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The effect of blood loss during surgery on the pharmacokinetics of perioperative antibiotic prophylaxis

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THE EFFECT OF BLOOD LOSS DURING SURGERY ON THE PHARMACOKINETICS OF PERIOPERATIVE ANTIBIOTIC PROPHYLAXIS

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Dedication

To my parents, Neal and Patsy Armstrong

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Abstract

The Effect of Blood Loss During Surgery on the Pharmacokinetics of Perioperative Antibiotic Prophylaxis

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These studies were designed to correlate the pharmacokinetics of perioperative prophylactic cefazolin with the degree of intraoperative blood loss in patients undergoing various types of surgery. Serial serum concentrations of cefazolin were also correlated with serum albumin levels, the duration and type of procedure, and the type of anesthetic. It was postulated that the results might help predict dosing regimens that will maintain therapeutic antibiotic levels perioperatively.

Thirty-six patients were included in this study of antibiotic prophylaxis. Seven serial serum blood levels of cefazolin were drawn during the operative procedure throughout an eight hour period. The pharmacokinetics of the serum cefazolin levels of those patients with a small blood loss was compared to those with a large blood loss. The patients were divided into three arbitrary groups of small blood loss as defined as less than 1000cc and large blood loss defined as greater than 3000cc and a third group of intermediate blood loss between these two.

The serum levels of cefazolin as determined by the area under the curve (AUC), were found to be significantly lower in those patients with a large blood loss, as compared to those patients with a small blood loss, 283 vs. 134, ($p<.05$). A large variation in the apparent volume of distribution was also noted in this study group. Only 1% of this variation in the volume of distribution could be attributed to differences in lean body mass. These results suggest a need for more frequent dosing of prophylactic antibiotics in those patients undergoing operative procedures in which there is substantial blood loss.

An Historical Outline of Antibiotic Prophylaxis

Antibiotic prophylaxis is strictly defined as the administration of an antimicrobial agent prior to bacterial contamination. Prophylaxis is to be distinguished from early therapy in that the latter is initiated immediately after the diagnosis of infection has been determined. An illustrative example would be the use of a prophylactic antibiotic prior to the inititiation of gastrointestinal surgery as the agent is administered prior to entering an internal viscus. In contrast, antibiotics administered prior to surgery to a patient presenting with pentrating abdominal trauma, would be considered early therapy as contamination has already occured in this case.[1] Careful review of the literature reveals that many of the early studies of antibiotic prophylaxis fail to make a clear distinction between these two concepts.

Early Antisepsis

For centuries surgeons have long recognized the significance and sought ways to reduce the devastating effects of post-operative infections. The earliest examples of attempts to prevent post-operative infections date back to the 1500's when cauterization was routinely performed after amputation of the extremities.[2] It is interesting to note that the field of bacteriology could have begun in the 1500's if the ideas of the Italian physician Girolamo Fracatoro had been accepted. Along with contributing to Copernicus' explanation of the solar system, in 1546 he

published an entire book on the spread of diseases by "tiny fast-multiplying bodies". Unfortunatly for Fracastorius, he was three hundred years ahead of his time.[3]

It was not until the 1800's when it became apparent that certain clinical factors could influence infection rates. One of the most notable experiments in antispetic clinical practice came in 1846 at the General Hospital in Vienna. Dr. Philipp Semmelweis noted a large discrepancy between the mortality rates from "childbed fever" (peurperal fever) in the maternity clinic between ward one (29.33%) and ward two (3%). The main difference between the two wards was that the medical students attended the patients on ward one directly after their anatomy class. Through a process of elimination the difference in mortality was found to be due to students carrying infections from their cadavers directly onto the patient wards. Subsequently, Semmelweis required everyone to wash their hands with clorinated water before births. This resulted in a reduction in the mortality rate to the same levels as ward two.[4] Tragically, this discovery was not heeded and did not find a place in surgery until decades later.[5]

Despite observations such as this, many were reluctant to accept a bacterial origin of infection. Up until this time the causative agent of infections had remained elusive. One popular theory of infection invoked an ephimeral "miasma" which existed in the air.[6] Another theory attributed wound infection to an adverse chemical reaction of oxygen in the air to the exposed tissues.[7]

It was not until the 1870's that the germ theory began to gain wide acceptance. In 1840 Henle had revived the germ theory with his text; "On

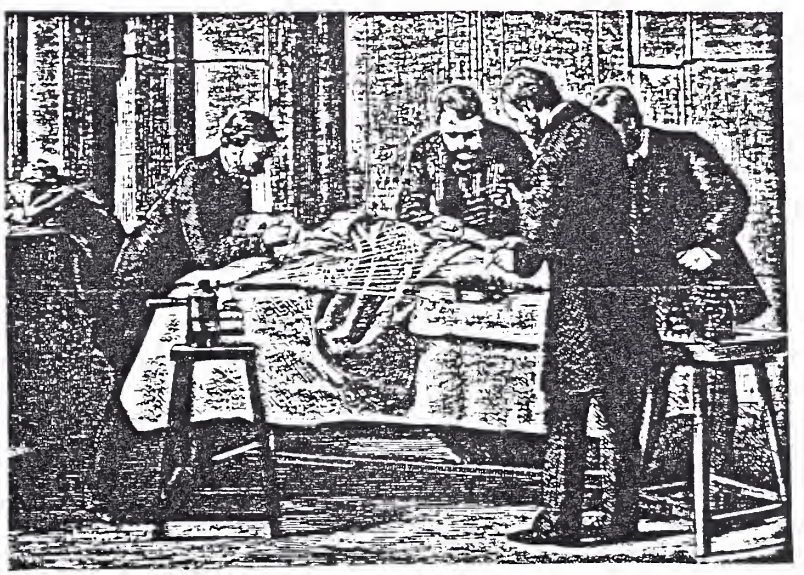
Miasmas and Contagions".[8] A student of Henle's, Robert Koch, became one of the first professors of hygiene and bacteriology. Along with his influence in promoting a bacterial cause for infections, Koch was also noted for inventing new methods of culturing bacteria, the development of tuberculin, and bichloride of mercury as a bactericidal agent. His studies led to the formulation of "Koch's law", which serves as the basis for all modern bacteriology.[9]

Another great figure in the field of bacteriology was Louis Pasteur. Pasteur studied a variety of issues closely related to the medical field. One of his interests was in the field of microorganisms which cause disease in humans. Pasteur proved that fermentation was caused by fungi or bacteria.[10] He is credited with having discovered staphylococci and streptococci along with explaining the toxic effects of bacteria. He was a strong proponent of bacteria as the causative agents in various infections.[11] The work of Pasteur and Koch issued in the "Golden age of bacteriology".[12] Their work set the foundation for future research.

The concepts of bacteriology had immediate applications in the field of surgery. It was at this time that some surgeons began to attempt methods to reduce the incidence of bacterial infection. Joseph Lister became one of surgery's outstanding pioneers in the field of antisepsis. Lister was the first to use a surgical antiseptic solution. The year 1867 saw the advent of Lister's carbolic spray as a means of antisepsis.[13] It is interesting to note that despite the vast evidence in favor of the concept of bacteriology, even at this time the field of bacteriology had its skeptics. Florence Nightingale, who is well noted for her two books whose main points emphasized soap, cleanliness, fresh air, and one patient to a bed, knew nothing about bacteria and doubted Lister's theory that

bacteria even existed.[14]

Lister's paper on antisepsis was published in the Lancet and his ideas gained wide acceptance making him world renownd.[15,16] Refinements were made on Lister's original techniques and other antiseptic agents were discoverd such as iodine and bichloride of mercury.[17] The principles of antisepsis were later carrried to their logical conclusion of total surgical asepsis. Ernst von Bergman proposed the sterilization of surgical instruments.[18] Dr. Bernhard Kronig was instrumental in promonting the use of surgical gloves.[19] Johannes von Mikulicz-Radecki was one of the first to use a face-mask during surgery. Perhaps the ultimate extension of antisepsis is in the mouthwash which honors Lister's name, Listerine.[20]



The proper use of Lister's carbolic spray during surgery. (From Glaser, The Road to Modern Surgery, 1960 Lutterworth Press, London.)

The history of early antisepsis is significant as it describes the progression of a new understanding of infectious complications of surgery and fostered the development of measures to counter infection. These

discoveries transformed the field of surgery allowing new operations of even greater magnitude in a variety of anatomical locations previously considered prohibitive.[21]

Early Antibiotic Prophylaxis

It was not until 1939 that the first reported use of an antimicrobial for surgical prophylaxis appeared. Some of the initial studies of antibiotic prophylaxis are incongruous due to variations in the timing of antibiotic administration. Indeed, in many of these early studies the authors failed to make a clear distinction between early therapy and true prophylaxis. In a study by Jensen using topical sulfanilamide in open fractures it was found that the post-operative infection rate was reduced from 27% to less than 5%.[22] This study issued in the concept of prophylactic antibiotics in surgery along with several decades of controversy as to the details of prophylactic antibiotic usage along with questions as to the validity of the concept as a whole.

In 1945 as the First World War was drawing to a close Meleney published a report on the use of prophylactic topical and systemical sulfonamides in surgery.[23] It was hoped that this study would suggest a possible role for preventing infection in the enormous number of injured soldiers. Using abundant information gathered from civilian casualties Meleney was forced to the conclusion that overall the controls did better than those treated with the sulfonamides. Despite certain studies such as this indicating the ineffectiveness of these agents for prophylaxis, the search for the beneficial effects of prophylactic antibiotics continued.

With the advent of penicillin in the United States in 1942 a new

agent entered the arena of antibiotic prophylaxis. It initially found its main use in the field of surgical obstetrics.[24] By the late 1940's streptomycin and tetracycline became widely available and along with penicillin began to be prophylactically administered to parturient women.[25] These agents showed a clear benefit in reducing post-operative morbidity, especially in patients at high risk for infection.

Yet further support for the use of sulfonamides and penicillins came in the field of gastrointestinal surgery. In 1947 Griffin reported the benefit of these agents in reducing the rate of infection for appendectomy. During the period from 1935 to 1947 the mortality associated with an appendectomy was reduced from 7.6% to 0.9%. While these were many factors involved, this reduction was attributed in large part to the effects of prophylactic antibiotics.[26]

While the beneficial effect of prophylactic antibiotics in certain situations was apparent it was also becoming evident that the indiscriminate use of these agents could have detrimental effects. A study by Prince in 1946 examining the effects of prophylactic antibiotics for transurethral resection of the prostate showed a beneficial effect of the antibiotic in some cases yet also revealed the emergence of resistant strains of microorganisms such as Pseudomonas sp.[27] This was one of the earliest papers to discuss the difficulty of balancing the therapeutic effects of reducing the bacterial counts of certain strains while simultaneously avoiding the proliferation of competing strains.

By 1954 the use of prophylactic antibiotics for many procedures had gained wide acceptance, although many aspects of their effective administration had yet to be resolved. A study by McKittrich and

Wheelock examined the potential benefits of antibiotic prophylaxis against the hazards associated with the indiscriminate use of these agents.[28] They pointed out the risks of the selection of resistant strains, the expense involved in the routine use of antibiotics, and the potential toxicity of these agents. In their study examining the use of antibiotics in abdominal surgery they concluded that there was not sufficient benefit to justify the use of these agents when weighed against the negative aspects of antibiotic prophylaxis. In retrospect it is to be noted that their conclusions were flawed as they were not established on the premise that prophylaxis should be started prior to the initiation of surgery. Had they controlled for this factor their results would undoubtably have differed.

The evolution of the use of antibiotics during the treatment of burn cases revealed the importance of the method of administration along with the type of agent used. The use of systemic antibiotics for burn cases was reported as early as 1946. There were conflicting reports as to the efficacy of systemic antibiotics in burn cases at that time.[29,30] Part of this controversy centered around the fact that the systemic antibiotics are effective against early superficial wounds yet are less efficacious against major burns and sepsis in which they often select for resistant strains.[31] In 1960 it was noted that topical penicillin cream greatly reduced the rate of infection in burn cases.[32] In 1965 Moncrief and Moyer proposed the barrier technique to reduce the degree of bacterial colonization by applying prophylactic antibiotic coverage to the surface of the burnt tissue.[33] With further refinement of this technique by Lindberg and Polk, decreased post-operative morbidity and mortality were observed.[34]

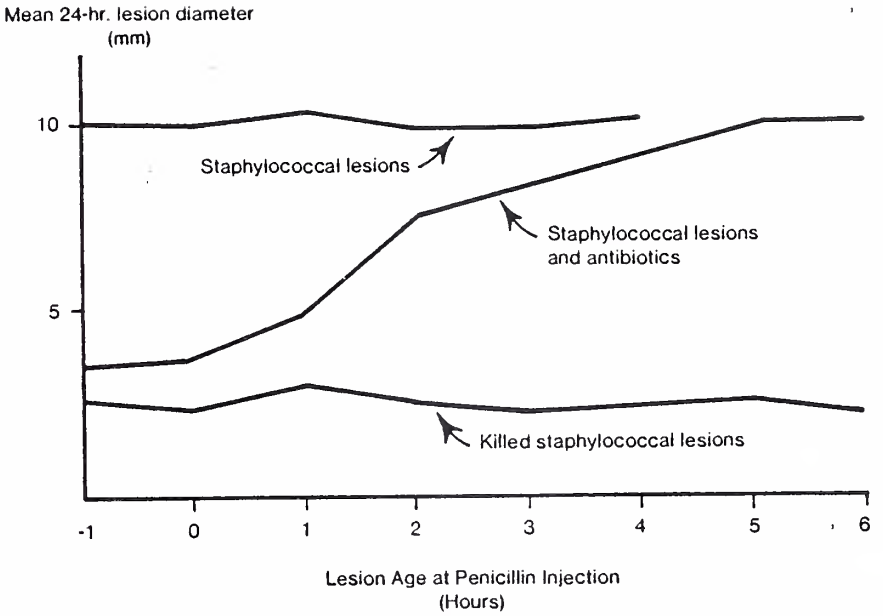
The 1960's was a period of further refinement in the use of antibiotic prophylaxis. Antibiotics were being used frequently, often with little regard to the actual risk of infection. In many cases "prophylactic" antibiotics were administered after surgery rather than before. There were those who criticized this indiscriminate use of these agents, although there was no official consensus as to the optimal point for initiating antibiotic administration.

Miles and the "Decisive Period"

A study by Miles assisted in clarifying this issue by showing that there was an optimal period during which antibiotics had their maximal prophylactic effect.[35] His study revealed that the antbiotics had to be present within a window period of three hours of the time of the initiation of the lesion. By three hours after the bacterial inoculation the antibiotics had lost the majority of the observed prophylactic action. Miles termed this phenomenon the "decisive period" for infection and defined it as that period of time after the primary lodgement of bacteria that manipulation of host defense factors could impact on the ultimate course of the infection.

An elegant study by Andriole and Lytton examining the effect of increased pressure on intradermal staphylococcal infections also demonstrated a critical period of increased tissue susceptibility to infection. This study demonstrated a window period, much like that descrebed by Miles, of approximately four hours beyond which increased tissue pressure failed to alter the degree of erythema and induration observed in the animal model. Studies such as these provided a framework for guiding the optimal timing of prophylactic antibiotic

administration.[36]



Effective period of preventive antibiotic action in experimental surgical lesions.
(From Conte, Antibiotic prophylaxis in surgery, 1984, Lippincott, Philadelphia.)

In the seventies and eighties many studies in particular types of surgery with specific agents chosen to counter the anticipated microorganisms encountered were undertaken. Although these studies were often of variable quality, they secured a role for antibiotic prophylaxis in specific types of surgery.[37] With the advent of wound classification surgeons had an official guideline for identifying those wounds that would most likely benefit from prophylactic antibiotics.[38] The advantages of prophylactic antiseptic wound irrigation were also explored and further refined.[39] Further studies during this period helped to define an optimum duration for the administration of antibiotics. While many surgeons were continuing prophylaxis for several days post-operatively, studies during this period revealed this was unnecessary and that prophylactic antibiotic coverage could be restricted to a shortened perioperative period.[40,41] During this period optimum methods

of administration for various procedures were also defined.[42] In conjuntion with the work of Miles and Andriole, it was discovered that perioperative short-term prophylaxis was most effective in procedures with significant bacterial contamination of the operative site and with subsequent high infection rates and in procedures frequently followed by serious infection.[43]

Modern Antibiotic Prophylaxis

The use of prophylactic antibiotics has developed over the past sixty years via a number of reports concerning various aspects of this topic which have gradually coalesced into the present regimes. From the large number of studies published, the basic elements determining effective antibiotic prophylaxis have emerged. It is now known that an effective prophylactic regimen should be directed against the most likely infecting organisms but need not eradicate every potential pathogen; rather the goal is to decrease their numbers below the critical level necessary to cause infection. Today, the type of antibiotic used, its duration of use, its spectrum of coverage, and designated time of administration are designed to suit the particular operation and the specific microorganisms which the surgeon anticipates encountering.[44] Through a continual process of investigating various aspects of antibiotic prophylaxis in different operations surgeons were able to gradually refine the process of prophylaxis to obtain optimal therapeutic results. This process of ultimate refinement is one which continues to this day.

Introduction

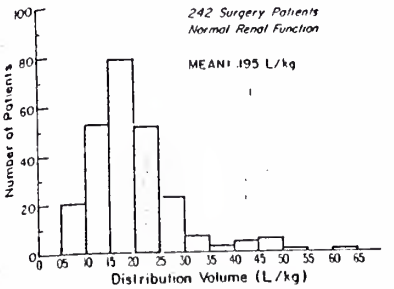
The main rational for the administration of prophylactic antibiotics is in the potential of these agents to reduce surgery-related wound infections. Certain factors have been clearly identified as risks for post-operative wound infections. Modifying factors which might influence surgeons to use antibiotics with either greater frequency or in instances in which they might not otherwise use them include; the implantation of prosthetic devices, remote infection, immunosuppressive agents, steroids, diabetes, radiation, obesity, and extremes of age.[45] Despite the recognition of these many factors which exert an influence on the effectiveness of antibiotic prophylaxis, to date there have been no definitive studies directly related to the effect of blood loss during the operative procedure on the serum levels of prophylactic antibiotics. A survey of the literature in fact reveals few articles relating simply to the effects of surgery itself on the levels of antibiotics, or other drugs for that matter. This is clearly an area which could benefit from further investigation.

Altered antibiotic dosing requirements in surgical patients

A wide variety of pathophysiologic changes can occur in the surgical patient. It is reasonable to assume that these alterations in normal physiology may contribute to altered kinetics of therapeutic agents administered.[46] It has been shown that there can be wide interpatient variations in antibiotic dosage requirements in surgery patients with normal renal function despite standard dosing. A study in 1980 by Zaske

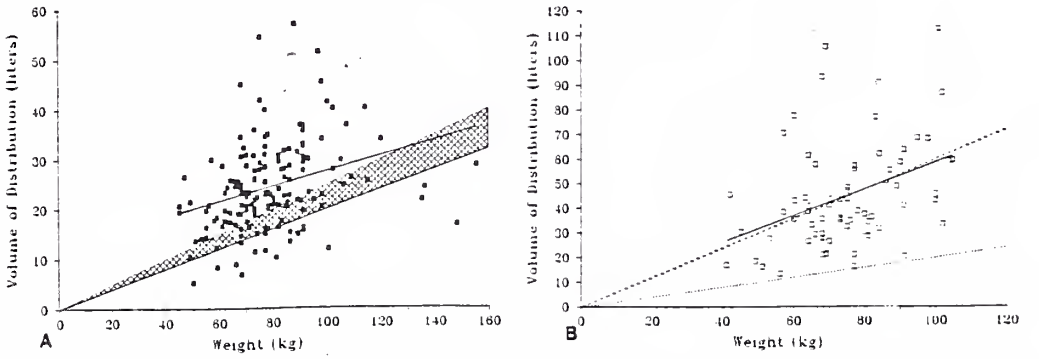
looked at 242 surgery patients who were treated with gentamicin for gram-negative pneumonia, peritonitis, urinary tract or soft tissue infections with clinical signs of sepsis. While the study did not directly address the issue of serum gentamicin levels during surgery it did emphasize the important fact that surgery patients with otherwise normal renal function can have wide variations in antibiotic half-lives, distribution volumes, and corresponding gentamicin dosage and interval of administration requirements. The study concluded that 47% of the patients actually required higher doses than those normally reccomended for non-surgical patients. The study also concluded the standard gentamicin dosing interval of eight hours resulted in either subtherapeutic or potentially toxic serum concentrations in three of every five patients, the majority of patients requiring more frequent dosing.[47]

The importance of this finding cannot be underestimated as other studies have shown that subtherapeutic antibiotic levels in surgical patients can have a significant impact on patient morbidity.[48,49] Recognizing that there were several factors commonly known to contribute to interpatient variability such as altered renal funciton, body temperature, and lean body mass, this study concluded that the distribution volume for gentamicin is not consistent in surgical patients who commonly have pathophysiological changes, such as peritonitis or congestive heart failure.



Variations in distribution volume in 242 surgery patients.
(From Zaske, D., Cipolle, J. Surgery, 1980, 87:2)

Reed has also elegantly demonstrated that this variation in the volume of distribution cannot be explained simply by differences in body weight among patients. In his study examining pharmacokinetic monitoring of antibiotics in surgical intensive care patients, he shows that predicted serum levels of antibiotics are often far below the actual pharmacokinetically determined values. Much of this variation is attributed to large alterations in the volume of distribution. It is futhermore noted that only 10% of differences in the apparent volume of distribution are related to alterations in body weight among the patients.[50]



Relationship between apparent volumes of distribution and body weight for (A) aminoglycosides and (B) vancomycin. (From Reed, L., Wu, A. J. of Trauma, 1989, 29:11)

Increased Need for Antibiotics in Burn Patients

Burn patients represent a special sub-category of surgical patients who have a variety of pathophysiologic derangements. In order to better understand the various factors affecting the serum antibiotic levels of patients during surgery, and the special case of burn patients, it is necessary to investigate the systemic effects which are most frequently observed in association with such trauma. There are numerous pathophysiologic changes accompanying burn trauma which can alter the

distribution of administered drugs, including cardiovascular changes, alterations in renal and hepatic function, and fluctuations in plasma protein concetrations.[51,52] Other complicating factors such as sepsis, drugs that induce or inhibit drug metabolism, hepatotoxic or nephrotoxic drugs, malnutrition, parenteral nutrition, preexisting systemic disease, and endogenous burn-induced substances can all contribute to alterations in drug distribution and response.[53]

There are two metabolic phases to the patient sustaining a burn injury. The acute phase, immediately after injury, is marked by decreased blood flow to the tissues.[54] This decreased blood flow is brought about by multiple factors such as; hypovolemia, depressed myocardial function, increased blood viscosity, and the release of vasoactive substances.[55] These factors will all contribute to altered pharmacokinetics of any drugs administered.

In the event of adequate resuscitation, a second phase of burn injury, begining 48 hours after the original insult has been identified as the hypermetabolic or recovery phase which is associated with increased blood flow to the organs and tissues.[56] During this time total body and hepatic oxygen consumption as well as glucose and protein turnover by the liver are increased.[57] These alterations should also affect the kinetics of therapeutic agents administered at this time.

The plasma concentrations of drugs which are highly protein bound may be deranged in burn patients as plasma protein concentrations have been observed to be altered in both the acute and recovery phases of burn injury.[58] It has further been noted that while the concentration of albumin is decreased, the concentration of the acute phase reactant,

alpha-acid glycoprotein is increased.[59] This relative change in the percent protein composition can result in contradictory effects on different therapeutic agents. While the half-lives of some agents such as the tricyclic antidepressants is increased, the binding of other agents such as certain antibiotics is decreased.[60]

Previous studies have demonstrated a need for higher than normal doses of certain antibiotics in burn patients in order to maintain therapeutic levels.[61] The two main factors dictating these increased requirements are the enhanced elimination of the drugs by the kidney resulting from burn-induced increases in the glomerular filtration rate[62] and the direct drug loss through the burn wound.[63] The loss of agents through the burn wound may be more significant in the infant than the adult because of the infants relatively high surface-area to body-weight ratio. In summary, there are multiple factors to be taken into consideration in obtaining optimum antibiotic dosage requirements in the burn patient. The situation is further confounded by the co-administration of drugs that can induce or inhibit the metabolism and excretion of other drugs. The pharmacology of this population is also complicated by changes in target-organ sensitivity induced by multiple endogenous substances released in response to, or as a consequence of, the burn injury, as well as from malnutrition, physical immobilization, and various iatrogenic factors.[64]

It has been shown that burn patients with normal renal function can have an increased need for antibiotic coverage irrespective of surgical blood loss. A 1978 study by Zaske examined the serum levels of amikacin in burn patients.[65] In contrast to other surgical patients, this study found that burn patients demonstrate a more rapid rate of elimination of

amikacin than expected. The half-life of the drug was decreased such that the standard recommended doses frequently resulted in subtherapeutic levels of amikacin. The study concluded that many burn patients require a more frequent dosing interval and consequently a higher daily dosage of amikacin. The author suggested the wide volume changes occuring in burn patients was one of the main factors contributing to these altered pharmacokinetics. This study emphasized that systemic antibiotic administration in the burn patient is complex and deserves special consideration, including close monitoring of plasma drug concentrations.

The Effect of the Duration of Surgery

It has been shown that the duration of an operative procedure can have a significant impact on the occurence of wound infection. In a study by Kaiser comparing the prophylactic use of cefoxitin to a triple combination of erythromycin, neomycin, and cefazolin in colorectal operations, it was found that among those patients undergoing a surgical procedure of less than four hours only 8.7% incurred a wound infection. In those patients whose procedures lasted greater than four hours the infection rate was found to be 18.5%.[66]

Duration of Surgical Procedures	Total Patients/Infections
<3hrs	0/46
≥3hrs ≤4hrs	4/46 (8.7%)
>4hrs	5/27 (18.5%)

The effect of the duration of surgery on the incidence of wound infection.
(From Kaiser, A. Ann. Surg., 1983, 198:4)

There are several factors which may contribute to these observations. It seems reasonable that an operation which lasts greater than four hours is one which is technically more difficult and more prone to infectious complications. It may also be that some procedures last greater than four hours due to complications which arise during the operation and which may also predispose to infection. Certainly, any prolonged period for bowel surgery provides a greater opportunity for infection from gastrointestinal microorganisms. It is also likely that a technically more difficult procedure or one in which there are complications will be associated with a greater intraoperative blood loss. Regardless of the cause of this increased incidence of wound infection, it illustrates the importance of adequate antibiotic coverage for prolonged operative procedures.

The Effect of Hemorrhagic Shock on Antibiotic Levels

Previous studies by Livingston and Miles have shown that dehydrational shock reduces the effectiveness of penicillin to control an intradermal Staphylococcus aureus infection.[67] It has been shown that hemorrhagic shock increases the susceptibility to wound infection with S. aureus that is not reduced despite resuscitation and standard prophylactic antibiotic use.[68] Furthermore it has been shown that this decreased ability to combat infection persists for up to 5 days after the hemorrhagic insult. These experimental observations serve as evidence that current conventional administration of prophylactic antibiotics may be ineffective in the presence of shock.[69]

Previous reports, especially in the field of penetrating abdominal

trauma, have already shown a positive correlation between perioperative shock or excessive blood loss and a higher risk of infection, even in the presence of appropriately administered antibiotics.[70,71] These studies have shown that hypotension is a significant risk factor for the development of late infectious complications in these injured patients. Still other reports of non-trauma patients have demonstrated that intraoperative hypotension increased the incidence of infectious complications that occur after operations on the colon and other areas of the gastrointestinal tract.[72]

A study by Livingston looked at quantitative correlations between the degree of shock and its influence on infection rates.[73] This study examined the effect of hemorrhagic shock on Sprague-Dawley rats with the dose and duration of antibiotic coverage necessary to reduce morbidity and mortality. These studies in an experimental animal model have shown that increasing both the dose and duration of antibiotic administration is more effective than standard short-course antibiotic prophylaxis in preventing experimental infection after hemorrhagic shock.

In this study Sprague-Dawley rats were injected with one of three concentrations of Staphylococcus aureus subcutaneously. Five treatment groups were then analyzed. The treatment groups consisted of one control and four consecutively larger doses of cefazolin. Results were derived by measuring abscess number, weight, and diameter seven days after inoculation.

The results of this study show that hemorrhagic shock significantly increased the susceptibility to infection compared with the unshocked controls; however, the magnitude of change was related to the number of

bacteria injected. At the lowest inoculum, shock resulted in a small increase in abscess number, with a minimal increase in size. With an inoculum of 10x8 bacteria, abscess diameter increased significantly compared with unshocked animals. After shock, inoculation with 10x10 S. aureus resulted in a mortality rate of 100% of the controls, compared with that of unshocked animals of 20%.

TABLE 2. *Effect of 10^8 S. Aureus, Hemorrhagic Shock, and CEF on Abscess Number, Diameter, and Weight*

	No Shock					Shock					
Abscess	Control	Short	Long	Mega	Mega-Long	Abscess	Control	Short	Long	Mega	Mega-Long
(n = 20)	20	9*	ND	3†	3†	(n = 20)	20	20	16	11	4‡
Diameter (mm)	11.0 ± 1.9	6.2 ± 1.3*	ND	2.8 ± 0.6†	2.3 ± 0.6†	Diameter (mm)	14.2 ± 2.2	9.9 ± 1.4*	5.6 ± 2.7†	5.6 ± 1.6†	4.2 ± 1.9†
Weight (mg)	401 ± 147	129 ± 40*	ND	17 ± 7†	24 ± 8†	Weight (mg)	511 ± 135	194 ± 71*	92 ± 82*	73 ± 46*	32 ± 30‡

* $p < 0.05$ *vs.* control.
† $p < 0.05$ *vs.* control and short.
‡ $p < 0.05$ *vs.* all other groups.
ND = not done.

The effect of hemorrhagic shock on abscess number, diameter, and weight.
(From Livingston, D. Ann. Surg., 1988, 208:4)

This study illustrates that as host defenses are depressed, fewer bacteria are necessary to create an infection. Miles has shown that the pattern of bacterial elimination is such that 95% of the bacterial challenge was eliminated by local host defenses during the first 4 hours of the decisive period.[74] Factors such as local vasoconstriction induced by epinephrine and hypovolemic shock led to actual bacterial growth instead of elimination during the decisive period, resulting in larger lesion size.

Since shock results in multiple immunologic derangements, it is probable that hemorrhagic shock alters local host defenses by interfering with initial bacterial elimination during the decisive period and affects systemic host defenses by decreasing the ability to kill the bacteria

remaining after the decisive period.[75] In accord with previous work by these authors, it has been demonstrated that, after shock, a standard prophylactic antibiotic regimen was ineffective in reducing infection, despite resuscitation and the presence of drug levels in the tissue greatly exceeding those recommended for killing bacteria.[76]

The literature reveals a variety of articles relating shock to infection rates. In evaluating patients in shock relative to normotensive patients in cases involving penetrating abdominal trauma, a study by Jones showed that despite receiving standard antibiotic prophylaxis, the infection rate in shock patients was 30% while that in the normotensive patients was 14%.[77] A study by Nichols showed similar results with infection rates in shock patients of 37% compared to 14% in normotensive patients.[78] Other studies have confirmed these findings and shown that the infection rate relates to the severity of the injury, blood loss, and possible decreased host resistance.[79]

These studies show that after shock, a standard prophylactic antibiotic regimen was ineffecive in reducing infection, despite resuscitation and the presence of drug levels in the tissue greatly exceeding those recommended for killing bacteria. The improved efficacy of higher doses may be due to a greater and sustained reduction in the bacterial inoculum during the decisive period, as opposed to standard doses and timing of antibiotics in which a nadir of ineffective or no antibiotic levels exist.[80]

Although recent randomized, prospective clinical trials comparing antibiotic regimens after abdominal trauma have not shown benefit from the administration of standard antibiotic regimens in patients sustaining

hemorrhagic shock,[81] a careful analysis of these studies demonstrates a 35% infection rate in patients who sustained hemorrhagic shock after trauma, compared with a 10% infection rate in normotensive patients. This discrepancy is comparable to the infection rate that results after abdominal trauma when no antibiotics are used and demonstrates that standard antibiotic dose and schedule are ineffective in patients who have sustained shock and bacterial contamination.[82]

In summary, standard antibiotic prophylaxis is sufficient to combat infection when host defenses are normal or when host defenses are abnormal and bacterial contamination is small. Depression of host defenses produced by shock accentuates the importance of antimicrobials in combating infection. In this setting, increasing the peak tissue antibioitic/MIC ratio by increasing antibiotic dose and duration of therapy decreases the incidence and magnitude of infection, and a longer course of antibiotics may be necessary for apppropriate prophylaxis against infection in this setting. Modification of antibiotic adminstration by increasing the doses and duration of drug decreases experimental S. aureus infection after hemorrhagic shock and deserves further examination in the clinical setting.

Cefazolin in Antibiotic Prophylaxis

Cefazolin was chosen for this study as it is one of the most commonly used agents for surgical antibiotic prophylaxis. An effective prophylactic regimen should be directed against the most likely infecting organisms, but need not eradicate every potential pathogen; rather the goal is to decrease their numbers below the critical level necessary to cause infection.

The use of therapeutic antibacterials can often be accomplished with great precision, since the offending organism is usually known, its behavior understood, and its sensitivity to the variety of agents available easily determined. In contrast, there are few situations in which prophylactic antibacterials can be chosen with equal precision and their overall value measured. Thus, the choice of a prophylactic antibiotic is based on previous experience with the type of organisms commonly encountered and the kinetic aspects of the drug chosen.

Cefazolin has excellent gram-positive coverage along with good anaerobic coverage. It has an extended serum half-life compared to the other first generation cephalosporins due to its extensive plasma-protein binding. It also has a lower cost than other second and third generation cephalosporins. For most procedures cefazolin has an advantage over the other first generation cephalosporins because of its longer half-life and the fact that it causes less pain with intramuscular injection. Indeed, cefazolin is the reccomended agent for a variety of surgical procedures including; prosthetic valve and other open-heart surgery, arterial surgery involving the abdominal aorta, orthopedic surgery such as total hip replacement, internal fixation of fractures, head and neck surgery, biliary

tract surgery, vaginal or abdominal hysterectomy, high risk cesarean section, second-trimester abortion, and all varities of traumatic wounds.

Studies have shown the ability of cefazolin to maintain sufficient tissue levels in surgical incisions. A study by Polk showed that cefazolin achieved and maintained, for a period reasonable for the completion of major abdominal operations, minimum inhibitory concentrations for all bacteria ordinarily thought to be within their spectrum. When compared to another first-generation cephalosporin, cefalothin, it was found that this drug attained, but did not maintain, wound levels consistent with effective antimicrobial activity, even in 2 gram doses.[83]

The frequency with which cefazolin is used and its favorable kinetic characteristics made it an ideal choice for the agent of prophylaxis in this study.

Materials and Methods

Patient groups: There were three groups of patients based on the degree of observed blood loss. The various surgical procedures observed in the study included; hand surgery, prosthetic implants (i.e., hip replacements, fracture patients, spine fusions, implant breast reconstructions, etc.), and burn surgery or trauma surgery. The types of surgery differed with regards to the anticipated degree of blood loss. While procedures such as hand surgery are associated with a minimal degree of blood loss, other procedures such as burn and trauma surgery tend to be associated with more significant degrees of hemorrhage.

This project received complete approval from the Yale University Human Investigation Committee. Although the study was approved for verbal consent alone, in all cases written or verbal consent was obtained and the details of the study were explained along with any potential risks. It was emphasized that patients were free not to participate and to withdraw from the study at any time should they so desire. Patients also received a personal copy of the consent form.

Patient evaluation parameters: Each patient was analyzed according to the following variables: age, sex, height, weight, type of anesthesia, liver function, and vital signs. In addition, renal function was evaluated with serum creatinine and blood urea nitrogen values which were obtained prior to surgery and post-operatively. The degree of urine output was further evaluated as evidence for adequate renal function. Serum protein and albumin levels were determined as cefazolin is known to be approximately 85% protein bound. Hematocrit and serum hemoglobin were also determined for each patient.

Prophylaxis: Cefazolin, the most commonly used perioperative prophylactic antibiotic, was used for these studies: cefazolin, at a dosage of 1 gram IV every eight hours, was first administered to the patient at induction of anesthesia in the operating room. This dose was diluted in approximately 8cc of normal saline and injected as a bolus just prior to the initiation of surgery. Serum levels were determined by drawing 6 ml blood samples in each patient at 5, 15, 45, 90, and 120 minutes after cefazolin administration, and then at four, and eight hours after administration. Any deviations in sampling from this schedule were noted at the time of surgery. Samples were drawn from either a heparin-lock intravenous catheter or, when available, from an arterial catheter. The samples were collected in standard red top tubes and refrigerated within two hours of drawing. The samples were then centrifuged and frozen within six hours of refrigeration.

Serum cefazolin levels were determined by standard bioassay. This technique in our observation is accurate within 95% confidence limits (5% standard error). Concentrations were measured by microbiological assay with the agar well diffusion method. All samples were run in duplicate to ensure consistency.

Estimating blood loss: Blood loss was recorded using estimates provided by the respective surgeon and anesthesiologist also taking into account other indicators of blood and fluid loss such as the volume of crystalloid administered, amount of packed red blood cells administered, and the volume of blood in the vacuum containers. This empiric system was deemed superior to other methods such as dye dilution techniques and red blood cell labeling which are cumbersome, and regardless are never used in common clinical practice, and would thus be inappropriate to the

standard clinical situation. Red blood cell labeling involves injecting patients with radioactive Cr^{51} and the calculated values using this method are affected by such factors as whether the blood sample is removed from the arteries or veins. Dye dilution techniques measure plasma volume rather than total blood volume and are subject to various complicated correction factors such as the presence of edema and variations in renal excretion of the dye. For patients with small blood loss of less than 1000cc, the surgeon's and anesthesiologist's estimates were averaged. For cases of large blood loss of greater than 3000cc, the number of units transfused was heavily weighed as a factor in determining the magnitude of total blood loss.

Assay Procedures

A standard bioassay procedure was employed to determine the serum levels of cefazolin present in each sample.[84] All concentrations were measured by microbiological assay with the agar well diffusion method. This technique is accurate within 95% confidence limits (5% standard error).[85] All blood samples were refrigerated within two hours of drawing in order to preserve the maximum bioactivity of the antibiotic. When all seven of the samples for an individual patient had been collected after eight hours they were spun to obtain the serum. The serum was carefully stored in labelled containers and immediately frozen. Specimens were frozen at -15 degrees celcius until they were assayed (<2 weeks). This period of storage did not affect the assay results. Reconstituted cefazolin has been shown to be stable for 24 hours at room temperature, 96 hours at 5 degrees celsius, and three months at -15 degrees celsius.[86] Studies have shown no significant change in the bioactivity in the cefazolin upon thawing provided it is within the time limits specified above.[87]

A running log was maintained on all samples which were coded according to the patient's name and unit number. Each sample was then given a unique sample code. This information was recorded both in the log and on a label placed on each storage vial.

The bioassay was performed by first preparing the standards from samples of reference cefazolin which were kept in sealed containers in a dessicator which was maintained at 4 degrees celcius as the powder is hygroscopic and tends to deteriorate after humidification.[88] The

standards were prepared as 1 mg/ml in a .1 M phosphate buffer at a pH of 8.0.

Working cultures for the bioassay were prepared by adding two drops of a Staphylococcus aureus (Boston strain) suspension to 25 ml of trypticase soy broth (TSB) in a 100 ml bottle. This was then incubated overnight at 37 degrees celcius. This culture was held at 4 degrees celcius between uses and was not used for a period exceeding two weeks. The agar plates were then prepared by dissolving Trypticase soy agar on a hot plate with stirring. The solution was then sterilized in the autoclave for 20 minutes at 15 pounds pressure and 121 degrees celcius. This was then stored in the refrigerator until needed. The autoclaved agar medium was then melted and allowed to cool to 45 degrees celcius. The working culture of Staph was then vortexed and diluted 1:4 in the Trypticase Soy Broth. Then .1 ml of the diluted Staph was added to 35cc of the agar medium. This was swirled and poured into a bioassay plate. The plates were left at room temperature until hard. The plates were then refrigerated until needed.

When the assay was to be performed the assay plates were allowed to warm to room temperature. Wells for antibiotic standards and for the patient's serum were punched out in duplicate using a standard 4 mm punch. Each well was filled with 20 microliters of appropriate samples and standards. The plates were incubated overnight at 37 degrees celcius. The zones of inhibition were then measured and the concentration of the drug in the patient's serum was calculated from linear regression analysis and the results were recorded.

Results:

Thirty-three patients were divided into three groups based on the degree of blood loss. The three groups were defined as those patients with less than 1000cc blood loss, those between 1000cc and 3000cc and finally, those patients with a blood loss in excess of 3000cc. These groups were designated as low, intermediate, and high blood loss categories. There were twenty-two, four, and seven patients in each respective group (figure 1).

The data from all patients was analyzed according to both a one compartment and a two compartment model of antibiotic distribution. The two compartment model is known to be the most accurate representation of the pharmacokinetic distribution of cefazolin. In a few cases the one compartment model was required to accurately describe the data. Approximately 25% of the patients required a one compartment model to evaluate their pharmacokinetic parameters. In these cases there was an insufficient number of data points in the alpha distribution phase causing the A and alpha terms to poorly fit a two compartment model. The fitting characterized the distribution and elimination of the drug to give the area under the curve, which represents an integrated marker of body exposure to drug.

By exposing the data to a rigid statistical analysis it was discovered that three of the patients had highly deviant serum values of cefazolin. In each case it was found that the observed serum decay profile could not be accurately described by either the one or two compartment model. In the case of patient #42 the serum values were essentially linear over time and failed to show a significant serum decay

profile. In patients #25 and #41 the data points were internally inconsistent. The data points appeared dichotomous such that the alpha distribution phase could not be correlated with the beta distribution phase. Due to the complicated logistics involved in obtaining the samples and performing the bioassay, several possible explanations for these variations are apparent. It could be that some of the samples were not pure blood as some were drawn from arterial lines which could have been inadvertantly flushed prior to drawing the sample. A significant complication involved some surgeons who would redose the antibiotic prior to the standard eight hour interval. Usually this complication was detected, however, it offers an explanation for the nearly linear serum values seen in patient #25. Finally, the bioassay is subject to a certain degree of variablity in its implimentation and errors are possible. On the reccomendation of our consultant pharmacologist, these three data points were removed from subsequent data analysis.[89]

All kinetic parameters were calculated to fit the concentration equation for a two compartment open system as outlined by Riegelman[90] in which the serum concentration, C, at any time, t, can be characterized by;

$$C = Ae^{-at} + Be^{-bt}$$

The coefficients A and B, along with the hybrid rate constants a and b were determined by computer analysis. The beta half-life, $T_{1/2}B$, was given by the equation; $T_{1/2}B = .693/B$. The volume of distribution into which the drug apparently distributes can be calculated in the two compartment system by the equation:

$$V_d = Da/(Ba+ab)$$

where D=dose administered, a and b are the hybrid rate constants and A and B are the exponential coefficients (table 1).

Using a Tukey Multiple Range test no statistically significant differences were found in the hybrid rate constants, the exponential coefficients, or the elimination rate constants among the three groups. There was a considerable amount of variation in these calculated parameters, as is commonly seen in patient studies. These calculated parameters showed differences in the coefficient of variation ranging from 22.3% to 92.2%. These values were used to calculate the area under the curve values for each patient in the two compartment model. Although statistically significant differences were not found in these parameters, the AUC values did show a significant degree of variability.

The area under the curve was calculated for each set of data at both eight hours and extrapolated out to infinity. An attmept was made to normalize the AUC value to patient body weight. This correction factor was generated by taking the original AUC and normalizing this value to that of a 70kg individual;

$$NAUC = AUC * \text{pt. body wt.}/70kg$$

This provided another value for analysis which assisted in compensating for changes in concentration due to differences in body weight. The average normalized area under the curve at eight hours (NAUC8) was noted to be 283, 285, and 134 for groups one through three respectively. Individuals within a group demonstrated wide degrees of variation. The range of values in group one spanned from 118 to 685 with a standard deviation of 116 and a coefficient of variance of 41%. In group two the values ranged from 241 to 368 with a standard deviation of 57 and a

coefficient of variance of 20%. In the third group the values ranged from 93 to 212 with a standard deviation of 33 and a coefficient of variatiance of 24% (table 1). In virtually every instance the area under the curve as described as either; AUC, NAUC, AUC8, or NAUC8, revealed data which followed the same pattern from high area values in group one through intermediate values in group two to low values in group three.

Concentration-time curves were generated for each of the three groups (figure 2). This graph shows group one with the highest serum concentrations over time and group three with the lowest serum concentrations, while group two was intermediate. The difference between groups one and three and groups two and three was found to be statistically significant. While the averages differed for groups one and two, this difference was not found to be statistically significant.

The degree of blood loss was also correlated to the area under the curve and this relationship was graphed (figure 3). In this analysis a linear relation was observed between the degree of blood loss and the AUC with a standard deviation of .00628. This line is described by the equation;

$$AUC = -.014(\text{deg. blood loss}) + 326.1$$

When the volume of distribution of the antibiotic was compared to the body weight of each patient it was discovered that only a small percentage of the variation in the volume of distribution could be attributed to differences in body weight. The R^2 for this relationship was found to be .013 thus indicating that only 1.3% of the observed variation in the apparent volume of distribution could be attributed to variations in patient body weight (figure 4). The standard deviation was found to be

15.2. The equation for this relation is described as;

$$Vd = .105(\text{pt. weight}) + 7.32$$

All patient characteristics were compared in order to rule out selection bias in any particular group. Average values such as BUN, creatinine, and HCT were compared and standard deviations were calculated for each. Basically, the three groups were noted to be homogeneous with regards to laboratory values, age, and weight (figure 5). However, group two which consisted of only four patients contained all males and did show minor variation with regards to certain laboratory values such as the hematocrit, serum hemoglobin, and body weight. One patient (#22) was excluded from serum creatinine, blood urea nitrogen, hemoglobin, and hematocrit determinations due to renal failure which gave highly deviant values.

Table 1.

	SUBJECT	CMPT	A	B	ALPHA	BETA	AUC	T1/2	V	KG	NAUC	AUC3	NAUC3
I	3	2	88.9	49.1	1.99	0.229	259	3.02	16.9	68.0	252	225	220
	5	1				0.300	294	2.31	11.4	65.3	276	267	251
	7	2	151.0	70.2	2.39	0.348	254	1.99	11.3	44.0	160	242	171
	9	1				0.704	283	0.98	5.01	63.5	257	282	256
	10	1				1.020	118	0.68	8.35	70.3	119	118	118
	11	2	364.0	89.1	11.7	0.527	200	1.31	9.49	70.0	200	198	198
	12	1				0.521	235	1.33	8.18	55.0	185	231	182
	13	2	128.0	84.5	2.87	0.262	368	2.65	10.4	70.3	370	327	329
	16	2	63.7	56.9	1.28	0.184	359	3.77	15.1	104.3	535	288	405
	17	2	232.0	57.3	3.38	0.123	539	5.63	15.1	52.2	402	363	288
	18	2	276.0	103.0	13.2	0.145	732	4.78	9.42	95.3	997	509	685
	19	2	241.0	92.0	16.2	0.377	259	1.84	10.2	67.5	250	247	239
	20	1				0.784	221	0.88	5.76	55.3	176	221	176
	21	2	102.0	109.0	4.23	0.350	335	1.98	8.53	65.0	311	317	296
	22	2	213.0	78.3	18.9	0.156	512	4.43	12.5	68.9	504	369	363
	23	1				0.449	201	1.54	11.1	68.0	195	195	190
	24	2	155.0	86.6	6.15	0.328	290	2.12	10.5	76.2	316	270	292
	28	2	93.7	79.3	3.60	0.212	403	3.27	11.7	60.0	345	333	239
	29	2	80.5	57.3	2.46	0.142	435	4.87	16.2	81.6	507	307	352
	30	2	57.7	81.2	1.80	0.172	504	4.02	11.5	67.1	483	385	370
	32	2	272.0	97.9	15.1	0.395	266	1.76	9.52	72.6	276	255	264
	34	2	133.0	44.7	2.69	0.138	373	5.03	19.4	80.0	426	266	297
MEAN		1.73	165.7	77.3	6.78	0.353	338	2.74	11.3	69.2	343	282	283
SD		0.46	90.3	19.5	6.01	0.235	140	1.51	3.52	13.3	190	81	116
CV%		26.4	54.5	25.2	88.7	65.7	41.4	55.2	31.3	19.2	55.6	28.7	41.0
II	4	2	144.0	80.7	10.4	0.556	159	1.25	11.3	118.3	270	157	257
	14	2	94.1	45.5	1.66	0.228	256	3.04	17.1	77.1	282	224	241
	15	1				0.322	236	2.15	10.9	97.5	398	264	368
	31	2	72.3	44.1	3.15	0.135	349	5.13	21.2	81.6	407	239	274
MEAN		1.75	103.5	56.8	5.07	0.310	263	2.39	15.1	93.3	339	221	285
SD		0.50	36.8	20.7	4.68	0.181	79.1	1.66	4.95	18.9	73.4	45.6	56.9
CV%		28.6	35.5	36.5	92.2	58.3	30.1	57.4	32.7	20.1	21.6	20.6	19.9
III	1	2	211.0	77.7	9.28	0.418	209	1.66	11.4	55.0	164	202	164
	2	2	157.0	70.7	5.72	0.345	232	2.01	12.5	46.3	153	219	154
	6	2	53.3	4.43	0.77	0.087	120	7.99	96.1	77.0	132	94	97
	33	2	73.6	81.5	6.73	0.352	242	1.97	11.7	50.0	173	229	166
	35	1				0.736	120	0.94	11.4	54.4	93.3	120	93
	37	2	205.0	61.7	16.0	0.302	217	2.29	15.3	45.0	140	199	132
	39	2	91.3	57.4	4.28	0.264	239	2.62	15.8	70.0	239	212	212
MEAN		1.33	140.0	59.2	7.70	0.373	190	2.31	26.4	54.6	143	177	134
SD		0.41	73.3	31.5	5.57	0.211	55.4	2.58	34.2	11.7	28.5	56	33
CV%		22.3	52.4	53.3	72.4	56.5	29.2	91.3	129	21.4	20.0	32	24

Figure 1.

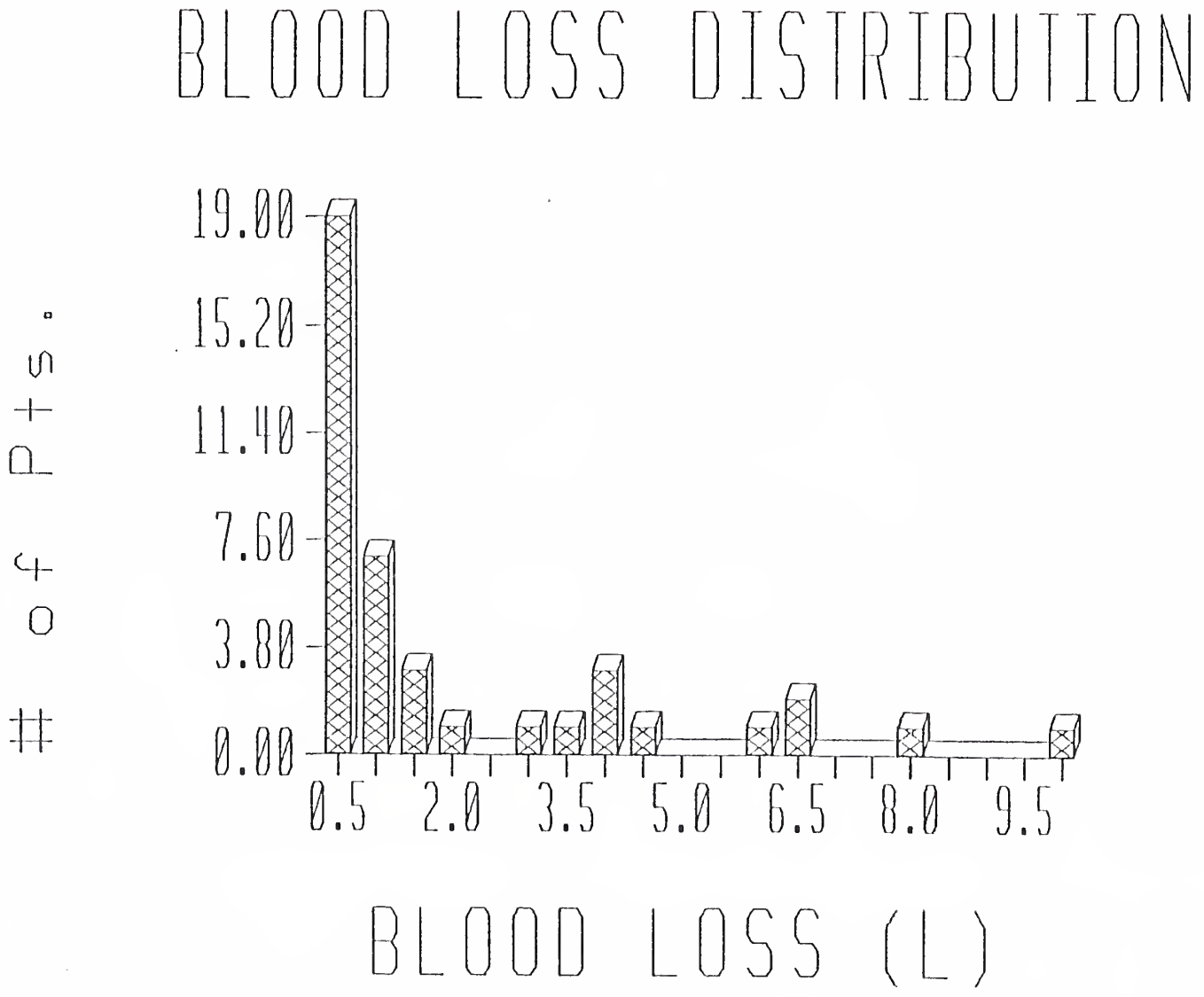

BLOOD LOSS DISTRIBUTION
# of Pts.
19.00
15.20
11.40
7.60
3.80
0.00
0.5
2.0
3.5
5.0
6.5
8.0
9.5
BLOOD LOSS (L)

Figure 2.

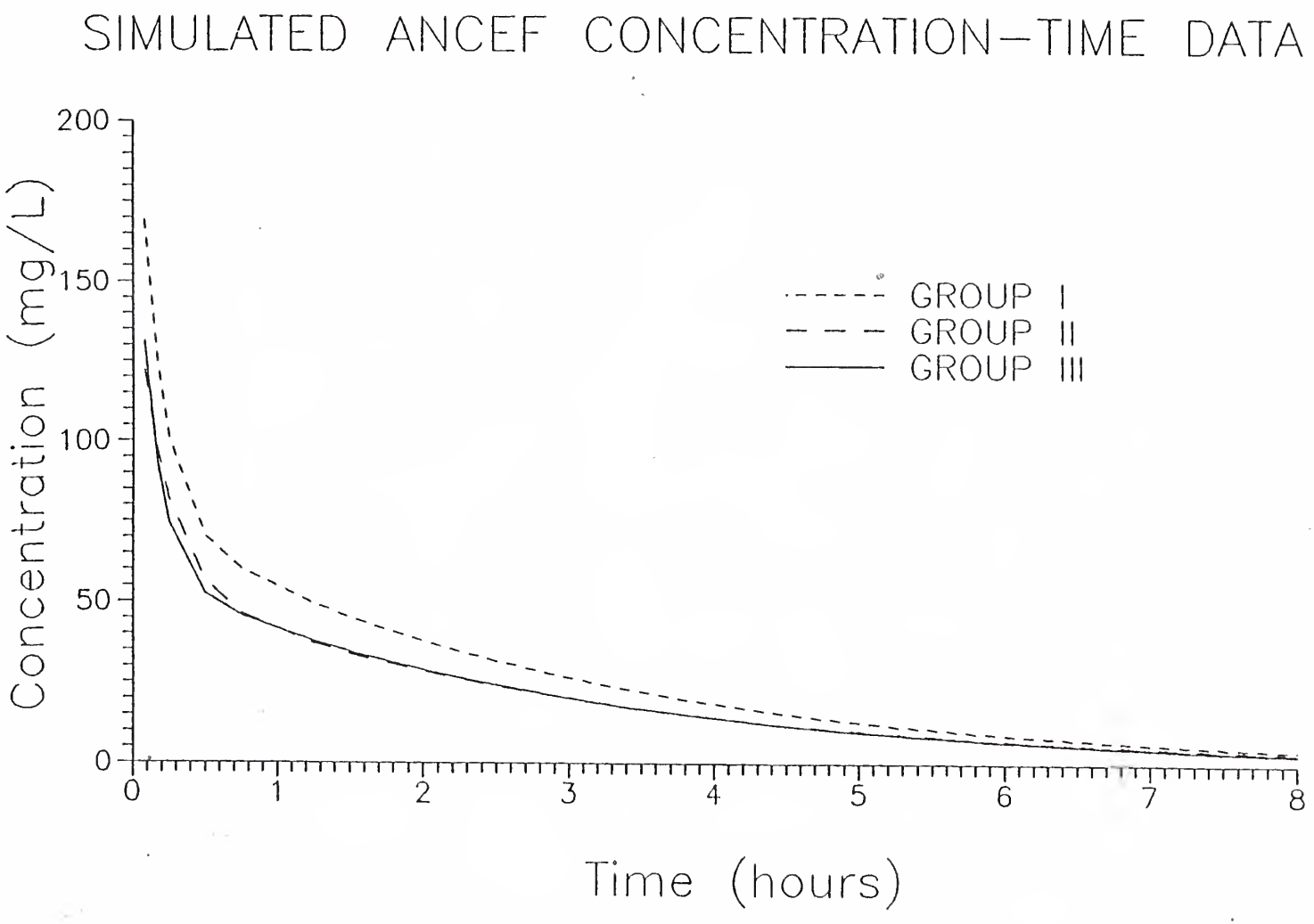

SIMULATED ANCEF CONCENTRATION–TIME DATA
Concentration (mg/L)
200
150
100
50
0
0
1
2
3
4
5
6
7
8
Time (hours)
GROUP I
GROUP II
GROUP III

Figure 3.

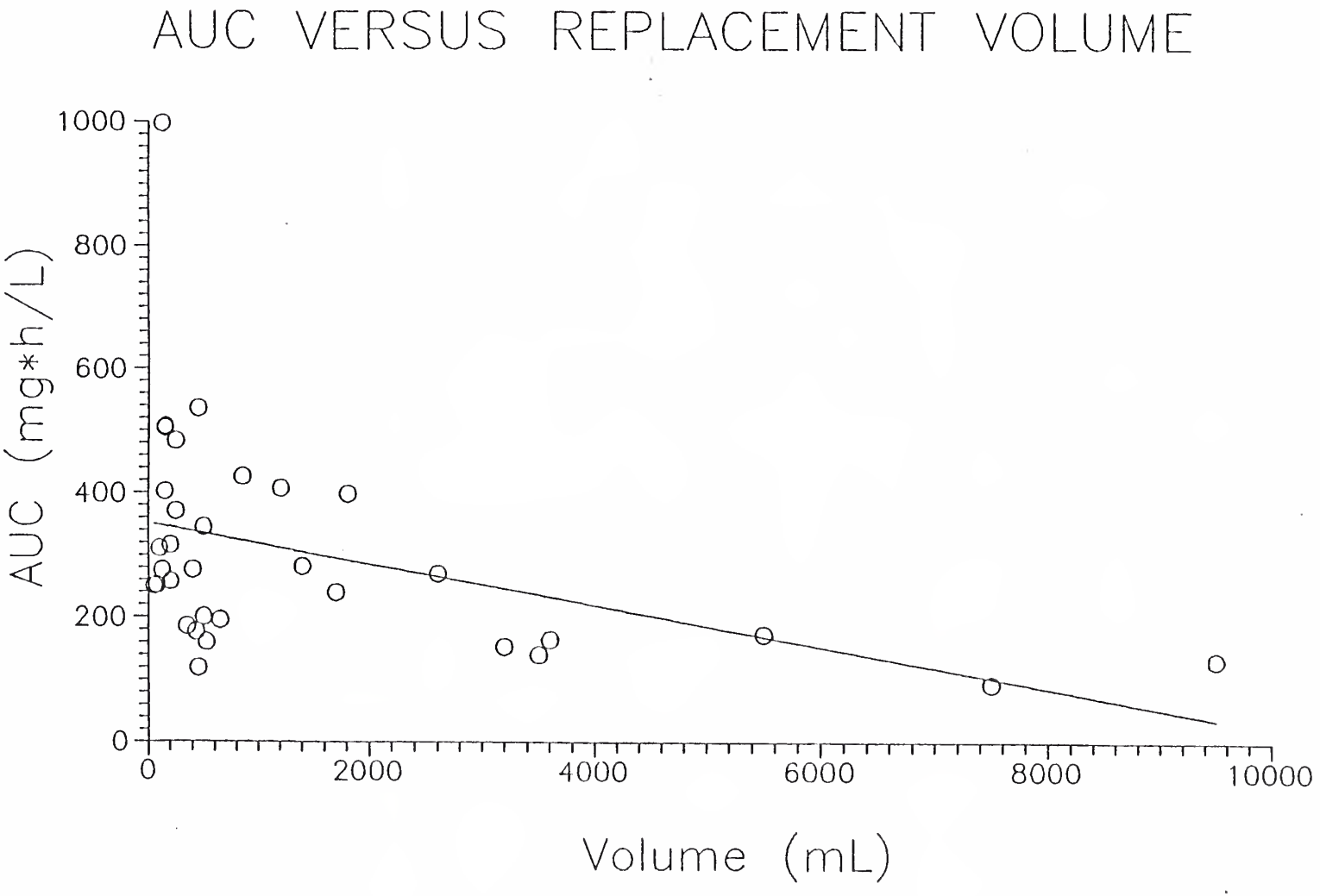
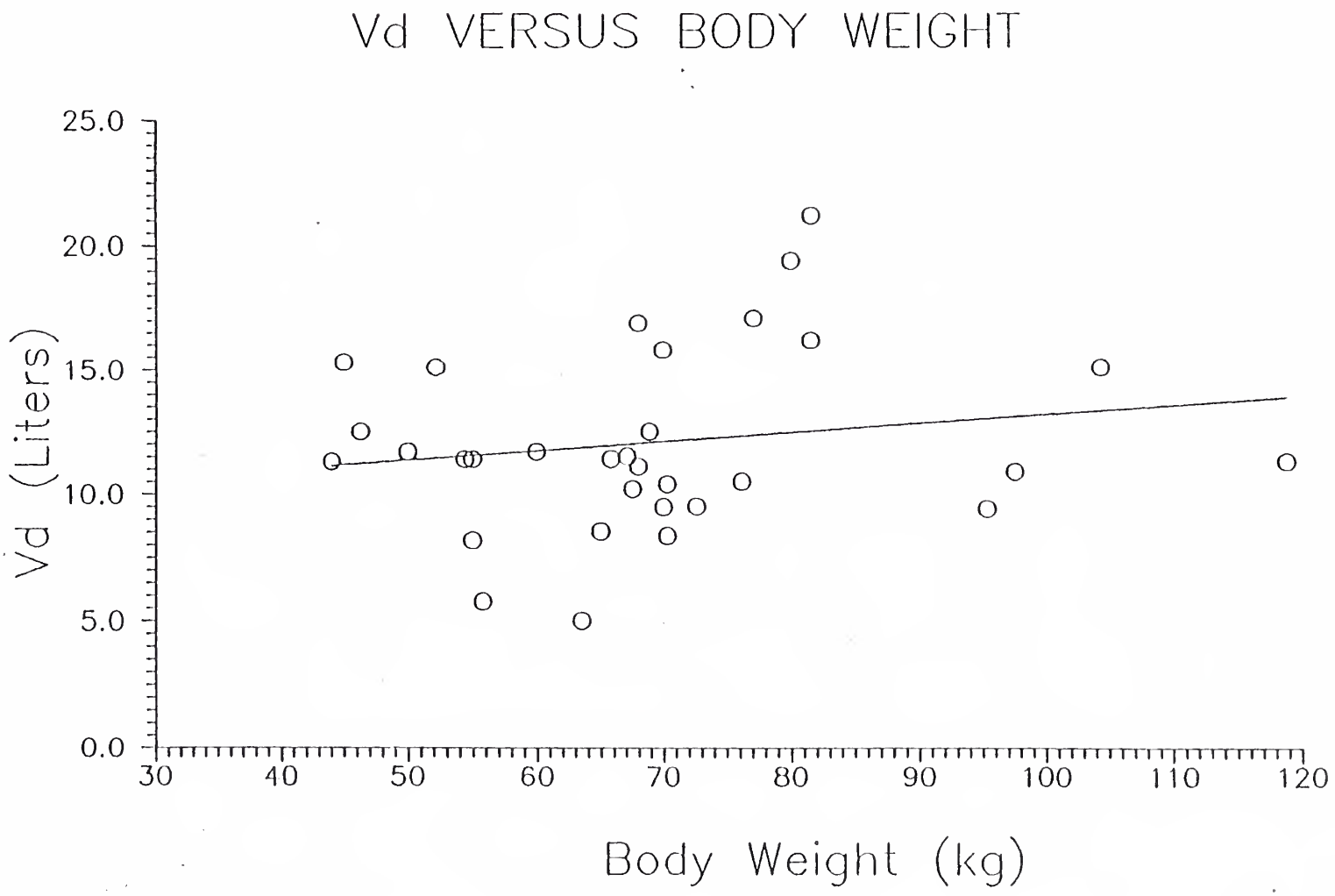

AUC VERSUS REPLACEMENT VOLUME
AUC (mg*h/L)
1000
800
600
400
200
0
0
2000
4000
6000
8000
10000
Volume (mL)

Figure 4.

patient values

	group #1			group #2			group #3		
	Avg.	Sd.	Cv%	Avg.	Sd.	Cv%	Avg.	Sd.	Cv%
serum protein	6.26	.95	15.2	5.57	.64	11.5	5.16	1.37	26.5
serum albumin	3.44	.64	18.7	2.83	.31	10.8	2.71	1.02	37.5
pre-op creat.	.94	.24	25.9	.88	.04	5.1	1.04	.69	66.2
post-op creat.	.96	.26	27.4	.96	.17	17.4	.93	.49	53.5
HCT	38.7	5.2	13.4	45.6	1.2	2.5	35.6	3.9	11.0
Hb	13.0	1.8	13.8	15.3	.33	2.14	12.1	1.4	11.8
pre-op BUN	13.95	5.4	38.7	16.2	3.3	20.2	15.1	11.1	73.6
post-op BUN	10.3	4.0	38.9	12.2	5.9	48.3	16.3	9.1	56.1
age	46.5	17.2	37.1	42.8	18.3	42.8	38.3	14.6	38.0
weight	69.2	13.3	19.2	91.4	17.2	18.8	64.2	18.7	29.2

Figure 5.


Vd VERSUS BODY WEIGHT
Vd (Liters)
25.0
20.0
15.0
10.0
5.0
0.0
30
40
50
60
70
80
90
100
110
120
Body Weight (kg)

Discussion:

In the present study all patients were analyzed according to either a one or two compartment model. The ideal pharmacokinetic model for cefazolin is known to be the two compartment model. In approximately 24% of these patients the data did not fit the two compartment model. In these cases there was an insufficient number of data points in the alpha distribution phase causing the A and alpha terms to poorly fit the two compartment model. This was not unexpected, even from the outset of the study. As this was a patient study and the samples were drawn during an operative procedure, it was decided to obtain the smallest number of samples possible while still providing enough samples to allow a detailed pharmacokinetic evaluation of the data. It was decided to draw a total of seven samples as this would allow sufficient data points to analyze the alpha and beta phases of the distribution curve. It was felt that more samples than this might contribute to unnecessary interference with the operative procedure and possibly present a confounding factor contributing to the total blood loss as each blood sample consisted of six milliliters of blood. As a result of this decision, in a small percentage of patients, due to either missed sample points or individual patient variation, there were too few data points to fit the data to the two compartment model. In such cases these patients were analyzed according to the one compartment model to determine the AUC values.

Dividing the patients into three groups was a somewhat arbitrary decision influenced by the distribution of patients with regard to blood loss. Figure one illustrates this patient distribution. The designation of small, intermediate, and large blood loss reflects an attempt to classify

groups in this study rather than giving definitions to degrees of blood loss. Certainly what is considered a small blood loss for one procedure might represent an excessive blood loss for a different operation. The choice of these three groups allowed group comparisons to be made between serum antibiotic levels and the degree of blood loss.

In an attempt to analyze the data on a more continuous scale, the blood loss was correlated to the area under the curve simultaneously for all three groups. The area under the curve (AUC) is considered to be one of the most sensitive pharmacokinetic parameters in analyzing such data. The AUC gives an integrated value which is representative of the total amount of agent in the body over a period of time. The AUC tends to be subject to less variation than other calculated pharmacokinetic variables such as the elimination half-life, the exponential coefficients, and the hybrid rate constants.

The normalized area under the curve at eight hours was compared among all three groups. Using the Tukey Multiple Range test it was found that the NAUC8 for group three was significantly smaller than that for either groups one or two. While the NAUC8 for group two was smaller than that for group one, this difference was not found to be statistically significant. This indicates that those patients with a blood loss in excess of three liters have significantly reduced levels of cefazolin when compared to a similar group of randomly selected surgical patients sustaining minimal blood loss. Although the differences between groups one and two were not statistically significant, they did follow the same pattern. It is likely that with a larger sample size in group two a statistically significant difference would in fact be revealed.

This difference in serum levels of cefazolin was also clearly

demonstrated in figure two. The average serum values for each group were graphed against the degree of blood loss. Group one shows the highest serum values of antibiotic while group two is intermediate and groups three shows the lowest serum values of antibiotic.

When these values are compared to a sample of healthy volunteers not undergoing an operative procedure, it is noted that the surgical patients have significantly reduced plasma antibiotic levels, especially during the first four hours of antibiotic administration. These control studies demonstrate cefazolin reaching peak concentrations in five minutes of 188 micrograms/ml. This value dropped to 16.5 mcg/ml at four hours, yet remained above the values seen in all three groups in this study up to this point. At eight hours the healthy volunteers had attained approximately the same antibiotic levels as the surgical patients in this study.[91]

One of the objectives of this study was to attempt to define an optimum redosing period for this agent if blood loss could be correlated to serum levels of the antibiotic. The degree of blood loss vs the area under the curve reveals a linear relationship between the amount of serum antibiotic in the circulation and the degree of blood loss. In order to accurately predict the optimal redosing schedule for patients with a significant degree of blood loss, the reference curve from healthy volunteers was carefully analyzed and revealed redosing at eight hours when the serum level was approximately 6 mcg/mcl. This is approximately three times the MIC of cefazolin for staphylococcus aureus, however, the MIC of cefazolin for Enterococcus and methicillin-resistant Sthapylococcus aureus is in excess of 30 mcg/ml.[92] Most patients in this study had values lower than this within the first four hours of antibioic

administration. Although all three groups in this study had comparable serum values to the healthy volunteers at eight hours, they had significantly lower values during the first four hours of this period.

It is clear from the graph of the degree of blood loss vs the AUC that the greater the blood loss, the less the antibiotic concentration. This would indicate a possible estimated redosing schedule based on the degree of blood loss. This is of course subject to a certain degree of variation in the individual patient.

Although there are benefits to maintaining high antibiotic levels in surgical patients, these must be weighed against the potential risk of toxicity along with the cost of extra antibiotic doses. It may be further argued that as long as the serum values remain above the therapeutic MIC concentrations, there should theoretically be no need to redose. Since all groups in the study did maintain values above the MIC for many of the commonly encountered organisms, this might appear a valid arguement. However, standard dosing regimens are such that serum levels several times that of the MIC are continually maintained. This is further supported by the fact that although serum levels are in excess of the MIC, diffusion into tissues or abscesses is dependent on first-order kinetics. The higher the initial serum value, the greater the degree of interstitial diffusion, and hence the higher the tissue levels. So it would seem that there are clear benefits to maintaining serum values which are several orders of magnitude greater than the MIC in order to maintain adequate tissue levels. The question of toxicity is not as significant with cefazolin, or any of the first generation cephalosporins which are associated with a high therapeutic index as compared to the ototoxicity and nephrotoxicity commonly seen with excessive doses of the

aminoglycosides.

Although this study attempted to examine an unbiased homogenous patient group, as with all patient studies there was a significant degree of interpatient variation. Although overall patterns were observed in each of the patient groups, there was a significant degree of variation. Some of the factors contributing to this variation include the fact that the degree of blood loss was an estimation and not a quantified value. It was felt that a combined estimate of the surgeon, anesthesiologist and researcher would provide a relatively accurate estimate of the total blood loss. Quantifiable techniques such as RBC labelling, weighing sponges, and dye dilution techniques were deemed inappropriate due to the technical difficulties in their implementation along with the confounding effects of plasma expansion with crystalloid and RBC infusions during the operative procedure. While this technique of estimation introduces a certain degree of variability it was combined with knowledge of the volume of crystalloid and units of RBC administered in order to give a more accurate estimate of the total degree of blood loss.

Another factor contributing to variation was the fact that individual patients were undergoing a variety of different operative procedures. There was also a certain amount of variation in the type of anesthesia which was administered. It is possible that these factors may have influenced the volume of distribution of the antibiotic. There are also interpatient variations in plasma binding, distribution, and renal excertion of the antibiotic. These effects were grossly controlled for by checking common laboratory values such as serum protein/albumin, and tests of renal function such as BUN, and creatinine both pre and post operatively.

The small sample sizes of the groups necessitated larger differences

in average values in order to obtain statistically significant differences. Perhaps if group two had more patients it would also have revealed statistically significant differences as did groups one and three. It would also have been helpful to observe a larger number of patients with blood loss in excess of three liters and to have perhaps a fourth group consisting of patents with a blood loss in excess of six liters, as is commonly seen in patients with extensive burns.

In conjunction with previous studies, it was found that this groups of surgical patients demonstrated wide variations in the apparent volume of distribution of the antibiotic (figure 4). Lean body mass is often considered to correlate with the estimated volume of distribution. While this relation may hold for certain patients, this study demonstrates that surgical patients are a special category undergoing unique pathophysiological variations which contribute to wide variations in the apparent volume of distribution. It is furthermore demonstrated that only 1.3% of this variation in the apparent volume of distribution could be attributed to differences in patient weight.

There are a few issues which this study raises that have yet to be solved. In the first place, the question of when to redose has not been completely answered. It has now been shown that significant blood loss leads to depressed antibiotic levels, however, there are significant interpatient variations in serum antibiotic levels regardless of the degree of blood loss. The graph of the area under the curve vs blood loss and the observation of significantly lower blood levels of antibiotic in those patients with a blood loss in excess of three liters helps to provide guidelines as to more appropriate dosing yet further clarification of exact dosing schedules remains.

Although this study did demonstrate decreased antibiotic levels as a function of blood loss, the study did not address the effect of this decreased level on the incidence of post-operative infection. While the serum levels of antibiotic are significatly reduced in patients with a significant blood loss, the effect of these decreased levels on the rate of post-operative infection has yet to be determined. Furthermore, due to the relatively low incidence of post-operative infection in such cases, it would be necessary to examine a much larger sample size with several hundred patients in order to determine statistically significant differences.

Conclusion:

This study demonstrates that integrated antibiotic levels can be described as a function of the degree of blood loss in a patient undergoing an operative procedure. It has also been shown that there is a linear relation between the degree of blood loss and the antibiotic levels with greater blood loss resulting in depressed serum antibiotic levels.

By dividing patients into three arbitrary groups it was discovered that those patients with a blood loss in excess of three liters had significantly reduced serum antibiotic levels when compared to groups one and two. While group two had lower serum antibiotic levels than group one, this difference was not found to be statistically significant. This was attributed to the small sample size in group two.

As demonstrated in previous studies of surgical patients, the patients in this study demonstrated wide variations in the apparent volume of distribution. Only a small percentage of this variation could be attributed to differences in patient weight. This variation was attributed to the variety of pathophysiologic changes commonly seen in surgical patients.

As a result of these findings more frequent antibiotic dosing is reccomended in those patients sustaining significant levels of blood loss during an operative procedure. Further studies remain to determine the optimum redosing schedule in those patients with a large blood loss.

The benefits of increased dosing of antibiotics must be weighed in consideration to the added expense to the medical industry. Future

studies quantifying the incidence of infection with relation to the degree of blood loss will be instrumental in deciding this issue. It is also noted that there are wide interpatient variations in antibiotic levels such that redosing in a minority of patients will result in excessive antibiotic levels.

Appendix I

Patient consent forms

Name: _____

Hospital Unit Number: _____

Patient Consent Form

Yale University School of Medicine - Yale-New Haven Hospital

The Effect of Blood Loss During Surgery on the Pharmacokinetics of Perioperative Antibiotic Prophylaxis

You are invited to participate in a study of antibiotic usage at the time of surgery. The antibiotic for this research will be cefazolin. This antibiotic is one of the most commonly used at the time of operations such as the one you are about to undergo. You have been chosen for this study because you are a candidate for one of the following types of surgery; hand surgery, prosthetic implants (i.e., hip replacement, implant breast reconstruction, fracture patients, spine surgery, etc.), or surgery for burns or other trauma.

In this study each subject will be given an injection of cefazolin just before the initiation of surgery just as is typically done in surgery every day. Seven blood samples will be drawn after the antibiotic is given to determine if

enough antibiotic remains in the blood stream. Many of these will be drawn while you are under anesthesia. The blood samples will be of five to seven milliliters each (about a teaspoon). The total amount drawn in all samples will amount to less than 1% (or one hundredth) of your total blood volume.

It is hoped this study will help us know whether surgeons have been giving antibiotics often enough during operations to do the best possible job in preventing infection.

You are free not to participate and if you do become a subject you are free to withdraw from this study at any time during its course. If you choose not to participate or if you withdraw it will not adversely affect your relationship with the doctors or this hospital or change your treatment. Over the last few years, these medications have been administered to most surgical patients by their physicians as a course of routine treatment whether they are in a study or not.

We have used some technical terms in this form. Please feel free to ask about anything you don't understand and to consider this research and the form carefully--as long as you feel necessary--before you agree to participate.

Authorization: I have read this form and decided that _____ will participate in the project described above. Its general purposes, the particulars of involvement and possible hazards and inconveniences have been explained to my satisfaction. My signature also indicates that I have received a copy of this consent form.

_____
Signature

_____
Relationship (self, parent, etc.)

_____
Date

_____
(Signature of principal investigator)

_____

If you have any further questions about this project or your rights as a research subject or if you have a research related injury, please contact the principal investigator, James A. Armstrong, at 787-4323 or Dr. Richard Stahl, at 785-2576.

Appendix II

Data collection form

The Effect of Blood Loss During Surgery on the Pharmacokinetics of Perioperative Antibiotic Prophylaxis

Patient Information Sheet

Researchers: Dr. Richard Stahl
Dr. Vincent Andriole
James A. Armstrong

Patients name:_____

Hospital unit #:_____

Patients address:_____

_____

_____

Phone:_____

Date of Birth:____/____/____

Weight:_____

Reason for hospitilization:_____

Type of surgery being performed:_____

_____

Other medications:_____

_____

Prophylactic antibiotic being used:_____

date of admission to hospital:____/____/____

date scheduled for surgery:____/____/____

date of release:____/____/____

Any past history of kidney disease?_____

_____

Surgery record:

anesthesia started:_____(am/pm)

surgery started:_____(am/pm)

surgery completed:_____(am/pm)

anesthesia completed:_____(am/pm)

Total volume of transfusions:_____

Total volume of crystalloid:_____

Anesthetic used:_____

Estimated blood loss:

Surgeon:_____

Anesthesiologist:_____

any complications:_____

_____

_____

test	before surgery	eight hours after surgery
BUN	_____	_____
Creatinine	_____	_____

Liver function tests:

hematocrit:_____

serum protein albumin:_____

hemoglobin:_____

RBC:_____

serum iron:_____

TIBC:_____

Plasma cephalosporin levels during surgery:
(measured from time of injection of antibiotic)

reading #	time	exact time	drawn via
0 -start-	_____		
1 (5 min)	_____	_____	_____
2 (15 min.)	_____	_____	_____
3 (45 min.)	_____	_____	_____
4 (90 min.)	_____	_____	_____
5 (120 min.)	_____	_____	_____
6 (4 hrs.)	_____	_____	_____
7 (8 hrs.)	_____	_____	_____

comments: _____

_____

_____

_____

patient history: _____

_____

_____

_____

_____

Appendix III

Compiled data

Report: cef.mat1

Code #	Date of birth	Type of surgery	Weight
1	'Mar 23 55	Debr. Sp. Wound	55.0 kg
2	Jul 26 34	Bilat. Mas. Im. Recon.	46.3 kg
3	Sep 21 39	L. Isch. Press. Sore R	68.0 kg
4	Nov 29 31	Repl. Abd. Aortic An.	118.8 kg
5	Jun 23 56	Pilonidal sinus tract	65.8 kg
6	Dec 20 48	Exc+SG Up. body burns	77.0 kg
7	Mar 19 27	Hemiglossectomy	44.0 kg
8	Aug 5 16	Pec. Flap Closure	54.4 kg
9	Dec 10 72	Nail L. Hip	63.5 kg
10	Nov 16 44	Post. Thor. Sp. Red.	70.3 kg
11	Mar 27 37	Peustow proc.	70.0 kg
12	Mar 8 50	L. Fron. Craniotomy	55.0 kg
13	Jun 23 48	Bil. Mastectomies	70.3 kg
14	Jun 20 62	L. Hip Currettage	77.1 kg
15	Sep 8 18	Lumbar Laminectomy	97.5 kg
16	Mar 19 23	Exp. Lap.	104.3 kg
17	Aug 17 46	R. Cheek Fix.	52.2 kg
18	Mar 3 27	Cholecystectomy	95.3 kg
19	Nov 1 72	SG. to L. Ant. Thigh	67.5 kg
20	Sep 4 68	Red. Mammoplasty	55.8 kg
21	Feb 8 57	Radial Arm Flap	65.0 kg
22	Jan 16 23	Pseudoaneurysm	68.9 kg
23	Jun 6 33	R. Temporal Lobectomy	68.0 kg
24	Apr 8 27	Lam. ankle athrodesis	76.2 kg
25	Oct 28 58	Pancreatectomy	82.0 kg
26	Aug 18 20	Exp. Lap.	87.0 kg
27	Sep 23 18	Lip. Res. Maxill.	77.1 kg
28	Dec 19 40	THR	60.0 kg
29	Oct 31 23	R. Carotid Endar.	61.6 kg
30	Mar 24 15	fem.-fem. bypass	67.1 kg
31	May 31 49	THR	81.6 kg
32	Oct 11 35	Fem. bypass	72.6 kg
33	Dec 26 75	Skin Grafts	50.0 kg
34	Nov 8 19	Pinnectomy, etc...	80.0 kg
35	Oct 25 60	Skin grafting	54.4 kg
36	Nov 6 37	Sig. Colostomy	61.2 kg
37	Sep 13 30	skin graft	45.0 kg
38	Jul 15 43	disarticulation, l. le	60.0 kg
39	Apr 28 42	excise burn,skin graft	70 kg
40	Apr 28 42	skin grafts	70 kg
41	Feb 6 38	excision+graft burns	80 kg
42	Apr 13 56	debridement of burns	100 kg

Code #	Prophylactic	Other medications	Date of surgery
1	Ancef	tobramycin	Jul 9 87
2	Ancef	Dalmane, Midazolan	Jul 14 87
3	Ancef	none	Jul 23 87
4	Ancef	K-tabs, Tagamet	Jul 23 87
5	Ancef	Valium	Jul 28 87
6	Ancef	Cefadyl, haldol	Jul 31 87
7	Ancef	Midazolan	Aug 4 87
8	Vancomycin/Genta	none	Aug 5 87
9	Ancef	none	Aug 6 87
10	Ancef	none	Aug 7 87
11	Ancef	Demerol, Dalmane	Aug 10 87
12	Ancef	Valium, Dalmane	Aug 18 87
13	Ancef	none	Aug 18 87
14	Ancef	Demerol	Aug 19 87
15	Ancef	none	Aug 25 87
16	Ancef	Amp., Gent.,Clind.	Aug 26 87
17	Ancef	none	Aug 27 87
18	Ancef	Procardia	Aug 27 87
19	Ancef	none	Aug 28 87
20	Ancef	Versed	Aug 28 87
21	Ancef	none	Aug 31 87
22	Ancef	Prozosin, Zantac	Aug 31 87
23	Ancef	Thorazine, DPH	Sep 1 87
24	Ancef	Capoten, Valium	Sep 1 87
25	Ancef	Heparin, Zantac	Sep 2 87
26	Gentamycin	none	Sep 2 87
27	Gentamycin, Oxac	Librium, Cleocin	Sep 3 87
28	Ancef	none	Sep 3 87
29	Ancef	Midazolan, Metamucil	Sep 4 87
30	Ancef	Coumadin, digoxin	Sep 9 87
31	Ancef	none	Sep 10 87
32	Ancef	none	Sep 10 87
33	Ancef	none	Sep 15 87
34	Ancef	Dalmane, Valium	Sep 29 87
35	Ancef	none	Nov 4 87
36	Gentamicin	none	Dec 4 87
37	Ancef	Tobramycin	Dec 18 87
38	Gentamicin, 60mg	Epi., dopa., Naficil	Jan 10 88
39	Ancef	none	Mar 8 88
40	Gentamicin	none	Apr 7 88
41	Ancef	ranitidine	Jul 9 88
42	Ancef	Pavalon	Mar 15 89

Code #	Vol. of transfusions	Vol. of crystalloid	Anesthetic used
1	20 u	17000cc	nitrous oxide,
2	6u	8000cc	Ethrane
3	0	1400 cc	Fentanyl, Ethra
4	4 u	5200 cc	Enflur, Duramor
5	0	2050 cc	spinal block
6	16 u	12,900 cc	ethrane
7	0	6100	Isoflurable
8	.5 unit	750 cc	Fentanyl
9	0	1200 cc	Enflourane
10	0	5500 cc	Fentanyl
11	0	4000 cc	Isofuran
12	0	2700	Vecuron
13	0	1000cc	Ethrane
14	0	3600cc	Enflurane
15	0	10,000cc	Isofuranyl
16	0	450cc	Ycuran
17	0	1700cc	Ethrane
18	0	1600cc	Enflur, Fentany
19	0	1600cc	Fentanyl, Ethra
20	0	2700cc	Ethrane
21	0	3900cc	Ephadrane, Fent
22	0	80cc	Fentanyl, spina
23	0	1450cc	Ethrane, Fentan
24	0	1200cc	Spinal block, W
25	0	8000cc	Midazol
26	0	4500cc	Isoflur, Fentan
27	0	4600cc	Ethrane
28	0	1700cc	Ethrane
29	0	2000cc	Fentanyl, Wyami
30	0	1700cc	Fentanyl, Verse
31	3u	4000cc	Wyamine, Vecuro
32	0	2400cc	Fentanyl, Panc.
33	11.5 u	6100cc	Fentanyl, Ketom
34	0	6200cc	Enflurane, Vecu
35	15u	4800cc	Fentanyl, Vecur
36	2u	4000cc	Fentanyl, Foran
37	7u	3500cc	nitrous oxide,i
38	7u	3500cc	n/a
39	34u	6900cc	ethrane
40	12u	4800cc	ethrane
41	12u	7000cc	ethrane
42	8u	6500cc	ethrane

File: ce.file2

Report: cef.mat4

Code #	Blood loss (sur)	Blood loss (anes)	Avg. blood loss
1	3600 cc	3600 cc	3600 cc
2	3200cc	3200cc	3200cc
3	75 cc	75cc	75cc
4	2600cc	2600 cc	2600 cc
5	125 cc	125cc	125 cc
6	9500cc	9500cc	9500cc
7	250 cc	800 cc	525 cc
8	150 cc	50 cc	100 cc
9	300 cc	100 cc	200 cc
10	450 cc	450 cc	450 cc
11	500 cc	500 cc	500 cc
12	300cc	400cc	350cc
13	250cc	250cc	250cc
14	1400cc	1400cc	1400cc
15	1800cc	1800cc	1800cc
16	450cc	450cc	450cc
17	150cc	150cc	150cc
18	100cc	150cc	125cc
19	50cc	50cc	50cc
20	200cc	660cc	430cc
21	100cc	100cc	100cc
22	150cc	150cc	150cc
23	800cc	500cc	650cc
24	200cc	200cc	200cc
25	1000cc	1000cc	1000cc
26	100cc	100cc	100cc
27	500cc	500cc	500cc
28	500cc	500cc	500cc
29	150cc	150cc	150cc
30	250cc	250cc	250cc
31	500cc	1200cc	1200cc
32	400cc	400cc	400cc
33	5500cc	5500cc	5500 cc
34	700cc	1000cc	850cc
35	7500cc	7500cc	7500cc
36	1000cc	1000cc	1000cc
37	3500cc	3500cc	3500cc
38	3500cc	3500cc	3500cc
39	17000cc	17000cc	17000cc
40	6000cc	6000cc	6000cc
41	6000cc	6000cc	6000cc
42	4000cc	4000cc	4000cc

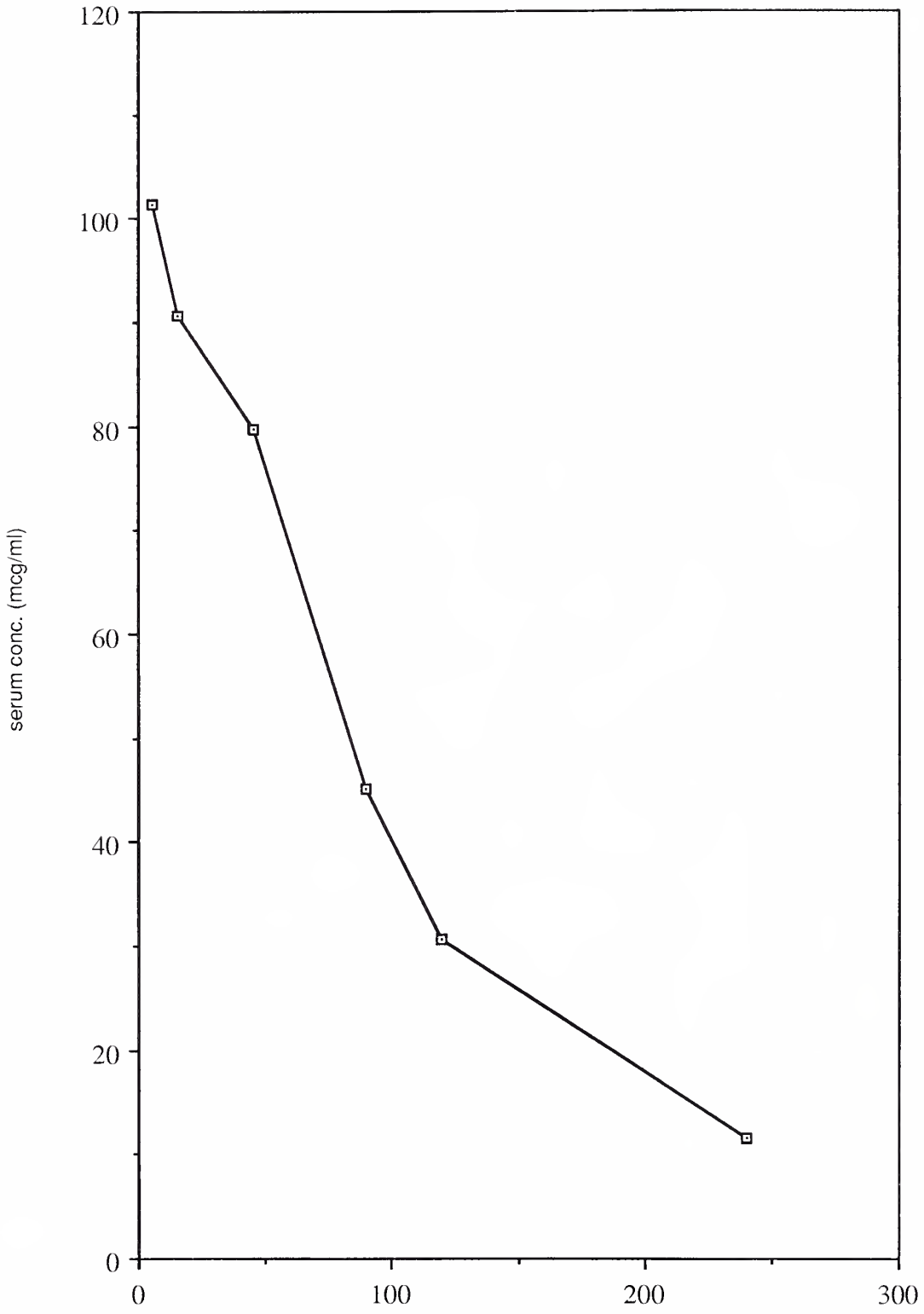
Code #	BUN	Creatinine	Hematocrit	ser. prot. al.	hemoglobin
1	7/8	.6/.3	32.2	4.6/-	10.6
2	13/-	.8/-	42	7.0/4.5	14
3	7/7	.6/.6	33.6	6.1/3.6	11.3
4	15/11	.9/1.1	44.1	5.1/2.5	15.3
5	9/9	.9/.9	42.6	5.8/3.7	14.6
6	25/20	1.2/1.0	33.2	2.9/1.3	11.3
7	20/5	.8/.7	30.7	7.6/4.4	10.6
8	7/6	.9/.9	27.2	4.9/2.5	9.1
9	12/12	.9/.9	42.8	5.2/2.6	14.2
10	16/10	.8/.9	41.4	5.5/2.6	14.3
11	16/5	1.0/1.0	45.8	7.6/4.0	15.4
12	13/7	.8/.8	41.2		14.3
13	13/13	.9/.9	43.7		14.5
14	21/20	.9/1.0	45.2		15.2
15	17/16	.9/1.1	47.3		15.7
16	20/12	.9/.9	36.5	6.7/3.4	12.0
17	17/7	.9/.9	40.3		13.7
18	20/20	1.6/1.8	33.6		11.1
19	11/11	.9/.9	40.4		13.8
20	5/5	1.1/1.1	42.3		13.9
21	10/10	.8/.8	38.4		13.0
22	71/97	97/12.9	24.1	6.7/3.8	7.9
23	20/-	.9/-	42.9		14.3
24	11/11	.9/.9	46.2	7.5/4.3	15.9
25	16/5	.9/.9	45.5	6.3/3.1	14.8
26	15/8	.8/.9	35.5	6.1/3.5	12.0
27	34/30	1.5/1.2	34.0	6.0/3.4	11.3
28	25/-	1.0/-	31.0	5.0/2.7	10.4
29	10/15	.8/.8	41.9	6.1/3.4	13.6
30	13/12	1.6/1.3	29.0	5.3/2.8	9.8
31	12/9.	.8/.7	45.7	5.3/2.9	15.4
32	6/-	.7/-	33.1		10.8
33	19/20	.7/.6	34.4	4.8/2.1	11.6
34	19/15	1.0/1.1	35.3		11.8
35	7/5	.6/.5	32.5	5.4/3.0	10.9
36	5/5	1.0/.9	32.0	5.1/7.6	10.1
37	5/6	.7/.6	36.4	5.3/2.3	12.4
38	16/19	.4/1.0	7.6	5.0/1.7	2.9
39	7/16	.9/1.3	31.6		10.6
40	8/6	.4/.6	47.5	5.6/2.4	16.0
41	14/27	1.1/1.6	41.6	7.0/2.5	14.4
42	39/28	2.8/1.5	36.3	4.3/3.3	12.7

Code #	1	2	3	4	5	6	7
1	172.8	90.9	55.1	42.4	39.4	10.8	5.6
2	167.6	99.4	65.1	42.2	29.1	19.0	5.3
3	122.8	102	60	no sample	34.5	18.2	8.3
4	137.9	80.8	57.8	31.5	25.0	10.6	29.5
5	94.3	80.1	70.4	50.6	46.1	27.3	9.6
6	51.6	51.7	34.0	21.4	14	5.8	2.3
7	181.9	145.5	68.1	39.2	44	16.2	61.0
8	3.45	3.31	3.31	3.06	2.68	2.06	1.41
9	187.9	161	126	64.6	58.8	8.6	8.9
10	123.3	86.4	52.9	21.4	18.2	16.7	<3
11	223.5	97.7	64.2	43.1	25.3	10.4	9.8
12	126.8	95.5	87.5	56.5	42.3	no sample	no sample
13	no sample	141.2	87.7	51.8	51.8	38.6	8.4
14	127.8	105.6	60.8	46.7	31.3	16.5	8.1
15	99.2	84	63.2	58.8	47.9	26.8	38.2
16	108.5	108.5	71.9	50.1	46.9	27.4	50.5
17	227.5	163.9	65.8	53.2	47.1	32.5	22.5
18	193.9	109.6	91.1	75.6	89.5	57.7	31.4
19	151.9	87.9	69.1	52.1	45.2	18.6	5.2
20	229.1	110.9	74.1	69.7	66	4.2	71.1
21	176.6	136.9	85.3	69.8	51.2	27	80.5
22	121.7	77.2	63.9	91.4	45.5	43.0	90.9
23	90.1	83.9	57.2	46.4	36.8	16.0	42.6
24	176.3	115.9	61.1	61.1	45	25.8	5
25	101.4	90.6	79.6	45.0	30.6	11.7	43.4
26	3.68	no sample	3.3	2.27	1.67	1.2	no sample
27	8.9	5.13	3.57	2.86	2.47	1.00	1.25
28	147	115.7	72.4	59.9	51.7	35	14.3
29	118.4	105.6	61.3	45.2	49.2	31.5	34.7
30	129.1	115.7	85.6	69.2	55.9	42.8	20
31	99.6	75.1	47.0	37.0	33.3	58.7	15
32	172.6	95.0	72.3	53.5	46.2	19.7	58.2
33	121.5	87.5	67.6	46.6	41.4	15.4	10.2
34	144.3	121.6	52.5	41.0	34.7	25.7	14.7
35	106.8	60.6	47.8	26.4	21.6	6.1	4.3
36	no sample	8.09	5.51	4.89	4.56	2.78	no sample
37	114.7	61.0	56.8	32.6	33	20.8	59.3
38	5.77	5.93	3.10	2.59	2.37	1.84	1.60
39	121.9	81.9	55.0	41.7	32.6	15	11.7
40	14.14	4.74	4.82	2.66	2.88	1.29	.86
41	109.7	102.1	81.6	56.8	31.6	13.1	no sample
42	66.1	60.9	47.7	52.2	47.4	49.6	no sample

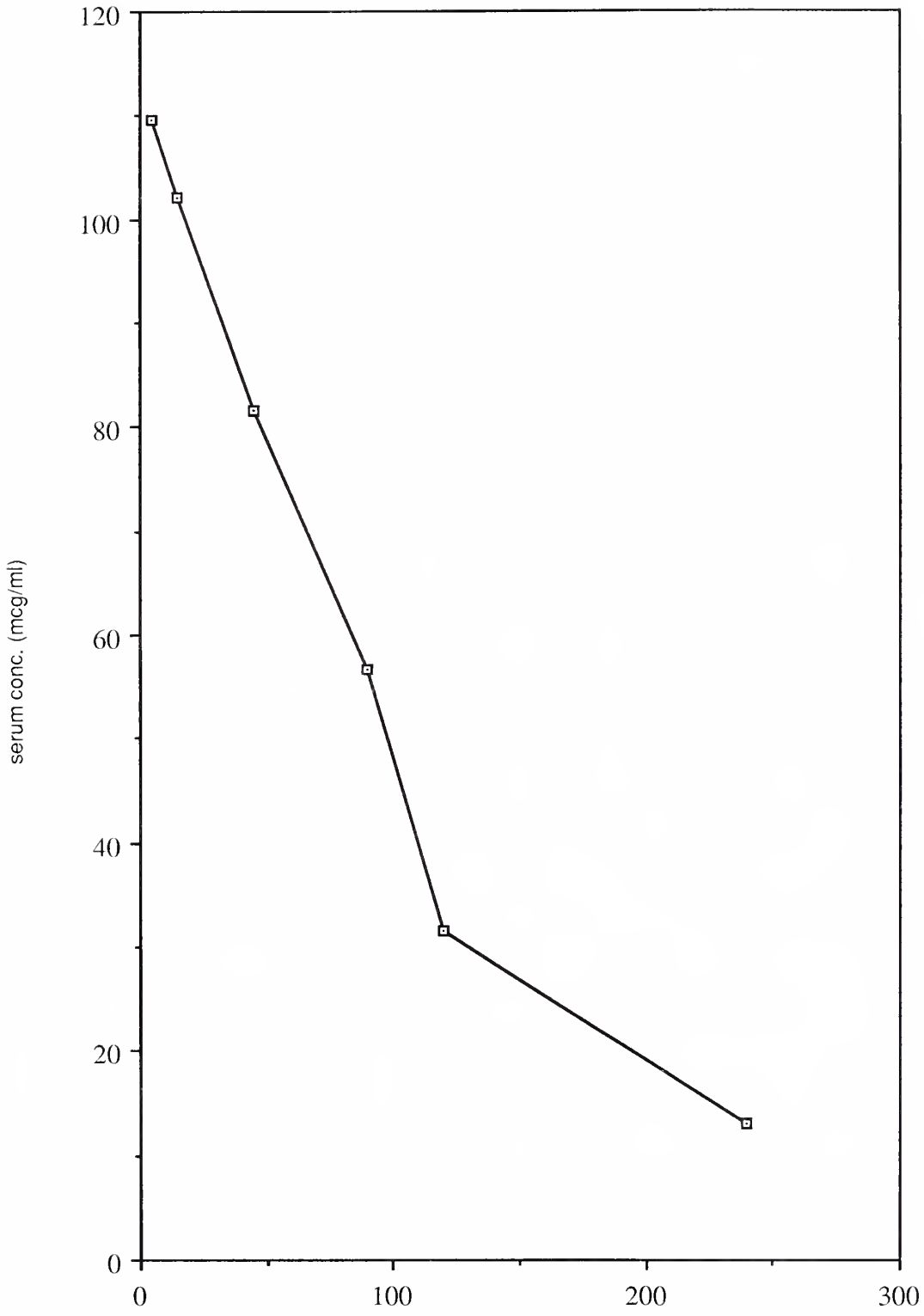
Appendix IV

Data deleted from study

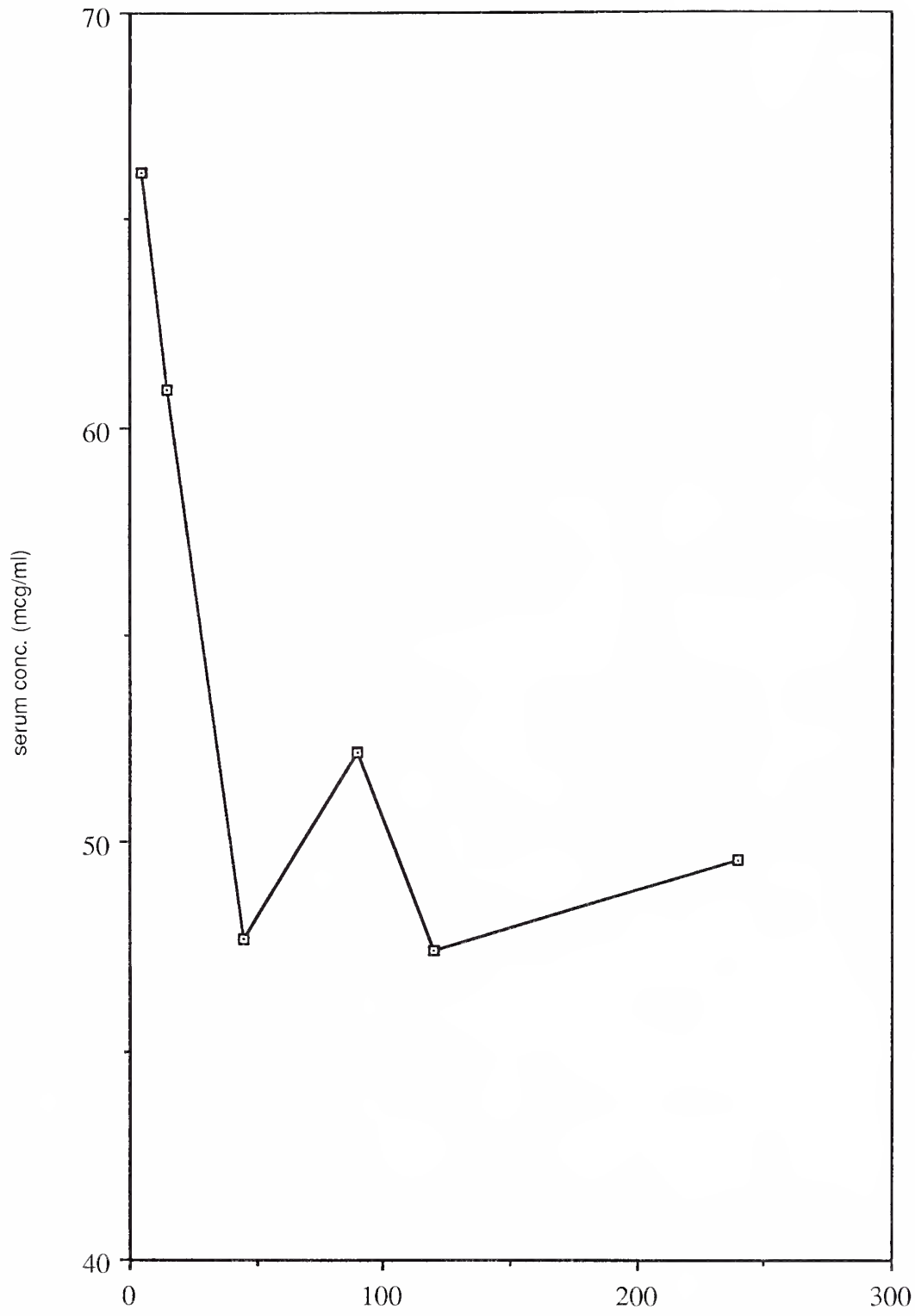
patient #25


120
100
80
60
40
20
0
0
100
200
300
serum conc. (mcg/ml)
time (minutes)

patient #41


120
100
80
60
40
20
0
0
100
200
300
serum conc. (mcg/ml)
time (minutes)

patient #42



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