Variable name: urine albumin			
(cont vs int)	Pre(c) vs Pre(i)	(2,20)	0.035
	Post(c) vs Post(i)	(2,20)	0.957
	1hr Post(c) vs 1hr Post(i)	(2,20)	0.233
Variable name: urine total protein			
(cont + int)	Pre vs Post	(2,20)	0.09
	Post vs 1hr Post	(2,20)	1.23
	Pre vs 1hr Post	(2,20)	1.55
(cont vs int)	Pre(c) vs Pre(i)	(2,20)	0.04
	Post(c) vs Post(i)	(2,20)	0.49
	1hr Post(c) vs 1hr Post(i)	(2,20)	0.61

legend: (c)=continuous work test results

(i)=intermittent work test results

* denotes significance at the $p<0.05$ level

4.0.3 Blood Lactate Results

The results of the post-hoc Scheffe comparisons for the variable blood lactate, indicate that no significant differences exist for the three collection times between the two work tests.

Such results were not consistent with the findigs of Holloszy et al (1977), and Christensen et al (1960). As previously reported, these authors stated that the duration and intensity of an activity are of primary importance in influencing blood pH and blood lactate changes.

Although the comparisons between the two work tests were not significantly different in this study, a comparison of the graphed mean values would indicate that the post intermittent exercise scores were higher than the post continuous exercise scores. The lower blood lactate values which were observed in the continuous work test are in agreement with the reported findings of Karlsson (1970) and Astrand (1963).

According to Astrand (1981), specific metabolic factors such as substrate availability, inhibition of the rate limiting enzymes and oxygen supply are considered to be regulatory mechanisms in the utilization of either the anaerobic or aerobic energy systems during a given activity. Astrand has stated that in the initial stages of exercise the principle source of energy is the anaerobic system, while the

aerobic processes contribute very little to the overall energy requirements. However, as the duration of the work continues to increase, the aerobic energy system becomes the preferential energy production pathway.

The blood lactate concentrations reported in the present study follow the expected results presented by Astrand (1977, 1981). During the work bouts of high intensity and short duration (the intermittent work test) there occurred a large production of lactate, whereas in the work bouts of moderate to high intensity for a prolonged duration (the continuous work test) the observed blood lactate concentrations appeared to be lower (however, the differences between the two work tests in this study were not significant). The difference in the lactate concentrations observed between the pre- and post-exercise blood samples measured in the continuous work test may be explained in a similar way. The anaerobic energy system is normally required to share the energy supply responsibilities during a continuous exercise procedure, when the workload is equal to or greater than seventy percent of the maximum. This requirement exists until the glycolytic supply of energy is either used up or inhibited by the accumulation of substrates from either glycolysis or other energy contributing processes (Astrand 1981, Astrand and Rodahl 1977).

Astrand has suggested that the utilization of anaerobically produced energy is inversely related to the duration of the exercise activity. The author presented the following quantitative relationships: after 5 min prolonged heavy exercise the anaerobic energy system is responsible for approximately 25% of the total energy production, while after 10 min of work the contribution of the anaerobic system has been reduced to approximately 15% of the total energy requirement.

In evaluating the results of the blood lactate measurements for the present study, one must consider that the reported observations only refer to the pre-, post- and one hour post-exercise results. The kinetics of the blood lactate molecule were not measured during either the continuous or intermittent exercise tests. This measurement may have been critical to the evaluation of the biochemical processes which affect the blood lactate molecule under different types of work tests. For example, in the intermittent work test the blood lactate which was produced during each 2 min ride on the bicycle ergometer may have been degraded by the specific lactate regulatory systems during the 2 min rest stages (McGilvery 1979, Lehninger 1972, Astrand 1981). Therefore, it would be incorrect to suggest that any blood lactate regulatory systems were responsible for differences which may have been observed between the intermittent and continuous work tests. The research design and analytical

techniques used to measure blood lactate in this study, did not allow for the analysis of blood lactate regulatory systems. Catheterization and subsequent continuous venous sampling may have been one technique which could have been used to measure the involvement of biochemical regulatory systems in the various types of exercise. According to Gass et al (1981), the potential for these systems to be effective during exercise must be realized.

4.0.4 Urine Albumin and Total Protein Responses

The analysis of variance procedures, used in this study, showed that a significant difference existed between the overall effects of the two exercise protocols, intermittent and continuous cycling, on the urinary outputs of UA and TP. However, the results of the post-hoc Scheffe tests did not show further significant differences within paired comparisons for either UA or TP (refer to Table 7).

With reference to the graphical presentation of the UA and TP results (Figures 3-6) it is evident that the post-intermittent exercise average values for both UA and TP were higher than the post-continuous exercise test average values. Likewise, the average value for BL was highest in the post-intermittent exercise blood sample. However, as no significant simple, pairwise correlations were found between either BL and UA, or BL and TP (Appendix A), a direct rela-

tionship between the post-exercise measurements of BL with either UA or TP, in either work test was not supported. Todorvic et al (1972), reported that no significant correlations were observed between BL and proteinuria in submaximal work tests where the exercise intensity was changed incrementally.

Although no "direct" relationship was observed between BL and either TP or UA, in either the continuous exercise test or the intermittent exercise test, a "conditional" relationship was observed. For example: both UA and TP were used as dependent variables in multiple linear regression equations which attempted to determine if, by combining the collection time measurements of BpH and BL, for each test independently, significant relationships would be observed (Tables 8-11). As a result, in each work test, post-exercise TP was significantly related to the combined measurements of pre- and post-exercise BpH and BL. Urine albumin was also significantly related to the combined measurements of pre- and post-exercise BpH and BL. However, the significant multiple regression relationships for the variable UA were only true for the intermittent work test results. No other significant multiple regression equation relationships were observed.

Todorvic et al (1972) reported that a positive significant correlation was observed between BL and proteinuria,

and a negative correlation was found between BpH and BL, following exercise where the intensity was held constant. (The reader will notice that these findings are contradictory to previously reported results by Todorvic regarding incremental exercise of varying intensity.) Based on the results of exercise at a constant work intensity, Todorvic stated that post-exercise proteinuria was directly related to workload intensity and workload duration. Such conclusions are consistent with previously reported studies which attempted to identify a cause-effect relationship between post-exercise blood acidity and proteinuria (Poortmans and Haralambie 1979, Castenfors 1977). However, as Todorvic also showed, the relationship between BL and proteinuria should be considered unstable, whereby the correlations were only observed to be significant within a limited range. For example, when the post-exercise blood lactate and blood pH levels became too high their correlational significance with the observed levels of proteinuria were non-existent. This observation is very important as it suggests that the possible relationships between blood acidity and proteinuria may not be linear, regardless of the type of exercise.

The significant conditional-relationships, reported in the present study, are consistent with those authors who have suggested that, post-exercise proteinuria may be related to specific changes in the ionic nature of either the fluid medium (blood), the protein molecule, or the renal

tissues, which may then affect the normal filtration of macromolecules. It would be incorrect however, to suggest that from the results of this study, or previous similar studies (Todorvic et al 1972, Javitt and Miller 1952) any single item of the macromolecular filtration process was more affected by exercise than any of the other items. Presently, the literature is inconclusive in stating which factors of macromolecular filtration are most affected by exercise-induced blood acidity (Ryan 1981). For example, although Todorvic had suggested that increased concentrations of circulating cations would be predominant in altering the physical properties of the macromolecular plasma proteins, previously presented research (Jones 1969, Chang et al 1975) regarding the glycoprotein constituents of the glomerular capillary indicate that, renal blood of a cationic nature, which may have the potential to modify the electrical properties of the plasma proteins, should also be considered to have the potential to neutralize the anionically-natured sialoproteins of the GCW.

Therefore, the significant relationships which have been reported by this study should be evaluated in the following way- a) these results are extremely conditional to the design of this study, b) the tests used to measure the blood acidity and urinary protein outputs were non-invasive and as such do not indicate precisely the systems which were operational within the exercising kidney, c) neither the regres-

sion nor the pairwise correlation procedures were used to show a cause-effect situation, these techniques were used only to provide a measure of potential relationship between indicators of blood acidity and the urinary output of protein.

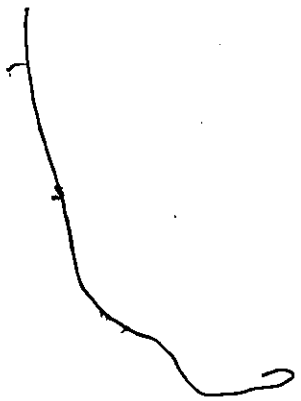


TABLE 8

MULTIPLE REGRESSION EQUATIONS FOR URINE ALBUMIN

INTERMITTENT WORK TEST

POST EXERCISE URINE ALBUMIN: PRE, POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.950 R^2 VALUE 0.902 F VALUE 9.265 *

POST EXERCISE URINE ALBUMIN: POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.507 R^2 VALUE 0.256 F VALUE 1.035

ONE HOUR POST EXERCISE URINE ALBUMIN: POST, 1hr POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.789 R^2 VALUE 0.622 F VALUE 1.649

ONE HOUR POST EXERCISE URINE ALBUMIN: 1hr POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.238 R^2 VALUE 0.056 F VALUE 0.181

* Denotes significant F values at $p < 0.05$

TABLE 9

MULTIPLE REGRESSION EQUATIONS FOR URINE ALBUMIN

CONTINUOUS WORK TEST

POST EXERCISE URINE ALBUMIN: PRE, POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.785 R^2 VALUE 0.618 F VALUE 1.614

POST EXERCISE URINE ALBUMIN: POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.283 R^2 VALUE 0.080 F VALUE 0.262

ONE HOUR POST EXERCISE URINE ALBUMIN: POST, 1hr POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.740 R^2 VALUE 0.547 F VALUE 1.207

ONE HOUR POST EXERCISE URINE ALBUMIN: 1hr POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.647 R^2 VALUE 0.419 F VALUE 2.169

* Denotes significant F value at $p < 0.05$

TABLE 10

MULTIPLE REGRESSION EQUATIONS FOR URINE TOTAL PROTEIN

INTERMITTENT WORK

POST EXERCISE URINE TOTAL PROTEIN: PRE, POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.931 R^2 VALUE 0.867 F VALUE 6.51 *

POST EXERCISE URINE TOTAL PROTEIN: POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.352 R^2 VALUE 0.124 F VALUE 0.426

ONE HOUR POST EXERCISE URINE TOTAL PROTEIN: POST 1hr POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.523 R^2 VALUE 0.274 F VALUE 0.377

ONE HOUR POST EXERCISE URINE TOTAL PROTEIN: 1hr POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.323 R^2 VALUE 0.104 F VALUE 0.348

* Denotes significant F value at $p < 0.05$

TABLE 11

MULTIPLE REGRESSION EQUATIONS FOR URINE TOTAL PROTEIN

CONTINUOUS WORK TEST

POST EXERCISE URINE TOTAL PROTEIN: PRE, POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.840 R^2 VALUE 0.706 F VALUE 2.396 *

POST EXERCISE URINE TOTAL PROTEIN: POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.215 R^2 VALUE 0.046 F VALUE 0.146

ONE HOUR POST EXERCISE URINE TOTAL PROTEIN: POST, 1hr POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.713 R^2 VALUE 0.508 F VALUE 1.031

ONE HOUR POST EXERCISE URINE TOTAL PROTEIN: 1hr POST (BLOOD pH, BLOOD LACTATE)

R VALUE 0.6419 R^2 VALUE 0.412 F VALUE 2.102

* Denotes significant F value at $p < 0.05$ level

Chapter V

CONCLUSION

5.1 SYNOPSIS

The purpose of this research study was to compare the relative exercise-induced alterations in blood pH (BpH) and blood lactate (BL) with the urinary output of total proteins (TP) and albumin (UA) in trained male athletes participating in separate intermittent and continuous work tests.

Three primary areas of concern were investigated:

(1) The comparison of the results for the dependent variables- BpH, BL, UA, and TP, in the intermittent versus continuous work test protocols specific to this study.

(2) The comparison of the results for the pre-, post- and one hour post-exercise measurements of the dependent variables in each of the work tests.

(3) The evaluation of both pairwise correlations and multiple regression equation measurements, for potential relationships which were specific to this study.

5.2 CONCLUSION AND RECOMMENDATIONS

5.2.1 Summary

(1) The analyses of variance procedures, used in this study, produced significant main effects comparisons for the between work test factor (level A) for the variables BL, UA and TP (refer to Tables 3-6). However, the interaction component (Level C) of the ANOVA was not significant for the variables UA and TP, but was significant for the variable BL.

(2) The analyses of variance procedures also produced significant main effects comparisons, for the between collection time factor of the two work tests combined (level B), for the variables BpH, Bl and TP (refer to Tables 3-6).

(3) Post-hoc Scheffe analyses of the significant results produced by the analyses of variance procedures, failed to provide significance for either BpH, UA or TP. However, this post-hoc technique was successful in establishing that significant differences existed between specific collection times for the variable BL, within both exercise tests. Further, no significant differences were observed between, the

collection times for the two work tests, (continuous pre- versus intermittent pre-, etc.) for any of the dependent variables.

(4) No significant pairwise correlations were observed between either of the indicators of blood acidity (BpH and BL), with either of the indicators of proteinuria (UA and TP), in either work test (refer to Appendix A).

(5) Specific multiple regression equations indicated that potential relationships for the variables TP and UA with pre- and post-exercise collection time measurements of BpH and BL existed for specific combinations of these variables (refer to Tables 8-11).

5.2.2 Concluding Remarks

This study had attempted to indirectly measure the mechanisms which have most often been related to the post-exercise observation of plasma proteinuria, namely blood acidity related to albuminuria. Based on the results of this study a global statement referring to the relationship between blood acidity and post-exercise proteinuria could not be made.

Although the specific multiple regression equations showed that a relationship was present between the combined pre- and post-exercise measurements of BpH and BL with the post-exercise urinary output of TP, and the UA results in the intermittent work test, the fact that these results are specific to this study should be emphasized.

From the results of the analysis of variance procedures and the the post-hoc Scheffe tests, one may conclude that this study was unable to provide evidence that a significant difference existed between the two exercise protocols in their ability to affect the physiological responses of blood pH, blood lactate, urine total protein, or urine albumin.

5.2.3 Recommendations

The following recommendations for future studies are suggested from the conclusions of this research study.

(1) Future studies should include more comprehensive techniques such as nephron micropuncture as a means of providing a direct analysis of the mechanisms involved in the transglomerular movement of macromolecules.

(2) Blood samples should be collected during the exercise sessions to measure lactate accumulation and degradation continuously (ie: through catheterization).

(3) Results of the urinary outputs of macromolecules should be related to the clearance values of these molecules in order to provide a quantitative measure of the magnitude of the result.

(4) In order to allow these more extensive measurements it is recommended that the subject population be made up of laboratory animals as opposed to humans.

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Appendix A

RAW DATA TABLES

Table A1: Raw Data For pH Measurements

Subject	Cont Pre	Cont Post	Cont 1hPost	Int Pre	Int Post	Int 1hPost
J.S.	7.285	7.285	7.235	7.255	7.229	7.299
G.T.	7.317	7.365	7.382	7.330	7.325	7.339
D.S.	7.375	7.315	7.332			
M.F.	7.363	7.314	7.310			
G.G.	7.361	7.382	7.391	7.352	7.232	7.416
M.V.				7.301	7.280	7.342
J.F.	7.315	7.346	7.361	7.323	7.146	7.352
G.B.	7.409	7.407	7.420	7.315	7.348	7.320
R.C.	7.328	7.205	7.338	7.350	7.258	7.419
N.M.	7.355	7.264	7.346	7.368	7.376	7.376
A.W.	7.316	7.287	7.368	7.374	7.303	7.403
D.K.	7.349	7.226	7.323	7.409	7.352	7.359
S.M.	7.350	7.297	7.350	7.345	7.158	7.338
L.M.	7.377	7.339	7.358	7.353	7.324	7.385

Table A2: Raw Data For Lactate Measurements

(Values in Millimoles per Liter)

Subject	Cont Pre	Cont Post	Cont 1hPost	Int Pre	Int Post	Int 1hPost
J.S.	0.830	1.991	0.940	1.078	7.880	1.920
G.T.	0.553	4.090	0.885	0.830	4.090	0.830
D.S.	0.785	1.770	0.885			
M.F.	0.199	2.650	1.580			
G.G.	1.350	16.150	1.710	1.290	11.390	2.320
M.V.				1.050	9.290	1.880
J.F.	0.774	3.096	1.050	0.664	10.950	4.150
G.B.	0.719	2.650	1.160	1.220	6.080	0.995
R.C.	0.940	10.700	2.489	1.844	10.314	2.212
N.M.	0.783	3.589	0.926	1.501	6.633	0.693
A.W.	0.802	3.042	2.350	1.009	7.233	1.438
D.K.	0.650	4.731	1.150	0.932	7.203	1.161
S.M.	0.727	4.728	0.982	1.095	10.479	1.980
L.M.	0.818	4.377	1.037	0.954	7.955	1.482

Table A3: Albumin Per 0.2 Grams Dried Urine

Subject	Cont Pre	Cont Post	Cont 1hPost	Int Pre	Int Post	Int 1hPost
J.S.	0.065	0.065	0.057	0.050	0.232	0.093
G.T.	0.028	0.030	0.130	0.173	0.166	0.138
D.S.	0.030	0.108	0.062			
M.F.	0.020	0.057	0.030			
G.G.	0.059	0.065	0.040	0.053	0.063	0.044
M.V.				0.063	0.181	0.071
J.F.	0.044	0.060	0.068	0.029	0.029	0.141
G.B.	0.025	0.030	0.045	0.063	0.065	0.051
R.C.	0.015	0.041	0.294	0.028	0.144	0.074
N.M.	0.102	0.052	0.126	0.041	0.220	0.085
A.W.	0.058	0.044	0.017	0.038	0.069	0.036
D.K.	0.044	0.078	0.173	0.090	0.142	0.062
S.M.	0.065	0.036	0.022	0.030	0.186	0.034
L.M.	0.034	0.034	0.044	0.042	0.042	0.062

Table A4: Total Protein Per 0.2 grams Dried Urine

Subject	Cont Pre	Cont Post	Cont 1hPost	Int Pre	Int Post	Int 1hPost
J.S.	0.116	0.116	0.075	0.080	0.294	0.145
G.T.	0.044	0.047	0.149	0.192	0.167	0.144
D.S.	0.041	0.120	0.064			
M.F.	0.021	0.060	0.031			
G.G.	0.061	0.091	0.040	0.063	0.116	0.080
M.V.				0.074	0.236	0.117
J.F.	0.075	0.087	0.097	0.040	0.037	0.175
G.B.	0.031	0.036	0.059	0.065	0.070	0.052
R.C.	0.036	0.095	0.296	0.037	0.216	0.123
N.M.	0.236	0.146	0.130	0.067	0.255	0.167
A.W.	0.060	0.094	0.060	0.042	0.134	0.065
D.K.	0.056	0.080	0.206	0.165	0.233	0.072
S.M.	0.063	0.069	0.060	0.065	0.189	0.091
L.M.	0.050	0.065	0.066	0.070	0.065	0.097

Table A5: Albumin Content (Grams Per 100 ml. Urine)

Subject	Cont Pre	Cont Post	Cont 1hPost	Int Pre	Int Post	Int 1hPost.
J.S.	0.582	0.829	0.634	0.469	3.800	2.060
G.T.	0.407	0.443,	1.296	1.723	1.965	2.687
D.S.	0.380	1.079	0.853			
M.F.	0.296	1.348	0.824			
G.G.	0.798	0.594	0.761	0.651	0.814	0.990
M.V.				0.699	2.152	0.967
J.F.	0.739	0.866	1.488	0.322	0.326	1.909
G.B.	0.337	0.403	0.594	0.824	1.044	0.850
R.C.	0.219	0.272	2.273	0.603	1.320	0.678
N.M.	1.447	0.586	1.894	1.718	1.494	2.668
A.W.	1.402	1.282	0.561	1.010	0.690	1.175
D.K.	1.049	0.580	2.262	2.007	1.473	1.188
S.M.	0.781	0.360	0.247	0.425	1.879	0.384
L.M.	0.683	0.570	0.825	0.402	0.655	2.036

Table A6: Total Protein (Grams Per 100 ml. Urine)

Subject	Cont Pre	Cont Post	Cont 1hPost	Int Pre	Int Post	Int 1hPost
J.S.	1.035	1.473	0.834	0.750	4.772	3.201
G.T.	0.639	0.698	1.495	1.907	1.977	2.822
D.S.	0.528	1.194	0.880			
M.F.	0.310	1.414	0.857			
G.G.	0.812	0.828	0.764	0.782	1.504	1.789
M.V.				0.821	2.812	1.594
J.F.	1.260	1.258	2.121	0.537	0.416	2.370
G.B.	0.418	0.487	0.772	0.857	1.132	0.867
R.C.	0.526	0.630	2.288	0.787	1.975	1.123
N.M.	3.349	1.645	1.954	2.786	1.732	5.226
A.W.	1.426	2.738	1.970	1.118	1.339	2.105
D.K.	1.336	0.595	2.693	3.680	2.417	1.370
S.M.	0.788	0.690	0.673	0.921	1.909	1.028
L.M.	1.005	1.090	0.487	0.678	1.017	3.201

Table A7: Dry Weights of Freeze Dried Urine

Subject	Cont Pre	Cont Post	Cont 1hPost	Int Pre	Int Post	Int 1hPost
J.S.	1.792	2.040	1.780	1.876	2.598	2.656
G.T.	2.615	2.670	2.002	1.987	2.605	1.563
D.S.	2.830	1.998	2.475			
M.F.	2.962	1.891	2.764			
G.G.	2.683	1.461	2.282	1.985	1.563	2.700
M.V.				1.774	2.384	2.725
J.F.	2.686	1.350	1.621	2.687	2.885	1.750
G.B.	2.425	2.685	2.376	2.902	1.942	2.667
R.C.	2.630	1.591	1.701	2.586	1.833	1.833
N.M.	2.838	1.803	2.405	1.676	1.358	3.319
A.W.	3.686	2.330	3.300	1.886	1.599	2.611
D.K.	1.430	1.487	2.052	2.230	1.660	2.300
S.M.	2.000	1.999	2.243	2.550	2.020	2.034
L.M.	2.010	2.013	1.476	1.937	2.005	1.320

Table A8: Wet Sample Size in Milliliters of Output

Subject	Cont Pre	Cont Post	Cont 1hPost	Int Pre	Int Post	Int 1hPost
J.S.	100	80	80	100	80	60
G.T.	90	80	100	100	110	40
D.S.	110	100	90			
M.F.	100	40	50			
G.G.	100	80	60	80	60	60
M.V.				80	100	100
J.F.	100	60	60	80	100	40
G.B.	90	100	90	110	60	80
R.C.	90	120	110	60	100	100
N.M.	100	80	80	20	100	50
A.W.	80	40	50	35	80	40
D.K.	30	100	80	50	80	60
S.M.	80	100	100	90	100	90
L.M.	50	60	100	100	65	20

Table A9: Physical Profiles of Subjects in Rugby

Subject	Weight (kg)	Maximum Heart Rate	VO2 Maximum (ml/kg.min)
J.S.	76.4	184	49.61
G.T.	70.5	184	53.64
D.S.	86.0	184	55.38
M.F.	52.2	191	65.25
G.G.	68.1	214	63.38
M.V.	97.0	204	42.77
J.F.	64.4	200	54.53
G.B.	92.0	187	50.67

APPENDIX 10

Correlation Matrix For Intermittent Work

		pH			Lactate			Total Protein			Albumin		
		Pre	Post	1h Post	Pre	Post	1h Post	Pre	Post	1h Post	Pre	Post	1h Post
pH	Pre	1.00											
	Post	0.40	1.00										
	1h Post	0.63	0.09	1.00									
Lactate	Pre	0.10	0.19	0.45	1.00								
	Post	-0.01	-0.78	0.39	0.20	1.00							
	1h Post	-0.26	-0.85*	0.10	-0.22	0.79	1.00						
Total Protein	Pre	0.61	0.61	-0.03	-0.02	-0.49	-0.56	1.00					
	Post	-0.46	-0.01	-0.45	0.14	-0.16	-0.23	0.15	1.00				
	1h Post	-0.09	0.32	-0.03	-0.03	-0.33	-0.23	0.26	0.14	1.00			
Albumin	Pre	0.53	0.72	-0.01	-0.01	-0.67	-0.67	0.93*	0.09	0.26	1.00		
	Post	-0.58	-0.05	-0.62	0.08	-0.25	-0.26	0.11	0.95*	0.19	0.08	1.00	
	1h Post	-0.20	0.34	-0.22	-0.31	-0.53	-0.20	0.27	0.12	0.87*	0.36	0.21	1.00

* denotes a significant correlation, where RHO=0

APPENDIX A II

Correlation Matrix For Continuous Work

		pH			Lactate			Total Protein			Albumin		
		Pre/Post/1h Post			Pre/Post/1h Post			Pre/Post/1h Post			Pre/Post/1h Post		
pH	Pre	1.00											
	Post	0.40	1.00										
	1h Post	0.66	0.61	1.00									
Lactate	Pre	0.10	0.13	0.08	1.00								
	Post	0.16	0.02	0.24	0.84*	1.00							
	1h Post	-0.13	-0.35	0.14	0.46	0.49	1.00						
Total Protein	Pre	-0.06	-0.34	-0.18	-0.08	-0.25	-0.20	1.00					
	Post	-0.43	-0.17	-0.20	0.03	0.32	0.29	0.51	1.00				
	1h Post	-0.35	-0.64	-0.11	-0.26	-0.06	0.33	0.36	0.17	1.00			
Albumin	Pre	-0.06	-0.27	-0.07	0.01	-0.18	0.01	0.80*	0.70	0.32	1.00		
	Post	-0.47	-0.01	-0.20	0.04	-0.32	0.21	0.30	0.89*	0.19	0.60	1.00	
	1h Post	-0.18	-0.60	-0.14	-0.13	0.15	0.17	0.33	-0.20	0.86*	0.05	-0.23	1.00

* denotes significant correlation, where RHO=0

Appendix B

THE QUANTITATIVE ANALYSIS OF LACTATE IN BLOOD

"The Boehringer-Mannheim Method"

"Equipment"

Beaker of ice water (crushed ice for blood samples)
1 ten ml. centrifuge tube for reagent blank
2 ten ml. centrifuge tubes for each blood sample
1 pipette with one ml. capacity
1 pipette with 200 micro-ml. capacity
1 Vortex test tube mixer
1 table top centrifuge
disposable pipettes
1 Bausch and Lomb Spectrophotometer
Spectrophotometric cuvettes
Chemicals for reagent preparation (see below)

Molecular Weights
Oxygen 15.999
Nitrogen 14.0067
Hydrogen 1.0079
Carbon 12.011

* When making solutions always use RE-distilled water.

"Solution A" 0.5 moles/liter Glycine
0.4 moles/liter Hydrazine

Glycine (NH_2-CH_2-COOH): Formula Weight 75.0662
0.5 x 75.0662 = 37.5331 grams/liter
37.5331 x 0.05 = 1.8766 grams/50 cc.
37.5331 x 0.1 = 3.7533 grams/100 cc.

Annhydrous Hydrazine ($H_2-N-N-H_2$): Formula Weight 32.05
0.4 x 32.05 = 12.82 grams/liter
12.82 x 0.05 = 0.641 grams/50 cc.
12.82 x 0.1 = 1.282 grams/100 cc.

"Solution B" 27 mmoles/liter NAD

Nicotine Adenine Dinucleotide: Formula Weight 663.4
0.027 x 663.4 = 17.9118 grams/liter
17.9118 x 0.01 = 0.1791 grams/10 cc.

"Suspension C" Lactate Dehydrogenase

The lactate dehydrogenase used in this experiment was the crystalline suspension in an ammonium sulphate solution.

L-Lactate:NAD Oxido-Reductase; E.C. 1.1.1.27

"Perchloric Acid" (HCLO4) circa 0.6 Normal

1 Normal = Mol. Wt. / (Valence x Spec. Grav. x % Acid)

1 Normal =100.5 / (1 x 1.67 x 0.70)
1 Normal for (HCLO4) = 85.97
1 Normal x 0.6 Normal = 51.582 grams/liter
100 ml. of 0.6 N (HCLO4) = 5.1582 grams of (HCLO4)

"Deproteinizing Blood Samples"

* After taking samples deproteinize immediately with (HCLO4).

(1) Pipette 1.0 cc. of ice cold perchloric acid into a centrifuge tube.
(2) Pipette 0.5 cc. of blood into the test tube containing the Perchloric acid.
(3) Mix well with Vortex.
(4) Centrifuge at 2,000 RPM. for five mintes, remove supernatant with a disposable pipette and place supernatant in new centrifuge tube, centrifuge again for five minutes, remove only the supernatant.

* If the blood was taken without enough time to centrifuge, then add 0.5cc. of blood directly to 1.0 cc. of perchloric acid and keep test tube in ice water.

"Preparing Samples for Spectrophotometric Analysis"

* Prepare two test tubes per blood sample and one reagent blank test tube per subject.

* Pipett the reagent solutions into test tubes according to the following schedule.

	Reagent Blank	Sample Test Tube
Solution A	2.00 ml.	2.00 ml.
Supernatant	--	0.20 ml.
(HCLO4:0.6N)	0.20 ml.	--
Solution B	0.20 ml.	0.20 ml.
Suspension C	0.02 ml.	0.02 ml.

Use the Vortex mixer to thoroughly mix each test tube and incubate for exactly one hour at twenty five degrees centi-

grade for one hour. After the incubation time pour solutions into the spectrophotometric cuvettes and read light absorbance of each blood sample against the reagent blank sample.

"Calculations of Lactate Concentrations"

C = (¢E x V / e x d x u) x F umoles/liter
Where ¢E = spectrophotometer reading
V = Assay Volume (=2.42 ml.)
e = Extinction Coeffecient cm2/umole (=6.22)
d = light path of Spectrophotometer (=1cm.)
u = volume of blood used in assay (=0.2 if undiluted)
F = dilution factor for blood

"Calculating the Dilution Factor"

* In this case we used 0.5 ml. of blood which has been deproteinized in 1 ml. of perchloric acid.

F = (blood vol. x S.G. x %H2O + HCLO4 vol.) / blood vol.
F = (0.5 x 1.06 x 0.8 + 1) / (0.5)
F = 2.848

The multiplication factor for calculating light absorbancy is set at a constant for the conditions of this experiment; yet the conditions change between pre and post-exercise blood samples, as post-exercise blood samples must be diluted.

((Assay vol. x Mol. Wt.) / (e x d x u)) x F

Where Assay vol. = 2.42
Mol. Wt. = 90.08 grams
This is the molecular weight of lactate, which has the formula (CH3-CH-OH-COOH)
* The multiplication factor for undiluted blood is 49.9 mg/100 cc.
* Multiply the spectrophotometer reading x 49.9 = [lactate]

Appendix C

A STEPWISE CALCULATION OF SERIAL SAMPLES OF LACTATE

* 1 Normal = 1 Molar (when valence is one)
1 Mole / Liter
mg% = mg/ x ml.

Find weight of Lactate:
CH3CHOHCOOH.....C3 = 12.011 x 3 = 36.03
H6 = 1.0008 x 6 = 6.00
O3 = 15.990 x 3 = 47.96
Lactate = c.90gm/liter

*90gm/liter : 90,000mg/liter
9gm/100ml.
9,000mg/100ml.
90mg/1.0ml.

* If 90mg of lactate is found in one milliliter of stock lactate solution, then 0.1ml. of stock solution equals 9mg.

* To make serial solutions use the following calculations:

S.S. = Stock solution of Lactate
HCLO4 = Perchloric acid, c. 0.6 Normal.

0.1 ml. of S.S. + 59.9 ml. of HCLO4
9mg of Lactate in 60 ml. of sol'n
9/60 = 0.15 mg/ml. of new sol'n
to find mg%, multiply the equation by 100
thus 0.15 mg/ml. x 100 = 15 mg%.

* The regression equation of y=ax+b may be used to calculate the standard curve for the samples.

Where: y = Optical Density at 340 nm.
x = mg%
a = slope = y2-y1/x2-x1 (rise over run)
b = y intercept (in this case '0')

Appendix D

WORKLOADS ON A BICYCLE ERGOMETER

Bicycle Setting Kiloponds	Workload Joules	Watts/ Minute	(Approx.) Watts	KPM
1.0	2942	49.019	50	300
1.5	4413	73.529	75	450
2.0	5884	98.039	100	600
2.5	7355	122.549	125	750
3.0	8826	147.058	150	900
3.5	10297	171.568	175	1050
4.0	11768	196.078	200	1200
4.5	13239	220.588	225	1350
5.0	14710	245.098	250	1500
5.5	16181	269.608	275	1650
6.0	17652	294.117	300	1800
6.5	19123	318.627	325	1950
7.0	20594	343.137	350	2100
7.5	22065	367.647	375	2250

1 KP. = 9.80 Newtons
= 49.019 Watts
= The force acting on the mass of 1 kg. at Normal Gravity.

1 KPM. = 9.80665 Joules
300 KPM. = 6 meters x 50 revolutions x 1 KP.

1 Joule = 0.10197 KPM.
1 KP. = 2942 Joules
1 Joule = 50 Watts/min.
* To find the Watts/min. use (KPM / 6.12) or (KP x 50).

Appendix E

METHOD TO CALCULATE VO2 MAXIMUM

Procedure:

The following sequence of events will be used for each subject with the option of altering initial workloads amongst different subjects.

(1) Have subject sit on bicycle ergometer, and take resting blood pressure and heart rate readings.

(2) Each workload will last for five minutes; this includes the resting state workload.

(3) Collect expired gas volumes in the last minute of each workload, rest inclusive.

(4) Subject should be on the mouthpiece by the third minute of each exercise session, allow tissot tank to wash out old gas by having the expired air flow through the tank.

(5) Read tissot tank thermometer, deflection scale, room thermometer, and barometer, expired gas will be analyzed with the previously mentioned analytical equipment.

(6) Upon completion of workload allow subject to rest for at least five minutes.

(7) VO2 maximum measurements may be calculated with the "VO2 maximum Calculating Program" designed by Wm. Montelpare, written in BASIC for Microcomputers.

Use 'Appendix D' to calculate workload settings for each subject.

Appendix F

SCHEDULE FOR COLLECTION AND ANALYSIS OF URINE SAMPLES

(1) Collect urine in 150 ml. urine collection containers according to the time table of; one sample immediately before exercise, one sample within five minutes after exercise, and one sample one hour after exercise.

(2) Place tightly covered urine samples into a styrofoam cooler containing dry ice or directly into the deep freezing unit in the laboratory (Room 306, Montpetit Hall, University of Ottawa).

(3) Freeze dry each sample using the vacuum type freeze drying apparatus, note the freeze drying process may be left overnight.

(4) Determine the total protein concentration by using the Bio-Rad Assay technique as outlined in Appendix F.

(5) If a total protein value is obtained then measure the albumin concentration by using the spectrophotometric technique as outlined in Appendix G.

(6) If no total protein value is obtained then discard the sample, (note) under normal conditions there should be no macromolecular proteins found in urine however low molecular weight proteins which have not been reabsorbed by the proximal and distal tubules may be present.

Appendix G

A RAPID TECHNIQUE TO MEASURE TOTAL PROTEIN

"The Bio-Rad Method"

Assay kits and reagents may be purchased from Bio-rad Ltd. Mississauga, Ontario. (Bradford, 1976)..

* Preparing the Protein Reagent:

(1) Dissolve 100mg of Coomassie Brilliant Blue G-250 dye in 50mg of 95% Ethanol.
(2) Add 100ml of 85% Phosphoric Acid.
(3) Dilute to one liter resulting in the concentration of 0.01% Dye, 4.7% Ethanol, and 8.5% Phosphoric Acid.

* When making serial solutions of protein, Bio-Rad Ltd. has suggested the use of Bovine albumin, whenever investigating for albumin.

* The technique to measure protein concentrations is extremely easy and quick.

(a) Add 0.1ml of solution (urine) to 5ml of Protein Reagent (the dye).
(b) Mix with either the vortex, or by inverting.
(c) Adjust the urine solution to 0.1ml by using the appropriate buffer (ie: 0.15M nacl solution).
(d) Use 3ml cuvettes to read the absorbancy at 590 nm.
(e) Samples may be read between two minutes and one hour after preparation.

* Making the 'saline buffer':

0.15M nacl = na 22.9898
cl 35.453
**Formula weight of nacl is 58.4428
Thus a 1M sol'n of nacl = 58.4428 gm/liter
a 0.15M sol'n = 8.76642 gm/liter.

Appendix H

MODIFIED SPECTROPHOTOMETRIC ANALYSIS FOR ALBUMIN

"Based on the Technique of Sigma Chemicals Company for: The Colourimetric Determination of Albumin in Serum"

Theoretical Principle:

The underlying theory for this technique's measurement of albumin is similar to that described in the "Bradford analysis of total protein". That is, when albumin is placed in a buffered environment containing the anionic dye "Bromcresol Green", there occurs a binding reaction between the dye and the albumin which changes the colour of the dye to "an intense blue". The blue dye is referred to as the albumin-BCG complex. The albumin-BCG complex is measured at c.630 nanometers on either a spectrophotometer, or a colourimeter. The following technique has been modified for the analysis of albumin in urine as opposed to the analysis of albumin in serum, for which the original technique was designed. According to Gustafsson (1978), changing the dilution factor between the "Bromcresol Green Dye" and the sample of urine is a acceptable procedure for measuring increases in measuring increased levels of albumin in urine.

Preparing The Reagents for The In-Vitro Diagnosis:

(1) albumin Colour Reagent, Stock Number 630-2
contains Bromcresol Green, 0.01%(w/v) in buffer: pH 4.0
Sodium Azide 0.04% added as preservative, surfactant added.
* Store at room temperature and keep protected from light.

(2) Protein in Standard Solution, Stock Number 540-10
Contains; Human albumin c.5 gm./100ml., and Human Gamma Globulin c.3 gm./100 ml.
Sodium Azide 0.05% added as a preservative.

(3) Sodium Chloride Solution of c.0.85% or 0.15moles/liter
0.15 NACL= NA 22.9898
CL 35.453
Formula Weight= 58.4428
If a one molar solution of NACL= 58.4428 gm./liter then:
* 0.15 molar solutionof NACL= 8.76642 gm./liter
* 0.85 % solution of NACL= 8.5 gm. of NACL per liter of H2O.

(4) Collect urine in standard urine collection jars provided with a screw on/off cap.
Place urine sample in deep freeze unit immediately.
Record volume of sample before freeze drying, with a vacuum type freeze drying unit, to a powder.

(5) To reconstitute urine: Dilute 0.2 grams of freeze dried urine to one milliliter with the appropriate NACL buffer.

This will be a Five-Fold Dilution of the urine sample.

Analytical Procedure:

* Use A Standard to Validate Calibration Curve *

(A) Label three or more test tubes; Blank, Standard, Test 1, etc.

(B) To all test tubes add 2.5 ml. of albumin colour reagent, this is the Bromcresol Green Dye.

(C) To "Blank" add 0.01 ml. of NACL.

(D) To "Standard" add 0.01 ml. of Protein Standard.

(E) To "Test 1", etc., add 0.1 ml. of five-fold diluted sodium chloride reconstituted urine solution.

(F) Mix with "Vortex", and allow to stand at room temperature for ten minutes.

(G) Read absorbancy at 630 +/- 5 nm. using the blank as a standard measurement.

In view of the fact that this technique was designed for serum, the choice of spectrophotometric wavelength was verified by the following experiments:

* Making Standard Solutions of Protein (albumin).

Using a stock solution of a known concentration of albumin, dilute the known concentration with 0.15 Molar NaCl Buffer, according to the following table.

References for Standard Solutions of Albumin

5.0 g. BSA / 100 ml.	NaCl= 5.0 gm./dl.=	5000	mg.%
4.5 g. BSA / 100 ml.	NaCl= 4.5 gm./dl.=	4500	mg.%
4.0 g. BSA / 100 ml.	NaCl= 4.0 gm./dl.=	4000	mg.%
3.5 g. BSA / 100 ml.	NaCl= 3.5 gm./dl.=	3500	mg.%
3.0 g. BSA / 100 ml.	NaCl= 3.0 gm./dl.=	3000	mg.%
2.5 g. BSA / 100 ml.	NaCl= 2.5 gm./dl.=	2500	mg.%
2.0 g. BSA / 100 ml.	NaCl= 2.0 gm./dl.=	2000	mg.%
1.5 g. BSA / 100 ml.	NaCl= 1.5 gm./dl.=	1500	mg.%
1.0 g. BSA / 100 ml.	NaCl= 1.0 gm./dl.=	1000	mg.%
0.9 g. BSA / 100 ml.	NaCl= 0.9 gm./dl.=	900	mg.%
0.8 g. BSA / 100 ml.	NaCl= 0.8 gm./dl.=	800	mg.%
0.7 g. BSA / 100 ml.	NaCl= 0.7 gm./dl.=	700	mg.%
0.6 g. BSA / 100 ml.	NaCl= 0.6 gm./dl.=	600	mg.%
0.5 g. BSA / 100 ml.	NaCl= 0.5 gm./dl.=	500	mg.%
0.4 g. BSA / 100 ml.	NaCl= 0.4 gm./dl.=	400	mg.%
0.3 g. BSA / 100 ml.	NaCl= 0.3 gm./dl.=	300	mg.%
0.2 g. BSA / 100 ml.	NaCl= 0.2 gm./dl.=	200	mg.%
0.1 g. BSA / 100 ml.	NaCl= 0.1 gm./dl.=	100	mg.%

Creating Microquantities with 100 ml. solutions

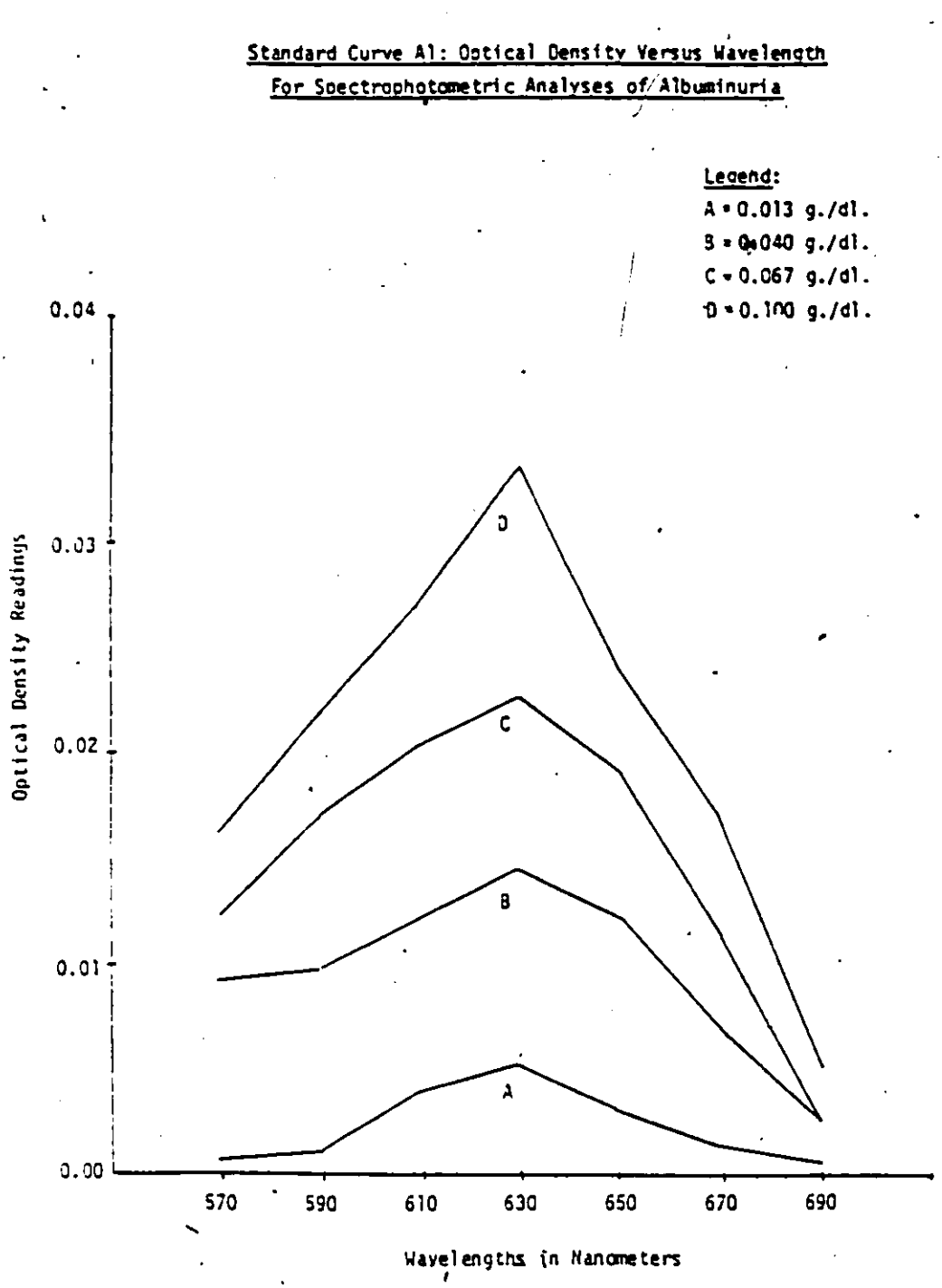
1 ml. of 100 mg.% sol. diluted to 7.5 ml.=13.33 mg %
1 ml. of 100 mg.% sol. diluted to 5.0 ml.=20.00 mg.%
1 ml. of 100 mg.% sol. diluted to 3.5 ml.=28.57 mg.%
1 ml. of 100 mg.% sol. diluted to 2.5 ml.=40.00 mg.%
1 ml. of 100 mg.% sol. diluted to 1.5 ml.=66.67 mg.%

The four standard solutions were tested for absorbancy at the seven wavelengths; 570, 590, 610, 630, 650, 670 and 690.

Curve A1 graphically displays the results which have been recorded in Table A12.

Standard Curve A1, presents a diagramatic comparison between the optical density measurements for the four

Standard Curve A1: Optical Density Versus Wavelength
For Spectrophotometric Analyses of Albuminuria



standard solutions of albumin, at four different wavelengths. Note that the total assay volume used in this technique was 2.04 ml. where 2.00 ml. was dye, and 0.04 ml. was urine sample. The volume of 2.04 ml. was required to ensure a one centimeter volume in the cuvette when being read on a spectronic 88. The dye to urine ratio of this assay was 50:1, which was similar to the dye to urine ratio for the Bradford Assay, used in the analysis of the urinary output of total proteins.

Table A12: Absorption Results for Albumin Standards

Wavelength	Concentrations in grams per deciliter			
Nanometers	0.013	0.040	0.067	0.100
570	0.0005	0.0095	0.0125	0.0165
590	0.0010	0.0100	0.0175	0.0225
610	0.0040	0.0125	0.0120	0.0280
630	0.0050	0.0150	0.0235	0.0350
650	0.0030	0.0125	0.0200	0.0250
670	0.0010	0.0070	0.0120	0.0175
690	0.0005	0.0020	0.0020	0.0050

Method Used To Calculate Albumin and Total Protein

Collection Procedure;

(1) Samples were collected in graduated collection jars.

(2) The volumes of the urine samples were recorded and the jars were placed in a deep freezing unit (-70 degrees Celcius).
The volumes of the samples do not represent the total urinary output in all cases as some of the the subjects only provided only partial quantity of their total output volume.

(3) All urine samples were freeze dried in a cannister type freeze drying unit for ninety-six hours.

(4) The freeze dried urine was then scraped out of the collection jars.

(5) Each sample of scraped urine was then weighed, the values have been reported in Table A7, in Appendix A.

(6) This weight value was corrected to a weight value per one hundred milliters of urine output.

Calculations For Urine Analysis

"0.2 gram samples were used in all measurements"

Sample A:

0.2 grams of freeze dried urine diluted to 1 ml. with 0.15 Molar Nacl Buffer (a five fold dilution).

A reconstituted urine sample of 0.04 ml. was used in each of the urine analytical tests.
The spectrophotometer readings were recorded and compared to their respective standard curves which referred

to the amount of albumin, and total protein per 0.2 gram these results have been presented in Tables A3 and A4, in Appendix A.

The value from the Standard Curve was then multiplied by the original weight value, and then divided by (0.2), the final value of this calculation referred to the output of either total protein or albumin per 0.2 gram sample of freeze dried urine. Next, the value calculated per 0.2 gram sample of freeze dried urine was multiplied by (100), and then divided by the original wet sample size (values in Table A8, of Appendix A).

This calculation referred to the concentration of either albumin or total protein in a 100 ml. output of urine.

Sample Calculation

Dry Weight = a
[] of albumin per 0.2 gram sample = b
original urinary output =c

(a x b / 0.2 x 100 / c) = Value per 100 ml. output

LET; Dry weight = 2.6830
[] of albumin per 0.2 gram sample = 0.059
Original Urinary Output = 100 ml.

(2.6830 x 0.059 / 0.2 x 100 / 100) = 0.79

Therefore 0.79 grams of albumin per 100 ml. of output.
** This calculating procedure was used for all samples.

Appendix I

ELECTROPHORETIC SEPARATION OF SERUM PROTEINS

"Using the Technique of Gelman Chemicals Company (1980)"

Equipment:

(Hardware):
1 Gelman Deluxe Electrophoretic Chamber #51212
1 Electrophoresis power supply
1 Serum Applicator
5 Staining and rinsing trays

(Reagents):
Tris-Barbital Sodium Barbital High Resolution Buffer, pH 8.8, #51104.
Ponceau S Solution, General Protein Stain, #51284
Gelman Sepra Clear II, #51283
5% Acetic Acid Solution

(Membranes):
Sepraphore III, Deluxe size Cellulose Acetate Membranes, #51003

Procedure; (using concentrated urine samples follow the same methodology as if using serum samples)

Preparing Reagent Solutions;

Tris-Barbital Sodium Barbital, pH 8.8. This high resolution buffer is used with the Gelman Deluxe Electrophoretic chamber. Dissolve one eighteen gram vial of high resolution buffer in 1200 ml. of distilled water.

General Protein Stain, Ponceau S Solution.

0.5% Ponceau S in 7.5% agueous trichloracetic acid. Purchased in 470 ml. volumes, pour 100 ml. into a staining tray and cover.

5% Acetic Acid Solution:

Add 50 ml. of glacial acetic acid to 950 ml. of distilled water. Pour 10ml. of this 5% acetic acid solution into each of three rinse trays and cover.

Gelman Sepra Clear II tm:

40% Aqueous N-Methyl Pyrrolidone solution required to clear the membrane after the migrated proteins have been fixed. Purchased in 470 ml. volumes. Pour 100ml. into a staining tray and cover.

Procedure to Separate Proteins:

Pour 100ml. of high resolution Barbital Buffer into a staining tray. Using forceps to avoid fingerprints mark one end of the cellulose acetate strip, then place the membranous strip on the surface of the buffer in the tray. Allow the membranous support medium to become completely saturated (this may be left overnight, but requires a minimum time of thirty minutes).

Fill each side of the electrophoretic chamber with 450 ml. of the high resolution Barbital Buffer. Remove membrane from the soaking tray and place on the absorbant pad, do not blot. After a few minutes, blot the Sepraphore III strip with a second absorbant pad. Place membrane in electrophoretic chamber.

Using a "serum applicator", make one application of 1.6 ul. of the sample to the membranous support medium. Hook up power supply to electrophoretic chamber, and adjust the voltage to 350 Volts. Electrophorese for 30 minutes (1-3 milliamps per second).

Procedure to Stain Migrated Proteins

When the thirty minute time limit has expired, turn off power supply to electrophoretic chamber immediately. Remove membbrane and float it on the Ponceau S solution in staining tray. When the strip is completely wet, submerge it in the stain for at least ten minutes.

Procedure to Rinse and Decolourize

Remove cellulose acetate membrane from the stain with forceps and place in the tray containing 100 ml. of 5% acetic acid solution, agitate tray to remove excess stain.

Transfer strip to second and third tray and continue to rinse until a white background is obtained. This process should take at least ten minutes.

Procedure to Clear Support Medium

With forceps remove strip from rinse tray and immerse in a tray containing 100 ml. of Sepra Clear II. Allow to clear for at least five minutes. Remove strip from clearing solution and place strip lengthwise on a glass slide. Pull a second glass slide across the membrane at a 45 degree angle to remove excess fluid and bubbles. Place slide in a 80-90 degree oven for approximately 20 minutes, this should produce a completely transparent background.

*** To quantitatively evaluate the protein migration use a densitometer at 525 nm.

Appendix J

INFORMED CONSENT LETTER

I _____, understand that the purpose of this research project was to investigate the effects of exercising continuously and intermittently on the amount of protein found in my urine.

I also understand that the term intermittent work applies to this research project as; cycling on a bicycle ergometer, at a constant speed for sixteen two minute trials, each trial being separated by a two minute rest. As well I understand that the term continuous work will apply in this study to, the act of riding a bicycle ergometer continuously for forty-two minutes at a workload which will range between 75%-80%, of my previously determined VO2 maximum.

I have agreed to perform a preliminary maximum oxygen consumption test, the results of which will be used to determine my work rate for the continuous and intermittent work tests. This preliminary VO2 maximum test will be performed on a convenient day prior to the experimental test day, and will follow the protocol of riding a bicycle ergometer against progressively more difficult workloads.

With respect to the experimental test days, I agree to provide the researcher with three seperate blood samples taken from a vein in my arm by a qualified laboratory technician, as well as three seperate urine samples which I will void voluntarily under the specified time schedule of, one of each samples prior to exercise, immediately after exercise, and one hour after exercise.

Finally I agree that I will be asked to perform to the best level of my ability, and that I may experience some uncomfortable feelings such as dizziness, nausea, and possibly cramps. Yet I fully realize that I reserve the right to discontinue my services as a subject at any time during this entire research project. As well I understand that the results of my performances will remain in the strictest confidence between the researcher and myself.

Appendix K

GLOSSARY OF TERMS AND DEFINITIONS

Anionic: a molecule which displays negative electrical charge characteristics, ie dissociated carboxylic acids.

Blood Acidity: normal pH measurements for blood range between (7.35 to 7.43). Values reported below the 7.35 level are referred to as acidic, where as those values which are observed above 7.43 are referred to as alkaline. As well, an upper limit of 7.6 and a lower limit of 7.1 has been suggested.

Bowman's Capsule: the cellular tunic like structure which encases the glomerular capillary bed of each nephron.

Bowman's Space: the inner compartmental area of the Bowman's Capsule. The Bowman's Space is a low-pressure area, which creates a diffussion gradient, promotig the movement of fluid across the glomerular capillary wall into the Bowman's Space.

Cationic: a molecule which displays positive electrical charge characteristics, the opposite of an anionically natured molecule, ie: sodium.

Fenestrae: are the spaces which separate the endothelial cells of the glomerular capillary lumen. These spaces are approximately 70.0 nanometers wide, which is relatively large as compared to the molecular radius of the plasma protein albumin (molecular radius 3.6 nanometers).

Glomerulus: refers to the renal afferent-efferent arteriole capillary bed within each nephron. The glomerulus contains the capillary bed, the Bowman's Capsule, and the Bowman's Space.

Glomerular Basement Membrane (GBM): the middle layer of the glomerular capillary wall. This structure is composed of three distinct layers, the lamina rara interna which lies adjacent to the endothelium, the central pseudo-collagenous

lamina densa, and the outer layer, the lamina rara externa. The GBM has no fixed pore structures, and holds a very weak negative charge.

Glomerular Capillary Wall (GCW): the tri-layer filtration structure of the glomerular capillary bed. The GCW is composed of an endothelium, which lines the inner capillary lumen, an elastic-like, pseudo-collagenous basement membrane, and a layer of epithelial podocyte cells, which protrude outward into the Bowman's Space.

Glomerular Filtrate (GF): is the fluid which has passed through the GCW and collected in the Bowman's Space of the Glomerulus. The GF is normally free of macromolecular substances such as hemoglobin or the plasma proteins.

Glomerular Filtration Rate (GFR): is a measure of the amount of filtrate which crosses the GCW per minute. The value has been determined in most species through tests with the low molecular weight substance Inulin, molecular weight 5200 daltons. In man the GFR is 130 ml/min. (based on two normal kidneys). In dogs the GFR value is approximately 43 ml/min. while in rats the GFR has been observed at approximately 2.0 ml/min.

Glycoproteins: are low molecular weight amino acid-carbohydrate structures. The glycoprotein which is most important to this study is, N-acetylneuraminic Acid (Sialic Acid), which has been located within both the endothelial and epithelial linings of the GCW.

Macromolecules: are substances which have a molecular weight of greater than sixty thousand Daltons, and a molecular radius of 3.5 nanometers or larger. albumin (molecular weight 66,000 Daltons, molecular radius 3.6 nm.), is a considered to be one of the smaller macromolecules.

N-acetylneuraminic Acid (Sialic Acid): this is a glycoprotein which has been primarily located as the foundation of a larger polysaccharides or proteoglycan structures. Sialic Acids are formed from N-acetyl mannosamine and pyruvic acid. The carboxylic acid terminal of the pyruvic acid component leads to the net negative charge of the Sialic Acid molecule.

Nephrons: are the functioning units of the renal filtration process. The primary features of the nephrons include the, glomerular capillary encased in the Bowman's Capsule, the proximal and distal tubule system, the periglomerular capillaries, and the vasa recta. Each of the over 1.3 million nephrons in each kidney is supplied blood through an afferent arteriole.

Permselectivity: the discriminative passive process which allows the movement of specific molecules across the capillary walls. In the glomerular capillary, this process is enhanced by the criteria of molecular charge and molecular size.

Renal Blood Flow (RBF): refers to the amount of blood which is filtered by the kidney, not to be confused with the relatively small amount blood supply which perfuses the outer layer of the kidney. The rate of renal blood, which is reported as a ml./min. value, is 500 ml./min., or approximately ten per cent of the cardiac output. Although it has been suggested that renal blood flow may be influenced by changes in normal cardiac output, the rate of renal blood flow is held nearly constant.

Serum Albumin: this macromolecular, plasma protein, made up of five hundred and eighty five amino acid residues, functions as a free fatty acid transport molecule. serum albumin, which is the same as urine albumin, has an approximate weight of 66,000 Daltons, and a molecular radius of 3.6 nanometers. albumin has been referred to as a polyanion in solution with its relatively low iso-electric point of 4.9.