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The Effect of Tourniquet Application On Systemic Coagulation

Christa Choate Hudalla

Department of Nurse Anesthesia
School of Allied Health Professions
Virginia Commonwealth University

This is to certify that the thesis prepared by Christa Choate Hudalla entitled **THE EFFECT OF TOURNIQUET APPLICATION ON SYSTEMIC COAGULATION** has been approved by her committee as satisfactory completion of the thesis requirement for the degree of Master of Science.

[redacted]
Director of Thesis

[redacted]
Committee Chairman

[redacted]
Committee Member

[redacted]
Department Chairman

[redacted]
School Dean

8/24/92
Date

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

by

Christa Choate Hudalla
Bachelor of Science in Nursing
Oakland University, 1985

Director: James Embrey, Ph.D
Associate Professor
Department of Nurse Anesthesia
School of Allied Health Professions

Virginia Commonwealth University
Richmond, Virginia
August, 1992

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Table of Contents

Page

List of Figures

List of Tables

Abstract

THE EFFECT OF TOURNIQUET APPLICATION ON COAGULATION AS MEASURED BY THE THROMBELASTOGRAM®

Christa Choate Hudalla, BSN, CCRN

School of Allied Health Professions--Virginia Commonwealth University, 1992.

Director: James Embrey, Ph.D.

Seven orthopedic surgery patients requiring the use of a tourniquet were studied. The hypothesis stated that tourniquet application does not affect coagulation. A total of 5 blood samples were drawn perioperatively (preoperative, 5 minutes after induction of anesthesia, 30 minutes after tourniquet inflation, 2 - 5 minutes after tourniquet deflation, and 30 minutes after tourniquet deflation. For each sample, TEG parameters (R, R + k, MA and α) were measured.

The value for each TEG parameter was compared by analysis of variance (ANOVA), then the samples were contrasted and examined by repeated measures ANOVA. None of the TEG parameters showed a statistically significant difference in the blood samples before, during or after tourniquet application. The hypothesis could not be

rejected at the $\alpha = .05$ level of significance. A $\underline{t}$ test was used to examine the effect of anesthesia on coagulation. The TEG parameters indicated a significant relationship between the TEG values R and R + k, and a near significant relationship between TEG values MA and α and the administration of anesthesia.

It was concluded that tourniquet use does not effect coagulation when applied 2 hours or less. However, anesthesia had a significant effect on TEG parameters R and R + k. The clinical significance of this effect was questionable since the type of anesthesia varied in some patients, and none of the patients in the study demonstrated symptoms of coagulopathy.

Chapter One

Introduction

Modern orthopedics has broadened its practice to include patients of all ages, for a wide variety of procedures. This has increased the need for anesthesia care providers to understand both the procedures and equipment that are employed today in orthopedics (Barash, Cullen & Stoelting, 1989). One device that is frequently used in orthopedic extremity surgery is the pneumatic tourniquet. These tourniquets allow surgeons better operating conditions because of dramatically decreased bleeding (Barash, Cullen & Stoelting, 1991). Complications as a result of the use of pneumatic tourniquets are rare (Palmer, 1986). However, one area that has not been studied is the effect of routine tourniquet use on the clotting mechanism.

Problem Statement

Does tourniquet use on a lower extremity effect coagulation in healthy persons undergoing orthopedic surgery?

Statement of Purpose

The purpose of this study was to investigate whether changes in coagulation occurred following tourniquet release and reperfusion of a lower extremity.

Hypothesis

The release of a tourniquet and reperfusion of the lower extremity does not effect coagulation as measured by the Thrombelastograph® (TEG).

Variables

Independent. The independent variable was the use of the pneumatic tourniquet.

Dependent. The dependent variable was the effect of reperfusion on coagulation as measured by the Thrombelastograph® (TEG).

Definition of Terms

Routine tourniquet application. The pneumatic tourniquet inflates with a compressed gas. The tourniquet pressure is both adjustable and displayed numerically on a gauge.

Effect on coagulation. The coagulation effect was analyzed by a Thrombelastograph® (TEG). Samples of venous blood were analyzed using four TEG parameters: Reaction

time (R), Clot formation time (R+K), Alpha angle (α), and Maximum Amplitude (MA).

Assumptions.

1. The TEG was correctly calibrated and functioned according to the manufacture's specifications.

2. All measurements were accurately recorded.

3. The patient did not have an undiagnosed coagulopathy.

4. Each patient served as their own control.

5. All blood samples were uniformly drawn and placed in the TEG within the 4 - 6 minute time period.

Limitations

1. Patients with known coagulopathy were excluded from the study.

2. The type of anesthetic technique differed between patients. Epidural block, or epidural block combined with general anesthesia was used in most cases. However, subarachnoid block combined with general anesthesia was used for one patient.

3. The sample was chosen from the operating room schedule between January 1 to May 1, 1992.

Delimitations

1. Patients under the age of 18 years and over the age of 65 years were excluded from the study.

2. The sample was limited to those patients scheduled for elective orthopedic surgery who required the use of a lower extremity tourniquet.

Conceptual Framework

Coagulation. Blood coagulation involves the conversion of blood from a free-flowing liquid to a semi-solid gel. If normal amounts of calcium, platelets and clotting factors are present, a series of reactions occur ending with the formation of fibrin (Petrovitch, 1991). Many conditions affect this complex process. Disease states, drug therapy, and other factors are able to cause either a coagulopathy or other abnormal coagulation profile (Ellison, 1977).

There are over 40 substances in the blood that effect coagulation. Some of these act as anticoagulants and some as procoagulants. Usually the anticoagulant activity predominates, and the blood substances are in their normal fluid state. Tissue damage, however, causes substances to be released from the (a) vascular wall, (b) platelets, and (c) blood proteins which cause increased procoagulant activity. As a result, procoagulant activity exceeds that of anticoagulant activity in the damaged area allowing clot formation to occur (Stoelting, 1987).

Normal blood coagulation is divided into four basic phases: (1) vascular spasm, (2) formation of a platelet plug, (3) procoagulant phase, and (4) restoration of vascular integrity (Price & Wilson, 1982).

1. Vascular spasm: Both nervous reflexes and myogenic contraction cause vascular spasm. Vascular spasm occurs in response to vessel wall damage; the more damaging the trauma, the more intense the vascular spasm. A crushing injury causes a more intense spasm than does the clean cut of a surgeon's knife. The obvious advantage of more intense spasm is the resulting decrease in the amount of hemorrhage. Amputating crush injuries illustrate the importance of vascular spasm, because lethal blood loss is frequently prevented by the intense vascular spasm that occurs (Petrovitch, 1991).

2. Formation of the Platelet Plug: Platelet function changes with defects in vascular integrity, hypercoagulability, hypocoagulability, and abnormalities in fibrinolysis. An adequate platelet count is essential for normal clotting to occur (Franz & Coetzee, 1981). The recommended platelet count for surgery is approximately 100,000 per cubic millimeter (mm^3). However, the presence of adequate platelet numbers does not

preclude platelet dysfunction as being a cause of coagulopathy (Mackie & Pittilo, 1985).

Platelets have unique characteristics that make them integral to the entire clotting process. They have actin and myosin molecules in their cytoplasm allowing them to contract when necessary. These molecules are similar to those found in muscle, and make platelets integral to the process of clot retraction. In addition, structures similar to muscle's endoplasmic reticulum, store calcium (Petrovitch, 1991). Other structures, contained in the cytoplasm, are responsible for the synthesis of various enzymes involved in the process of coagulation. Some of the more important substances released include (a) serotonin (increases the vascular phase), (b) adenosine diphosphate (ADP) (induces aggregation), (c) fibrin-stabilizing factor (strengthens fibrin fiber network of the clot), and (d) growth factor (stimulates endothelial growth and healing) (Packham & Mustard, 1977). In addition, fibrinogen and plasminogen, absorbed on the surface of platelets, play a role in inhibiting or accelerating clot lysis (Barrer & Ellison, 1977).

Vessel wall damage results in platelet adhesion (to the damaged region), aggregation

(platelets adhering to each other) and release of substances that augment the coagulation process. The function of the platelet plug is to decrease or stop hemorrhage. This occurs very rapidly in response to vascular damage. Thus, the platelet plug and vascular spasm are at work while the rest of the clotting mechanism (formation of a blood clot) takes place. The platelet plug differs from the blood clot in that the platelet plug often does not occlude small vessels. The damaged area specifically attracts platelets, leaving the rest of the vessel lumen open. However, compromised large blood vessels require a blood clot to stop hemorrhage (Mackie & Pittilo, 1985).

The best laboratory test of platelet function is the bleeding time. This test lends more information than a platelet count because it indicates whether platelet dysfunction is present rather than just the sheer number of platelets present. Deficient clotting factors also cause a prolonged bleeding time and must be considered in the differential diagnosis when a prolonged bleeding time is present (Moir, 1985).

3. <u>The procoagulant phase</u>: The procoagulant phase consists of the extrinsic coagulation pathway, the intrinsic coagulation pathway, and the common

pathway. The common coagulation factors involved in these pathways are shown in Table 1. Activation of the intrinsic pathway, so-called because it exists within the boundaries of the blood vessel, involves factors X, XI, IX, along with activating factors, XII, VIII, and V. Activation of the extrinsic pathway, however, requires tissue thromboplastin that is released from the surfaces of injured tissue located outside the blood vessel. Both clotting sequences lead to the activation of factor X. This is usually the rate-limiting step of the coagulation cascade; the rest of the process (the common pathway) takes only 10 - 15 seconds. This pathway culminates in the formation of fibrin (Petrovitch, 1991).

The intrinsic and extrinsic pathways are similar in many ways. Both pathways consist of a series of chain reactions, involving division of the clotting factor molecule. The larger portion becomes an active enzyme, and splits a portion of the next clotting factor, thus activating it. This process progresses to the formation of fibrin unless it is interrupted (Petrovitch, 1991).

Table 1

Coagulation Factors Necessary for Maintenance of Normal Hemostasis

Factor	Synonym
I	Fibrinogen
II	Prothrombin
III	Tissue thromboplastin
IV	Calcium
V	Proaccelerin
VII	Serum prothrombin conversion accelerator
VIII	Antihemophilic Factor
IX	Christmas Factor
X	Plasma thromboplastin
XII	Hageman Factor
XIII	Fibrin stabilizing Factor

Note. From Human Anatomy and Physiology (p. 467) by A. P. Spence and E. P. Mason, 1983, Menlo Park, CA: Benjamin/Cumming.

Calcium is integral to the clotting process, and is required for all reactions except for two steps in the intrinsic pathway (see Figure 1).

The intrinsic pathway differs from the extrinsic pathway in several ways. The triggering mechanism for each pathway is different. While the extrinsic pathway requires tissue thromboplastin and calcium for activation, the intrinsic pathway requires the activation of factor XII and/or platelets caused by trauma to the blood or contact with collagen. The intrinsic pathway is also slower than the extrinsic pathway, taking 2 - 6 minutes to produce a blood clot. Finally, there are many controls present that limit the intrinsic pathway, whereas the extrinsic pathway is limited only by the amount of tissue thromboplastin released from the tissues and the quantity of factor X, VII, and V in the blood (Guyton, 1991).

4. Restoration of vascular integrity: The final phase of normal blood clotting consists of processes that stabilize the fibrin clot and secure hemostasis. These processes involve fibrin stabilizing factor, thrombin and clot retraction.

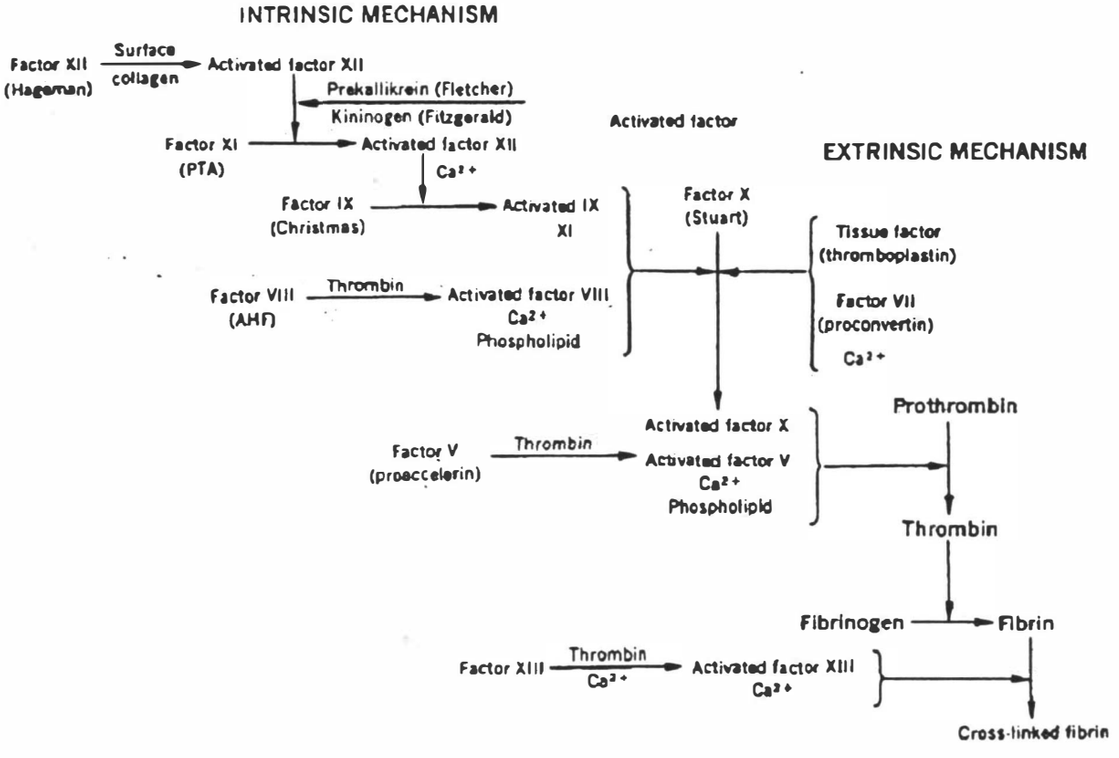

Figure 1. Coagulation cascade and clot formation.

Note. From Anesthesia and Co-Existing Disease (p. 577) by R. K. Stoelting, S. F. Dierdorf, & R. L. McCammon, 1988, New York: Churchill Livingstone.

The blood clot consists of platelets, clotting factors and blood proteins. Fibrin threads, formed in the procoagulant phase, make up the network structure holding the blood clot together. Platelets incorporated in the clot secrete a substance called fibrin stabilizing factor (FSF) (Roath & Francis, 1985). This substance strengthens the fibrin threads by establishing covalent bonding.

Thrombin's positive feedback effect on factors V and VIII accelerates the processes in the coagulation cascade. Thrombin's feedback effect on factor V is of importance because it accelerates the conversion of prothrombin to thrombin. This causes the formation of both more thrombin and fibrin. The process can reactivate itself again and again unless it is terminated by the body's regulatory processes (Spence & Mason, 1983).

Clot retraction contributes to the ultimate state of hemostasis by mechanically pulling the walls of the compromised blood vessel together. It also acts to express most of the serum from the clot, leaving a fairly strong solid mass that prevents hemorrhage. Clot retraction requires platelets, and requires their ability to contract.

The process begins a few minutes after the clot is formed and takes approximately 30 to 60 minutes to complete (Roath & Francis, 1985). Figure 2 depicts the entire process.

5. Clot lysis During the clotting process, a blood clot often occludes the entire vessel. Life expectancy would certainly be reduced if hemostasis permanently closed every injured blood vessel. Clot lysis is the process that clears the blood vessel (hopefully after healing has occurred), restoring blood flow (Silver & McGregor, 1983).

During the procoagulant phase, a large amount of plasminogen, a protein present in the blood, is incorporated into the blood clot. It requires activation to cause clot lysis. This is accomplished by thrombin, enzymes released by tissue and the vasculature, and factor XIIa (the activated form of factor XII (Roath & Francis, 1985). Within a day after clot formation, activators cause the formation of enough plasmin to start dissolving the clot. This process occurs more readily in small vessels than in larger ones. The corresponding re-opening of the blood vessel

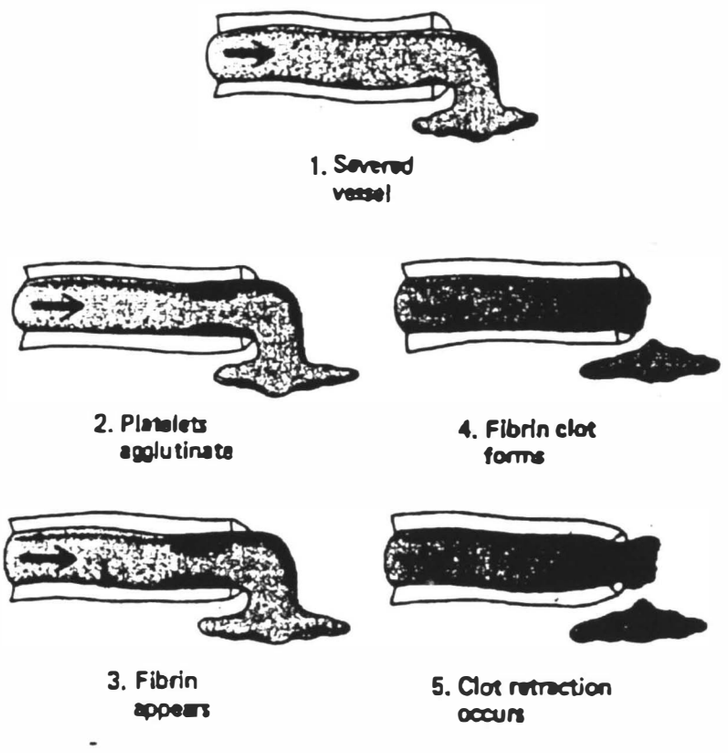

Figure 2. The clotting process.

Note. From Textbook of Medical Physiology (p. 77) by A. C. Guyton, 1991, Philadelphia: Saunders.

occurs gradually allowing the healing process to finish before completely removing the protective clot (Hirsh, 1977).

This well-designed and intricate hemostatic system functions daily to protect the body from life-threatening hemorrhage. However, without the regulatory processes that exist to control it, this very system that protects the body from

hemorrhage could also cause such extensive thrombosis as to cause death.

Regulation of coagulation. Regulation of the hemostatic process requires the combined endeavors of blood flow, endothelial vessel lining, fibrin, macrophage systems and natural anticoagulants in order to prevent pathologic clotting. Once blood clot formation is initiated, the resulting clot would continue to grow if the process was not interrupted (Stoelting, 1987).

1. Blood flow: Most of the coagulation factors circulate in an inactive form in the bloodstream. When they become activated, normal blood flow dilutes them and washes them away from the site of injury so their concentrations can not rise high enough to promote further clotting. Thus, clot formation occurs only where the blood is not moving. This helps to keep the site of clot formation localized to the site of damage (Stoelting, 1987).

2. Endothelial lining: The smooth endothelial surface that lines blood vessels prevents the activation of both the platelets and the intrinsic coagulation pathway (Stoelting, 1987). Normal endothelial cells secrete prostacyclin, an anticoagulant. It reduces platelet ADP release, inhibits platelet aggregation, and prevents the

growing platelet mass from extending beyond the site of injury onto normal endothelium.

3. Natural anticoagulants:

Anticoagulants are also important in maintaining homeostasis. Natural anticoagulants not only prevent clot formation but also help prevent clot spread once it has formed. Some of the more common anticoagulants are (a) antithrombin III, (b) heparin, (c) protein C, and (d) protein S (Petrovitch, 1991).

Antithrombin III combines with and inactivates thrombin. Macrophages then recognize and terminate the inactive complex. Heparin, which is produced by many different cells of the human body, combines with antithrombin III increasing the affinity of antithrombin III for thrombin by as much as a thousandfold (Petrovitch, 1991).

Disorders of coagulation. Surgical patients are predisposed to coagulopathy for many reasons (Barash et al, 1989). These coagulopathies can be grouped into hypocoagulable and hypercoagulable states.

1. Hypocoagulability: This refers to any condition that predisposes a person to abnormal

bleeding. There are several factors that predispose a person to a hypocoagulable state. The effects of anesthesia, drug therapy, hypothermia, and hemodilution that often occur during surgery, can produce a hypocoagulable state, and is considered in this discussion (Giddings & Evans, 1985).

Thrombocytopenia is a common cause of abnormal bleeding in the surgical patient and may result from many causes. Thrombocytopenia occurs through decreased production (chemotherapy), increased utilization (DIC), increased destruction (splenomegaly) or massive blood transfusion (dilutional) (Gilman, 1990). In addition, extensive tissue damage may cause abnormal platelet consumption. For instance, massive tissue damage that occurs with crush injuries or burns, causes so many aggregates to form that thrombocytopenia ensues (Slater, 1985).

Dilutional thrombocytopenia results from massive blood transfusion. Stored blood contains few viable platelets and causes a dilutional thrombocytopenia that can significantly affect hemostasis once approximately 10 units of blood

have been given (Stoelting, Dierdorf, & McCammon, 1988)

However, the most common platelet disorders are caused by pharmacologic agents. Medications such as anti-inflammatory agents, (ASA, Indomethacin, phenylbutazone), prostaglandin synthesis inhibitors (ibuprofen), antidepressants (amitriptyline) and other drugs such as phenothiazines, ethanol, dipyridamole, dextran, propranolol, and diphenhydramine are well known for causing functional platelet defects (Stoelting, 1987).

It is not clear to what extent inhalational and intravenous narcotic anesthetics alter platelet function (Gotta, Gould, Sullivan, & Goldinger, 1980). Enflurane and fentanyl appear to produce only insignificant prolongation of bleeding time (Fauss, Meadows, Bruni & Qureshi, 1986). Halothane, isoflurane, nitrous oxide, and local anesthetic agents, on the other hand, interfere with platelet aggregation (Borg & Modig, 1985).

Normally, a balance exists between the fibrinolytic and coagulation systems. Pathologic fibrinolytic activity can cause a bleeding tendency during surgery, and usually occurs during, or after major operations (Silver & McGregor, 1983). Hemodilution, stress of surgery,

and anesthetic agents play a role in pathologic fibrinolysis. Inhalational, intravenous and local anesthetic agents accelerate the process of fibrinolysis. However, according to Muravchick (1991) enhanced fibrinolysis in the surgical patient is probably a generalized response to perioperative stress, not a drug-specific phenomenon.

Hemodilution can be a factor in the hypocoagulable states often seen in surgical patients. Colloids such as dextran and hetastarch are more likely to remain in the intravascular space for prolonged periods of time. Therefore, large volumes of colloids may cause coagulation defects, related to dilution of both clotting factors and platelets. Dextran also decreases platelet adhesiveness which impairs the ability to form the platelet plug (Stoelting & Miller, 1989).

Commonly associated with general anesthesia, hypothermia may be a factor in anesthesia-related disorders of hemostasis. For every degree Celsius decline in temperature, the viscosity of the blood increases 2 - 3 %. In addition, platelets sequester in the portal circulation as the body temperature falls. If a sufficient number of platelets are removed from the circulation in this

manner, a hypothermia induced bleeding diathesis occurs (Schwartz, 1988). This coagulopathy reverses completely with return of body temperature to normal values (Valeri et al., 1986).

2. Hypercoagulability: Hypercoagulability is a hypothetical concept implying that prethrombotic changes can be detected in blood and are important for the development of thrombosis or can be used to predict thrombosis (Hirsh, 1977). According to Silver (1990), there are four basic situations that favor inappropriate thrombus formation (a) a reduced rate of blood flow, (b) reduced vascular endothelial protective activity, (c) blood hypercoagulability, and (d) impaired fibrinolytic activity.

According to Yardumian and Machin, (1985), there are several factors that may cause a hypercoagulable state. These include (a) platelet disorders, (b) an increase in activated coagulation factors, (c) a decrease in anticoagulants (antithrombin III), (d) defective fibrinolysis, cigarette smoking, and (e) the use of contraceptive agents. In addition, various disease states cause a hypercoagulable state (Franz & Coetzee, 1981). Because normal

coagulation is required for participation in the study, only those factors inducing hypercoagulability in the surgical patient are discussed. This also includes the effects of surgical trauma and blood loss (Eldrup-Jorgensen et al., 1989).

Surgical trauma induces a hypercoagulable state for several reasons. The stress caused by surgical trauma results in adrenergic stimulation which increases both platelet aggregation and adhesiveness. Adrenergic stimulation also causes the release of factor VIII and increased factor VIII activity systemically. In addition, tissue trauma causes the release of procoagulants at the operative site, as well as a rise in the levels of α_1-antitrypsin (a potent antiplasmin). Elevated levels of α_1-antitrypsin may be responsible for the decreased fibrinolysis seen postoperatively (Tuman, Spiess, McCarthy, & Ivankovich, 1987). In addition, generalized declines in antithrombin III activity occurs, both during surgery and postoperatively (Franz & Coetzee, 1981).

Surgical blood loss causes a loss of coagulation factors and platelets. Combined with other factors, such as hemodilution and drug effects, surgical patients seem predisposed to a

hypocoagulable state. However, the coagulation system appears to be stimulated during progressive blood loss. This may be caused by the increased levels of renin, angiotensin, and catecholamines that occur during periods of blood loss. According to Tuman et al. (1987) it is likely that the tissue trauma (with release of tissue thromboplastin), and elevations in serum catecholamine levels offset the hypocoagulable tendency caused by the hemodilution and loss of coagulation factors seen in progressive blood loss.

The Thrombelastogragh® machine. The Thrombelastograph® (TEG) machine was developed by Hartert in 1947 (Bjoraker, 1991). Preliminary results of its use were published in 1948. The mechanically operated optical system provided a continuous graphic observation of blood or plasma, and measured the entire coagulation process *in vitro*. The resulting recording (see Figure 3) was termed a thrombelastogram (TEG).

The thrombelastograph machine tests the blood sample by four basic parameters (a) R (reaction time), (b) R + k (clot formation time), (c) α (alpha angle) and (d) MA (maximum amplitude) (see Figure 4). The reaction time represents the rate of thromboplastin generation and is influenced by

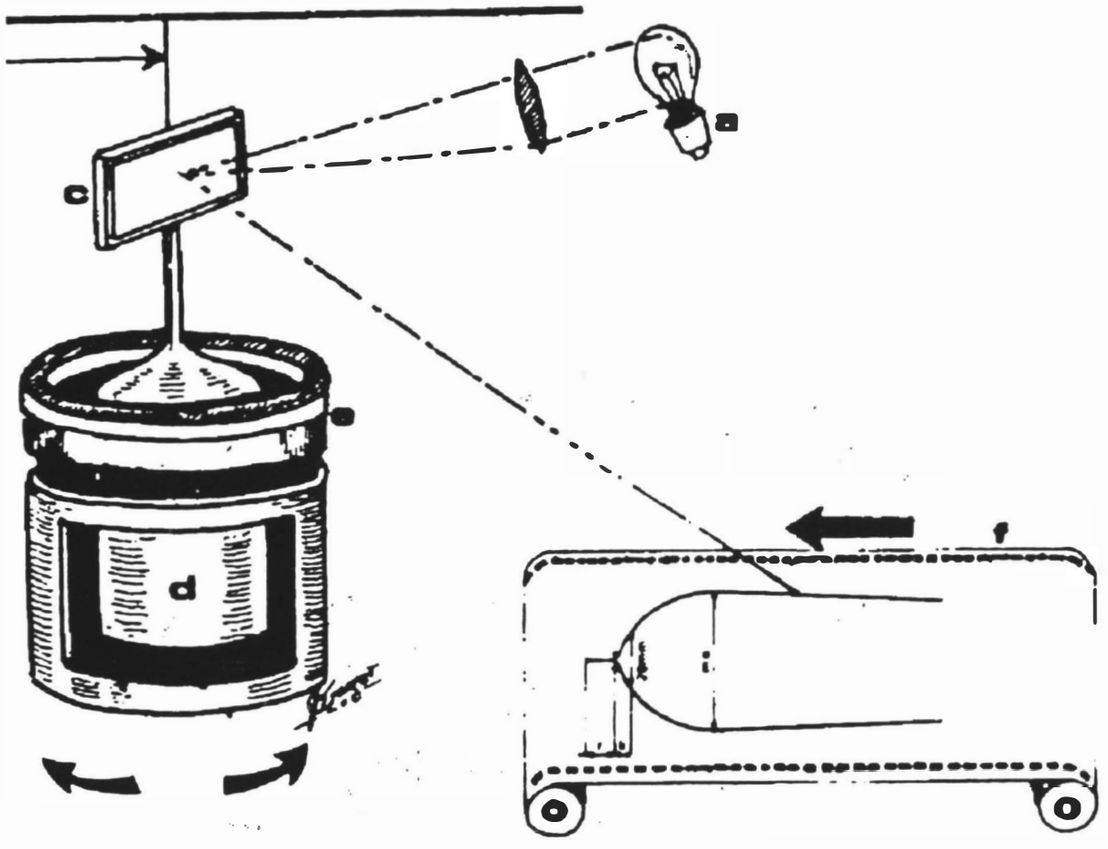

Figure 3. Schematic of TEG mechanics.

Note. From "The Thrombelastographic diagnosis of hemostatic defects" by Franz, R. C. & Coetzee, J. C., 1981, Surgery Annals, 13, p. 77.

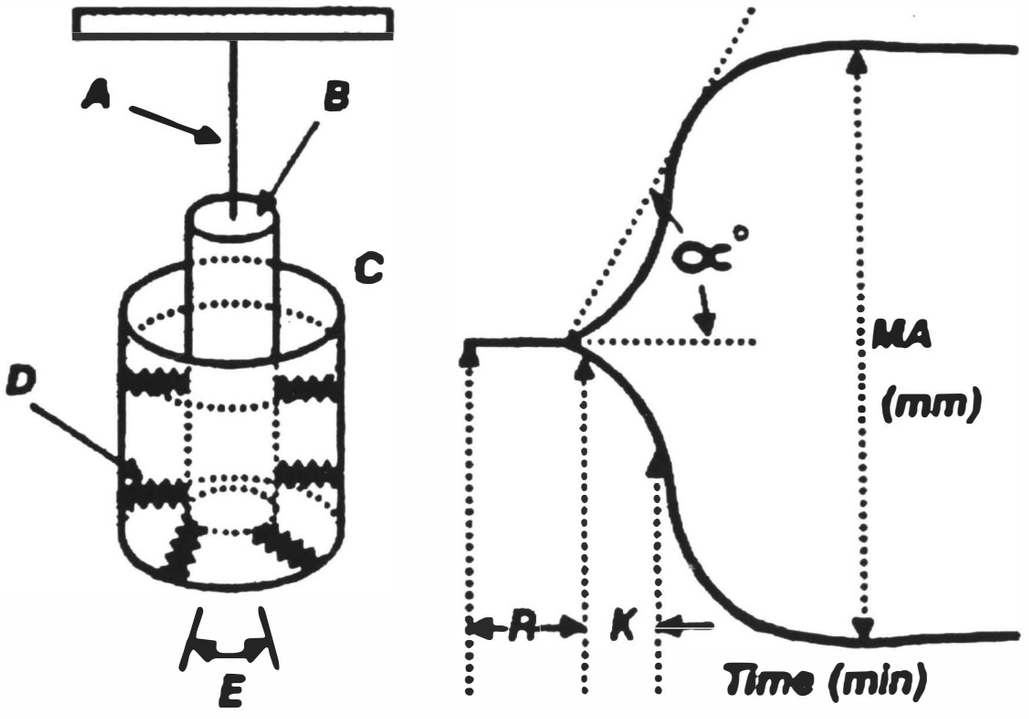

Figure 4. TEG parameters.

Note. From "The Thrombelastograph Coagulation Analyzer" by D. G. Bjoraker, 1991, Anesthesiology Review, 18(4), p. 35.

factors VIII, IX, XI, and XII. The reaction time begins when the pin is inserted into the blood filled cup and proceeds to the point where the amplitude of the graph reaches 2 millimeter (mm). Both K and the α are indexes of the rate of fibrin formation and cross-linking. The clot formation time (K) begins when the amplitude of the tracing is 2 mm in width and ends when the amplitude reaches 20 mm.

The K interval measures the rapidity of fibrin formation; whereas, α represents the rate of clot growth and fibrinogen function. Finally, MA, or maximum width, reflects the clot strength and relates to the modulus of shear elasticity (Bjoraker, 1991).

Evaluation of TEG results. Evaluation of the TEG tracing requires a knowledge of the normal values for each parameter. The normal R value is between 12 and 16 mm. TheR + K normal value ranges from 20 - 24 mm. Maximum amplitude normally measures between 50 and 70 mm, and the α angle is normally greater than 50°.

1. Hypocoagulability: Thrombelastographic hypocoagulability is diagnosed by characteristic changes seen in the TEG tracing that affect the size of TEG variables. Platelet disorders are diagnosed classically by a normal R value, prolonged R + k value and a reduction in MA. Defects in thromboplastin generation are shown by a prolongation in R. This may be seen in hemophilia, heparin therapy, or high levels of circulating anticoagulants. Deficiencies in the intrinsic pathway factors (XII, XI, IX, and VIII), circulating coagulants, thrombocytopenia and qualitative defects in either platelets and/or fibrinogen cause a coagulation defect that is shown by a prolonged R + K. A decreased α is seen

with thrombocytopenia, whereas a decrease in MA indicates either thrombocytopenia, a qualitative platelet defect, or increased circulating anticoagulants.

2. Hypercoagulability: An accelerated onset and rate of fibrin formation characterizes hypercoagulable states. Thrombelastography interprets hypercoagulability as an accelerated rate of clotting and increased firmness of the formed clot (Franz & Coetzee, 1991). However, a hypercoagulable TEG implies an increased tendency for clotting and not thrombogenesis, or actual formation of clots.

The essential features of TEG hypercoagulability are a reduction in both R, R + K, and an increase in MA (see Figure 5). However, if the hypercoagulability is plasma related, then R is decreased proportionally more than K, and MA remains less than 60 mm. If the hypercoagulability is platelet related, then the thrombelastograph exhibits a greatly decreased R and K and an MA more than 60 mm (Franz & Coetzee, 1991).

Tourniquets in orthopedic surgery. Pneumatic tourniquets are routinely used in extremity surgery because the amount of blood loss is often decreased, thereby improving surgical operating conditions (Gruendemann &

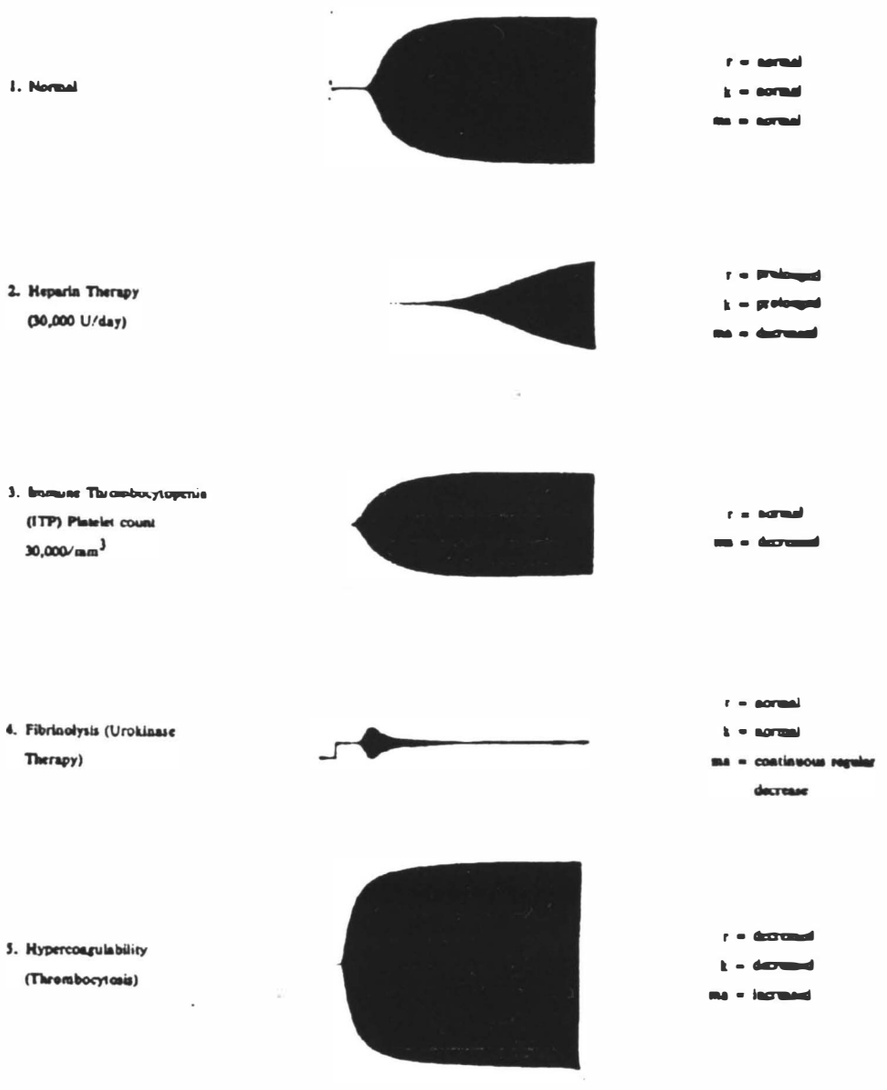

Figure 5. Characteristic TEG patterns.

Note. From "The thrombelastographic diagnosis of hemostatic defects" by Franz, R. C. and Coetzee, J. C., 1991, Surgery Annals, 13, p. 82.

Meeker, 1987). The tourniquet pressure and length of time the tourniquet is inflated varies according to local practice.

1. Metabolic effects of tourniquet ischemia: The complete arrest of circulation to the limb produces anoxia or ischemia both under and distal to the tourniquet. As tourniquet time continues to increase, the tissues of the extremity become ischemic with a progressively decreased pH and PaO_2, and increased $PaCO_2$ and lactate (Klenerman, Biswas, Hulands, & Rhodes, 1980). When the tourniquet is deflated, reperfusion of the extremity "washes out" the products of anaerobic metabolism, and they enter the general circulation (Lynn, Fischer, Brandford, & Pendergrass, 1986).

Ogawa et al. (1990) found that hypoxia exerts a complex effect on endothelial function. Hypoxic tissue was found to have an increased endothelial permeability and a disturbance in coagulant properties. Procoagulant activity on the cell surface was found to be increased by hypoxia, producing a hypercoagulable state. This could provide insight as to what may occur as a result of limb ischemia caused by tourniquet application.

The degree of anaerobic metabolites released after tourniquet ischemia depends, in part, on

whether the tourniquet is applied to a upper or lower extremity. One study found a threefold higher increase in end-tidal carbon dioxide levels following the release of a lower extremity tourniquet when compared to the release of a upper extremity tourniquet. This was attributed to the difference in skeletal muscle mass in the upper and lower extremity (Dickson, White, Kinney & Kambam, 1990).

2. Tourniquet time: The longer the tourniquet is in place the greater are the biochemical changes in the effected limb (Hargens et al., 1987). The time for the intramuscular acid-base balance to return to normal is directly proportional to the tourniquet time. After 1 hour of ischemia, recovery of normal acid-base balance occurs in the limb within 20 minutes. Recovery increases to 40 minutes for tourniquet periods of 2 - 4 hours. However, after 5 hours, recovery of acid-base balance can require 2 to 3 hours (Klenerman et al., 1980). Similarly, the time for intramuscular oxygen tension to achieve baseline values following tourniquet release are 10 minutes following 1 hour of ischemia, and increase to 12 to 15 minutes after 2 hours, and 15 to 20 minutes after 3 hours tourniquet ischemia. This may

reflect either preferential arteriovenous shunting to other tissues or increased oxygen consumption in the muscle following the tourniquet's release (Heppenstall, Balderston, & Goodwin, 1979).

Tourniquets applied for long periods cause more severe and lasting damage to the muscle lying beneath the tourniquet than to the muscles distal to it. The time a tourniquet is inflated, and whether an intermittent release of the tourniquet is used during long inflation times appear to be critical factors in determining the severity of muscle damage. Elevated creatinine phosphokinase (CPK) and lactic acid values, seen in human studies beyond 2 hours of ischemia, reflect muscle changes at that time (Shaw & Murray, 1982).

Tourniquets are often deflated for a period of 10 - 20 minutes after 1.5 to 2.0 hours of inflation and every hour thereafter regardless of the tourniquet pressure used. This tourniquet deflation time interval allows for removal of anaerobic metabolic byproducts and return of normal tissue oxygen levels. Many sources state that this "rest period" is required if greater than 2 hours of tourniquet time is necessary (Shaw & Murray, 1982). However, this point is controversial (Klenerman et al., 1980).

Since injury to soft tissue beneath a tourniquet appears to be a direct effect of applied pressure and unduly high pressures may produce injury more rapidly, the lowest pressure that maintains the objective of a bloodless field should be used. These minimum pressures for hemostasis are not well defined, however. For a standard tourniquet, commonly recommended cuff pressures for the arm are between 250 - 300 millimeters mercury (mm Hg) and between 300 - 400 mm Hg for the leg (Shaw & Murray, 1982).

3. The effects of tourniquet deflation: The body has the ability to minimize the effects of the released ischemic metabolites upon reperfusion. First, upon tourniquet deflation, the large volume of blood contained in the venous side of the circulation (50% as opposed to 1.5% in the arterial system) has a dilutional effect on the released ischemic metabolites so that changes in the acid-base status of the individual are minimized. Second, there is an efficient buffering capacity of the blood that helps to minimize acidosis caused by metabolites (Klenerman et al., 1980). This buffering capacity is reduced by anemia, hypovolemia, metabolic acidosis and pre-existing vascular disease (Palmer, 1986).

Summary

Hemostasis and coagulation refer to a complex series of reactions that lead to the control of bleeding through the formation of a platelet and fibrin clot at the injury site. The thrombelastogram provides a simple, and reliable method for evaluating the kinetics of blood clot formation and is suitable for assessing blood coagulation in the operating room environment.

It is well known that tourniquet application causes a variety of physiologic changes in the distal extremity due to anaerobic metabolism and ischemia. It is largely unknown as to whether the mechanism of coagulation is altered by these changes and is the basis of this study. Perhaps the anesthetic or surgical management of these patients could be improved by research in this area.

Chapter Two

Review of Literature

While there have been numerous studies performed on the effects of tourniquet use, none have examined what influence, if any, tourniquet use might have on coagulation. A review of literature from anesthesia, surgical and other journals may allow one to theorize if any relationship exists. Because the surgical patient is also effected by the stress of surgery and anesthesia while the tourniquet is in use, these effects on the hemostatic system are also discussed.

The Effects of Surgery on Coagulation

The occurrence of acquired coagulation defects during surgery has made the monitoring of clotting important to those involved in patient care (Howland, Schweizer, & Gould, 1974). O'Brien, Etherington and Jamieson (1971) examined intraoperative changes in platelet function. Ten patients, whose ages ranged from 17 - 85 years, were studied. All patients had major thoracic surgery.

Platelet responsiveness was analyzed by a two stage testing of platelet aggregation. Stage 1 involved the ability of platelets to change shape and become "sticky" so they adhered to one another. This was induced by the addition of ADP. Stage 2 involved a "release reaction" in which ADP was released from within the platelet. This further elevated the ADP concentration around the platelet, increasing platelet adhesiveness, aggregation, and encouraging the formation of a platelet plug. Stage 2 was induced by collagen, but can also be induced by either endogenous or exogenous ADP or epinephrine.

Blood samples were obtained: (1) 1 - 4 days before the operation, (2) immediately before the operation, (3) during the induction of general anesthesia (after administration of each anesthetic drug), and (4) intraoperatively. The timing of intraoperative samples was not specified.

Ten milliliters (ml) of citrated blood was collected and centrifuged within 10 minutes of collection. Two milliliters (ml) of the resulting platelet-rich plasma was stirred at 37 °C in an aggregometer to which either ADP or a "dilute" suspension of "ground-up collagen". If complete aggregation occurred, no further addition was made. If disaggregation occurred, ADP 1.5×10^{-5} Molar (M) was added exactly 2 minutes later. This technique permitted examination of platelet activity.

Platelet response to ADP decreased steadily with time in 9 of 10 patients (90%), until at least 1 hour after the operation, and returned to normal by the next day. Platelet response to collagen also "decreased temporarily at the end of the operation" (p. 742). It was shown that some aspect of a long operation caused a temporary decrease in both platelet aggregation and platelet release of ADP. Platelet count and volume did not change.

The authors recognized that the anesthetic agents used may have been responsible for the changes seen in platelet function. However, further decreases in platelet response were "often observed" after the end of the anesthetic. This supported the conclusion that anesthesia was not the cause of the observed platelet defects.

Franz and Coetzee (1981) studied the effects of surgery on the hemostatic profile of 20 patients undergoing a standard retropubic prostatectomy. Blood for TEG analysis and coagulation studies was obtained before, during, and 10 days after surgery. It was not stated when intraoperative blood samples were obtained or how they were drawn.

The results of the study demonstrated an increase in TEG hypercoagulability postoperatively. This coincided with a decline in plasma fibrinolytic and antithrombin III activity, as well as, a rise in the levels of α_1-antitrypsin (a potent antiplasmin). It was concluded that these findings were consistent with similar studies documenting

changes in both fibrinolysis and in overall coagulability in response to surgical stress. The TEG was recommended as an acceptable test for revealing hypercoagulability.

Butler (1978) studied 50 patients before, during and after elective abdominal surgery. The patients were divided into 3 groups (a) Group 1 (n = 26) had upper abdominal surgery (not involving splenectomy), (b) Group 2 (n = 14) had a splenectomy (in whom the platelet count was within the normal range before operation, and (c) Group 3 (n = 10) had a splenectomy with preoperative thrombocytopenia.

Serial samples of 0.36 ml, free-flowing, venous blood were taken by venipuncture using a two-syringe technique. The test sample was anticoagulated with sodium citrate solution. Blood samples were obtained (a) 1 day before operation, (b) after induction of anesthesia, (c) 1 hour after incision, (d) end of operation, (e) 1 day after operation, (f) 3 - 4 days after operation, and (g) 7 days after operation. The platelet count, fibrinogen concentration, factor VIII activity, as well as Thrombelastograph® values were analyzed.

Intraoperative TEG values demonstrated increased coagulability with a corresponding decrease in R. This hypercoagulability was significantly greater in Group 1, which exhibited a shortened R + k as well as a marked shortening of R. Postoperatively, all Groups demonstrated a significant shortening of R + k time and an increase in MA,

which was most significant in the patients with preoperative thrombocytopenia.

The MA reflected the thrombocytopenia seen preoperatively in Group 3 patients, but returned to normal postoperatively. One day after surgery, the K and MA values were found to be elevated in all groups, but more so in Group 3. The fibrinogen concentration was found to correlate better with MA than the platelet count postoperatively. Factor VIII activity was found to be elevated intraoperatively, but postoperative levels were found to be higher than intraoperative levels. The TEG variables, R and R + k were found to correlate best with factor VIII activity.

The hypercoagulability seen during surgery was attributed to the adrenergic stimulation caused by surgical trauma which is known to augment factor VIII activity, increase platelet adhesiveness, and induce platelet aggregation. Thrombelastograph values taken postoperatively indicated hypercoagulability. This hypercoagulability was not necessarily associated with actual thrombus formation, but rather, should be regarded as "a sequel" to the hemostatic changes occurring as a result of surgery.

<u>The effect of progressive blood loss</u>. Tuman et al. (1987) studied the coagulation effects of progressive blood loss on 87 adults undergoing general anesthesia for a variety of operations (orthopedic, abdominal, cranial,

thoracic, and gynecologic). None of these patients had preoperative alterations in coagulation or liver function. Blood coagulation profiles were examined using TEG.

Blood samples were obtained (a) 5 minutes before induction of anesthesia, (b) 15 minutes after induction, (c) after each 5% increment loss of estimated blood volume (EBV), (d) at the end of surgery, and (e) 2 hours postoperatively. Patients with an estimated blood loss (EBL) exceeding 0.15 EBV were given packed red cells and crystalloid solution. Patients with EBL less than 0.15 EBV received only crystalloid solution.

Thrombelastographic analysis revealed a trend toward hypocoagulability after induction of general anesthesia when compared to the preinduction state. This finding was reflected by prolongation of both R and K values, and a decreased α and MA values. Moderate blood loss (greater than 0.1 - 0.15 times the EBV) induced a hypercoagulable state by TEG analysis (decreased R and K values, increased α and MA values) when compared to postanesthesia values. Additional increases in coagulability were seen in patients with EBL of 0.10 to 0.15 times the EBV. Two hours after the operation, TEG parameters remained hypercoagulable when compared to the same values following induction of anesthesia.

It was concluded that the decreased coagulation activity seen immediately after the induction of general

anesthesia might correspond to a decreased level of stress and lower serum catecholamine levels when compared to the unanesthetized preoperative state. In addition, general anesthetics were known to have effects on blood coagulation, especially platelet function. This may have been a factor in the postanesthesia hypocoagulability.

It was hypothesized that blood loss (with the corresponding losses of coagulation factors and platelets), coupled with hemodilution (due to crystalloid replacement), would probably produce a hypocoagulable state. On the contrary, the study found no evidence for hypocoagulability after progressive blood loss. In fact, the coagulation system appeared to be stimulated during progressive blood loss.

It was surmised that increased levels of renin, angiotensin, and release of catecholamines was likely during progressive blood loss. Platelet adhesiveness was known to increase significantly after angiotensin as well as catecholamine administration. These factors might have produced the hypercoagulable state seen in most of the patients experiencing moderate to massive blood loss.

The Effect of Anesthesia on Coagulation

Gotta et al. (1980) studied 30 patients undergoing major operations to assess alterations in platelet aggregation and function during anesthesia. Patients

received either nitrous oxide, oxygen and enflurane; or nitrous oxide, oxygen and fentanyl anesthesia. Preoperative platelet counts, PT, and aPTT were normal in all patients. All patients were evenly matched as to age, sex and type of operation.

All blood samples were obtained via a 16-gauge central venous pressure line. The first 10 ml of the blood samples were discarded. All samples were analyzed for platelet aggregation as well as thrombelastography.

Untreated whole blood (0.3 ml) was pipetted into the TEG cuvette for analysis. The piston was lowered into the sample, and "covered" with mineral oil. The samples for platelet analysis were prepared by centrifuging whole blood for 10 minutes, removing 2.5 ml of platelet poor plasma from the top of the sample, then centrifuging the remaining plasma for 20 minutes. After 1 - 1.5 hours, platelets were placed in an aggregometer at 37 °C. Adenosine diphosphate (2×10^{-5} M concentration), and collagen (0.26 mg/ml) were added to each sample. Platelet aggregation was determined using Born's turbidimetric technique, which used a mechanical device to chart alterations in optical density as an index of the number of platelets in suspension. The optical density decreased in proportion to the amount of platelet aggregates in a sample because aggregation decreased the number of platelets in suspension.

The results indicated that, with one exception, all platelet specimens were aggregated by the ADP solution and this response was not altered either by anesthesia or operation. Similarly, collagen-treated specimens showed no significant alteration in the pattern of aggregation during the study period. Thrombelastography confirmed the absence of changes in either clot formation or the development of clot tensile strength.

Borg and Modig (1985) studied the effects of local anesthetics on platelet aggregation in healthy donors (N = 12). Blood samples were stored at room temperature for 1 hour. They were then prepared by a centrifugation technique into platelet-rich plasma with a platelet count of 300,000 per microliter (μL). All 12 platelet-rich plasma samples were then exposed to lidocaine, bupivacaine and tocainide, "of different concentrations" and over "different incubation times" (p. 740). Then platelet aggregation was tested using a 1 micromole (μmol) solution of ADP and a 1 microgram per milliliter (μg/ml) collagen solution. Testing was completed within 3 hours of preparation, since platelet reactivity was stated to gradually decrease with storage of the plasma.

It was found that lidocaine, bupivicaine, and tocainide caused inhibition of platelet aggregation whether or not aggregation was induced by ADP or collagen. It was indicated that the concentrations of local anesthetics

needed to cause distinct and reproducible inhibition were far greater than those usually used clinically during continuous lumbar epidural anesthesia (LEA). However, in all of the anesthetics, longer incubation times (such as is seen in LEA) produced significantly greater platelet dysfunction than short incubation times.

Of the three local anesthetics tested, lidocaine was found to be the most effective in preventing platelet aggregation; whereas, tocainide was the least effective. Higher concentrations of local anesthetic agents caused a greater inhibition of platelet aggregation. However, it was noted that the inhibitory effects of the local anesthetics were partially or totally overcome by high concentrations of ADP or collagen (10 μmol and 10 μg/ml). These concentrations of ADP and collagen would not be attainable physiologically, however.

The study concluded that the reported findings lent strong support to an antithrombotic effect of local anesthetics, particularly lidocaine. However, it cautioned against generalizations to clinical practice since higher concentrations of local anesthetics than those used clinically were needed to cause decisive and reproducible inhibition of platelet aggregation. On the other hand, the increased inhibitory effect, seen with prolonged exposure to local anesthetics, might be of clinical importance since local anesthetics have contact with the blood for long

periods of time in clinical situations such as epidural anesthesia.

Fauss, Meadows, Bruni and Qureshi (1986) investigated the effects of isoflurane and nitrous oxide on platelet aggregation both *in vivo* and *in vitro*. Venous blood samples (25 - 30 ml) were obtained, centrifuged to isolate platelets, then the platelet-rich plasma (PRP) was diluted with platelet-poor plasma to a PRP of a 150,000 - 200,000/μL platelet count.

The *in vitro* experiments were carried out on healthy volunteers (*n* = 17) with two blood samples drawn from each person. After identical preparation of the blood sample, a 3.5 ml platelet sample was placed in a Petri dish and then stored in a airtight chamber at 37 °C. Samples were then exposed to carbon dioxide (CO_2), and humidified oxygen (O_2). They were then divided into groups (a) Group 1 (isoflurane treated), (b) Group 2 (nitrous oxide treated), (c) Group 3 (isoflurane/nitrous oxide treated) and (d) Group 4 (control group). A 0.45 ml sample was withdrawn after 30 minutes and transferred to an aggregometer where platelet aggregation was measured. The agents used to stimulate aggregation were ADP (2.5 - 5.0 μM), and collagen (0.19 or 0.08 mg/ml). The aggregation response was recorded for 7 minutes after the addition of either aggregating agent.

The *in vivo* effects were also studied. Two samples of venous blood were obtained from 9 patients undergoing

elective surgery. The patients were divided into 2 groups: (1) control group (blood sample taken 1/2 hour before induction of anesthesia) and, (2) experimental group (blood sample taken 1/2 hour after induction of anesthesia. The experimental group was maintained with 1.5% Isoflurane and 66.6% nitrous oxide. The blood samples were centrifuged and prepared in the same manner as above, then placed in the aggregometer and tested.

In both the *in vitro* and *in vivo* studies, nitrous oxide (80%) and isoflurane (1.5%) demonstrated a small but significant inhibition of ADP-induced platelet aggregation. There was not a significant inhibition of aggregation noted in the collagen-treated samples. The *in vitro* addition of isoflurane to the nitrous oxide treated samples did not accentuate the platelet inhibition caused by nitrous oxide alone.

It was concluded that since none of the patients in the study developed any symptoms of abnormal bleeding, the degree of platelet dysfunction caused by nitrous oxide and isoflurane might not be clinically significant. It was suggested that in patients with mild congenital or acquired platelet disorders, the effects of these drugs might be important.

Effect of anesthetic technique. Donadoni, Baele, Devulder and Rolly (1989) studied the influence of anesthetic technique in 88 ASA II and III patients

undergoing total hip replacement. The subjects were divided into groups depending on anesthetic technique (a) Group I (general anesthesia), (b) Group II (epidural anesthesia) and (c) Group III (epidural and general anesthesia).

Blood was collected after venipuncture in tubes containing 0.1 volume of 3.8% of sodium citrate. Several laboratory studies were performed (PT, aPTT, fibrinogen, plasminogen, antithrombin III (AT III), protein C, α_2-antiplasmin (α_2AP), factor VIII activity, and von Willebrand activity. Samples were taken before the induction of anesthesia, at the end of surgery, on the first postoperative morning, and on the seventh postoperative morning.

The results demonstrated no significant differences in (a) the perioperative blood loss, (b) the amount of fluid administered or (c) the amount of blood transfused between the epidural group, and the other two groups. The aPTT was prolonged in all groups immediately after surgery when compared to the initial value, but this was only slightly significant in Group I. Fibrinogen, AT III and plasminogen were decreased significantly at the end of surgery in all three Groups, but AT III and plasminogen returned to normal faster in Group II than in the other Groups. Seven days after surgery, the plasma AT III and plasminogen levels were significantly higher in Group II than in the other Groups. Because AT III was considered the most important

physiological antagonist of activated factors II, IX, X, XI, XII, its fast return to normal was stated to possibly play a beneficial role in the prevention of DVT.

It was concluded that the influence of the anesthetic technique could not easily be separated from the influence of the surgical technique, positioning of the patient and fluid replacement. The study demonstrated the incidence of deep vein thrombosis was lower after epidural block: 45% after general anesthesia, 25% after epidural anesthesia, and 38% after both. Whether these effects are due to changes in the blood chemistry, or to a decrease in platelet aggregation (caused by the local anesthetic) remains speculative.

The effects of hypothermia and acidosis. Ferrara, McArthur, Wright, Modlin and McMillen (1990) performed a retrospective study examining 45 massively transfused trauma patients with similar Injury Severity Scores. The mean patient age was 28.5 years and the male/female ratio was 3:1. These patients had no significant head injuries or concurrent medical diseases.

Fluid resuscitation was performed in the following manner: Crystalloid infusion 3 times the blood volume lost was transfused through two 14 or 16 gauge intravenous catheters. When it was evident that blood products were needed, a third large bore site was usually placed centrally. Central venous pressure, urine output,

insensible loss, and ongoing losses were monitored and replaced. All patients in this study were treated within these guidelines and had central venous pressures that indicated adequate cardiac filling.

Charts were reviewed for age, sex, mechanism of injury, Injury Severity Score, vital signs on admission, time from injury to resuscitation initiation, duration of shock (systolic blood pressure less than 80 mm Hg), presence of acidosis, operative findings and procedures, perioperative core temperature, and 24-hour postoperative temperature. The number and type of all blood products given were recorded.

The overall mortality was 33%, with the cause of death in six patients, irreversible shock. All patients who died had documented coagulopathy. In five of those patients, uncontrollable bleeding was the cause of operative death. When survivors were compared to nonsurvivors, no difference was found in age, male/female ratio, or Injury Severity Score. However, nonsurvivors received more transfusions of PRBC than survivors, and were more likely to have a penetrating injury. Laboratory studies indicated that nonsurvivors had a higher mean PT than survivors. Nonsurvivors were also more acidotic (pH 7.04 ± 0.06 vs 7.18 ± 0.02), and more hypothermic than survivors (31 ± 1°C vs 34 ± 0.7°C). The combination of severe hypothermia, acidosis,

and clinically severe coagulopathy was, in particular, more frequent in nonsurvivors than in survivors.

The authors indicated that nonsurvivors were significantly more acidotic and hypothermic. Hypothermia was associated with severe coagulopathy in 85% of the nonsurvivors. Hypothermic, acidotic patients were found to develop significant bleeding disorders despite adequate blood, plasma, and platelet replacement. Coagulation studies performed on these patients were normal, except for the PT, which was prolonged. However, the PT values were well within the range of surgical hemostasis.

The study suggested that the total volume of transfusion, severity of acidosis, hypothermia, and the development of clinical coagulopathy statistically distinguished nonsurvivors from survivors. The combination of acidosis and hypothermia with coagulopathy had the highest mortality (90%). Once coagulopathy had developed, FFP and platelet transfusions were ineffective in correcting it. It was stated that hypothermia was an underappreciated cause of lethal coagulopathy in the patient requiring massive transfusion.

The Thrombelastograph® as a Monitor of Coagulation

A comparison of TEG to other tests. Howland, Schweizer and Gould (1974) compared intraoperative coagulation measurements against TEG values from 158 patients. A

combination of TEG, activated clotting time (ACT), and activated partial thromboplastin time (aPTT) were performed 154 times; TEG, aPTT and Hemochron® (another brand of ACT) was conducted 74 times. The initial screening sample was obtained immediately before operation, usually from a central venous pressure (CVP) catheter, and subsequent samples were drawn, when indicated, either as a follow-up of therapy or because of abnormal bleeding.

The TEG was found to detect the most clotting problems requiring therapy (whether the clotting abnormality was hypercoagulability or hypocoagulability) in all the tests performed. The best overall TEG variable for detecting both coagulopathy appeared to be the R-time. Although the aPTT was found to be "relatively useless" in detecting hypercoagulability, it was slightly superior to the TEG in determining hypocoagulable states. In evaluating the speed of obtaining results from the different methods used, the study revealed that the ACT and Hemochron® had the advantage of obtaining data rapidly (less than 5 minutes), whereas the aPTT required 30 to 40 minutes and the TEG at least 20 minutes to obtain results. However, the ACT and Hemochron® did not provide any diagnostic information. Only the TEG and aPTT assisted in determining the cause of clotting abnormalities.

Kang et al. (1985) studied TEG effectiveness in both detecting coagulation changes and guiding blood product

replacement therapy in 66 patients who underwent liver transplantation. All patients exhibited a coagulopathy preoperatively. Two groups of patients were studied. Group 1 was monitored and treated according to laboratory tests [PT, aPTT, thrombin time (TT), reptilase time, factor I, II, V, VII, VIII, IX, X, XI, XII levels, fibrin degradation product (FDP) level, euglobulin lysis time (ELT) and platelet count] and Group 2 was monitored and treated according to TEG variables. The specific number of patients in each group was not identified. All patients had two indwelling 8.5 French catheters, a pulmonary artery catheter, and an arterial line. Normal saline was used to flush the pressure monitoring catheters.

Coagulation tests were obtained (a) before induction of anesthesia, (b) 30 minutes after incision, (c) 5 minutes and 30 minutes into the anhepatic stage, and (d) 5 and 30 minutes after reperfusion of the donor liver. Post perfusion blood samples were then taken (a) every 2 hours, and (b) after every 6 liters of administered blood volume.

A blood sample of 0.36 ml was taken for the TEG test. Depending on the value obtained, the coagulopathy was treated. For example, a prolonged R (R > 15 mm) was treated with 2 units of fresh-frozen plasma, and a decrease in MA (MA < 40 mm) was treated with 10 units of platelets. When the coagulation defects did not improve, 6 units of cryoprecipitate was administered.

The results reflected a generally poor correlation between conventional laboratory tests and the TEG values. This was attributed to deviations in the individual data and other major physiologic changes that occurred during liver transplant of which standard laboratory tests might not be particularly sensitive.

The amount of blood transfused to those patients monitored with TEG was compared to those patients who were monitored with standard laboratory tests. The TEG-monitored Group received fewer units of packed red blood cells (17 ± 12.9 units vs. 26.7 ± 23.8 units), fresh-frozen plasma (18.3 ± 12.5 vs. 26.7 ± 24.1 units) and a decrease in the total fluids infused (20.2 ± 11.2 liters vs. 31.4 ± 19.2 liters) when compared to the other Group. However, the TEG monitored Group received more platelets (20.8 ± 12.8 vs. 14.1 ± 13.7 units) and cryoprecipitate (17.2 ± 8.5 vs. 10.2 ± 4.5 units). The number of blood donors the patients from either Group received were similar (67.9 ± 43.9 vs. 71.4 ± 63.4). The study concluded that the TEG was a reliable monitor of coagulation.

Spiess et al. (1987) studied the use of TEG as an indicator of post-cardiopulmonary bypass coagulopathies in 38 patients who had either coronary artery bypass or valve replacement surgery. Blood samples were taken (a) before cardiopulmonary bypass (CPB), (b) 45 minutes after the start of bypass, (c) 30 minutes after administration of protamine,

and (d) postoperatively. Blood samples were evaluated by aPTT, PT, ACT, platelet count, fibrinogen, FDP, and TEG. The samples were obtained from an arterial catheter after discarding 10 ml of blood.

The results of the study showed a weak, positive correlation between the TEG and routine coagulation tests pre-bypass; however, post-bypass test results reflected no correlations. It was stated that the TEG measured the interaction between the platelet surface and the coagulation cascade which was changed during CPB. Routine laboratory tests also were not able to measure these changes. In addition, the postoperative data suggested the TEG was a better predictor of postoperative bleeding (80%) than the ACT coagulation profile (50%).

It was concluded that the R value of the TEG was the variable most sensitive in detecting the heparin effect. However, while the TEG could be successfully used to effectively monitor low dose heparin therapy, it provided no discrimination between adequate and inadequate heparinization and was, therefore, not recommended as a monitor of heparinization during cardiopulmonary bypass.

Importance of the TEG. Lee, Taha, Trainor, Kavner, and McCann (1980) compared TEG with aPTT while monitoring heparin therapy in patients (N = 60) with deep vein thrombosis (DVT). Of these patients, Group 1 (n = 41) received heparin via a continuous infusion pump, and Group 2

(n = 19) had an intermittent infusion. The patient's heparin dosage was adjusted based upon the R value obtained from the TEG. A R value of 1.5 to 2 times normal (normal = 12 - 16 mm) was selected as desirable for each patient. The TEG and aPTT were performed twice daily.

The results indicated that heparin dosages were decreased in 27 of the 41 patients (65.9%) being treated with continuous infusion to maintain the desired R value. Of the 19 patients receiving heparin intermittently, only 4 (21.1%) needed their dosages reduced below the initial level. In 1,107 aPTT tests performed on these patients, only 26% showed a therapeutic effect at these reduced dosages of heparin. The remaining 74% revealed little or no change from the baseline values prior to heparin therapy.

It was demonstrated that the incidence of serious bleeding in patients receiving heparin therapy was 20 - 25%. However, there were no bleeding complications in the patients treated with heparin. The extreme sensitivity of the TEG to the effects of heparin allowed effective anticoagulation with less drug. The system was recommended as a practical and informative means of detecting coagulation problems.

Zuckerman, Cohen, Vagher, Woodward and Caprini (1981) compared the TEG with common coagulation tests (hematocrit, platelet count, PT, PTT, fibrin and FDP). Two groups were selected (a) Group 1 (n = 141) comprised normal volunteers,

and (b) Group 2 (n = 121), patients with cancer. Group 2 patients were selected because malignancy was known to induce a hypercoagulable state.

The results indicated that TEG variables were more accurate than the laboratory variables in differentiating between the two Groups. Only 72.3% of Group 2 patients were correctly classified by the standard laboratory tests, whereas 96.7% of these patients were identified by TEG variables. This was attributed to the higher rate of false negatives and decreased sensitivity of standard laboratory tests to the fact that standard laboratory clotting measurements end with the formation of the first fibrin strands. However, the TEG measured the process of hemostasis in whole blood from the initiation of clotting to the final stages of clot lysis or retraction. Although there was a strong correlation between the TEG variables and the common coagulation laboratory tests in this study, the TEG variables contained additional information that went beyond the first stages of clot formation. This additional information made the TEG test more sensitive to changes in the hemostatic balance of coagulation and related systems.

The Physiologic Effects of Tourniquet Use

Tourniquet ischemia. Tountas and Bergman (1977) studied the ultrastructural (appearance of the mitochondria and sarcoplasmic reticulum) and histochemical changes caused by

ischemia in both human and monkey muscle. Ten patients, aged 19 - 73 years and undergoing operations on the hand, were studied. These procedures were performed with a tourniquet in place for 1 - 2 hours. Specimens of human muscle were obtained every 30 minutes of tourniquet time. In addition, two monkeys were studied and subjected to continuous tourniquet ischemia for a period of 6 hours. Biopsies of muscle and nerve were also taken at one half hour intervals. Neither the tourniquet pressure nor anesthetic technique were mentioned.

Specimens were studied by both light and electron microscopy. Biopsies of human muscle obtained at operation showed no significant changes in muscle structure with tourniquet times of 1 - 2 hours. The animal study generally coincided with human studies for shorter tourniquet times. However, biopsies of primate forearm flexor muscles for periods longer than 2 hours revealed evidence of significant alterations. Intracellular edema and other changes leading to cell death occurred after 3 hours of tourniquet time. Tourniquet times greater than 4 hours resulted in permanent structural and functional damage to muscle and nerve.

The investigators performed an additional experiment to examine whether ischemic muscle changes in the monkey could be delayed if blood flow was restored for 30 minutes after 3 hours of ischemia. The tourniquet was then re-inflated for 2 additional hours. The authors concluded that restoration

of circulation after a period of ischemia had a myoprotective effect; it was found to prevent ultrastructural changes in the muscles for at least the succeeding 2 hour period.

Heppenstall et al. (1979) studied pathophysiologic effects of ischemia distal to a tourniquet on the hind limb of the dog. Twenty-four, adult, mongrel dogs of equal sex distribution were anesthetized with pentobarbital (20 mg/kg) and intubated. The tourniquet pressure used was 350 mm Hg. The dogs were divided into 4 groups (a) Group 1 (1 hour ischemia), (b) Group 2 (2 hour ischemia), (c) Group 3 (3 hour ischemia), and (d) Group 4
(3 successive 1 hour intervals of ischemia separated by 10 minutes of tourniquet release and reperfusion).

Once anesthetized, a GoreTex® membrane was inserted into the right quadriceps muscle distal to the tourniquet with the use of a large bore needle. An anoxic solution was then perfused through the membrane, allowing for measurement of O_2, CO_2, and pH. Samples of the solution were obtained at 15 minutes, 1 hour, and 2 hours following deflation. In addition, the lactic acid level was measured 1 minute after deflation. A biopsy of the affected muscle was taken at 2 and 24 hours post-deflation. After 2 weeks of recovery, the procedure was repeated on the opposite leg.

The results revealed that a severe state of tissue hypoxia, hypercarbia, and acidosis occurred with tourniquet

ischemia. A linear decrease in oxygen tension was noted. Intramuscular oxygen decreased from a resting value of 22 ± 4 Hg, to 0 by 25 minutes of ischemia. The amount of time to return of normal oxygen tension was proportional to tourniquet time: 10 minutes after 1 hour ischemia, 12 - 15 after 2 hours, and 15 to 20 after 3 hours. In general, the values recorded for Group 4 were very similar to Group 1 and 2, even though a total of 3 hours of tourniquet time was used in Group 4. The muscle biopsy samples revealed early degenerative changes after 1 hour of ischemia, that progressed with 2 hours of ischemia. These degenerative changes were noted to increase 24 hours later. Any permanent effects were not mentioned.

It was concluded that tourniquet time should not exceed 1 to 1.5 hours if complications are to be avoided. If further time is required, the tourniquet should be intermittently released for 10 minutes at every 1-hour interval before reinflation of the tourniquet to permit metabolic byproducts to be removed and allow normal tissue oxygen levels to return.

Patterson and Klenerman (1979) studied the effect of pneumatic tourniquets on the ultrastructure of skeletal muscle in rhesus monkeys. The actual number of subjects and tourniquet pressures were not mentioned. After induction of general anesthesia, a Kidde® tourniquet cuff was applied to the upper thigh of the right lower limb for periods lasting

from 1 to 5 hours. Biopsies were taken from the muscle lying both under the tourniquet, and distal to the tourniquet, immediately before and after tourniquet release. Samples from the opposite limb were used as controls. After biopsy, a minimum of 15 muscle fibers were prepared, then examined under a Philips® EM 300 electron microscope.

The results showed that the time a tourniquet is left in place is a critical factor in determining whether or not severe damage occurs to the muscle lying underneath. Tourniquets applied for long periods caused more severe and lasting damage to the muscle lying beneath the tourniquet than to muscles lying distal to it. It was concluded that 3 hours is probably close to the limit of time that a muscle can tolerate sustained tourniquet compression and higher tourniquet pressures may produce detrimental effects more rapidly. Therefore, the lowest effective pressure that produces a satisfactory bloodless field should be used.

Systemic effects of tourniquet application. Klenerman et al. (1980) studied the systemic and local effects of tourniquet application in adult rhesus monkeys and in patients undergoing total knee replacement. All subjects were anesthetized under general anesthesia.

Animal studies: The number of animal subjects was not given. The right internal carotid artery was cannulated for blood pressure measurement and the right internal jugular for blood sampling. In

addition, a catheter was placed in the femoral vein of the experimental limb, and passed retrograde to the area of tourniquet application. An infant-sized Kidde® tourniquet cuff was used at 300 mm Hg in all animals. Samples were taken from the right atrium over a period of 1 minute to establish controls for acid-base status and potassium. After release of the tourniquet, samples were taken simultaneously from both the internal jugular route and the femoral vein for 2 hours.

Results revealed that the acid-base balance took longer to recover with longer tourniquet times. For instance, 20 minutes were required for recovery after 1 hour of tourniquet time while 40 minutes were required after 2-4 hours of tourniquet time or inflation.

Clinical studies: Nine patients (3 men and 6 women), average age 68 years, undergoing total knee replacement were also studied. General anesthesia was used for all patients. The right internal jugular vein was cannulated, and a catheter placed into the atrium for blood sampling. An Esmarch's bandage was used to exsanguinate the limb, then a 10 cm Kidde® tourniquet cuff was inflated at a pressure of

twice the pre-induction systolic pressure. Baseline values were again taken before tourniquet deflation. Immediately before deflation, samples of blood were taken from the IJ and femoral vein of the operated limb. When the tourniquet was released, samples were taken simultaneously from the femoral needle and IJ for 15 minutes, then intermittently from the jugular cannula for 2 hours. Tourniquet time ranged from 70 to 186 minutes.

The results of both of the animal and human studies suggested that the longer the tourniquet was in place, the greater were the biochemical changes in the limb. When the tourniquet was released, samples taken from the right side of the heart showed little or no change in acid-base status. Although the potassium levels leaving the ischemic limb were elevated, none of the samples measured from the right atrium showed a significant rise in potassium. The authors indicated that this is probably due to a dilutional effect by the large volume of blood on the venous side of the circulation. In addition, the pH of the blood samples remained stable after reperfusion of the extremity, and was attributed to both a dilutional effect as well as the efficient buffering capacity of the blood.

The authors concluded that systemic changes produced by tourniquet use for up to 3 hours were not marked and were

readily reversible, providing that the blood pressure and acid-base status of the subject was stable. The sampling technique was criticized by the authors because of a well-known streaming effect of blood within the vena cava. This effect had been known to affect the measurement of venous oxygen in estimations of cardiac output. In addition, the local acid-base balance was found to recover in less than 40 minutes with tourniquet times of as much as 3 hours. The authors stated that the practice of releasing the tourniquet at 2 hours for a period of 5 to 10 minutes to allow a "breathing period" may not be appropriate. Three hours are recommended as the upper time limit for safe use of a tourniquet.

Lynn et al. (1986) studied the systemic responses to tourniquet release in 15 children, ASA I - III, aged 6 months to 15 years. Of the children studied, lower extremity tourniquet was used in 13, and upper extremity tourniquet in 2. Seven children had bilateral lower extremity surgery. In 5 patients, tourniquets were used serially (the second tourniquet was inflated after the first had been deflated). In one patient, tourniquets were inflated and deflated simultaneously.

After induction of general anesthesia, the extremity undergoing surgery was exsanguinated with an Esmarch bandage and the tourniquet inflated to a pressure ≥ 75 mm Hg higher than the child's awake systolic blood pressure. Heart rate

and rhythm, blood pressure, temperature, respiratory rate and arterial blood samples were observed at specified times (a) after induction of anesthesia (control), (b) immediately preceding tourniquet deflation, and (c) 1, 3, 5, and 10 minutes after deflation of the pneumatic tourniquet. When surgery involved two extremities, tourniquets were released sequentially (with one exception), and data were collected at the time each tourniquet was deflated.

The release of pneumatic tourniquets resulted in a 5 - 10 mm Hg transient decrease in blood pressure, lasting less than 10 minutes. Tourniquet release allowed ischemic metabolites to enter the general circulation, causing a mixed respiratory and metabolic acidosis. While the respiratory acidosis was quickly compensated in patients with one tourniquet in use, the metabolic acidosis persisted for more than 10 minutes after two tourniquets were released. The degree of metabolic acidosis increased as ischemic time and the ischemic area increased (lower limb greater than upper limb). The largest change in pH was seen when bilateral lower extremity tourniquets were released simultaneously. It is interesting that when bilateral tourniquets were used sequentially, lactate levels (which had increased after the first tourniquet release) remained elevated during the inflation time of the second tourniquet. This lactic acidosis did not clear until after the second tourniquet had been deflated. The authors indicated that

this might represent leakage of acids from the second tourniqueted extremity via bone circulation.

Tissue hypoxia, ischemia and coagulation. Eriksson, Replogle and Glagov (1986) studied reperfusion of skeletal muscle after a period of ischemia in 29 anesthetized, spontaneous breathing cats. Occlusion of blood flow was accomplished by a tourniquet inflated at 300 mm Hg, and was confirmed by a microscope focused on the area. Occlusions of 1, 2, 4, 6, 8,and 10 hours were studied, with a minimum of 4 cats in each group. Just before release of the tourniquet, the exposed vessels of the leg were observed under a microscope. At the end of observation, muscle biopsies were taken and the specimens prepared for electron microscopy.

The results revealed that the blood did not clot in the occluded vessels during the period of tourniquet use. Clot formation was noted after several minutes after tourniquet deflation and limb reperfusion. Following tourniquet release, arterioles occluded for more than 2 hours increased their diameter transiently by about 50%, and reperfusion of the extremity occurred. The diameter of most arterioles returned to baseline within 5 minutes. Venules displayed some dilation, but of less magnitude and longer duration than arterioles. In addition, the flow velocity seemed to increase in all vessels upon reperfusion and returned to baseline after about 5 minutes. However, this did not

prevent the re-occlusion of flow that was seen in muscles occluded longer than 6 hours.

Upon tourniquet deflation, blood flow ceased (in one muscle occluded for 6 hours and in all muscles occluded longer than 6 hours), after a 3 to 55 minute period of reperfusion. These muscles were noted to have numerous tiny emboli and thrombi in the vasculature during reperfusion that eventually seemed to cause the obstruction of flow. Within minutes these processes progressed to a complete obstruction of the entire vascular tree.

The authors stated these findings may imply one of two things. Either there is either a progression of the ischemic injury after the reestablishment of flow, or the injury to the tissue activated coagulation, and only required more platelets, fibrin, and other components (supplied during the period of reperfusion) to occlude the vascular tree.

Ogawa et al. (1990) studied the effect of hypoxia on capillary endothelial cell function and coagulant function. Microvascular endothelial cultures, grown in culture dishes, were exposed to hypoxia by placing them in an incubator attached to a hypoxia chamber. The chamber regulated the oxygen concentration, humidity and CO_2 content of the environment. At intervals throughout the experiments, the oxygen content of the culture medium bathing the cells was

determined by analyzing the dissolved gas. The pH of the medium was kept constant throughout the experiment.

The results demonstrated that hypoxia directly regulated the coagulant properties of microvascular endothelium. Considerable shifts in oxygenation were required for endothelial permeability to be disturbed enough to interfere with the maintenance of hemostasis. It was concluded that the effects of hypoxia on coagulant function related to a redirection of protein biosynthesis with the suppression of certain proteins and induction of others.

Summary

Many articles have been written about tourniquet ischemia and the variety of chemical changes induced by anaerobic metabolism and ischemia. It is largely unknown as to whether coagulation is affected by these changes, and is the basis of this study. The Thrombelastograph® is a sensitive instrument that monitors the entire coagulation process. The TEG values obtained can be used to diagnose many coagulation abnormalities and provide useful information to aid in the anesthetic management of patients who require the use of a tourniquet.

Chapter Three

Methodology

Design

The purpose of this study was to investigate whether changes in coagulation occur following tourniquet use on the lower extremity. The study design was quasi-experimental (Polit & Hungler, 1985).

Population, Sample, and Setting

The study population consisted of orthopedic surgery patients presenting to a mid-atlantic, university, teaching hospital. All patients were scheduled for elective surgery and required the use of a tourniquet on the lower extremity. A sample of convenience or sequential sample was employed.

Blood samples were collected perioperatively. The first sample was collected preoperatively; whereas, all other specimens were collected after the patient had been anesthetized in an operating room suite.

Orthopedic patients who met inclusion criteria were admitted to the research sample. The following criteria were used:

1. Patients must undergo orthopedic surgical procedures involving the use of a thigh tourniquet.

2. Patients in the study must have baseline normal clotting status and no history of coagulopathy.

3. Patients in the study must be between the age of 18 and 65 years.

4. Patients in the study could not have had recent (less than one week) exposure to quinidine, aspirin, rifampicin, heroin, heparin, coumadin, and nonsteroidal anti-inflammatory drugs.

5. Patients in the study must be within 20% of their ideal body weight.

6. Patients in this study must sign a informed consent form.

Morbidly obese, hypertensive (systolic blood pressure > 200 mm Hg, diastolic blood pressure > 110 mm Hg), trauma patients, and patients with recent cytotoxic treatment for malignancy (within 6 months of the study) were excluded.

Protocol

Patients were selected from the operating room schedule as they met sampling criteria. Informed consent was obtained prior to initiating the study.

On the contralateral arm to the primary peripheral intravenous catheter (IV), an 18 gauge intravenous catheter

was placed in the antecubital fossa with a low rate IV of Dextrose 5% and water to keep the vein open.

The TEG machine was warmed for 30 minutes prior to drawing the first blood sample. Blood samples were drawn in the following manner, using a two syringe technique:

1. 5 ml of blood was drawn off and discarded.

2. 0.7 ml of blood was drawn in a TB syringe for sampling. Of this blood, the first 0.35 cc were discarded. The last 0.35 cc of this blood was put into the TEG cuvette 4 - 6 minutes after venipuncture. The piston was lowered and raised 2 times and left in the lowered position. Four drops of mineral oil were placed on the surface of the sample to prevent premature drying of the sample. The TEG graph was then marked with an initial reference point. Marking the TEG graph also initiated simultaneous CTEG analysis of the TEG tracing.

Blood samples were collected at the following intervals:

Sample 1. Prior to induction (In the holding area)

Sample 2. 10 minutes after induction

Sample 3. 30 minutes after tourniquet application

Sample 4. 3 - 5 minutes after tourniquet deflation

Sample 5. 30 minutes after tourniquet deflation

Sample 6. A postoperative hemoglobin and hematocrit were drawn and recorded.

A total of five blood samples were taken for analysis on all patients in the study. Five milliliters of blood was withdrawn and discarded prior to each test sample since tissue contaminants and intravenous fluid could potentially confound the results.

Patients received either general anesthesia, epidural anesthesia, or a combination of both. One patient underwent spinal anesthesia combined with general anesthesia. All patients undergoing general anesthesia received specific agents: Midazolam, sodium thiopental, sublimaze, vecuronium and/or succinylcholine, and isoflurane and nitrous oxide for inhalation. The use of morphine sulfate (MS) was optional. The dose of MS was restricted to 10 mg or less since large dosages have been associated with hypocoagulability. Intravenous local anesthetics were not allowed on study patients during the procedure since they have also been associated with hypocoagulability. Monitoring was carried out in the routine manner. The blood pressure cuff was placed on the arm opposite the site where the blood samples were drawn.

Instrumentation

Native whole blood coagulation was measured by the Haemoscope Thromboelastograph® Machine (TEG). The TEG records the process of blood coagulation, including fibrinolysis. The result is a measure of the kinetics of

clot formation and quality. The Computerized Thrombelastogram® Analyzer (CTEG) is a computer software system linked electronically to the TEG machine. Because the CTEG monitors the TEG graph produced by the TEG machine, it provides an analysis of each parameter in numeric and graphic form.

The TEG is sensitive to all the interacting cellular and plasmatic components in the blood that may affect the rate or structure of a clotting sample. The overall profile can be qualitatively interpreted (TEG graphic tracing) or quantitatively interpreted (TEG parameters: R, R + K, MA and α) in terms of the hypocoagulable, normal or hypercoagulable state of the sample, and the degree of lysis (Haemoscope, 1991).

The TEG had two cylindrical sampling cuvettes allowing two measurements to be carried out simultaneously. Those cuvettes were maintained at a temperature of 37 ° Celsius. When in operation, each cuvette is rotated around a vertical axis at an angle of 4.5°. Inside the cuvette, a cylindrical, stainless steel piston is freely suspended from a torsion wire leaving a space of 1 millimeter (mm) between the piston and the cuvette. A mirror attached to the torsion wire reflected the light from a slit lamp onto a strip of photographic film.

As long as the sample remains fluid, the piston remains motionless, making a straight line on the graph. As the

blood clot develops, fibrin strands begin to form. These fibrin strands attach the cuvette to the piston, and the oscillations of the cuvette are transmitted to the piston via the connecting fibrin strands. Unintentional oscillation of the piston is eliminated by an oil-filled damping device.

As the fibrin formation and elasticity of the blood clot progresses, the oscillations of the piston increase proportionately. This results in a progressive increase of the deflections of the reflected light source, and production of the characteristic TEG tracing.

An electromechanical transducer converts the rotational movement of the piston into an electrical signal that is amplified. This electrical signal drives an electromechanical pen motor that records the rotational movement on a chart called the thrombelastogram. The graph moves at a speed of 2 mm/min.

Data Analysis

All numeric data were obtained from the CTEG. The data were analyzed by an analysis of variance (ANOVA) or a repeated measures ANOVA for all samples. The accepted alpha level of significance was .05. A *t* test was used to examine any effect anesthesia might have had on coagulation.

Chapter Four

Results

The sample population for this study consisted of 9 patients undergoing orthopedic lower extremity surgery that involved the use of a pneumatic tourniquet. See Table 2 for demographic data. A thigh tourniquet was utilized in all cases at a pressure of 350 mm Hg. The surgical procedures performed on the sample population were represented by 4 anterior cruciate ligament reconstructions, 1 repair of nonunion femur fracture, 1 repair of ruptured achilles tendon, 1 ankle fixation, and 2 total knee replacements. Each patient utilized in the study was screened according to the sample admission criteria. No deviations from these criteria occurred. Each patient served as their own control. Five blood samples were drawn:

Sample 1 (S1) preoperative

Sample 2 (S2) 10 minutes after induction of anesthesia

Sample 3 (S3) 30 minutes after tourniquet inflation

Sample 4 (S4) 2-5 minutes after tourniquet deflation

Sample 5 (S5) 30 minutes after tourniquet deflation

Table 2

Demographic Data

Patient	Age	Sex	$EBL_{(ml)}$	$TT_{(min)}$	Anesthesia
1	33	M	500	101	GA
2	44	F	50	125	GA
3	22	M	50	105	GA
4	23	M	50	128	E_1/GA
5	33	M	100	123	SP/GA
6	57	F	100	91	E_2
7	27	M	50	120	E_1/GA

Note. GA = General Anesthesia
E_1 = Epidural (lidocaine 3 ml and fentanyl only)
E_2 = Epidural (lidocaine test dose and maintenance)
SP = Spinal (pontocaine 2 ml and epinephrine)
EBL = Estimated blood loss
TT = Tourniquet time

Thrombelastographic parameters R, R + k, MA, and α were measured for each sample. Mean values were determined for each TEG parameter for the four sample times (see Table 3). The data were analyzed by a repeated measures analysis of variance (ANOVA) after excluding S1. Thus, there were four repeated measures included in the analysis of data; all performed while patients were under anesthesia. Two of the

Table 3

Arithmetic TEG Means

Sample	R (mm)	R + k (mm)	MA (mm)	α Angle (°)
S1	37.1 ± 13.7	57.4 ± 21.3	51.3 ± 8.60	25.8 ± 13.5
S2	28.3 ± 9.80	37.4 ± 19.6	61.3 ± 16.8	35.1 ± 18.4
S3	32.0 ± 11.6	45.2 ± 14.3	56.3 ± 11.3	35.1 ± 12.7
S4	16.1 ± 12.6	29.9 ± 18.0	57.9 ± 18.1	36.9 ± 20.6
S5	12.8 ± 13.0	29.5 ± 18.5	61.3 ± 9.10	42.1 ± 9.70

female patients were not considered in the data analysis since their blood clotted before it could be placed into the TEG cuvette.

Reaction Time (R)

An analysis of variance (ANOVA) did not reveal any significant differences between the length of R over time [$F(3, 18) = 1.690$, $p = .025$]. These relationships are presented in Table 4.

Although the overall effect of tourniquet use was not significant, mean differences between the length of R and the blood samples were examined. The mean differences of the blood samples were then examined as

Table 4

Effect of Tourniquet Use on Reaction Time (R)

Source	SS	df	Mean Sq	F-ratio	p
R/Time	636.260	3	212.087	1.690	.205
Error	2259.310	18	125.517		

contrasts. An ANOVA revealed that the contrast between S2 and S4 was significant ($p < .05$). These contrasts were presented in Table 5.

Reaction Time and Rapidity of Fibrin Buildup (R + k)

An analysis of variance did not reveal any significant differences between the length of R + k over time [$F(3, 18) = 1.529$, $p = .241$]. These relationships are displayed in Table 6. Although the overall effect of a tourniquet use was not found to be significant by R + k, mean differences between the length of R + k and the blood samples were examined. These mean differences were then examined as contrasts. An ANOVA did not reveal any significant contrasts between blood samples. These contrasts are shown in Table 7.

Table 5

Contrasts of Samples for R at Different Sample Times

Sample	Mean Sq	F-ratio	p
S2 - S3	355.716	1.321	.294
S3 - S4	620.401	1.741	.235
S4 - S5	92.893	0.361	.570
S3 - S5	1,193.423	6.189	.047*
S2 - S5	246.036	1.058	.265

Note. * = $p < .05$

Table 6

Effect of Tourniquet use on Rapidity of Fibrin Buildup (R + k)

Source	SS	df	Mean Sq	F-ratio	p
R + k	636.260	3	320.961	1.529	.241
Error	3,778.179	18	209.899		

Table 7

Contrasts of Samples for R + k at Different Sample Times

Sample	Mean Sq	*F*-ratio	*p*
S2 - S3	567.000	1.274	.302
S3 - S4	1,056.571	2.238	.185
S4 - S5	85.751	0.184	.683
S3 - S5	1,744.321	4.618	.075
S2 - S5	322.321	1.425	.278

Maximum Elasticity of Clot (MA)

An analysis of variance did not reveal any significant differences in the width of MA over time [$F(3, 18) = 0.364$, $p = .780$]. These relationships are presented in Table 8. Although the overall effect of a tourniquet use was not significant for MA, mean differences between the width of MA and the blood samples were examined. These mean differences were then examined as contrasts. However, an ANOVA did not reveal any significant contrasts between blood samples. These contrasts are displayed in Table 9.

Table 8

Effect of Tourniquet use on MA

Source	SS	df	Mean Sq	F-ratio	p
MA/Time	123.321	3	41.107	0.364	.780
Error	2,034.179	18	113.010		

Table 9

Contrasts of Samples for MA at Different Sample Times

Sample Time	Mean Sq	F-ratio	p
S2 - S3	72.321	0.185	.682
S3 - S4	51.571	0.568	.479
S4 - S5	60.036	0.399	.551
S3 - S5	0.321	0.002	.970
S2 - S5	63.000	0.283	.614

Rate of Clot Growth and Fibrinogen Function (α Angle)

An analysis of variance did not reveal a significant difference in the α angle over time [$F(3, 18) = 40.905$, $p =$

.881]. These relationships are presented in Table 10. Mean differences between the α angle and the blood samples were then examined. An ANOVA did not reveal any significant contrasts (see Table 11).

Table 10

Effect of Tourniquet use on the α Angle

Source	SS	df	Mean Sq	F-ratio	p
α Ang/Time	122.714	3	40.905	0.221	.881
Error	3344.411	18	185.801		

The Effect of Anesthesia on TEG Results

Samples 1 and 2 were compared for each TEG variable using a t test to see if the administration of anesthesia had an effect on coagulation in the experiment. These relationships are presented in Table 12. The results revealed a significant difference between both the length of R and R + k for S2 as compared with S1. The MA and α angle approached significance.

Table 11

Contrasts of Samples for α angle at Different Sample Times

Sample	Mean Sq	F-ratio	p
S2 - S3	112.000	0.287	.612
S3 - S4	46.286	0.591	.471
S4 - S5	69.143	0.159	.704
S3 - S5	228.571	0.528	.495
S2 - S5	20.571	0.045	.839

Table 12

Sample 1 Compared with Sample 2

Effect of Anesthesia on blood samples as measured by TEG

Variable	Mean Difference	df	t	p
R(1) - R(2)	13.500	6	2.687	.036*
R + k(1) - R + k(2)	19.429	6	3.182	.019*
MA(1) - MA(2)	-9.571	6	-1.970	.096
α(1) - α(2)	-11.071	6	-2.123	.078

Note. *p < .05

Chapter Five

Discussion

The purpose of this study was to determine whether tourniquet use effects blood coagulation as measured by TEG. The hypothesis stated that tourniquet use does not affect TEG results. The study failed to reject the hypothesis at the $p = .05$ level of significance. None of the TEG parameters revealed a statistically significant difference in the blood samples before, during or after tourniquet application, but further examination of the R-time measurement demonstrated one significant contrast between samples.

TEG Parameters

Of the 20 total data comparisons that were completed between the 4 TEG variables (R, R + k, MA, α) and the 5 samples, there was only one contrast, an R variable, that revealed a value that was significant (S3 - S5). Because of the large number of comparisons made, p was adjusted by the Bonferroni correction, to assure that this one significant value was not due to chance. A more severe criterion was

selected (p = .01). Thus, the contrast between S3 and S5 was not significant at p = .047.

Comparison to Previous Studies

There have been no previous studies completed that compared the effect of tourniquet use on blood coagulation. However, Ogawa et al. (1990) studied the effect of hypoxia on capillary endothelial cell function and coagulant function. They concluded that hypoxia directly regulated the coagulant properties of microvascular endothelium by redirecting protein biosynthesis; suppressing certain proteins, and inducing others.

In this study, the TEG results were not compared to routine coagulation tests as routine coagulation tests measure only a part of the coagulation cascade. The TEG measured the entire coagulation process; the TEG gave different results than expected from routine coagulation tests.

Limitations

The small sample size was a major limitation of this study. Statistical significance may be more definitive with a larger sample size. In addition, the results may have been different if patients did not have to undergo the stress of surgery and the affects of anesthesia. The anesthetic did statistically effect TEG variables R and

R + k when S1 was compared with S2 by *t* test in this study. The fact that previous studies have documented that certain anesthetics can affect coagulation raises questions as to whether different types of anesthetic techniques may have skewed results in some way. There was no way to control for differences in blood loss, hemodilution (due to fluid replacement) and temperature, which differed between patients.

Difficulties

Obtaining samples of blood was a major difficulty in this study. Obtaining a free-flowing blood sample is essential so as not to artificially activate coagulation. However, the blood appeared to be so hypercoagulable after tourniquet deflation that samples were difficult to obtain. In two subjects, the study had to be aborted because the sample could not be obtained from the intravenous sampling catheter or from any other site. In addition, the relatively long period of time required to get measurements for all the TEG variables (at least 20 minutes) limited data collection due to heavy use of the machine by other anesthesia providers.

Recommendation for Future Studies

Based on the results of this study, it is recommended that a greater number of patients be included. It may be

beneficial to study subjects who are not associated with both surgery and anesthesia since these factors makes data interpretation more difficult. Perhaps a similar study on healthy patients not undergoing surgery and/or anesthesia would help to separate these effects from any effects that might be caused by tourniquet use. If anesthesia is used, the same technique should be used for all patients to avoid confounding results. In addition, sampling may be made easier by using an arterial line primed with 0.9 normal saline.

Summary

The TEG parameters of R + k, MA, and α angle provided similar results. For the TEG parameter of R, the contrast between S2 and S4 seemed significant at first, but after Bonferroni correction it was determined that the contrast was not significant. Based on the data from the study, the hypothesis failed to be rejected at the .05 level of significance. When TEG parameters were compared for S1 and S2 to determine the effect anesthesia had on coagulation, a significant difference existed between both the length of R and R + k for S2 as compared with S1. It was concluded that anesthesia had a significant effect on coagulation as measured by R ($p = .036$) and R + k ($p = .019$), and that tourniquet use does not appear to alter TEG values for inflation times less than approximately 2 hours.

References

References

Barash, P. G, Cullen, B. F. & Stoelting, R. K. (1989). Clinical anesthesia. Philadelphia: Lippincott.

Barash, P. G., Cullen, B. F. & Stoelting, R. K. (1991). Handbook of clinical anesthesia. Philadelphia: Lippincott.

Barrer, M. J. & Ellison, N. (1977). Platelet function. Anesthesiology, 46, 202-211.

Bjoraker, D. G. (1991). The Thrombelastograph® D coagulation analyzer. Anesthesiology Review, 18 (4), 34-40.

Borg, T. & Modig, J. (1985). Potential anti-thrombotic effects of local anaesthetics due to their inhibition of platelet aggregation. Anaesthesiology Scandinavia, 29, 739-742.

Butler, M. J. (1978). Thrombelastography during and after elective abdominal surgery. Thrombosis and Haemostasis, 39, 488-495.

Dickson, M., White, H., Kinney W. & Kambam J. R. (1990). Extremity tourniquet deflation increases end-tidal PCO_2. Anesthesia and Analgesia, 70, 457-458.

Donadoni, R., Baele, G., Duvulder, J. & Rolly, G. (1989). Coagulation and fibrinolytic parameters in patients undergoing total hip replacement: Influence of the anaesthesia technique. Acta Anaesthesiology Scandinavia, 33, 588-592.

Eldrup-Jorgenson, J., Flanigan, P., Brace, L., Sauchuk, A., Mulder, S. G., Anderson, C. P., Shuler, J. J., Meyer, J. R., Durham, J. R. & Schwarcz, T. H. (1989). Hypercoagulable states and lower limb ischemia in young adults. Journal of Vascular Surgery, 9 (2), 334-339.

Ellison, N. (1977). Diagnosis and management of bleeding disorders. Anesthesiology, 47, 171-180.

Eriksson, E., Replogle, R. L. & Glagov S. (1986). Reperfusion of skeletal muscle after warm ischemia. Annals of Plastic Surgery, 18 (3), 224-229.

Fauss, B. G., Meadows, J. C., Bruni, C. Y. & Qureshi, G. D. (1986). The in vitro and in vivo effects of isoflurane and nitrous oxide on platelet aggregation. Anesthesia and Analgesia, 65, 1170-1174.

Ferrara, A., MacArthur, J. D., Wright, H. K., Modlin, I. M. & McMillen, M. A. (1990). Hypothermia and acidosis worsen coagulopathy in the patient requiring massive transfusion. The American Journal of Surgery, 160, 515-518.

Franz, R. C. & Coetzee, J. C. (1981). The thrombelastographic diagnosis of hemostatic defects. Surgery Annals, 13, 75-107.

Giddings, J. C. & Evans, B. K. (1985). Drugs affecting blood coagulation and hemostasis. International Anesthesiology Clinics, 23, 103-122.

Gilman, A. E. (1990). The pharmacological basis of therapeutics, (8th Ed.). (1990). New York: Pergamon Press.

Gotta, A. W., Gould, P., Sullivan, C. A. & Goldinger, P. L. (1980). The effect of enflurane and fentanyl anaesthesia on human platelet aggregation in vivo. Canadian Anaesthetists Society Journal, 22 (4), 319-322.

Gruendemann, B. J. & Meeker, M. H. (1987). Alexander's care of the patient in surgery. St. Louis: Mosby.

Guyton, A. C. (1991). Hemostasis and blood coagulation. Textbook of Medical Physiology. Philadelphia: Saunders, 1991, 76-86.

Haemoscope, (1991). Thrombelastograph® Operations Manual and CTEG User's Guide. 5836 Lincoln Avenue Morton Grove, Illinois 60053.

Hargens, A. R., McClure, G., Skyhar, M. J., Lieber, R. L. (1987). Local compression patterns beneath pneumatic tourniquets applied to arms and thighs of human cadavera. Journal of Orthopedic Research, 5 (2), 247-251.

Heppenstall, R. B., Balderston, R. & Goodwin, C. (1979). Pathophysiologic effects distal to a tourniquet in the dog. The Journal of Trauma, 19 (4), 234-238.

Hirsh, J. (1977). Hypercoagulability. Seminars in Hematology, 14 (4), 409-425.

Howland, W. S., Schweizer, O. & Gould, P. (1974). A comparison of intraoperative measurements of coagulation. Anesthesia and Analgesia, 53, 657-663.

Kang, Y. G., Martin, D. J., Marquez, J., Lewis, J. H., Bontempo, F. A., Shaw, B. W., Starzl, T. E. & Winter, P. M. (1985). Intraoperative coagulation changes in blood coagulation and thrombelastographic monitoring in liver transplantation. Anesthesia and Analgesia, 64, 888-896.

Klenerman, L., Biswas, M., Hulands, G. H. & Rhodes, A. M. (1980). Systemic and local effects of the application of a tourniquet. The Journal of Bone and Joint Surgery, 62(3), 385-388.

Lee, B. Y., Taha, S., Trainor, F. S., Kavner, D. & McCann, W. J. (1980). Monitoring heparin therapy with thrombelastography and activated partial thromboplastin time. World Journal of Surgery, 4, 323-338.

Lynn, A. M., Fischer, T., Brandford, H. G. & Pendergrass, T. W. (1986). Systemic responses to tourniquet release in children. Anesthesia and Analgesia, 65, 865-872.

Mackie, I. J., & Pittilo, R. M. (1985). Vascular integrity and platelet function. International Anesthesiology Clinics, 23, 3-21.

Moir, D. (1985). Investigation of bleeding disorders. International Anesthesiology Clinics, 23, 37-47.

Muravchick, S. (1991). The anesthetic plan: From physiologic principles to clinical strategies. New York: Mosby.

O'Brien, J. R., Etherington, M. & Jamieson, S. (1971, October). The refractory state of platelet aggregation with major operations. The Lancet, 741-743.

Ogawa, S., Shreeniwas, R., Brett, J., Clauss, M. Furie, M. & Stern, D.M. (1990). The effect of hypoxia on capillary endothelial cell function: Modulation of barrier and coagulant function. British Journal of Haematology, 75, 517-524.

Packham, M. A. & Mustard, J. F. (1977). Clinical pharmacology of platelets. Blood, 50 (4), 555-565.

Palmer, A. K. (1986). Complications from tourniquet use. Hand Clinics of North America, 2(2), 301-305.

Patterson, S. & Klenerman, L. (1979). The effect of pneumatic tourniquets on the ultrastructure of skeletal muscle. The Journal of Bone and Joint Surgery, 61B (2), 178-183.

Petrovitch, C. (1991, October). Preoperative evaluation of coagulation. Paper presented at the meeting of the American Society of Anesthesiologists, San Francisco, CA.

Polit, D. & Hungler, B. (1985). Essentials of nursing research: Methods and applications. Philadelphia: Lippincott.

Price, S. A. & Wilson, L. M. (1982). Pathophysiology, New York: McGraw-Hill.

Roath, S. & Francis, J. L. (1985). Normal blood coagulation, fibrinolysis and natural inhibitors of coagulation. International Anesthesiology Clinics, 23, 23-35.

Schwartz, A. (1988, October). Perioperative hypothermia. Paper presented at the meeting of the American Society of Anesthesiologists, San Francisco, CA.

Shaw, J. A. & Murray, D. G. (1982). The relationship between tourniquet pressure and underlying soft-tissue pressure in the thigh. The Journal of Bone and Joint Surgery, 64A (8), 1148-1152.

Silver, D. & McGregor, F. H. (1983). Management of the surgical patient. Philadelphia: J.B. Lippincott.

Silver, D. (1990). Hypercoagulable states. Journal of Vascular Surgery, 11 (6), 846-847.

Slater, N. G. (1985). Acquired bleeding disorders. International Anesthesiology Clinics, 23, 73-87.

Spence, A. P., & Mason, E. P. (1983). Human anatomy and physiology. Menlo Park, CA: Benjamin/Cummings.

Stoelting, R. K. (1987). Hemostasis and blood coagulation. In H. Ewan (Ed.), Pharmacology and physiology in anesthetic practice. Philadelphia: Lippincott.

Stoelting, R. K., Dierdorf, S. F. & McCammon, R. L. (1988). Anesthesia and coexisting diseases (2nd ed.) New York: Churchill Livingstone.

Stoelting, R. K. & Miller, R. D. (1989). Basics of anesthesia (2nd ed.) New York: Churchill Livingstone.

Tountas, O. R. & Bergman, R. A. (1977). Tourniquet ischemia: Ultrastructural and histochemical observations of ischemic human muscle and of monkey muscle and nerve. Journal of Hand Surgery, 2 (1), 31-37.

Tuman, K. J., Spiess, B. D., McCarthy, R. J. & Ivankovich, A. D. (1987). Effects of progressive blood loss on coagulation as measured by thrombelastography. Anesthesia and Analgesia, 66, 856-863.

Valeri, R. C., Feingold, H., Cassidy, G., Ragno, G., Khuri, S. & Altschule, M. D. (1986). Hypothermia induced reversible platelet dysfunction. Annals of Surgery, 205 (2), 175-181.

Yardumian, A., & Machin, S. J. Hypercoagulable states. International Anesthesiology Clinics,23, 141-155.

Zuckerman, L., Cohen, E., Vagher, J. P., Woodward, E. & Caprini, J. A. (1981). Comparison of thrombelastography with common coagulation tests. Thrombosis Hemostasis. 46. 752-756.

Appendix A

Informed Consent Form

Appendix A

Informed Consent Form
The Effect of Tourniquet Application
On Systemic Coagulation

Introduction:

You are being asked to participate in a research study because the procedure that you are scheduled for involves the use of a tourniquet. This device allows your surgeon better operating conditions because of dramatically decreased bleeding, which improves operating conditions. Complications as a result of the use of the tourniquet are rare. One area that has not been studied is whether or not routine tourniquet application effects coagulation. This is the purpose of the study: To find out if there are any changes in your blood's ability to coagulate, or form a clot, caused by the tourniquet application.

What the study involves:

The subjects in the study are orthopedic surgery patients scheduled to have a lower extremity tourniquet

_____
Pt. initials

under general anesthesia. All subjects in the study will have a total of five blood samples taken via an IV, or intravenous catheter, which will be inserted in the holding area prior to surgery. One blood sample will be taken sleep. Five milliliters of blood preoperatively, and the other four after the patient is will be withdrawn prior to each sample taken for testing. This is important since tissue contaminants, intravenous fluid, etc., can confound the results.

Benefits:

The purpose of this study is to ascertain whether or not tourniquet application affects the body's clotting mechanisms. With better understanding of any clotting problems that might be caused by tourniquet application, perhaps future patients may benefit from improved anesthetic or surgical management.

Risks, Inconveniences, Discomforts:

There is essentially no risk to the patient undergoing this study. There is a small risk of infection, but no more than would be expected with routine IV therapy. The total amount of blood used in sampling is small. A thromboelastograph, which will be used to test the blood samples, is available at no cost to the patient at MCV.

_____

Pt. initials

Alternative Therapy

If you do not wish to participate in this study, your surgery will be performed without any blood sampling or collection of data for the study.

Cost of Participation

There are no extra costs for participation in this study. However, you will still be responsible for all routine anesthesia costs.

Pregnancy

While there is no additional risk as the result of this study to pregnant patients, general anesthesia itself can be hazardous to an unborn fetus. Please notify your anesthetist if there is any chance that you could be pregnant.

Research Related Injury

I understand that in the event of any physical and/or mental injury resulting from my participation in this research project, Virginia Commonwealth University will not offer compensation.

If injury occurs, medical treatment will be available billed to me or appropriate third party insurance.
at the MCV Hospitals. Fees for such treatment will be

Confidentiality of Records

I understand that my name and identity will be kept

_____
Pt. Initials

confidential. I understand that the results of this study may be published, but that my identity will not be revealed.

Withdrawal

If you have any questions please feel free to ask them. You may leave the study at any time without jeopardizing your anesthesia care.

Current Telephone Numbers

Human Research Committee (804) 786-0868
Christa C. Hudalla (804) 786-9808 or (804) 786-1324
Dr. M. Nakatsuka (804) 786-1324

The nature, purpose, method, risks, benefits, and alternatives have been thoroughly explained to me. After being given this information, I do hereby give consent to participate in the research project.

All subjects will receive a copy of this consent form.

_____	_____
Signature	Date
_____	_____
Witness Signature	Date
_____	_____
Investigator Signature	Date

Appendix B

Data Collection Form

Christa C. Rudolle
Department of Nurse Anesthesia

DATA COLLECTION FORM

Christa C. Rudolle
Department of Nurse Anesthesia

Date: __/__/__
Num: _____
Age: _____
Sex: M [ ] / F [ ]

Wt: _____ KG
Ht: _____ CM
Dx: _____

Medical History:

Surgical History:

Pre-op Labs
Hbg/Hct: __/__
PLT: _____
PT/PTT: __/__ __/__

Sampling	Time	% ET Forane	Vital Signs BP	P	T	TEG Result Rmn	RK	MA	Ang	
Initial										
10m Post Induction										
30m Post Trn. Inflation										
05m Post Trn. Deflation										
30m Post Trn. Deflation										

Length of Procedure:
Total Tourniquet Time:
Tourniquet Pressure:
Procedure / Surgical Site:
Anesthetic Technique:
Position:

EBL:
HBG / HCT:
Type / Crystalloid:
Colloid:

General Comments:

Appendix C

Raw Data

Appendix C

Raw Data
Raw Data: TEG Results

TEG RESULTS: Patient #1				
Sample	*R(mm)*	*RK(mm)*	*MA(mm)*	*Ang(°)*
S1	14.0	21.0	66.0	54.5
S2	21.5	27.0	70.0	53.0
S3	16.5	21.5	62.0	59.0
S4	1.5	8.0	84.0	63.0
S5	5.0	14.0	61.0	44.0

TEG RESULTS: Patient #2				
Sample	*R(mm)*	*RK(mm)*	*MA(mm)*	*Ang(°)*
S1	30.5	48.5	50.0	24.5
S2	27.0	41.0	57.5	28.5
S3	35.5	45.5	75.5	46.0
S4	16.0	25.0	70.0	40.5
S5	35.5	46.0	75.5	45.0

TEG RESULTS: Patient #3				
Sample	*R(mm)*	*RK(mm)*	*MA(mm)*	*Ang(°)*
S1	56.0	79.5	45.0	18.0
S2	34.5	56.0	42.5	17.0
S3	33.5	53.0	39.5	22.5
S4	38.5	56.0	37.5	22.5
S5	22.0	48.0	48.0	25.0

TEG RESULTS: Patient #4				
Sample	*R(mm)*	*RK(mm)*	*MA(mm)*	*Ang(°)*
S1	45.0	74.5	43.0	17.0
S2	35.0	55.5	49.0	23.0
S3	45.0	60.5	57.0	30.0
S4	13.0	40.0	41.0	22.0
S5	3.0	10.0	61.5	49.0

TEG RESULTS: Patient #5				
Sample	*R(mm)*	*RK(mm)*	*MA(mm)*	*Ang(°)*
S1	45.0	74.5	43.0	17.0
S2	35.0	55.5	49.0	23.0
S3	45.5	60.5	57.0	30.0
S4	20.5	40.0	41.0	22.0
S5	3.0	49.0	61.5	49.0

TEG RESULTS: Patient #6				
Sample	*R(mm)*	*RK(mm)*	*MA(mm)*	*Ang(°)*
S1	39.5	61.0	55.5	20.0
S2	9.5	18.5	90.5	34.0
S3	21.5	34.5	55.5	30.5
S4	2.5	6.5	70.5	67.5
S5	2.0	8.5	68.5	50.0

TEG RESULTS: Patient #7				
Sample	*R(mm)*	*RK(mm)*	*MA(mm)*	*Ang(°)*
S1	29.5	42.5	57.0	30.0
S2	35.3	8.5	70.5	67.5
S3	26.5	41.0	47.5	28.0
S4	21.0	34.0	55.0	20.5
S5	19.0	31.0	53.0	32.5

Vita