National Library
of Canada

Acquisitions and
Bibliographic Services Branch

395 Wellington Street
Ottawa, Ontario
K1A 0N4

Bibliothèque nationale
du Canada

Direction des acquisitions et
des services bibliographiques

395, rue Wellington
Ottawa (Ontario)
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Canada

University of Alberta

Preclinical Assessment of the Multi-YAG Laser for Intracorporeal Lithotripsy of Human Urinary Calculi

by

Timothy Armin Wollin 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Experimental Surgery

Department of Surgery

Edmonton, Alberta

Spring, 1996

ISBN 0-612-10765-5

Canada

University of Alberta

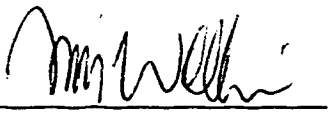
Library Release Form

Name of Author: Timothy Armin Wollin

Title of Thesis: Preclinical Assessment of the Multi-YAG Laser for Intracorporeal Lithotripsy of Human Urinary Calculi

Degree: Master of Science

Year this Degree Granted: 1996

(Signed) 

18405 95A Avenue

Edmonton, Alberta

Canada, T5T 3V7

January 4, 1996

University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Preclinical Assessment of the Multi-YAG Laser for Intracorporeal Lithotripsy of Human Urinary Calculi" submitted by Timothy Armin Wollin in partial fulfillment of the requirements for the degree of Master of Science in Experimental Surgery.

Ronald B. Moore, Supervisor

Malcolm S. McPhee

John Tulip

John D. Denstedt

Date Dec 21/95

To my loving wife Brenda
and my wonderful son Daniel.

Abstract

The dual wavelength Nd:YAG (multi-YAG) laser is a multi-purpose surgical laser designed to emit 1440 nm light in the pulsed mode for ablation of tissue. A three-part experimental study was undertaken to define the optimal parameters for laser lithotripsy and also to investigate the safety of this laser for fragmenting ureteral calculi.

Urinary calculi were first ablated in an *in vitro* study (n=120). Lithotripsy was then performed in an *ex vivo* pig model (n=45) and in nine female swine in order to study the acute and chronic tissue effects of treatment.

Fragmentation efficacy was significantly affected by the pulse energy ($p < 0.001$). The most efficacious and rapid fragmentation occurred at energies between 0.6 J and 0.9 J/pulse. *Ex vivo* lithotripsy caused low grade acute tissue effects in 37/42 cases and moderate to high grade injury in five ureters. There were no chronic high grade injuries in the *in vivo* model. These results suggest that the multi-YAG laser can efficiently ablate urinary stones with acceptable levels of acute and chronic tissue injury.

Acknowledgement

I am very thankful to have had this opportunity to pursue a Master of Science degree in experimental surgery. It has been a wonderful learning experience which I believe will continue to influence my career in urologic surgery. This project and thesis would not have been possible without help from a number of people. I would first like to thank my supervisors, Dr. R.B. Moore, Dr. J. Tulip, and Dr. M.S. McPhee. Each of them provided much guidance and encouragement during the course of this work. Although a busy clinician and clinical investigator, Dr. Moore always made himself available for consultation and discussion. His direction and advice were extremely valuable, but at the same time, he left me with abundant freedom to design and perform this project on my own. Dr. Tulip provided his expertise in lasers. Without his initial innovative research in the design and development of the multi-YAG laser, this study obviously would not have taken place. Dr. McPhee was not only a valuable source of advice and information, but because of his sincere commitment to research over the years, he has made it possible for students like myself to enjoy and benefit from a year of research during our clinical training. Despite a personally tragic year for himself, he continued to provide inspiration and encouragement. His retirement is well-deserved, however, he will be greatly missed by the urologic community.

Dr. J.D. Denstedt not only took time out of his busy schedule to be a part of the examination committee, but his knowledgeable critique of the initial project proposal and finally the thesis was greatly appreciated.

I would like to thank Dr. W.A. Mourad from the Division of Pathology at the University Alberta Hospitals for generously providing his time and expertise in the interpretation of

the histological data. Ms. Shelley Irwin also worked hard to organize and prepare the specimens for interpretation.

I am very grateful to Mr. Kevin Brown and Mr. Jack Demare for their technical support and surgical assistance. Their advice regarding the surgery and care of the experimental animals was very valuable.

Mr. John Hanson from the Department of Epidemiology at the Cross Cancer Institute graciously gave his time and abilities to carry out the statistical analysis for the project.

I would like to acknowledge and thank several businesses for their support and aid in carrying out this project. R. Laborie Surgical Ltd. (Mississauga, ON) generously contributed two ureteroscopes which were used to perform laser lithotripsy during each phase of the study. Their support of academic medicine is greatly appreciated. Louis C. Herring and Company, Analytical and Consulting Chemists (Orlando, FL), provided me with abundant stone material which allowed me to design a project with enough experimental levels and repetitions to make the results statistically valid. They also donated their services by performing crystallographic analysis on stones obtained from this region. Their laboratory supervisor, Mr. David Fletcher, was a pleasure to deal with. Finally, Gainers Burns Foods (Edmonton, AB), and Agriculture and Agri-Food Canada freely provided me with the porcine kidneys and ureters which were used for the *ex vivo* segment of the study. They should be commended for their support of academic research in this way. Dr. Mark Bielby of Agriculture and Agri-Food Canada was extremely accommodating and very helpful in allowing me access to the animal plant.

Ms. Joyce Spicer, formerly from the Department of Audio Visual Services at the Cross Cancer Institute, and Ms. Dawne Colwell, from the Department of Photographic Services

at the Surgical Medical Research Institute, provided their artistic expertise by creating the medical illustrations within the thesis.

I am also very thankful to Mrs. Susan Skelton for her help in the typing and editing of the thesis. Her willingness to assist in this way was greatly appreciated.

A special thanks is given to Dr. G.G. Miller from the Department of Surgery at the Cross Cancer Institute. Although busily involved in his own research, Dr. Miller always made himself available for discussion and counsel concerning this project.

Finally, I would like to acknowledge and thank the Edmonton Civic Employees Fund for providing the financial support for this research project. I appreciate their commitment to continuing medical education and research.

Table of Contents

List of Tables

List of Figures

List of Plates

List of Abbreviations

ALA	Aminolevulinic acid
ANOVA	Analysis of variance
AP	Apatite
cm	Centimeters
COD	Calcium oxalate dihydrate
COM	Calcium oxalate monohydrate
CPD	Calcium phosphate dihydrate
CT	Computerized tomography
CW	Continuous wave
°C	Degrees Celsius
EHL	Electrohydraulic lithotripsy
ESWL	Extracorporeal shockwave lithotripsy
F	French
Ho:YAG	Holmium: Yttrium aluminum garnet
HpD	Hematoporphyrin derivative
hrs	Hours
Hz	Hertz (cycles per second)
J	Joules
kg	Kilogram (10^3 grams)
lbs	Pounds
µm	Micrometer (10^{-6} meters)
µsec	Microsecond (10^{-6} seconds)
mg	Milligram (10^{-3} grams)
mJ	Millijoule (10^{-3} joules)
mL	Millilitre (10^{-3} litres)
mm	Millimeter (10^{-3} meters)
msec	Millisecond (10^{-3} seconds)
Nd:YAG	Neodymium: Yttrium aluminum garnet
nm	Nanometer (10^{-9} meters)
ns	Nanosecond (10^{-9} seconds)
PCNL	Percutaneous nephrolithotomy
sec	Seconds
UA	Uric acid
W	Watts

Chapter 1

Introduction to Urinary Lithiasis, Lasers, and Laser Lithotripsy

1.1 Introduction

Urinary stone disease affects approximately 0.5 to 2.0 per cent of the North American adult population (Johnson, 1979: Hiatt, 1982; Sierakowski, 1987; Frangos, 1987; Boyce, 1956). Surgical intervention may be required for stones that become impacted and do not pass spontaneously through the urinary collecting system. Although the majority of these calculi can be managed with extracorporeal shockwave lithotripsy (ESWL), some patients will fail this therapy and require some form of intracorporeal fragmentation. In addition, stones that become impacted in the distal ureter are often better treated with ureteroscopic lithotripsy and extraction. Ultrasonic and electrohydraulic lithotripsy are well established intracorporeal techniques but over the past decade, the photofragmentation of calculi with laser energy has been studied and is now another recognized modality.

The clinical application of laser lithotripsy began in 1986 with the pulsed coumarin dye laser (Watson, 1986; Dretler, 1987a). Since then, the rhodamine dye laser (Schmidt, 1993), the Q-switched Neodymium:yttrium aluminum garnet (Nd:YAG) laser (Hofmann, 1990), the alexandrite laser (Benizri, 1993), and the holmium:YAG (Ho:YAG) laser (Watson, 1993; Denstedt, 1994, Matsuoka, 1995) have all been described for clinical use. Successful fragmentation using these various laser systems is reported to occur in 75 to 95 per cent of patients (Begun, 1994).

The multi-YAG laser is a new, powerful, multiple purpose, dual wavelength Nd:YAG laser. It is an attractive laser for medical applications because it has dual wavelength capabilities which allow the user to select the wavelength appropriate for the desired tissue effect. Its unique 1440 nm wavelength produces superficial cutting or ablation of tissue

while the standard 1060 nm wavelength induces an area of deep thermal coagulation. This allows the operator to cut and coagulate with the same instrument. The stone-fragmenting properties of the multi-YAG laser are of particular interest because this could define another application for which the laser could be used. The overall aim of this project is to study the characteristics of multi-YAG laser lithotripsy and to assess whether this can be performed safely within the urinary tract. In order to critically study and evaluate this new laser for the purpose of urinary stone ablation, it is important to have a basic understanding of urinary stone disease, lasers, and the outcomes of basic and clinical research related to laser lithotripsy.

1.2 Epidemiologic Aspects of Urinary Lithiasis

Archeological excavations in the early twentieth century demonstrated that humans have been afflicted with urinary lithiasis since at least 7000 BC (Goldman, 1990). Hippocrates made note of the disease in his famous oath for the physician, "I will not cut, even for the stone, but leave such procedures to the practitioners of the craft" (Drach, 1992). Despite significant advances in the diagnosis and especially in the therapy of renal stone disease, the incidence of the disorder has increased by more than 60 per cent over the past 25 years and continues to rise, especially in industrialized nations (DeVita, 1993). Prior to industrialization, bladder calculi were the most frequent urinary stone, commonly composed of uric acid and magnesium ammonium phosphate (struvite) (Pak, 1990). Today, in North America and other industrialized countries, the upper urinary tract is the most common site of stone formation and these stones are composed predominantly of calcium oxalate (Pak, 1990).

Large population-based epidemiologic studies have determined the incidence of calcium urinary lithiasis to be 0.7 to 1.6 per cent in the U.S. (Johnson, 1979; Hiatt, 1982;

Sierakowski, 1987). Other authors have found a geographic variation in the incidence and have reported rates of 1.8 to 2.2 per cent in the southeastern U.S. which are consistently higher than other regions in the country (Frangos, 1987; Boyce, 1956). This has been attributed to a higher ambient temperature and is consistent with other studies documenting a higher incidence of stone disease in countries and regions with hot climates (Prince, 1960; Robertson, 1990). However, there are additional reports showing a relatively low incidence in regions where the climate is also hot such as Africa, Central and South America and areas of Australia populated by aboriginals (Drach, 1992). This may be secondary to genetic differences, but others believe that populations have to reach some moderate-to-high standard of living before ambient temperature plays a significant role in increasing the incidence of stone formation (Robertson, 1990). Affluent societies tend to eat more animal protein which increases urinary excretion of calcium, oxalate, and uric acid while reducing urinary pH and citrate excretion, and these are all risk factors for urolithiasis. A significant difference in the incidence of renal stones has been noted between the Caucasian and African-American races in the U.S. which is supportive of a genetic influence. (Frangos, 1987; Boyce, 1956).

The incidence of renal calculi occurs more commonly in males than females with a ratio between 2:1 and 3:1 (Frangos, 1987). This is thought to be secondary to a higher urinary excretion of calcium,. oxalate and uric acid, and a slightly lower excretion of citrate in the male sex (Robertson, 1990). Age-adjusted incidence rates are highest in the third to fifth decades and the disease is uncommon in those under 15 years (Drach, 1992; Frangos, 1987; Robertson, 1990; Scott, 1990). Children are believed to be protected by their relatively low excretion of calcium and because of a higher excretion of polyanionic inhibitors (Robertson, 1990). Finally, there may be an association between urinary stone disease and occupation. Studies have shown that there is a higher incidence of stone formation in those whose jobs are more sedentary (Frangos, 1987; Robertson, 1990;

Scott, 1990). However, the relationships involved are complex and may also reflect influences of socioeconomic status, dietary habits, and the working environment (Frangos, 1987).

1.3 Physiochemistry and Theoretical Basis for Urinary Stone Formation

Urinary calculi are composed of crystalline substances and the majority are comprised of mixtures of different compounds. For convenience, stones are often categorized into six major groups: calcium oxalate, calcium phosphate, purines and their salts, cystine, bacterial-induced stones, and others (Mandel, 1987). In North America, calcium oxalate and calcium phosphate are the most common components. According to two large series looking at the composition of urinary stones within the American population (Prien, 1949; Mandel, 1989), the admixture of calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD), and calcium phosphate (apatite-AP) occurred at a frequency of 5-22 per cent and COM and COD had a frequency of 19-22 per cent. Pure COM, pure COD, and pure AP occurred at a frequency of 14-22 per cent, 0.4-5 per cent, and 3-7 per cent respectively. The next most common group consisted of struvite and AP at a rate of 9-15 per cent and pure uric acid had an occurrence of 5-7 per cent. All other groups occurred at frequency of two per cent or less.

The exact mechanism of stone formation is not known. However, saturation and supersaturation of urine with certain chemical components, nucleation of the substances into a crystalline array, continued growth of crystals into calculi by agglomeration, epitaxis, matrix adhesion, and crystal retention, and inhibition of growth by urinary inhibitors are all believed to have a role in the overall process of urinary stone formation (Mandel, 1987).

A simple solution of water and a salt becomes *saturated* when enough salt is added so that crystals form and precipitate out of solution. The extent to which an individual compound will dissolve is determined by the *solubility product* of that particular compound (Jenkins, 1991). The *solubility product* is relatively simple to define for a solution of pure water, but is more difficult with a complex polyionic solution such as urine since it is greatly dependent on the pH and temperature of the given solution. In addition, many organic molecules such as urea, uric acid, citrate, and complex mucoproteins are all present in urine and all mutually affect the solubility of other substances (Drach, 1992). As a result, the solubility of a given compound in urine is generally much greater than if it was dissolved in water. In this situation, when the solubility product has been exceeded, but the substance is still in solution, the urine is said to be *supersaturated.*

When supersaturation reaches a point where the active ions no longer flow randomly in a completely dissociated fashion, they nucleate, or crystallize to form a lattice structure characteristic for that crystal (Drach, 1992). The level at which nucleation occurs is referred to as the *formation product.* When nucleation occurs spontaneously in a pure solution it is called homogeneous nucleation. The formation of urinary calculi most likely occurs through a heterogeneous nucleation. This process is influenced by the urine chemistry which can either enhance or retard the growth of the respective nuclei (Mandel, 1987).

Once crystal nuclei form within the urine, it is possible for larger crystal masses to grow through the process of aggregation or agglomeration. If sufficient numbers of crystals are present, collisions between crystal nuclei increase and binding together through electrostatic interactions may then occur. These crystalline clumps can frequently be seen within the center of calculi, especially calcium oxalate, uric acid and brushite stones (Mandel, 1987).

Epitaxial mediated crystal growth may also contribute to the continued growth of a crystal within the urinary tract. This can be defined as the oriented overgrowth of one crystalline material onto a substrate crystalline lattice (Mandel, 1987). Therefore, if a crystal's underlying structure closely resembles another crystal's structure, the former may actually grow on the surface of the latter resulting in overall growth of the crystal and creation of a multi-component stone. This theory is supported clinically where a strong association between hyperuricosuria and calcium oxalate stone formation has been noted (Jenkins, 1991; Coe, 1974). It is hypothesized that urate or uric acid seeds may function as nucleation sites for subsequent calcium oxalate crystallization (Mandel, 1987; Coe, 1992). In keeping with this theory, Coe and Raisen have shown that when calcium stone-formers are treated with allopurinol, to decrease the level of uric acid in the urine, the rate of new stone formation decreases (Coe, 1973). Conversely, others believe that epitaxis plays a very small role in the overall process of urolithiasis (Meyer, 1981).

Urinary calculi also contain a variable amount of organic material called matrix. The matrix content of most calculi is about 2.5 per cent and chemical analysis has revealed it to be about 65 per cent protein, ten per cent nonamino sugars, five per cent hexosamine (nitrogenous sugars) and ten per cent bound water (Boyce, 1985). The macromolecules matrix substance A, Tamm Horsfall mucoprotein, and hyaluronate have also been identified. (Mandel, 1987). Investigations using the scanning electron microscope have demonstrated the presence of fibrous bridges between crystals suggesting that matrix may act as a ground substance upon which crystallization can take place (Stacholy, 1985). However, others believe that matrix is simply an incidental finding because crystallization occurs in the presence of urinary macromolecules (Finlayson, 1961). Therefore, the exact role that matrix plays in the formation of urinary calculi remains uncertain.

Another factor believed to be important in crystal growth is the retention of crystals within the urinary tract with their subsequent growth in a supersaturated urinary environment. The most likely location for the initial crystal to form is in the renal papillae because this is where urine is most concentrated (Drach, 1992; Mandel, 1987; Finlayson, 1978). Crystal retention and growth is then theorized to occur by either a free particle or fixed particle mechanism. The free particle theory implies that nucleation occurs within the tubular lumen causing microscopic obstruction of the collecting tubule. Further growth to a macroscopic stone occurs through the processes of aggregation and agglomeration. Conversely, the fixed particle theory suggests that crystal formation and growth is unlikely to occur within the relatively swiftly flowing urine without some form of fixation (Finlayson, 1978). Proponents believe that crystals either form within the cells of the distal renal collecting tubules or become adherent to the renal collecting duct cells where growth continues (Mandel, 1987). Regardless of the overall mechanism, the end result of crystal retention is equivalent; a microcrystallite is allowed to grow to a macroscopic size, and depending on when it is released, it may or may not lead to gross urinary tract obstruction.

Although, calcium stone-formers tend to excrete more oxalate and calcium in their urine compared to normal persons, there is considerable overlap between the two groups (Drach, 1992). Many investigators believe that this is related to the presence of organic and inorganic urinary inhibitors of crystallization. Amino acid or small peptide inhibitors were first described by Howard and colleagues (Howard, 1967). Their studies demonstrated the presence of at least two low-molecular weight peptides in urine which were able to significantly increase the solubility of calcium in urine. They believed that these two fractions might account for most of the inhibitor potential in urine of some people. However, these experimental protocols were repeated by Robertson and associates and they were unable to show the same inhibitory activity (Robertson, 1969).

They concluded that the absence of peptide inhibitors was not a primary cause of stone formation in their patients.

Urinary mucopolysaccharide (uromucoid) is present in the urine of most people but may also play a role as an inhibitor. In stone-formers, this substance contains more sulfhydryl groups and this is believed to lead to increased calcium binding and a higher rate of stone formation (Foye, 1976). Other organic inhibitors include citrate, urea, and certain amino acids, specifically alanine (Drach, 1992; Mandel, 1987; Jenkins, 1991). Inorganic inhibitors which have been reported are related to the calcium phosphate or calcium oxalate systems. The most significant appears to be pyrophosphate, but magnesium and zinc have also been studied [Drach, 1992; Mandel, 1987; Jenkins, 1991).

In addition to these physicochemical theories for stone formation, a number of biochemical risk factors have been identified that are felt to contribute to the overall pathophysiology of urinary lithiasis. These abnormalities can be classified as environmental or metabolic risk factors (Preminger, 1994; Pak, 1985). The environmental factors can usually be controlled by the patient through dietary habits and include low urinary volume, low urinary magnesium, or an increased urine sodium, sulfate, or phosphate. The metabolic risk factors are caused primarily by a fundamental metabolic disturbance and this results in a high urinary calcium, oxalate, or uric acid, low urinary citrate, or an abnormally high or low pH.

Therefore, the entire process of urinary stone formation involves a combination of the above factors and theories. Once a crystal forms under the appropriate conditions of supersaturation, it is either retained within the urinary tract where growth continues, or it passes through and exits the system without consequence. Crystal growth occurs via agglomeration, epitaxis, matrix adhesion, or a combination of these. Finally, the presence

or absence of urinary inhibitors helps explain why some individuals with supersaturated urine for lithogenic components never form stones while others do

1.4 Medical Management of Urinary Lithiasis

Urinary stone disease may be conveniently classified as metabolically active, surgically active, or inactive (Seftel, 1990). Metabolically active disease includes radiologic evidence of stone growth, new stone formation within the past year, or documented passage of stone material within the same time period. Surgically active urolithiasis implies that the patient has acute or sub-acute obstruction of the urinary tract, possibly with symptoms or infection, and insinuates that some form of intervention will be required if spontaneous passage does not occur. Finally, if none of these criteria are present, the individual is considered to have inactive urinary lithiasis. Treatment of these clinical entities is dependent on a number of factors and can be broadly grouped into medical or surgical therapy.

The last decade has seen unparalleled progress in the surgical management of stone disease. When intervention is required, noninvasive or minimally invasive therapy can now be performed successfully in greater than 95 per cent of patients. Consequently, the main focus of medical management has swayed away from treatment and turned to the prevention of new stone formation or further growth of established renal calculi (Jenkins, 1991).

It has been demonstrated that approximately 50 per cent of first-time stone formers will have a recurrent stone within five years of the initial episode (Williams, 1963). Also, a metabolic or environmental etiology of nephrolithiasis can be found in about 95 per cent of patients evaluated for their stone disease (Preminger, 1994). As a result, many authorities

believe that first-time stone formers need evaluation. Conversely, 50 per cent of people will not form new stones following the initial event, and others have shown that a significant number of single stone formers treated conservatively with an increased fluid intake and avoidance of dietary excess, will remain metabolically inactive (Hosking, 1983). Therefore, a detailed evaluation for every patient may not be indicated. Regardless, patients that are high-risk for stone formation should undergo a full metabolic evaluation. Patients at risk include those with metabolically active stone disease, age under twenty years, nephrocalcinosis, chronic diarrheal or malabsorptive states, the presence of cystine, uric acid, or struvite stones, and a history of gout, pathologic fracture, or osteoporosis (Preminger, 1994).

An extensive ambulatory evaluation for recurrent stone formers or high-risk patients has been devised by Pak and associates (Pak, 1980) and has been reviewed and outlined by others (Drach, 1992; Preminger, 1994; Seftel, 1990). The protocol entails two outpatient visits and is completed over a 2 to 3 week period. The assessment includes serum biochemistry and urine evaluation on a regular and a calcium-restricted diet and also involves a "fast and calcium load" study (Pak, 1975). On completion of these studies, patients with stone disease can be placed into one of thirteen diagnostic groups based on the specific physiologic derangements (Table 1.1). An abbreviated metabolic evaluation for single stone-formers with none of the above risk factors has also been described (Preminger, 1994). The goal of this protocol is to identify those patients with underlying systemic disease which require specific and selective therapy such as, primary hyperparathyroidism, distal renal tubular acidosis, gout, cystinuria, and recurrent urinary tract infections.

Regardless of the underlying etiology or biochemical abnormality, all patients should follow certain conservative medical recommendations for prophylaxis against further stone

formation or stone growth. These include increasing fluid intake to maintain a urine output of greater than two litres per day and avoiding dietary excesses of sodium or oxalate (Preminger, 1994). Limitation of dairy products, in those suspected of having absorptive hypercalciuria, and reducing animal-protein intake, for patients with hyperuricosuria, is also suggested. These measures alone have been shown to eliminate metabolically active stone disease in a significant proportion of patients with idiopathic calcium urolithiasis and is often referred to as the "stone clinic effect" (Hosking, 1983). Patients should be re-evaluated with a repeat 24-hour urine collection in approximately four to six months. If an abnormality persists or the patient becomes metabolically active, some believe that extensive evaluation and selective medical therapy should be instituted at that time (Preminger, 1994). Others suggest that re-emphasis of the importance of increased oral fluid intake and dietary restriction be done first (Hosking, 1983).

For patients who undergo extensive ambulatory evaluation, treatment is administered selectively according to which diagnostic group they fall under (Table 1.1). These therapeutic guidelines and recommendations have been previously described and reviewed by other investigators and will not be repeated here (Resnick, 1990; Preminger, 1994; Seftel, 1990; Drach, 1992). The main role of selective medical management is prevention of new metabolically active disease. Its impact on prevention has been studied and shown to be significant. Preminger and associates studied 54 patients receiving potassium citrate for mild to moderate stone disease (less than one stone per patient per year) and compared them to 423 patients reported in the literature who were treated with conservative measures alone. They found that the stone formation was significantly less for the potassium citrate patients. The remission rate was 96 per cent for these patients compared to 61 per cent for those following conservative management (Preminger, 1985a). The same group also studied 103 patients treated with selective medical therapy and showed that this treatment appeared to reduce the need for surgical management (Preminger,

1985b). During the three years before beginning medical treatment, 58 per cent of patients with existing stones underwent surgical procedures. While on selective medical therapy, only two per cent of these patients required an operation for the removal of a new calculus.

1.5 Surgical Management of Urinary Lithiasis

Because medical management for existing stones or acute episodes is limited, surgical intervention plays an important role for these patients. Ever since the initial report of extracorporeal shock wave induced destruction of kidney stones by Chaussy et al. in 1980 (Chaussy, 1980), a revolution in the surgical management of the patient with urolithiasis has taken place. Open surgical procedures to extract stones from the kidney and ureter are now required in only one to two per cent of those affected (Begun, 1994; Assimos, 1989; Boyle, 1987). Extracorporeal shockwave lithotripsy (ESWL), percutaneous nephrolithotomy (PCNL), and ureteroscopic techniques are now the mainstays of surgical intervention.

The accepted indications for surgical intervention in urinary stone disease are situations of an obstructed solitary kidney, bilateral ureteral obstruction, infection behind an obstructed system, continuous unrelenting pain, or stone diameter greater than six millimeters (Spirnak, 1990a). Otherwise, awaiting spontaneous passage is indicated because this is obviously the least invasive form of treatment. Concerning expectant management, 70 to 90 per cent of all stones less than or equal to four millimeters in size will pass spontaneously in approximately two to three weeks time (Drach, 1992; Marberger, 1994; Ueno, 1977; Morse, 1991). Only ten to fifteen per cent of stones greater than six millimeters will pass spontaneously and essentially none will pass that are greater than eight millimeters in diameter (Morse, 1991). Animal and human studies, indicate that high

grade obstruction is tolerated for at least two weeks without permanent renal damage (Leahy, 1985). Therefore, continued obstruction by itself does not necessarily exclude expectant management. However, with the low morbidity associated with modern day surgical therapy, the alternative of waiting prolonged periods for spontaneous passage of a calculus has become less attractive to some patients and urologists. As a result, some have now suggested treating symptomatic stones greater than five millimeters in diameter that have not passed within one week, and even smaller ones if they are associated with repeated episodes of renal colic (Marberger, 1994).

Extracorporeal shockwave lithotripsy, PCNL, and ureteroscopic stone extraction are the main techniques for the surgical intervention of urinary lithiasis. ESWL is the most common modality used because of its success and ease of use. However, PCNL and ureteroscopic extraction are indicated in certain situations and are therefore also important. As the name implies, ESWL fragments stones with shockwaves generated outside of the body. Percutaneous nephrolithotomy and ureteroscopy can extract calculi without fragmentation, but most often some form of intracorporeal lithotripsy is required. Each of these forms of treatment plus the different techniques of intracorporeal lithotripsy will now be discussed in order to understand the advantages and disadvantages of each system.

1.5.1 Extracorporeal shockwave lithotripsy (ESWL)

The Dornier Company is a German pioneer aerospace company. They became interested in shock wave physics after it was noted from early research that the pitting that took place in the exposed metal of aircraft bodies was secondary to shock waves produced from raindrops and micrometeorites colliding with the aircraft (McCullough, 1992). Through a collaborative effort involving the Dornier Company and the University of Munich, research was initiated on the development of a clinical lithotriptor for urinary

calculi. In December 1980, the first report of shock wave lithotripsy in humans was described by Chaussy and colleagues (Chaussy, 1980) and by October 1984, the Dornier HM-3 (Human Model three) became commercially available after more than 1000 patients had been treated in Munich and Stuttgart (McCullough, 1992). By 1986, more than 130 Dornier units were operational world wide and by 1990, at least nine manufacturers were developing second-generation machines (Spirnak, 1990a). Hundreds of thousands of patients have been treated over the last decade and ESWL therapy truly represents a revolution in the surgical management of urinary stone disease.

Extracorporeal shockwave lithotripsy is a technology that uses focused shock waves generated outside of the body to disintegrate renal and ureteral calculi (Zhong, 1994). Because the fluid content of the body is high, little energy loss occurs until the wave reaches the stone and this results in gradual fragmentation of the stone (Segura, 1990c). It is believed that when the shock wave contacts the stone, a compression wave forms along the anterior surface of the stone causing anterior fragmentation (Wilson, 1990). Additionally, some of the shock wave traverses the stone and is reflected off the posterior surface of the stone creating tensile stress and additional crumbling along this surface.

Shock wave lithotriptors can be categorized as either electrohydraulic, electromagnetic, or piezoelectric, depending on how the shock waves are generated. All share four main components: An energy source, a focusing device, a coupling medium, and a stone localization system (Wilson, 1990). The electrohydraulic lithotriptors use a spark gap generator to produce a shock wave within a liquid medium. Fifteen thousand to 25,000 volts are discharged across the electrodes and this causes a sudden expansion of the surrounding fluid, leading to the formation of a spherically expanding shock wave (Zhong, 1994). The origin of the shock wave is designated as the primary focal point, or F_1, and the wave is focused at F_2, the location of the stone. Shock waves from an electromagnetic

lithotriptor are generated within a shock tube by electric currents which move a metallic membrane (McCullough, 1992). The waves are then focused to the stone site (F_1) with an acoustic lens. Finally, piezoelectric lithotriptors create shock waves by exciting thousands of ceramic elements arranged on a spherical dish with a high-frequency, high-voltage electric pulse (McCullough, 1992; Zhong, 1994). The shock waves are focused on the stone (F_1), which is at the geometric center of the spherical dish. The original, first-generation Dornier HM-3 lithotriptor used a large water bath to couple the shock waves to the patient (McCullough, 1992). More recently, the second-generation and third-generation machines use either an enclosed water cushion, a small exposed pool of water, or a totally contained shock tube (Zhong, 1994). Either fluoroscopy or ultrasonography is used for stone localization.

The indications for ESWL include the standard indications for surgical stone removal. In addition, numerous centers now consider "asymptomatic" caliceal stones an indication for treatment since many of these have been shown to cause intermittent symptoms (Spirnak, 1990a; Mee, 1988; Anderson, 1983; Brannen, 1986). The only absolute contraindications to treatment are coagulopathies which cannot be corrected and pregnancy (McCullough, 1992). In some situations, patient weight greater than 300 lb. is also considered a contraindication because the machine warranty is not effective for a weight greater than 297 lb. (Spirnak, 1990a).

As experience with ESWL has increased, it has become clear that clinical outcome is dependent on the stone size, stone location, stone composition, and on the type of machine used. The first clinical data reported by Chaussy and coworkers in 1982 showed a success rate of 91.5 per cent in 59 patients treated with a stone in the renal pelvis or calyx (Chaussy, 1982). The size of the stones treated and the length of follow-up to achieve a stone-free rate were not noted in this early study. Subsequent reports using the

original HM-3 lithotriptor for renal calculi in the United States have revealed that for stones less than 1.5 cm, ESWL monotherapy results in stone-free rates of 85 per cent with a retreatment rate of 16 per cent using an average of 1,200 shocks per treatment (Zhong, 1994; Drach, 1986; Lingeman, 1986; Graff, 1988). As the stone size increases above 2 cm, stone-free rates diminish to approximately 50 per cent at three months (Drach, 1986).

Stone location is another important consideration in the overall outcome of ESWL. Stone-free rates are 60 to 70 per cent for stones in the lower calyces, 75 to 80 per cent for calculi in the middle and upper calyces, and 85 to 92 per cent for renal pelvic and upper ureteral stones (Wilson, 1990). In the ureter below the pelvic brim, stone-free rates with the HM-3 lithotriptor have been reported to be 60 to 80 per cent with a 13 per cent retreatment rate (Wilson, 1990; Cass, 1992). Because ureteroscopic extraction of stones located in a similar position has a success rate of 83 per cent to greater than 95 per cent, some authors suggest that ureteroscopy should be the first choice of therapy for lower ureteral calculi (Cass, 1992). Finally, the overall stone-free rate for lower caliceal calculi is lower than for other renal calculi and is reported to be 64 to 71 per cent depending on the stone burden treated (Drach, 1986; Graff, 1988; Nicely, 1992). Some investigators have suggested that primary percutaneous nephrolithotomy may be more appropriate for these calculi. From their own experience and through a meta-analysis of previously published data, Lingeman and colleagues found a stone-free rate of 90 per cent for initial percutaneous nephrolithotomy of lower caliceal stones compared to 59 per cent for ESWL monotherapy (Lingeman, 1994). In addition, the success of percutaneous therapy was not dependent on stone burden.

Stone composition also has a role in the clinical effectiveness of ESWL. It is well known that stones made up of calcium oxalate monohydrate (COM), calcium phosphate dihydrate (CPD), or cystine, require more shock waves at a higher intensity to achieve

fragmentation (Zhong, 1994; Dretler, 1988b). Basic analysis has shown that COM and CPD stones have a higher density than other common stones making them "harder" (Dretler, 1988b). Cystine is not considered to be a "hard" stone with respect to its linear density. Its difficulty in fragmentation is thought to be secondary to a higher organic composition. Therefore, the acoustical properties of the stone may not differ enough from the surrounding body tissues and this does not allow sufficient tensile forces to develop to cause fragmentation (Spirnak/1990a).

The first-generation Dornier HM-3 lithotriptor represents the gold standard in terms of efficacy for urinary calculus fragmentation (Wilson, 1990). Second- and third-generation machines have now been manufactured in an attempt to lower the anesthetic requirements and make the machines more versatile. The differing characteristics of these lithotriptors also influence the overall clinical result. As noted previously, the Dornier HM-3 is associated with stone-free rates of 85 per cent, and retreatment rates of 16 per cent using approximately 1200 shocks per treatment. Zhong and Preminger have reviewed the differing modes of shock wave lithotripsy (Zhong, 1994). They found that as the shock-wave configuration is changed to reduce the pain during lithotripsy, the number of shock waves and the retreatment rates to achieve fragmentation and a stone-free rate increases. The second generation modified Dornier HM-3 and the Dornier HM-4 were found to require an average of 2100 shocks to maintain a stone-free rate of 56 to 82 per cent. The retreatment rate was 22 to 37 per cent for these machines. There is also a decrease in fragmentation efficiency with the electromagnetic and piezoelectric devices. The former were found to require a mean of 3,600 shocks to achieve stone-free rates of 69 to 80 per cent and were associated with retreatment rates of seven to 21 per cent. Finally, the piezoelectric lithotriptor used an average of 3,500 shocks per treatment, with a 74 to 80 per cent stone-free rate and a 30 per cent retreatment rate.

The high-energy shock waves from ESWL can cause significant biologic effects and are associated with some complications. Transient gross hematuria occurs in nearly 100 per cent of patients and usually resolves within 24 hours. It is believed to be secondary to blunt parenchymal trauma rather than the action of the stone on the urothelium since nearly all patients undergoing lithotripsy for biliary calculi also experience transient hematuria (McCullough, 1992; Spirnak, 1990a). Significant bleeding leading to blood transfusion is rare. Only one patient in 467 required a transfusion in a study reported by Riehle and colleagues (Riehle, 1986).

Parenchymal trauma is also suggested by radiologic studies performed before and after lithotripsy which show evidence of subcapsular hematoma, intrarenal hematoma, loss of corticomedullary junction demarcation, and perirenal fluid collection (Rubin, 1987; Kaude, 1985). Clinically significant hematomas are rare in patients with a normal clotting mechanism and have a reported incidence of 0.66 per cent (Knapp, 1988). In one study of patients with perirenal hematoma following ESWL, hypertension, especially uncontrolled hypertension, and pretreatment urinary tract infection were found to be significant risk factors (Knapp, 1988). Prior use of nonsteroidal anti-inflammatories as a risk factor is debatable (Riuz, 1990, Lingeman, 1989b). Finally, significant increases in serum and urine enzymes, as well as transient nephrotic-range proteinuria has also been observed after ESWL and found to return to normal within a few weeks (Zhong, 1994).

Urinary tract obstruction has been reported to occur in up to 30 per cent of patients after ESWL and its incidence is directly related to the size of the initial calculus undergoing fragmentation (Spirnak, 1990a). The accumulation of multiple fragments within the ureter, a condition known as *Steinstrasse*, or "stone street", occurs in less than five per cent of cases and requires intervention in six to 35 per cent (McCullough, 1992).

Indications for intervention in situations of post-ESWL obstruction are the same as for any patient presenting with obstruction secondary to ureteral calculi.

Septic complications develop in 0.5 to one per cent of patients following ESWL (McCullough, 1992; Spirnak, 1990a). Those with struvite calculi are especially at risk and should be treated with specific broad-spectrum antibiotics prior to and following shock wave therapy. The use of ureteral stents and nephrostomy tubes when a larger stone burden is anticipated can help reduce infectious complications by providing adequate drainage. Damage can also occur to adjacent organs during lithotripsy. The lungs are especially prone to injury if subjected directly to high-energy shock waves (McCullough, 1992). This complication is more likely to occur in myelodysplastic children or others with unusual anatomy and can be avoided by shielding the lungs with Styrofoam. Several cases of pancreatitis have been reported and transient gastrointestinal erosions have also been described. There have been no reports to date regarding rupture of aortic or renal artery aneurysms, but there have been cases of iliac vein and iliac artery thrombosis following ESWL therapy.

The long-term complication of hypertension secondary to ESWL is a controversial topic and has not been completely resolved as of yet. Some series have a reported an incidence of new-onset hypertension following ESWL of eight per cent (Lingeman, 1987; Williams, 1988). However, other investigators have found an incidence of one to three per cent (Liedl, 1988; Chaussy, 1988), similar to the expected incidence in an age-matched population followed for 40 months (Spirnak, 1990a). A long-term prospective trial is currently under way to help answer this question definitively. Finally, the mortality rate of ESWL was found to be 0.02 per cent in more than 62,000 patients treated (Spirnak, 1990a). None of the deaths were felt to be directly related to the lithotripsy.

Over the past fifteen years, ESWL has proven itself to be an effective and safe treatment for renal and ureteric calculi. The main advantage is its non-invasive nature. Patients may or may not require some form of anesthesia, but the treatment is usually performed on an outpatient basis with minimal amounts of discomfort. The treatment is safe with a low incidence of significant short-term complication and there have been no proven chronic complications thus far. Extracorporeal shockwave lithotripsy will therefore continue to be the first-line of therapy for most renal and ureteral stones that require therapy. However, experience has now shown that there are certain clinical situations where PCNL and ureteroscopy yield superior results compared to ESWL.

1.5.2 Percutaneous Nephrolithotomy (PCNL)

Rupel and Brown performed the first successful percutaneous stone removal through a surgically established nephrostomy tract in 1941. However, it was not until 1976 that Fernström and Johansson described the first percutaneous extraction of a renal stone through a *percutaneously* established nephrostomy tract (Spirnak, 1990b). By the late seventies and early 1980's, as clinical experience increased and available instrumentation developed, percutaneous lithotomy eventually replaced open surgical lithotomy as the treatment of choice for renal and upper ureteral calculi. However, at the same time, the technology for extracorporeal shock wave lithotripsy was also being developed. By 1984, the "golden age" of percutaneous nephrolithotomy ended as shock wave lithotripsy was introduced to the world (Spirnak, 1990b). Nevertheless, widespread use of ESWL has not rendered the technique of percutaneous lithotomy obsolete. Instead, the indications for percutaneous stone surgery have been refined. Today, percutaneous lithotomy is considered appropriate management for patients with a large stone volume, staghorn calculi, cases involving specific stone compositions, and in some cases, obesity. (Segura, 1990b; Lingeman, 1989c). About one per cent of patients will fail ESWL therapy and will therefore also be candidates for percutaneous lithotomy (Spirnak, 1990b). Finally, as

mentioned previously, there is also evidence that lower caliceal calculi may be better treated with initial PCNL (Lingeman, 1994).

Stone-free rates for ESWL when stones are less than two centimeters in diameter are approximately 85 to 90 per cent (Zhong, 1994; Drach, 1986; Lingeman, 1986). This rate drops to less than 65 per cent for stones greater than two centimeters and more importantly, the retreatment rate and morbidity increases significantly (Lingeman, 1986; Lingeman, 1989a). Lingeman and colleagues have now shown that these large stones are better managed with percutaneous extraction, either alone or in combination with ESWL (Lingeman, 1989a). Patients with stones three centimeters or greater had a stone free rate of only 30 to 67 per cent when treated with ESWL alone compared to 75 to 85 per cent when treated with PCNL and 92 per cent for those treated with a combination of PCNL and ESWL. The retreatment rates for these patients was 60 to 75 per cent for the ESWL patients and only 30 to 35 per cent for the PCNL patients.

Staghorn calculi are commonly composed of struvite which forms as a result of urinary tract infection with urease-splitting bacteria. These large stones may be managed by ESWL alone but stone-free rates are only twenty to 50 per cent and therapy is associated with ureteral obstruction in 30 to 50 per cent of patients (Stanely, 1994; Lingeman, 1989a). In addition, time to achieve a maximal stone-free rate is longer and may be as long eight months (Winfield, 1988). This is an undesirable result because residual fragments from these infected stones are known to have a propensity to progress and often lead to recurrent urinary infections. Therefore, because of the poorer outcome and increased morbidity, there is a growing body of evidence suggesting that staghorn stones be treated with percutaneous lithotomy with or without ESWL.

Stanley and Winfield have reviewed the overall results of PCNL for the treatment of staghorn stones. For PCNL monotherapy, maximal stone clearance was achieved with an average of 2.0 procedures in 304 patients studied (Stanely, 1994). The presence of residual stones following therapy ranged from 10 to 40 per cent compared to 34 to 56 per cent for those with ESWL monotherapy. Combined PCNL and ESWL has also been described and reviewed by the same authors (Stanely, 1994). In these cases, maximal debulking of the stone is accomplished with percutaneous methods followed by ESWL for residual fragments. The morbidity of passing stone fragments is significantly reduced by percutaneous debulking as well as the presence of the nephrostomy tube. For 586 patients treated with this combined therapy, the average number of procedures required to achieve maximal clearance was 2.62, and the incidence of residual stone fragments following therapy was 15 to 48 per cent. Complication rates were similar to, but lower than percutaneous surgery alone.

As noted previously, stones composed of calcium oxalate monohydrate (COM), calcium phosphate dihydrate (CPD), or cystine, are resistant to fragmentation with ESWL. Patients with COM calculi often require additional ESWL treatments and ureteroscopic manipulation of residual fragments. Therefore, it is recommended that if the this stone composition is known beforehand, these patients should be managed with primary percutaneous stone removal (Lingeman, 1989b). When cystine stones are subjected to ESWL, they either do not fragment, or if they do, the particles are generally large and are difficult to pass. Consequently, cystine stones that are not amenable to medical dissolution are also better treated with percutaneous lithotomy (Stanley, 1994). Finally, some authorities also recommend percutaneous extraction for uric acid calculi because monitoring the passage of the radiolucent fragments following ESWL may be difficult with larger stones (Lingeman, 1989b).

Successful percutaneous nephrolithotomy requires the completion of three separate yet interdependent procedures: Percutaneous access and nephrostomy tube placement, tract dilatation, and percutaneous stone removal. The techniques used to accomplish these procedures have been described in detail elsewhere (Spirnak, 1990b; Lingeman, 1989b; Segura, 1992). Stones that are one centimeter in maximal diameter or less can usually be extracted through a 30 Fr. nephrostomy tract with various grasping instruments or stone baskets without fragmentation (Spirnak, 1990). Larger calculi require intracorporeal fragmentation using ultrasonic, electrohydraulic, pneumatic, or laser lithotripsy. These techniques will be discussed in detail later.

Bleeding is the most common complication following PCNL and hemorrhage sufficient to require a blood transfusion is the most common major complication. It is more likely to occur when treating staghorn calculi compared to simple percutaneous lithotripsy and extraction (Lingeman, 1989b). Out of 110 nonstaghorn cases, 5.5 per cent of patients required transfusion compared to 12 per cent in patients who had a staghorn calculus removed (Lingeman, 1989b). Life-threatening renal hemorrhage from arteriovenous fistulas or false aneurysms have been reported to occur in 0.005 per cent (Patterson, 1985). Significant bleeding is best managed with a balloon tamponade catheter as described by Kaye (Kaye, 1986). If bleeding persists, then angiography is indicated with selective embolization. Rarely, partial or simple nephrectomy is required in cases of intractable bleeding.

The possibility of contiguous organ damage also exists with percutaneous surgery. Injuries to the pleura, liver, spleen, duodenum, and colon have been reported (Reddy, 1985; Lang, 1987; Lee, 1987). Pneumothorax and hydrothorax are potential sequelae any time a supracostal tract is employed. Should intervention be required, aspiration should suffice since lung injury with persistent leak is rare (Lingeman, 1989b). Colonic

perforation has a reported incidence of 0.2 to 0.5 per cent (Spirnak, 1990b). Although immediate operative intervention has been described, conservative management with parental antibiotics and staged nephrostomy tube removal has been uniformly successful if the injury is confined to the retroperitoneum. Factors that increase the risk of colon injury during percutaneous nephrolithotomy are staghorn calculi; anterior calyceal puncture; previous extensive renal operation; horseshoe kidney; kyphoscoliosis; massive weight loss; hepatomegaly; or splenomegaly (Lingeman, 1989b).

Extravasation of contrast from the renal collecting system is quite common following percutaneous lithotripsy and is best managed by the placement of a nephrostomy tube. Intraperitoneal perforation with extravasation of irrigant can cause hyponatremia or fluid overload and this can be life-threatening if not recognized (Lingeman, 1989b). Post-operative temperature elevation occurs in ten to 25 per cent of patients, and is more common following therapy for struvite calculi. Broad-spectrum parenteral antibiotics are recommended for these patients 48 hours preoperatively and for 24 to 36 hours following surgery to help decrease the incidence of septic complication (Spirnak, 1990b). The incidence of perinephric abscess or other suppurative complications are low and reported to be less than one per cent (Lang, 1987). Finally, death has been reported in association with percutaneous nephrolithotomy with an incidence of 0.046 per cent (Lang, 1987; Lee, 1987). The deaths were secondary to hemorrhage, overwhelming sepsis, and cardiopulmonary complications.

In vivo animal studies have been performed to assess the long-term results to the kidney from percutaneous surgery. Webb and Fitzpatrick performed open nephrostomy tube placement and tract dilatation to 24F in dogs and examined the kidneys six weeks post-operatively (Webb, 1985). Grossly, they found only a small dimple on the surface with a fine linear scar through the parenchyma on cross section. Microscopically, they estimated

that less than 140 nephrons were injured. Additionally, creatinine clearance studies were normal compared to the contralateral kidney. Clayman and colleagues performed antegrade nephrostomy in a porcine model and dilated the tracts to 24F with Amplatz dilators and to 36F with balloon dilators (Clayman, 1987). They estimated that total renal damage was less than 0.15 per cent of the total cortical surface. There was no significant difference in the amount of injury caused by the two different techniques.

Clinical studies investigating the long-term effects of percutaneous nephrolithotomy have shown similar positive results. Mayo and associates studied fifteen patients with seventeen stone-containing units and performed differential renal function studies pre- and postoperatively (Mayo, 1985). They found no significant change in the creatinine clearances overall, but there was a significant improvement in the function of those units containing infectious stones where preoperative function was reduced. In another report, a consecutive series of eleven patients were found to have no significant morphologic damage on serial CT scans and functional studies showed no adverse effects (Ekelund, 1986). Therefore, despite the invasive nature of PCNL, these clinical investigations and the above animal studies confirm that there does not appear to be any significant chronic effects on the kidney itself.

1.5.3 Ureteroscopic Management of Urolithiasis

Although ESWL and PCNL can effectively treat renal and upper ureteral calculi, stones in the distal and mid ureter are more difficult to treat and tend to have lower stone-free rates when treated with these techniques. Furthermore, certain patients may not be candidates because of morbid obesity, irreversible coagulopathies, radiolucent stones, and previous ESWL failures (Begun, 1994). This population is often better managed with ureteroscopic techniques of stone fragmentation and extraction. Young is credited with performing the first ureteroscopy when he passed a rigid cystoscope into the dilated ureter

of a patient with posterior urethral valves [Young/1929]. However, it wasn't until the late 1970's when Goodman and Lyon independently reported ureteroscopy using techniques to dilate the distal ureter that routine endoscopic evaluation of the ureter became practical (Spirnak, 1990c).

First-generation ureteroscopes were produced using rod-lens optics within lenses that were interchangeable and placed directly down a working sheath (Spirnak, 1990c). Second-generation instruments were designed so the lens and eyepiece were one unit and could not be removed. In addition, the eyepiece was offset at 30 or 90 degrees making it easier on the operator to visualize the ureter. Over the last five to ten years, technology has continued to improve. Using fiberoptic technology, 6 to 8F ureteroscopes are now available that can be passed through the ureteral orifice with greater ease and in most cases, without dilatation. More flexible, semi-rigid instruments have also been developed that allow greater degrees of curvature without compromising the visual field. Finally, flexible instruments are also available which allow one to maneuver the instrument into regions which would be difficult or impossible to see with the rigid variety. Modern flexible ureteroscopes are either passively deflectable or actively deflectable. The former are less expensive but do not allow for purposeful movement of the tip and therefore depend on a pre-placed guide wire for positioning within the urinary tract (Huffman, 1992).

Stones located throughout the ureter are accessible by ureteroscopy but the retrieval rate is lower and the incidence of complications is higher for stones in the proximal ureter or renal pelvis (Spirnak, 1990c; Begun, 1994; Clayman, 1991). The stone composition does not affect the success of ureteroscopy itself. However, if a cystine or uric acid stone is identified, attempts at medical dissolution should be made first before attempting a surgical extraction (Spirnak, 1990c).

Stones that are approximately five millimeters in size may be extracted intact using a variety of baskets or grasping instruments (Huffman, 1992). The removal of larger calculi will usually require some form of fragmentation or dilatation of the intramural ureter. Like PCNL, ultrasonic lithotripsy, electrohydraulic lithotripsy (EHL), the pneumatic lithoclast, and laser lithotripsy are available for intracorporeal lithotripsy.

Ultrasonic lithotripsy is based on a piezoelectric effect. An electrical charge is applied to a piezoceramic crystal and this causes vibration of the crystal and liberation of ultrasound waves. When this energy is transmitted down a metal probe, it causes the probe tip to vibrate making it an excellent tool for stone fragmentation when in contact with a calculus (Spirnak, 1990b). All stones may be fragmented using this modality but high density stones are more difficult and time-consuming to treat. Stones with smooth, round surfaces, such as uric acid or COM, also seem to be more difficult to fragment compared to those with a rough surface. Alken and associates reported the first series of patients in whom ultrasonic lithotripsy was used in the kidney (Alken, 1981). Since then, it has been shown to be a safe and effective means of achieving intracorporeal lithotripsy.

In the ureter, the ultrasound probe is advanced through the working port of the ureteroscope and placed in contact with the stone. Lithotripsy is then accomplished under direct vision until adequate fragmentation has occurred. Results from a number of clinical trials evaluating the effectiveness of intraureteral ultrasonic lithotripsy have recently been reviewed by Begun (Begun, 1994). The overall success rate using a number of different probes (2.7F to 7.5F) was 79.2 per cent in 1333 patients studied. As expected, lower ureteral calculi had a higher success rate when the results were stratified. Rates were 86 to 96 per cent for the lower ureter, 51 to 94 per cent for the mid ureter, and 38.5 to 91 per cent for the upper ureter.

One of the advantages of ultrasonic lithotripsy is that there is very little risk of tissue injury. *In vivo* animal studies have shown that ultrasonic lithotripsy is safe and can be performed with minimal tissue injury (Howard, 1974). Another advantage of ultrasonic lithotripsy is that manual extraction of residual fragments is eliminated when larger, hollow probes are used and attached to suction. Smaller probes without suction must be used for intraureteral fragmentation. Finally, the equipment required for this technique is relatively inexpensive. Once the initial set up fee of approximately $10,000 (U.S.) has been paid, there is little maintenance since the probes are reusable with an indefinite life-expectancy (Clayman, 1991). The disadvantage of ultrasonic fragmentation is its limited ability to fragment harder calculi such as COM, CPD, and cystine (Begun, 1994). Longer times and ancillary procedures are often required in these situations. Also, the rigidity of this instrument makes it much more difficult to use in the ureter through a ureteroscope.

The principles of electrohydraulic lithotripsy (EHL) are similar to extracorporeal lithotripsy. A shock wave is generated as a result of an underwater electrical discharge from the electrode tip of the EHL probe. Intense heat and vaporization of water results and this brings about a cavitation bubble and the formation of a compression wave (Spirnak, 1990b). Furthermore, as the gas bubble cools and collapses, a second hydraulic shock wave is formed which adds to the force of the initial wave (Clayman, 1991). These waves radiate spherically and will fragment urinary calculi when the shock wave comes in contact with the different density of the stone. Under direct vision, the EHL probe is placed in contact with the stone and then discharged until fragmentation occurs. Electrohydraulic lithotripsy was first used to fragment bladder stones but its application has now been extended to renal calculi (Clayman, 1983) and ureteric stones with micro-instrumentation.

Early reports describing this technique in the ureter were discouraging due to a high rate of injury (Raney, 1978). These injuries most likely resulted because the probes used at this time were 6 to 9F in size and constant contact with the ureter was difficult to avoid. More recently, 1.9 to 5F probes have been developed and as a result, intraureteral EHL can now be performed with a significantly lower and acceptable rate of injury (Clayman, 1991). Begun has reviewed the results of ureteral stone fragmentation with EHL (Begun, 1994). The cumulative overall success rate for fragmentation in 388 patients was 89.4 per cent.

Electrohydraulic lithotripsy has been more successful in fragmenting harder calculi that ultrasound. Also, the small, flexible probes can be used in flexible scopes or ureteroscopes. This allows easier access to stones within renal calyces and the ureter. Like ultrasonic lithotripsy, the equipment is relatively inexpensive to begin with ($7,000 to $13,000 U.S.), but each probe has a life-expectancy of only 50 to 60 seconds (Clayman, 1991). This is dependent on the strength and duration of the electric discharge. Although smaller probes are now available and are associated with fewer complications, EHL is still capable of significant tissue injury if the probe is discharged against the ureteral wall.

The Swiss Lithoclast is a new and unique technology that has been applied for intracorporeal lithotripsy. This tool uses a rigid probe to fragment stones under direct vision. The probe is connected to a hand piece within which is contained a small metal projectile or "bullet". The unit is fueled by compressed air which propels the projectile against the metal probe at a frequency of twelve cycles per second, thus activating the probe (Denstedt, 1992). Therefore, the mechanics of this device are similar to a pneumatic jackhammer and it has recently been reported for use within the kidney and ureter. Denstedt and associates used the lithoclast to fragment six renal calculi and 17 ureteral stones. Three staghorn calculi and three calculi greater than three centimeters in

diameter were successfully fragmented. For the ureteral stones, 15 of 17 ureteral calculi were successfully fragmented (Denstedt, 1992). The two failures occurred because of very mobile calculi within a capacious ureter. This did not allow direct contact of the probe on the stone. In another study, 27 renal calculi were treated with the Swiss Lithoclast and ESWL (Schulze, 1993). Forty-one per cent of patients were free of stones at time of discharge and 59 per cent had residual fragments. The stone-free rate at three months was not reported. In the same study, 96 ureteral stones were also treated (Schulze, 1993). Seventy-nine per cent had complete fragmentation and 15 per cent had residual fragments which cleared within one week for a total stone-free rate of 96 per cent. These initial reports show that this instrument is an effective tool for fragmenting all types of urinary calculi in a rapid manner. In addition, there appears to be a large margin of safety with this device. *In vivo* investigations have revealed no significant acute or long-term effects when the lithoclast was activated directly onto bladder and ureteral urothelium of pigs (Denstedt, 1995).

The main advantage of the pneumatic lithoclast is that it is based on simple technology that provides a reliable, effective, safe, and inexpensive means for intracorporeal lithotripsy. In addition, this instrument does not appear to be limited by the stone composition. Both of the above investigators found that even extremely hard calculi were rapidly fragmented. A potential disadvantage with the lithoclast is that the rigidity of the probes limits its use to rigid and semi-rigid endoscopes (Denstedt, 1992).

It is clear that none of these modes of intracorporeal lithotripsy is superior to the others. Each has its own advantages and disadvantages and they should probably used in complement with one another depending on the clinical situation. The ideal instrument for intracorporeal lithotripsy would combine the advantages of EHL, ultrasound, and the pneumatic lithoclast while eliminating the disadvantages. First, this tool should be safe

with a low risk for tissue injury. The device would also need to be small enough in diameter to be used in rigid and flexible, small caliber endoscopes. It should also be effective so that all types of urinary calculi could be ablated with success. Finally, this instrument should be inexpensive to use so that multiple patients could be treated without having to spend large sums of money on maintenance of the tool and supplies for it.

For the reasons stated above, applying laser technology to the treatment of urinary calculi is appealing. The energy can be transmitted through fiberoptic cables making lasers very convenient for use within endoscopes. These fibers can easily be passed up small caliber ureteroscopes and flexible scopes. Therefore, it is now possible to use laser energy to fragment stones in notoriously difficult locations such as the renal calyces. It is also possible to transmit enormous amounts of power through the laser fiber. When this energy is applied to a stone, it potentially enables the user to fragment even the most dense calculi. Finally, unlike some of the above modalities, the laser fibers used for stone fragmentation can be used multiple times making this technique cost-effective once the initial cost of the laser has been absorbed.

1.6 Laser Lithotripsy

1.6.1 Historical Background and Laser Physics

The theoretical basis for the laser was conceived by Dr. Albert Einstein in 1916 (Hecht, 1986). Einstein theorized that if an electron were in an excited state and collided with a photon having the proper energy, the electron would drop to a lower energy state while emitting another photon of the same energy that would move in the same direction. This would result in two identical photons traveling together in the same direction and same phase (Winburn, 1987). This concept was called stimulated emission but practical use of

this theory did not occur until the 1950's. At that time, scientists in the United States and the former Soviet Union independently conceived the idea of using stimulated emission in the microwave region of the electromagnetic spectrum. However, it was Dr. Charles H. Townes and colleagues at Columbia University who actually invented such a device and called it a "maser", an acronym for "microwave amplification by stimulated emission of radiation" (Gordon, 1955).

While work on new masers continued, other physicists began investigating the possible extension of maser techniques to the infrared and optical regions of the electromagnetic spectrum. Dr. Townes began a collaborative effort with Dr. Arthur L. Schawlow, a Bell Laboratories researcher, and together they were able to establish the fundamental principles of such an "optical maser". Their work was published in a landmark paper in 1958 (Schawlow, 1958) and their design was eventually called a *laser*, for light amplification by stimulated emission of radiation. While most researchers, including Schawlow and Townes, believed that the best material to construct a laser would be a gas, Dr. Theodore H. Maiman, a physicist with Hughes Research Laboratories, experimented with synthetic ruby crystals (Hecht, 1986). In mid-1960, using a ruby crystal of one centimeter dimensions, coated on two parallel faces with silver, and then irradiated by a high-power flash lamp, Dr. Maiman successfully created the world's first laser beam (Maiman, 1960).

Over the next five years there were abundant reports describing new laser material. The helium-neon laser, was created in 1961 by Dr. A. Javan (Javan, 1961). It continues to be one of the most popular lasers today. In 1964, Geusic and colleagues developed the Neodymium: yttrium aluminum garnet (Nd:YAG) laser (Guesic, 1964). Because of its excellent thermal and optical qualities, Nd:YAG has become one of the standard solid-state laser materials used today and has many applications in the medical profession

(Hecht, 1986). The argon laser was developed in 1964 by Bridges (Bridges, 1964) and in the same year, Dr. K. Patel used carbon dioxide (CO_2) for a lasing medium (Patel, 1964). The CO_2 laser continues to have widespread use today in medicine and industry. Finally, the organic dye laser, which is used in laser spectroscopy and also for laser lithotripsy in urology, was designed in 1966 by Drs. Sorokin and Lankard (Sorokin, 1966).

Laser light has unique characteristics that distinguish it from non-laser light. It is monochromatic, highly collimated, and coherent. Monochromatic light is virtually of a single wavelength and therefore has the same energy. Conversely, light from a simple incandescent light bulb consists of all colors with wavelengths ranging from the ultraviolet portion of the spectrum to the infrared region. The specificity of the wavelength and energy of monochromatic light means that laser light will have an identical effect on the target whether that be tissue vaporization or drilling metal. Highly directional or collimated light implies that the beam is parallel with a minimal angle of divergence (Dorros, 1991) (Figure 1.1). As a result, the diameter of the laser beam changes only slightly over long distances allowing it to maintain its intensity or brightness. Finally, coherence means that all the waves of the laser beam are in phase with one another (Figure 1.2). Longitudinal, or temporal coherence, and transverse, or spatial coherence are both exhibited in laser light (Dorros, 1991). The former refers to a fixed phase relationship along the axis of the beam, over time, and transverse coherence refers to unity across the beam, in space.

In 1913, Niels Bohr made two important postulates in quantum physics (Dorros, 1991). First, the electrons of an atom may exist only in certain energy levels or orbits surrounding the nucleus. The lowest energy state of an atom is called its atomic ground state. Secondly, if c ergy or some form of electromagnetic radiation is exerted onto an atom, it

can absorb this energy and elevate its electrons to higher "excited" orbits or energy levels. This is called *stimulated absorption* of radiation (Dorros, 1991) (Figure 1.3). This excited state is extremely unstable so that the electrons will quickly return to a lower energy level and ultimately to the ground state. In the transition from a higher energy state (E_b) to a lower one (E_a), a photon of light is emitted from the electron and its energy is equal to that given up by the electron. This event is called *spontaneous emission* of radiation (Figure 1.3).

The photon emitted during this transition is a form of electromagnetic radiation and is capable of propagating in any direction (Polanyi, 1985). It has a frequency and a wavelength that is related to the energy difference of the two energy states. This relationship is described in the famous Bohr equation:

$$E_{photon} = E_b - E_a = hf = hc/\lambda$$

(where h= Plancks constant, 6.6×10^{-34} J-sec
c= velocity of light, 3.0×10^{8} m/sec)

Therefore, these postulates explained two types of radiative transitions within atoms; stimulated absorption of radiation and spontaneous emission of radiation.

Albert Einstein was the first who realized that there must be a third type of radiative transition within atoms. He called this transition *stimulated emission*. While all three of these energy transitions occur in a laser, stimulated emission is responsible for the actual laser beam.

Stimulated emission can occur when an atom is in a excited state (E_b) and is waiting to spontaneously fall to a lower energy level (E_a). During this time, if it is exposed to a photon whose energy is exactly the same as the energy the atom is waiting to emit (E_b-E_a), the atom can be stimulated to prematurely emit a photon (Dorros, 1991). If this

occurs, the emitted photon and the passing photon will exit the atomic system and be absolutely identical in that they have the same wavelength, frequency, energy, phase and direction (Figure 1.4). It is this process which allows a laser to emit its monochromatic, highly collimated and coherent light.

For stimulated emission to occur, there must be more atoms in a given excited state than in the next lower state (Dorros, 1991). This phenomenon is called a population inversion and its presence is mandatory for laser light to be generated. Otherwise, any photons emitted will just be absorbed by a lower energy state and stimulated emission doesn't occur. Stimulated absorption with subsequent spontaneous emission will instead predominate.

A population inversion is an abnormal condition and must be created by pumping the active medium of the laser. The active medium is the material that actually emits the laser light when stimulated (Doros, 1991). It may be in the form of a gas, solid, or liquid and the wavelength of light emitted is unique to that element. Energy is therefore provided to the active medium so that its atoms become excited. Eventually, the majority of atoms occupy a higher energy state. When one of the "excited" atoms spontaneously drops to a lower energy level and emits a photon, this photon is then able to stimulate another "excited" atom to emit. These two photons stimulate two other atoms to give four photons which then become eight and a cascade effect is begun. Pumping the active medium can be achieved through an optical source by using a flashlamp, with electrical discharge, chemical excitation, electron beam energy, ionizing radiation, alternating magnetic fields, or by using one laser to pump another.

Once a population inversion is achieved and stimulated emission occurs, it is only those photons emitted along the axis of the active medium which take part in the laser beam.

The rest of the photons are lost to the environment as heat In a single pass along the axis of the laser, not all the atoms will be stimulated to emit photons. To further amplify the effect of stimulated emission, the photons are reflected back and forth along the axis of the active medium so they can stimulate more emission. This type of "optical feedback" is achieved by placing mirrors at the ends of cavity containing the active medium (Dorros, 1991). One of the mirrors is 100 per cent reflective and the other is partially reflective. This allows a certain percentage of light to escape as the laser beam. Typically, a photon makes about 50 round trips before leaving the laser cavity. This allows it to stimulate many atoms to emit photons, thus amplifying the laser light (Dorros, 1991). Therefore, a laser consists of an active medium which is housed within a resonance cavity with reflective mirrors placed at the ends. The active medium is pumped with energy which excites the atoms to a point of creating a population inversion. Stimulated emission of photons occurs along the axis of the active medium and cavity. Through the feedback mechanism of the reflective mirrors, this light is amplified, and the emitted laser light is thus monochromatic, highly directional, and coherent.

Lasers that operate with a constant beam are known as "continuous wave" (CW) lasers. A continuous beam of laser light is emitted while the energy source is continually supplying a population of excited state electrons for stimulated emission and amplification. Conversely, "pulsed" lasers emit light energy in a very short duration of time in a single pulse or a train of pulses. A pulse is achieved by temporarily pumping the active medium to an excited state and then allowing a quick discharge of energy (Winburn, 1987). This results in a single burst of coherent light to be emitted with a pulse duration that is usually less than one millisecond (Fuller, 1985).

"Q-switching" is one technique used to create a pulsed laser. This is usually accomplished by placing a fast shutter between the active medium and one of the mirrors (Fuller, 1985).

With the shutter closed, a buildup of energy occurs which far exceeds the usual lasing threshold levels. This "excess" energy is then discharged in an extremely short period when the shutter is open. Q-switching is able to produce pulse durations in the nanosecond to femtosecond range depending on the specific technique used. Because power is indirectly related to time (power = energy/time), one can see that the shorter the pulse duration, the higher the power associated with the pulse. As a result, pulsed lasers, and in particular Q-switched lasers, are capable of producing enormous peak powers while emitting less average energy overall.

1.6.2 Laser-Tissue Interactions

Lasers have many applications in medicine and surgery. The interactions that occur between the laser and tissue are responsible for the therapeutic effect of the laser, but they are also a potential cause for complication. These interactions are very complex and are influenced not only by the laser parameters of power, spot size, pulse duration and wavelength, but also by tissue properties (Nelson, 1991).

The most important operating parameter of the laser is the power density. This is the power, measured in watts, divided by the area of the laser spot size in centimeters squared (W/cm^2). When the time factor is considered ($W \cdot s/cm^2$), then the energy density, or fluence, is determined. The effects of power and time are directly proportional, while those of spot size have an inverse square relationship. Therefore, if either power or time is doubled, then the power density and energy density will increase two-fold. However, if the diameter of the laser beam is doubled, these parameters will decrease by a factor of four. If it is halved, they will increase fourfold. The interactions that result will therefore vary significantly depending on the diameter of the laser fiber and on where the operator holds the fiber in relation to the tissue.

Once laser light enters tissue, it can be absorbed, scattered, or transmitted (Nelson, 1991). Absorption is the most important laser-tissue interaction and without it there is no tissue effect. Scattering occurs when laser light is deflected from particles and structures within the tissue. Because scatter changes the direction of the incident laser beam, this "lost" energy can lead to surrounding tissue damage (Nelson, 1991). Finally, light is transmitted through tissue when neither absorption or scattering occurs.

Absorption is the process whereby energy from the laser light is transferred to the molecules making up the tissue. The energy-absorbing molecules in tissue are called chromophores. Each one is unique based on its molecular structure and is only able to absorb certain light energies. Therefore, different molecules will selectively absorb different wavelengths of light, and the amount of energy absorbed will vary according the wavelength of the light. For example, hemoglobin absorbs violet, blue/green, and yellow light very well. Absorption declines in the red region of the spectrum such that red light is scattered and transmitted thereby giving hemoglobin its characteristic red color (Nelson, 1991). Conversely, water appears clear because it has no absorption in the visible portion of the spectrum but only absorbs light in the infrared regions. These unique tissue properties provide the rationale for using different lasers with different wavelengths to bring about varying therapeutic effects.

Once the laser energy is absorbed, the tissue must dissipate it in some way. The dispersion of this energy is what brings about the different biological effects that are seen clinically (Nelson, 1991). As the excited molecule relaxes, it most commonly releases thermal energy, but photochemical reactions, and fluorescence are other possibilities.

Heat deposited by a laser can cause thermal injury to tissue through the processes of hyperthermia or coagulation (Jacques, 1992). These processes are rate dependent and

their effects on tissue accumulate as a function of time. Hyperthermic injury involves a long exposure to a mildly elevated temperature which may be as low as 40-45 ° C. The effects of this exposure are not immediately apparent but usually require at least 24 hours for necrosis to develop. On the cellular level, this type of thermal injury causes denaturation and irreversible aggregation of macromolecules which ultimately leads to cell death (Jacques, 1992). Conversely, coagulation, or photocoagulation, occurs with a short exposure to higher temperatures of 60-100 ° C. The effects are usually immediate and visible with the tissue turning a white or gray color (Stein, 1994). Further heating to over 100° C will result in vaporization of tissue water with subsequent carbonization and liberation of smoke and gas. As a general rule, the lower the temperature for thermal injury, the more important is exposure time as a factor in the accumulation of injury. The higher the temperature, the less important is exposure time, and the more important is the peak temperature (Jacques, 1992).

With continuous wave laser action, heat radiates in all directions from its initial point of absorption and leads to successive zones of carbonization, vacuolization, and edema (Ben-Bassat, 1976). Conversely, pulsed laser energy interacts with tissue in a way that is dependent on the duration of the laser pulse. The shorter the duration of the laser pulse, the more the laser energy will be confined to a progressively smaller target area (Anderson, 1983). This phenomenon is also related to the thermal relaxation time of the target chromophore. The thermal relaxation time is the time required for heat generated from absorbed light by the chromophore, to cool to one half of the original value immediately after the laser pulse (Nelson, 1991). If the laser pulse is equal to or less than the thermal relaxation time of the target molecule, then the spatial confinement of heat will be maximized and little if any thermal energy will dissipate to surrounding tissue. This concept has been applied clinically in the treatment of the port-wine stain, a congenital skin lesion. The flashlamp-pulsed dye laser has been used to treat this entity because its

wavelength of 585 nm is preferentially absorbed by hemoglobin. This causes thrombosis and thermal damage of the abnormal blood vessels in the dermis. In addition, the 450 μ sec pulse duration closely matches the thermal relaxation time of dermal blood vessels and this confines the thermal damage to the targeted area with little dissipation of heat to the overlying epidermis (Nelson, 1991).

A photochemical laser-tissue interaction can occur when laser energy excitation leads to the rearrangement of molecular bonds to bring about the formation of a new molecular structure. This type of interaction occurs with the use of photosensitizers. These can be endogenous or exogenous chromophores that absorb certain wavelengths of light and then transfer the energy to surrounding molecules to cause a molecular reaction. The best example of this interaction is the use of hematoporphyrin derivative (HpD) for the treatment of various malignant conditions (Nelson, 1991). This substance is a photosensitizer which by some unknown mechanism is preferentially retained within malignant cells. When these cells are exposed to red light at a wavelength of 630 nm in the presence of oxygen, the excited HpD creates a highly cytotoxic singlet oxygen molecule which leads to irreversible cell damage and death. A wide variety of tumors have been treated including cancers of the skin, female genital tract, esophagus, lung, bladder, eye, breast, and head and neck (Nelson, 1991).

Fluorescence occurs when light energy is absorbed by tissue and is then dissipated as the re-emission of longer wavelength light. This process is dependent on the electric bond structure and the chemical composition of the irradiated tissue (Bhatta, 1994). This interaction can be used to monitor compounds that occur naturally or have been added exogenously. For example, HpD has fluorescent properties which can be used to detect and localize malignant tissue that is undetectable by standard methods of diagnosis (Benson, 1982). The natural compound 5-aminolevulinic acid (ALA) is a precursor in the

heme biosynthetic pathway and can also be used for this purpose. When administered exogenously in excess amounts, 5-ALA induces intracellular accumulation of endogenous protoporphyrin IX. Like HpD, protoporphyrin IX has fluorescent properties and is preferentially retained within malignant tissue. This 5-ALA-induced protoporphyrin IX fluorescence has recently been used to diagnose flat urothelial dysplasia or carcinoma in situ (Kriegmair, 1994).

Plasma formation is another laser-tissue interaction which can occur but differs from the others in that the physical processes involved are associated with short laser pulses. As noted previously, Q-switched lasers are capable of producing huge peak powers over a short pulse duration. When these lasers are focused on a small spot of tissue, a "plasma" is generated. This is a gaseous cloud rich in free electrons which produces an intense photoacoustic shock wave that carries significant kinetic energy (Nelson, 1991). Plasma formation is the mechanism by which some lasers are able to fragment urinary calculi (Nishioka, 1987).

1.6.3 Historical Background to Laser Lithotripsy

The first attempt at laser lithotripsy was reported in 1968 by Drs. Mulvaney and Beck. Using an *in vitro* study, they showed that various urinary calculi could be fragmented with the ruby and CO_2 lasers (Mulvaney, 1968). Little work was reported over the next decade until 1978 when Fair described fragmentation of urinary calculi using stress waves generated by an optico-acoustic transducer (Fair, 1978). A thin film of aluminum confined between glass and brass was irradiated with a high-intensity pulsed laser and this produced shock waves of sufficient energy to easily fragment calculi. It was also noted that by shortening the laser pulse, higher peak pressures were generated. Fair believed this would lead to improved fragmentation.

In 1981, Tanahashi (Tanahashi, 1981) and Pensel (Pensel, 1981) independently reported using the continuous wave Nd:YAG laser to fragment bladder calculi in dogs. However, because continuous wave lasers fragment calculi through thermal processes whereby the stone itself is vaporized or melted, excess heating of surrounding tissues occurred. For this reason, attention was turned to using pulsed laser energy for fragmenting stones.

In 1983, Watson and colleagues investigated using the Q-switched Nd:YAG laser for fragmenting urinary stones (Watson, 1983). They found that when a series of 15 ns, 1 J pulses repeated at 10 Hz were focused on a calculus, all types of stones could be fragmented regardless of color or composition. However, this laser appeared to be limited because the high powered Q-switched pulses could not be transmitted through flexible glass fibers without consistently damaging the fibers. In the same study, a non-Q-switched Nd:YAG laser with a pulse duration approximately 10,000 times longer was examined. Using 800 mJ pulses of 100 μsec duration at 40 Hz, Watson demonstrated that dense oxalate stones fragmented in 30 seconds. However, fragmentation was associated with charring of the stone surface suggesting that some heating of the stone did occur. When the laser was focused directly on cadaveric renal pelvis, it caused a visible burn after 30 seconds and perforation after 50 seconds. Therefore, this laser appeared to be intermediary in its effect on stones between the Q-switched pulsed laser and the continuous wave laser. Watson concluded that lasers could be used for calculus fragmentation without injuring the urinary tract provided that they were not aimed directly at the tissue for longer than a few seconds and that they were used in sufficiently short enough pulses that would not allow excessive heat build up.

Therefore, by 1983, some basic principles and problems of using the laser for stone fragmentation were known (Dretler, 1988a). Continuous wave lasers were inappropriate for laser lithotripsy because they created too much heat and caused thermal tissue damage;

pulsed lasers appeared to act on stones by creating a shock or a stress wave that overcame the tensile strength of the stone; the shorter the laser pulse duration, the higher the pressure of the stress waves; and effective use of lasers for calculi depended on the ability to transmit the energy through optical fibers.

In order to find a laser with more ideal characteristics, Watson and colleagues began studying the pulsed dye lasers. They believed that these lasers had potential because their wavelengths could be adjusted. An ideal wavelength would be one where there was maximal absorption by the pigments in the stone with a lesser degree of absorption by tissue pigments. Theoretically, this would minimize the risk of injury to tissue while maintaining the stone-ablating qualities of the laser. Consequently, the stone-fragmenting properties of the pulsed dye laser were studied by varying the wavelength, pulse duration, and fiber size and the initial results were published in 1987 (Watson, 1987b). Optimal stone ablation occurred when coumarin green dye was used in the laser to produce a wavelength of 504 nm, with a pulse duration of 1 μsec, using a 200 micron diameter silica coated quartz fiber.

Once the efficacy of this laser was established, the same researchers investigated the safety of the pulsed dye laser in an *in vivo* animal study (Watson, 1987a). They found that tissue injury was minimal with only mild inflammatory changes occurring at the site of fragmentation. When the fiber tip was abutted against the ureteral wall and discharged at energies of 25 to 30 mJ per pulse, injury was again minimal and noted to consist of inflammation and purpura limited to the lamina propria and superficial muscle fibers depending on the number of pulses used.

A similar *in vivo* study was performed by Nishioka et al. (Nishioka, 1988). This group used the same pulsed dye laser but fragmented biliary calculi within the common bile duct

of pigs. They found minimal tissue injury which consisted microscopically of mild to moderate inflammatory infiltrate composed of lymphocytes, eosinophils, and polymorphonuclear leukocytes.

Study of the Q-switched Nd:YAG laser resurfaced in 1988 when Hofmann and colleagues were able to successfully transmit the powerful pulses through flexible fibers. A specially formed fiber end was designed that focused the laser pulses at the fiber tip. They not only confirmed that this laser was capable of fragmenting urinary calculi, but also demonstrated experimentally, that no significant macroscopic or microscopic tissue damage occurred when the laser was discharged directly onto pig urothelium (Hofmann, 1988).

More recently, two other laser systems have been described for laser lithotripsy; the solid-state alexandrite laser and the holmium laser (Watson, 1994). One study, reporting preliminary *in vitro* and *in vivo* results of lithotripsy with the alexandrite laser, seemed to demonstrate efficient stone ablating properties with no evidence of tissue injury when the laser was fired directly onto the ureter and bladder of rabbits (Mattioli, 1991). Another *ex vivo* study in swine found that the 250 μm quartz fiber tended to fragment and become embedded in the ureteral wall when a short pulse duration of 350 ns was used (Strunge, 1991). With a longer 1 microsecond pulse, fiber fragmentation did not occur, but histological damage was seen to the level of the muscularis mucosa. The holmium:YAG (Ho:YAG) laser has similar wavelength characteristics as the pulsed 1440 nm Nd:YAG laser. It was recently studied in an *in vivo* animal model and found to easily cause ureteral perforation and necrosis compared to other lithotriptors when placed blindly within the ureter and discharged (Piergiovanni, 1994). Like the multi-YAG laser, this laser is an excellent "tissue cutting" laser because of its absorptive properties. Therefore, these results are not unexpected. Clinically though, fragmentation would be performed under direct visualization with the fiber in contact with the stone, and irrigating solution would

buffer the ureteral wall so that these results may not be directly transferable to human trials.

1.6.4 Clinical Results of Laser Lithotripsy

Clinical experience using the pulsed dye laser for the fragmentation of ureteral calculi was first reported in 1986 and 1987 (Dretler, 1986; Watson, 1986; Dretler, 1987a). In these initial studies, investigators used the coumarin dye laser with a 200-250 μm fiber and energies ranging from 25-40 mJ per pulse. The fiber was placed in contact with the stone through a 9 to 11.5F ureteroscope. Dretler and associates reported successfully fragmenting 16 of 17 ureteral calculi for an overall success rate of 94 per cent (Dretler, 1987a). Eight calculi were ablated with laser alone, while seven required stone basket extraction of the fragments and one required subsequent ESWL when fragments were flushed into the renal pelvis. During the procedure, the laser was inadvertently discharged into the ureteral wall in six patients with no sequelae. However, two late strictures did occur in the intramural portion of the ureter at the site of ureteral balloon dilatation. Watson and Wickham described an 89 per cent successful fragmentation rate of 37 ureteral calculi. No complications were reported (Watson, 1986).

Since these initial publications, there have been numerous other series reporting clinical outcomes with the pulsed dye laser for laser lithotripsy (Table 1.2). The coumarin dye laser with a wavelength of 504 nm has been used almost exclusively in these reports. In one study, a very similar laser using rhodamine dye was utilized which emits at a wavelength of 594 nm (Schmidt, 1993). Fiber sizes used were 200, 250, or 320 microns and energy settings ranged form 30 to 140 mJ, depending on the size of the fiber used. Including only those reports which specifically investigated the clinical efficacy of the pulsed dye laser for lithotripsy, a cumulative total of 2481 stones have been treated since 1986 and reported in the literature.

The majority of the patients treated in these reports represent a select sample population in that they had complicated stones that were impacted. Most had failed conservative management or other standard treatment options such as ESWL. The lack of standardization in terms of energy settings, laser fibers, the use of ancillary procedures, and a definition of successful therapy, makes comparison of individual studies somewhat difficult. However, some important observations can be made from the cumulative data.

These data are summarized in Table 1.2. Results are classified according to whether patients required laser only, laser plus manual extraction of fragments, laser plus some ancillary procedure (ESWL, EHL, repeat ureteroscopy, etc.), or whether they had a treatment failure. Complete fragmentation without any other form of therapy occurred in 653/2481 (26.3%) of stones treated. Successful fragmentation with manual extraction of residual fragments took place in 1371 of the stones (55.2%). Laser fragmentation followed by an ancillary procedure was required for 316 calculi (12.7%) and 141 stones (5.7%) were considered failures. If we consider "laser alone" and "laser plus manual extraction" as successful therapy and "laser plus other" as partially successful, then the overall success rate of the pulsed dye laser is 81.6 per cent and the partial success rate is 12.7 per cent. These are very good results for a group of difficult patients that, for the most part, had already failed one form of therapy.

Stone composition and stone mobility within the urinary tract were the most common causes for failure with the pulsed dye laser. Stones composed of COM, cystine, and CPD were the most difficult to fragment and most often associated with failed fragmentation. Excessive stone mobility with subsequent treatment failure occurred more frequently in those series with an unselected patient sample. These patients were more likely to have stones that were not impacted and were therefore more prone to being propelled up the

ureter with fragmentation. Investigators often commented that improved fragmentation could be achieved by holding these calculi within a stone basket to prevent migration. Technical difficulty with the pulsed dye laser was an infrequent cause of failure and was only reported in one early series when a prototype machine was being used (Dretler, 1988c).

Four studies stratified their results with respect to stone location in the urinary tract (Dretler, 1990; Govier, 1990; Dretler, 1991; MacDermott, 1993). Successful fragmentation occurred in 58/103 (56.4 %) of upper ureteral calculi, 76/110 (69.1%) of mid ureteral stones, and in 223/254 (88.2%) of stones in the lower ureter. Most authors blamed difficulties with ureteroscopy and stone visualization on the decreased rate of success for calculi in the upper collecting system.

In 1990, the larger 320 μm size fiber was introduced for clinical use. It was believed that better fragmentation could be achieved because higher energy could be transmitted down the larger diameter fiber. Five of the above studies specifically reported their results according to the fiber size used (Vandeursen, 1991; Dretler, 1991; MacDermott, 1993; Baba, 1993; Boline, 1993). Using the same criteria, successful and partially successful fragmentation using the 320 μm fiber was accomplished in 384/470 (81.7%) and 52/470 (11.1%) of calculi respectively. Treatment failure occurred in 34/470 (7.2%). There was no difference compared to the 200 μm fiber where successful fragmentation was achieved in 1819/2220 (81.9%) of stones, and partially successful fragmentation occurred in 255/2220 (11.5%). Treatment failure occurred in 144/2220 (6.5%) of calculi.

These cumulative data seem to indicate no difference in the overall success with the larger laser fiber. However, investigators repeatedly stated that the increased energy transmitted through the larger fiber improved the efficiency of the pulsed dye laser for stone

fragmentation without compromising the margin of safety (Vandeursen, 1991; Dretler, 1991). In an effort to show this objectively, the number of pulses for fragmentation was compared for the two sizes of fiber. Dretler and Bhatta (Dretler, 1991) found that for COM calculi 51-100 mm^2 in size, 2371 pulses were required for fragmentation with a 200 μm fiber at 60 mJ compared to 522 pulses when a 320 μm fiber at 140 mJ was used. Similarly, Vandeursen and associates demonstrated that the mean number of pulses to fragment COM stones greater than 4 mm with the 200 μm/60 mJ fiber was 3500 compared to 1053 for the 320 μm/140 mJ fiber (Vandeursen, 1991).

The cumulative complication rate for pulsed-dye laser lithotripsy was 7.4 per cent (157/2133 patients). The most common problem encountered was ureteral perforation which occurred in 74/2133 (3.5%) patients. All were treated conservatively with ureteral stenting and only one person developed urinoma formation which required percutaneous drainage (Boline, 1993). The other most frequently encountered complications in order of decreasing frequency were sepsis/infection (1.7%), abrasions of ureter with no perforation (1.4%), ureteral stricture formation (0.6%), and miscellaneous others (0.4%). Concerning the problem of stricture formation, fourteen strictures occurred in total and 13/14 of these occurred in the intramural portion of the ureter, well below the site of laser fragmentation. None were felt to occur as a direct result of the laser energy on the ureter and since the majority were located at the ureterovesical junction, it was believed that these strictures resulted because of ureteral dilatation and ureteroscopy. Interestingly, since the introduction of smaller caliber ureteroscopes (7.0 to 8.0F) where ureteral dilatation is often not required, only one stricture has been reported (Psihramis, 1992). It occurred in a patient following balloon dilatation of the ureterovesical junction.

The reported clinical experience with the Q-switched Nd:YAG laser is not as extensive. Hofmann and Hartung have described the outcome in 189 patients treated with this laser

(Hofmann, 1990). Using an eight nanosecond pulse duration with single pulse energies of 20-80 mJ, they were able to achieve complete fragmentation of calculi the laser alone in 161/189 (85%) patients. Laser plus manual extraction of fragments was required in 18/189 (10%), and 10/189 (5%) were failures due to dense calculi. Complications occurred in 14/189 (7.4%) patients. These consisted of ureteral perforation in two patients and mild to moderate hematuria in the remaining twelve patients. A total of ten additional patients were felt to have had unintentional direct irradiation of the ureter with the laser without any adverse effect.

Benizri and associates compared the Q-switched alexandrite and pulsed dye laser for lithotripsy of ureteral calculi and found no difference in the clinical outcome (Benizri, 1993). Each successfully fragmented 45/47 (95.7%) and 97/102 (95%) stones treated respectively. The overall complication rate was 3.25 per cent with three ureteral perforations and one mucosal abrasion. No complication was believed secondary to the lasers themselves.

The Ho:YAG laser has also been reported for clinical use (Watson, 1993; Denstedt, 1994; Matsuoka, 1995). Watson and Smith treated 33 patients with ureteric calculi. Twenty seven of these patients were rendered stone free in one session (82%) while the remainder required a second ureteroscopy or ESWL. Two patients sustained trauma at the fragmentation site which was felt secondary to the laser energy. Only one required stenting. Denstedt and associates have performed Ho:YAG laser lithotripsy on 22 patients with ureteral or renal calculi. Forty-five per cent were fragmented using the laser alone and an additional 55 per cent were stone-free when the laser was used in combination with another modality. One patient suffered a ureteral perforation and another developed a stricture which was not felt to be related to the laser therapy. Finally, Matsuoka and colleagues used the holmium laser to treat five renal calculi, one bladder calculus, and 32

ureteral calculi. The stone-free rate was 87 per cent at six months. Ureteral damage secondary to the laser occurred in two patients. Both had minor perforations of the ureter, and both responded well to ureteral stenting. Neither patient developed stenosis or stricture formation.

1.6.5 Summary

Laser photofragmentation of urinary calculi is therefore an established method for intracorporeal lithotripsy in humans. The pulsed dye laser is by far the most common laser used for this purpose in North America but experience is increasing worldwide with the Q-switched Nd:YAG laser, the alexandrite laser and the Ho:YAG laser. The clinical results reported above reveal that lasers are efficient at fragmentation and appear comparable to other forms of intracorporeal lithotripsy. As new lasers are developed and become available for experimental and clinical use, they will need to be critically compared to the safety and efficacy data that has already accumulated.

As alluded to earlier, the multi-YAG laser is a new, multiple purpose, dual wavelength Nd:YAG laser. It was designed and developed at the University of Alberta (Tulip, 1992; Wong, 1990) and has now been produced commercially as the Zeiss OPMILAS 144 Surgical Laser System (Carl Zeiss, Inc., Princeton, NJ). This Nd:YAG laser is distinctive in that it emits light at 1440 nm in addition to the standard 1060 nm wavelength. Both wavelengths can be transmitted down fiber optic cables as small as 200 μm. The 1060 nm laser light is minimally absorbed by water and tissue pigment (Figure 1.5). As a result, it is able to penetrate tissue deeply and is therefore capable of wide thermal coagulation. Conversely, the 1440 nm wavelength has a large absorption coefficient in water. Because tissue is greatly composed of water, the majority of the laser energy is absorbed within one millimeter and this results in superficial ablation or cutting. Therefore, the dual wavelength capabilities allow the user to select the wavelength appropriate for the desired

tissue effect, making it an attractive laser for medical applications. This laser has been studied for applications in neurosurgery (Martiniuk, 1989) and orthopedics (Nishioka, 1992; Maes, 1994) and is currently being investigated in urology for the treatment of benign prostatic hypertrophy and transitional cell carcinoma of the urinary tract.

We believe that this powerful laser would also be a valuable tool for fragmenting urinary calculi. In 1990, Moore and colleagues used synthetic urinary calculi and showed that the multi-YAG laser is capable of rapid and effective stone ablation (Moore, 1990). Based on the successful results of this study, we have studied these stone-fragmenting properties of the multi-YAG laser further in a three-part experimental project. The overall aims of this study were to investigate the optimal parameters for multi-YAG laser lithotripsy and also to assess whether this can be performed safely within the urinary tract.

Part one of the project consisted of an *in vitro* study designed to study the efficacy and rate of stone fragmentation with the multi-YAG laser. The objectives of these investigations were to determine the optimal parameters for stone ablation. Based on these results, an *ex vivo* animal study was undertaken to investigate the acute tissue interactions associated with multi-YAG laser lithotripsy in the ureter. A large sample was studied using a wide range of clinically relevant laser parameters to determine the optimal settings for intracorporeal lithotripsy with respect to safety. Once it was established that intracorporeal stone ablation could be performed safely, an *in vivo* animal study was initiated to investigate the acute and chronic tissue effects of multi-YAG laser lithotripsy in a live animal. Our working hypothesis was that the multi-YAG laser would be able to fragment human calculi in an efficacious manner and that this could also be done intracorporeally without causing significant acute or chronic complications. If supported by the following preclinical studies, then a phase I clinical trial would be warranted and also justified.

Table 1.1: Diagnostic categories for urinary lithiasis

	Serum			Urine							
Diagnostic Groups	Ca	P	PTH	Ca Fasting	Ca Load	Ca Restricted	UA	Ox	Cit	pH	Mg
AH-1	N	N	N/↓	N	↑	↑	N	N	N	N	N
AH-2	N	N	N/↓	N	↑	N/↑	N	N	N	N	N
AH-3	N	↓	N/↓	N	↑	N/↑	N	N	N	N	N
Renal Hypercalciuria	N	N	↑	↑	↑	↑	N	N	N	N	N
Primary Hyperparathyroidism	↑	↓	↑	↑	↑	↑	N	N	N	N	N
Unclassified Hypercalciuria	N	N/↓	N	↑	↑	↑	N	N	N	N	N
Hyperuricosuria	N	N	N	N	N	N	↑	N	N	N	N
Gouty Diathesis	N	N	N	N	N	N	N/↑	N	N/↓	↓	N
Enteric Hyperoxaluria	N/↓	N/↓	N/↓	↓	↓	↓	↓	↑	↓	N/↓	N/↓
Hypocitraturia	N	N	N	N	N	N	N	N	↓	N	N
Renal Tubular Acidosis	N	N	N/↑	N/↑	N	N/↑	N	N	↓	↑	N
Hypomagnesuria	N	N	N	N	N	N	N/↓	N	↓	N	↓
Infection Lithiasis	N	N	N	N	N	N	N	N	N/↓	↑	N

Abbreviations: Ca, calcium; P, phosphorous: PTH, parathyroid hormone; UA, uric acid; Ox, oxalate; Cit, citrate; Mg, magnesium; AH, absorptive hypercalciuria.

(From Preminger, 1994)

Table 1.2: Clinical results of pulsed dye laser lithotripsy.

Investigator	No. Stones Treated	Laser Settngs (energy/fiber size)	Laser Alone (%)	Laser+ ME (%)	Laser/Other (%)	Failure (%)
Watson and Wickham, 1986	37	NS	33/37 (89)	-	-	4/37 (11)
Coptcoat et al., 1988	120	30-60 mJ/200 μm	91/107 (85)	-	-	16/107 (15)
Dretler, 1988	157	60 mJ/ 250 μm	-	106/157 (68)	38/157 (24)	13/157 (8)
Schoberg, 1989	22	30-50 mJ/250 μm	-	20/22	-	2/22
Watson and Wickham, 1989	250	NS	-	240/250 (96)	5/250 (2)	5/250 (2)
Dretler, 1990	222	60 mJ/250 μm	141/222 (64)	30/222 (13)	33/222 (15)	18/222 (8)
Gautier et al., 1990	325	60 mJ/250 μm	238/325 (73)	-	80/325 (25)	7/325 (2)
Govier et al., 1990	50	NS	-	46/50 (92)	3/50 (6)	1/50 (2)
Grasso and Bagley, 1990	33	60-90 mJ/200-320 μm	-	31/33 (94)	-	2/33 (6)
Higashihara et al.,1990	30	40-50 mJ/ NS	24/30 (80)	-	2/30 (7)	4/30 (13)
Zerbib et al., 1990	46	40-80 mJ/250 μm	22/46 (48)	14/46 (30)	-	10/46 (22)
Dretler and Bhatta, 1991	72	140 mJ/ 320 μm	51/72 (71)	8/72 (11)	11/72 (15)	2/72 (2)
Fugelso and Neal, 1991	204	30-60 mJ/NS	-	139/204 (68)	48/204 (24)	17/204 (8)
Grasso et al., 1991	80	80-120 mJ/200-400 μm	-	80/80 (100)	-	-
Loughlin and Sharpe, 1991	26	30-50 mJ/250 μm	18/26 (70)	4/26 (15)	-	4/26 (15)
Vandeursen et al., 1991	104	80-140 mJ/ 200-320 μm	85/104 (82)	-	-	19/104 (18)
Bolton et al., 1992	23	60 mJ/ 250 μm	19/23 (83)	-	4/23 (17)	-
Evans et al., 1992	28	30-60 mJ/ 250 μm	16/28 (57)	12/28 (43)	-	-
Psihramis, 1992	122	NS/250 μm	-	107/122 (88)	10/122 (8)	5/122 (4)
Baba et al., 1993	35	60-120 mJ/320 μm	-	32/35 (91)	-	3/35 (9)
Boline and Belis, 1993	115	40-120 mJ/320 μm	-	92/115 (80)	23/115 (20)	-
MacDermott and Clark, 1993	175	140 mJ/320 μm	-	134/175 (77)	18/175 (10)	23/175 (13)
Schmidt and Eisenberger, 1993	54	30-120 mJ/200-300 μm	32/54 (59)	6/54 (11)	16/54 (30)	-
Grasso and Bagley, 1994	176	60-200 mJ/200-500 μm	-	172/176 (97)	-	4/176 (3)

Abbreviations: ME, manual extraction; NS, not specified

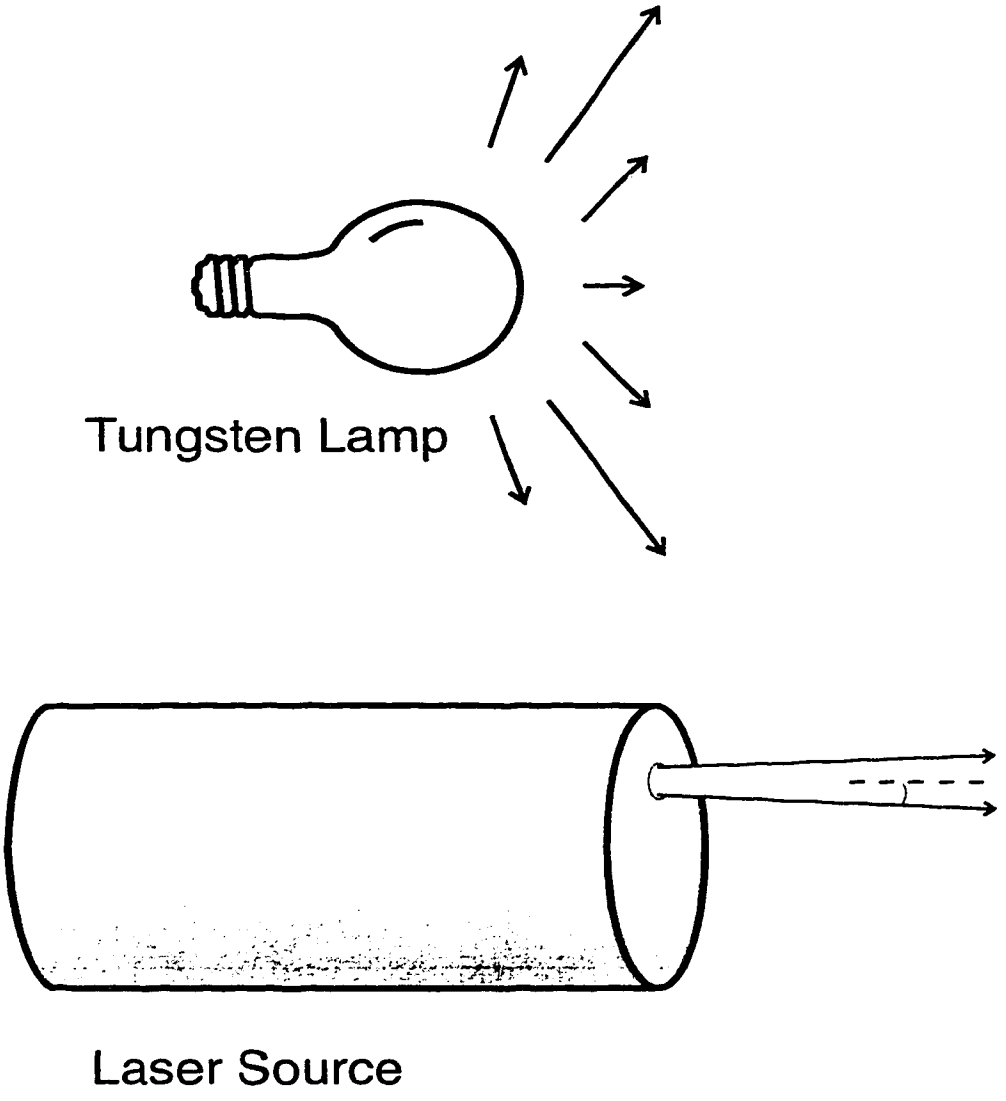


Figure 1.1: Laser light is highly directional or collimated. The laser beam has a very small angle of divergence so that the diameter of the beam changes only slightly over long distances.

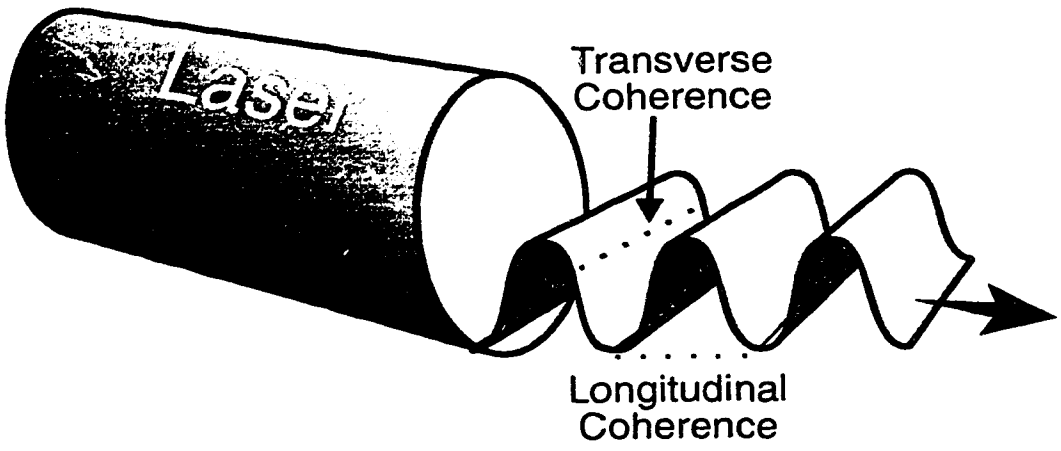


Figure 1.2: Laser light has longitudinal and transverse coherence. This implies that there is a fixed phase relationship of the photons along the axis of the beam, over time, and also across the beam in space.

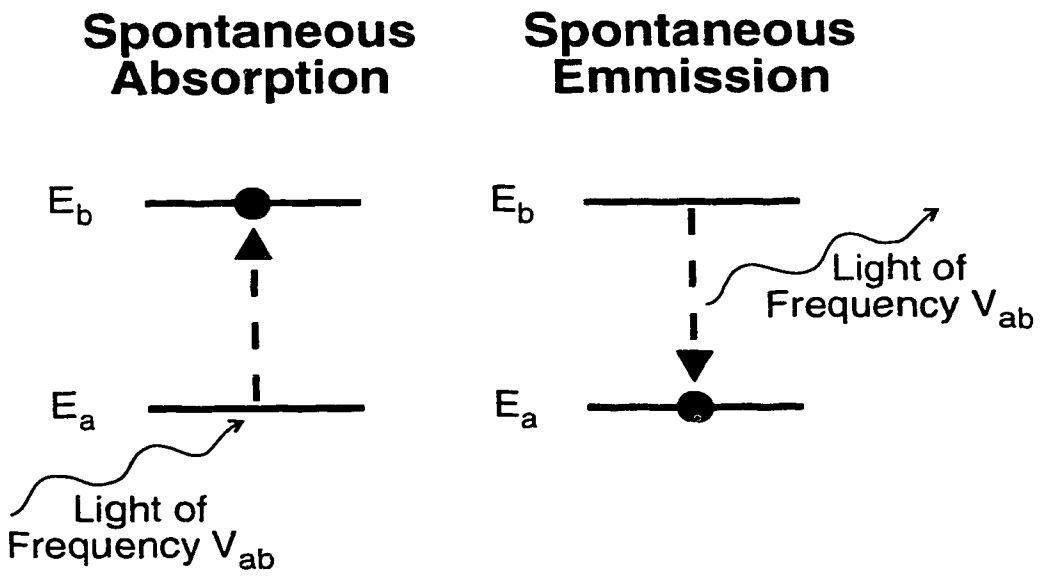


Figure 1.3: Energy diagram illustrating spontaneous absorption and emission of radiation.

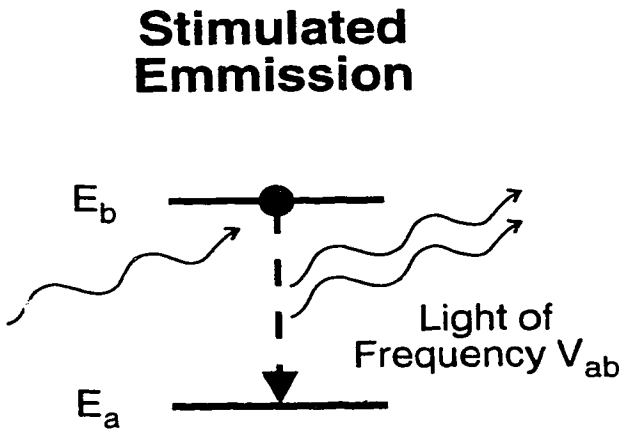


Figure 1.4: Energy diagram illustrating stimulated emission of radiation.

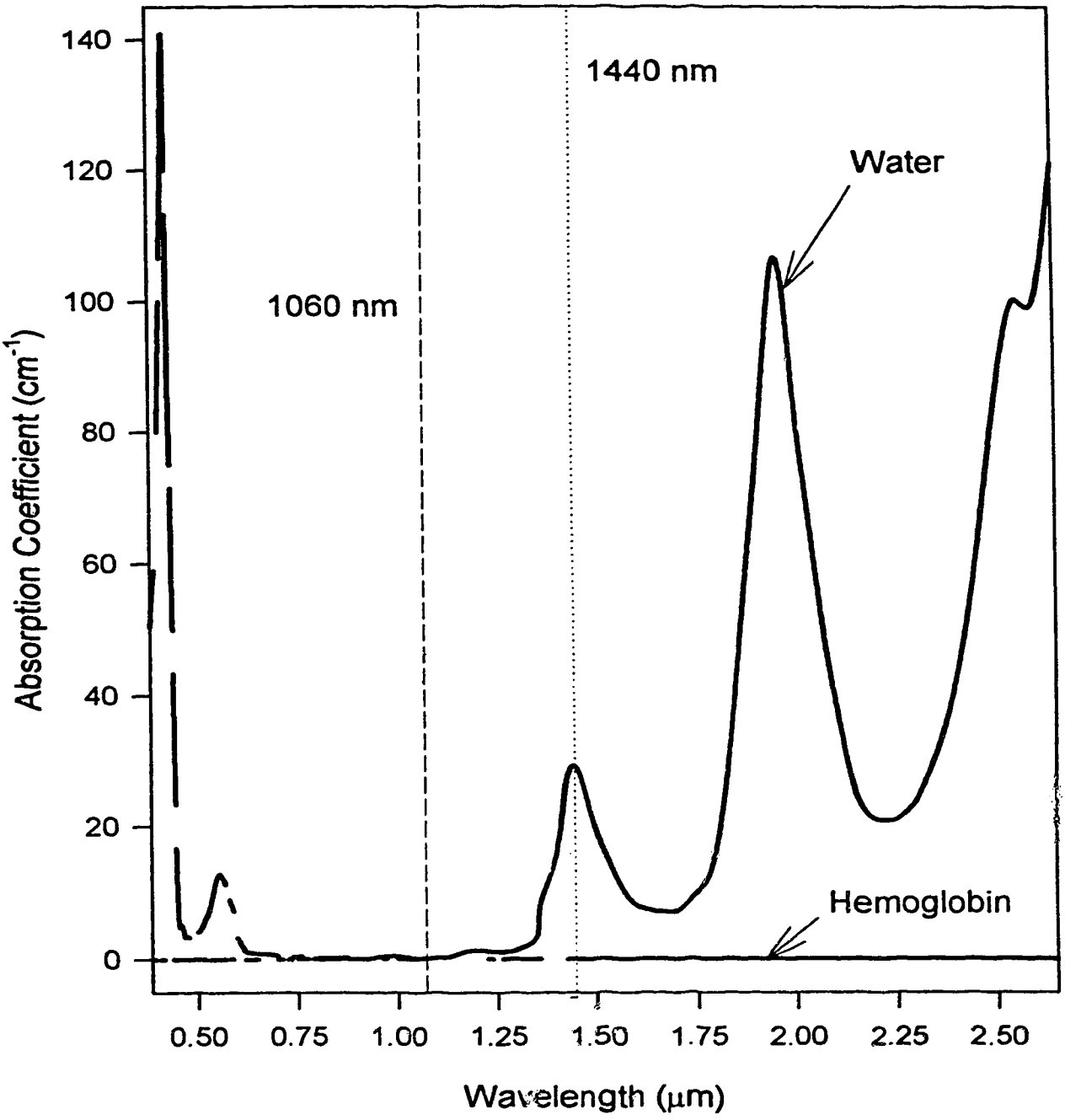


Figure.1.5: Absorption patterns of the dual wavelengths of light emitted from the multi-YAG laser with respect to the absorption coefficients of hemoglobin and water (from Smith, 1974 and Van Assendelff, 1970).

Chapter 2

In Vitro Fragmentation of Human Urinary Calculi with the Multi-YAG Laser

2.1 Introduction

Apart from the *in vitro* studies performed by Moore and associates (Moore, 1990), there have been no other studies investigating the stone-fragmenting properties of the multi-YAG laser. In order to eventually study the tissue effects of the multi-YAG laser, it is necessary to first have an understanding of the laser and how it interacts with the calculus itself. Based on Moore's results, we have performed *in vitro* investigations to study the photofragmentation of human urinary calculi with the multi-YAG laser. This would not only help establish the optimal parameters for stone ablation, but would also help determine those laser settings that would eventually require assessment in a safety study. By observing and measuring how the pulse energy and pulse frequency affect fragmentation, this information might also be useful in understanding the underlying mechanism of stone ablation. Therefore, the objectives of this study were to determine if human urinary calculi could be fragmented, and if so, we wanted to establish the optimal parameters for stone ablation.

2.2 Material and Methods

2.2.1 Specimens

The composition of the stone greatly influences the efficiency of laser lithotripsy. Calcium oxalate monohydrate (COM), calcium phosphate dihydrate (CPD or brushite), and cystine stones are the most difficult to fragment by laser lithotripsy while uric acid (UA), magnesium ammonium phosphate (struvite), and calcium oxalate dihydrate (COD) are

much more easily fragmented (Dretler, 1987b; Atala, 1990). Therefore, both a "soft" stone group and a "hard" stone group were studied. Sixty UA calculi (UA composition = 93%) made up the "soft" set and 60 COM stones (COM composition = ≥89%) were used for the "hard" group. All stones were from humans and were supplied by Louis C. Herring and Company (Orlando, FL) and also by local urologists. Stone composition was assessed by an integrated crystallographic analysis at Louis C. Herring and Company.

2.2.2 Laser

The Zeiss OPMILAS 144/60 Plus Surgical Laser System (Carl Zeiss, Inc., Princeton, NJ) was used for laser photofragmentation. In the 1440 nm pulsed mode, pulse energy settings of 0.3 to 3.5 Joules (J) can be used in 0.1 J increments with a fixed pulse duration of 650 μsec. Pulse frequency can be adjusted from 5 to 50 Hz in 1 Hz increments and the maximum average power that can be achieved is 60 watts.

Stone fragmentation was examined at pulse energies of 0.3, 0.6, 0.9, 1.2, and 1.5 J and pulse frequency was varied at 5, 10, and 15 Hz. Laser energy was transmitted to the stone through a 400 μm fiber (Fused Silica [LFS]; Radiant Communications Corporation, South Plainfield, NJ) and the pulse duration was constant at 650 μsec.

2.2.3 Experimental Method

All calculi were first weighed to the nearest 0.1 mg and sorted into four weight groups. The calculi from these groups were then assigned to different experimental levels in a block randomized design thus ensuring that stone weight was homogeneous for the different levels. Four UA stones and four COM stones from each weight group were randomly assigned to each experimental level for a total 120 treatments. The mean stone weight for each experimental level is shown in Table 2.1 and 2.2. There was no significant differences in the mean stone weights for both the UA or the COM groups.

Each stone was placed on a screen sieve with 1.5 mm spacing and submerged in 0.9% normal saline within a 250 cc glass beaker (Figure 2.1). Laser lithotripsy was performed under direct visualization using a 7.5F semi-rigid ureteroscope (Karl Storz Endoscopy-America, Inc., Culver City, CA). Fragmentation was performed with the laser fiber abutted against the stone to ensure that the fiber to stone distance remained consistent for each calculus. Treatment was continued until each stone was completely fragmented to particles less than 1.5 mm. The total energy required for fragmentation was recorded. This method was kept standardized throughout the whole study.

2.2.4 Fragmentation Efficacy

The lithotripsy process was assessed quantitatively by dividing the total energy required for fragmentation by the weight of the stone. This calculation describes the amount of energy required to fragment a unit mass of stone and was called the fragmentation efficacy (J/mg). It serves as a measure for the effectiveness of laser fragmentation for the different experimental variables studied.

2.2.5 Fragmentation Rate

The fragmentation rate is a calculated value which can be used as another measure of the effectiveness of lithotripsy at the different experimental levels. It was determined by first dividing the total energy required for fragmentation by the pulse energy. This value was then divided by the stone weight and inverted to provide an indexed result describing the milligrams of stone fragmented per pulse of laser energy (mg/pulse).

2.2.6 Statistical Analysis

The mean fragmentation efficacy was determined for each experimental level for both the UA and COM stone groups. These were compared with a two-way analysis of variance

to determine if there was a difference in the energy required for fragmentation with respect to pulse energy and pulse frequency. Similarly, the mean fragmentation rates were calculated for each experimental level in each stone group and a non-parametric two-way analysis of variance was used to compare these values. In both cases, comparisons within the two different stone groups were made using Duncan's Multiple Range tests (α=0.05) to ascertain any significant differences between the individual dependent variables. Finally, the complete data for all 120 stones was analyzed with multiple regression analysis in order to determine if the stone composition contributed significantly to the effectiveness of fragmentation. All statistical analyses were performed using SAS/STAT software, version 6.09 (SAS Institute Inc., Cary, NC).

2.3 Results

2.3.1 Subjective Observations

At the pulse energies and pulse frequencies studied, the multi-YAG laser was able to fragment all calculi. During photofragmentation, a drilling effect was seen such that the fiber bore down into the stone while emitting a fine spray of dust from the stone surface. To accomplish fragmentation, multiple small holes were drilled into the surface of the stone. As these coalesced, fragmentation or cleavage lines developed in and through the stone. As the stone broke apart, these individual pieces were further fragmented in the same manner until they fell through the sieve. It was our impression that the efficacy of fragmentation for these smaller particles decreased as the pulse frequency and pulse energy were increased. It was much more difficult to keep the laser fiber focused on the stone fragments because of the increased stone movement that occurred at the higher frequencies and pulse energies.

Stone ablation could still be performed when the fiber was not in direct contact with the stone. However, beyond one millimeter, there was only movement of the stone but no effective fragmentation. The laser fiber itself sustained little to no gross damage at the lower energy levels but at 1.2 and 1.5 J, the fiber tip was slowly eroded away. Despite this, stone fragmentation was still achieved with the same efficacy and rate.

The overall fragmentation was relatively slow at the lower energy levels but much more rapid with higher pulse energies. For the softer UA stones, complete fragmentation to particles less than 1.5 mm took approximately 15 to 20 minutes at 0.3 J and 5-10 minutes at 1.2 and 1.5 J. For the COM stones, fragmentation at 0.3 J took up to 60 to 90 minutes compared to 10 to 15 minutes at 1.2 to 1.5 J. Time for fragmentation was not measured and compared between groups because there were many variables that made this measurement inaccurate.

Cystine and struvite stones were also fragmented but not considered for statistical analysis. As expected, struvite calculi were easily and rapidly fragmented with minimal fiber damage. Photofragmentation of cystine stones could also be done without much difficulty. However, ablation was associated with charring of the stone and laser fiber surfaces and there was a burning odor present during fragmentation. In addition, damage to the fiber tip was more rapid, especially at higher energy and frequency settings.

2.3.2 Fragmentation Efficacy

The mean fragmentation efficacies at each experimental level for UA calculi are shown in Figure 2.2. The pulse frequency did not significantly effect the fragmentation efficacy ($p= 0.4069$) but the pulse energy did ($p= 0.0013$). The total energy required for fragmentation at 0.3 J/pulse was significantly more than the amount needed at pulse energies of 0.6 J to

1.5 J ($p<0.05$). Therefore, from 0.6 to 1.5 J/pulse, there was no improvement in the effectiveness of stone ablation with respect to energy.

The same data for COM stones is shown in Figure 2.3. Once again, the pulse frequency did not significantly affect the fragmentation efficacy ($p= 0.2560$) but the pulse energy did ($p= 0.0001$). The amount of energy required to fragment a milligram of stone was significantly greater for 0.3 and 0.6 J/pulse ($p<0.05$) compared to pulse energies of 0.9 J to 1.5 J. For these harder calculi, the plateau in fragmentation efficacy did not occur until 0.6 J. Compared to UA stones, COM calculi required an overall greater amount energy per unit mass of stone fragmented ($p= 0.0001$) (Figure 2.4).

2.3.3 Fragmentation Rate

The mean fragmentation rates at each experimental level for UA stones are shown in Figure 2.5. Overall, the pulse energy significantly affected the fragmentation rate ($p=0.0001$) while the pulse frequency did not ($p=0.7158$). A linear relationship between the pulse energy and rate of fragmentation is depicted such that an increase in the pulse energy was associated with an increased fragmentation rate. Using multiple comparisons, this association was found to be statistically significant ($\alpha=0.05$). The same data for COM stones is shown in Figure 2.6. Like the UA calculi, the mean fragmentation rates were not significantly affected by the pulse frequency ($p= 0.1233$) but pulse energy did have a significant effect ($p= 0.0001$). A similar positive linear relationship is demonstrated between the fragmentation rate and pulse energy and this association was found to be significant ($\alpha= 0.05$).

2.4 Discussion

The multi-YAG laser is a relatively new surgical laser with the ability to operate at the standard 1064 nm wavelength (continuous or pulsed) or with a pulsed 1440 nm wavelength. The dual wavelength capability allows the multi-YAG laser to both coagulate and ablate tissue thus making it a multiple purpose machine. Previous studies have demonstrated these tissue interactions by investigating the laser's effect on rabbit brain (Martiniuk, 1989) and bovine cartilage (Nishioka, 1992). This study demonstrates that when the multi-YAG laser is operated at a 1440 nm wavelength, it is able to effectively fragment human urinary calculi. Both soft and hard density calculi were amenable to fragmentation.

This study was not designed to determine the mechanism of stone fragmentation with the multi-YAG laser, but based on our subjective observations during these investigations, our knowledge of the physical characteristics of the multi-YAG laser, and previous *in vitro* studies, we can postulate that both thermal and plasma-mediated interactions are involved. The 1440 nm wavelength is well absorbed by water. Therefore, it is quite conceivable that water held within the stone undergoes thermal vaporization and this leads to localized ablation of the calculus. Our observation that the laser appeared to drill through the stone seems to support this theory.

Observations during lithotripsy also suggested that visible plasma formation was not necessary for successful fragmentation with the multi-YAG laser. Plasma formation did occur, but its incidence was variable and was noted more frequently when the laser was fired for greater than two to three second bursts. In these situations, the tip of the laser fiber tended to become embedded in the superficial surface of the calculus and at this point multiple bright flashes of light suggestive of plasma formation were often witnessed. In

general, when the laser was discharged for shorter periods, effective fragmentation occurred, but no light flashes were seen.

Previous *in vitro* studies using the multi-YAG laser were done in 1990 by Moore and coworkers (Moore, 1990). In addition to fragmenting synthetic calculi, barometric and high-speed cinematographic analysis was performed on the fragmentation process. When the multi-YAG laser was discharged under water, high-speed refractive photography demonstrated a pressure wave propagating from the laser fiber with a forward velocity of 2660 μm/msec and a lateral velocity of 1260 μm/msec. The pressure changes associated with this shock wave were measured and were found to be 82.28 pounds per square inch (5.6 atm) when the fiber was 1 mm away from a piezoelectric crystal. Compared to extracorporeal shock wave lithotriptors which are capable of producing pressures of approximately 1000 atm at F_2, (Spirnak, 1990a), this amount of pressure is significantly less. However, the tensile strength of most urinary calculi has been shown to vary from 0.8 to 34.3 atm (Spirnak, 1990a) and this is in the range of pressure that is generated by the multi-YAG laser.

Therefore, based on these experimental findings, it is probable that a shock wave energy component is also involved in the mechanism of stone fragmentation with multi-YAG laser lithotripsy but probably plays a minor role compared to the thermal effects noted above. A possible scenario therefore, is that localized stone ablation occurs secondary to thermal vaporization of water within the stone and shock wave energy contributes to the process by causing small stress fractures within the stone. These combined interactions would ultimately lead to fragmentation of the calculus.

The overall action of the multi-YAG laser on urinary calculi appears to be very similar to the holmium laser. This laser has a wavelength of 2100 nm and therefore also has a high

absorption coefficient in water. In vitro studies investigating the stone ablating properties of the holmium laser have shown that all types of urinary stones can be fragmented, including cystine calculi (Watson, 1993). Like the multi-YAG laser, the holmium laser has an action on stones that is partly drilling and partly fragmentation and this mechanism is thought to be secondary to laser energy absorption by water in the stone and on the surface of the stone (Watson, 1994).

The tunable dye laser is the most common laser used to fragment urinary calculi in North America. In contradistinction to the multi-YAG and Ho:YAG lasers, it causes fragmentation solely by acoustic shockwave energy through a plasma-mediated mechanism. Nishioka and colleagues have studied and proved this in an elaborate study investigating the temporal and spectral characteristics of the bright flash of light, or plasma, that takes place with stone fragmentation (Nishioka, 1987). The plasma was found to be composed of neutral and singly charged calcium ions and without it, no stone damage occurred. They therefore theorized that as the microsecond laser pulse is absorbed by pigments in the stone, microscopic heating occurs on the surface of the stone, and this leads to liberation of free calcium electrons which thus forms the plasma. The plasma then becomes the primary absorber of the laser energy, expands with further heating, and then contracts after completion of the laser pulse. This expansion and contraction produces an acoustic shock wave which both damages the stone and causes a brisk recoil of the laser fiber.

Finally, laser lithotripsy with the Q-switched Nd:YAG laser is also believed to be plasma-mediated (Hofmann, 1988). The nanosecond pulses of this laser cause significantly higher peak powers than the pulsed dye laser. This increased intensity is believed to result in plasma formation within the liquid around the stone which is thought to be initiated by the physical process known as a dielectric breakdown [Nishioka/1987].

Our results demonstrated that the pulse energy of the laser and the stone composition significantly affected the fragmentation efficacy while the pulse frequency did not. With respect to the pulse energy, an increase in pulse energy was associated with an increased fragmentation efficacy. However, a plateau in the efficacy is reached for both soft and hard density stones. These findings imply that there is a threshold for fragmentation efficacy beyond which there is no improvement in the effectiveness of fragmentation despite an increased amount of energy being applied to the calculus. If photofragmentation is accomplished mainly through a thermal-mediated mechanism, then it may be that only a fixed mass of stone is able to be fragmented per pulse of energy based on the amount of water held within the crystalline structure of the stone and on the depth of penetration of thermal energy for that stone. Beyond a certain point, there would be no further absorption of laser energy and the remaining energy is dissipated to the surrounding medium.

The threshold for fragmentation efficacy appears to be higher for COM stones compared to the softer UA stones since the plateau in fragmentation efficacy occurs at higher pulse energies for the COM calculi. The tensile strengths of softer stones such as UA would be expected to be significantly less than those for harder density calculi like COM. When these forces are significantly greater than what can be generated with the multi-YAG laser, then it is likely that the blast effect of the laser has an insignificant effect on stone ablation. Therefore, as the stone density and tensile strength of the stone increase, the mechanism of fragmentation and the fragmentation efficacy would be more thermal dependent and this might translate into a higher overall threshold for fragmentation efficacy.

Although there was a slight improvement in the fragmentation efficacy with increased frequency, this trend did not attain statistical significance. However, it is interesting that

there appears to be some interaction between pulse energy and pulse frequency. Figure 2.2 shows that for UA stones, the fragmentation efficacy curves for the different pulse frequencies intersect just above the pulse energy of 0.9 J. Below this point, an increase in frequency is associated with an increase in the effectiveness of fragmentation and thus its contribution with the pulse energy is additive. Beyond this intersection point however, the inverse is true. Once this level has been reached, any additional power supplied to the calculus in the form of increased pulse frequency appears to be "wasted", and this energy is either scattered or transmitted to the surrounding fluid medium. There is also a complete inversion of the fragmentation efficacy curves for COM stones (Figure 2.3) but this does not occur until the pulse energy of 1.2 J has been reached.

This interaction may actually be directly related to the size of the particle being fragmented. Our observations during lithotripsy revealed that fragmentation seemed subjectively much more inefficient as the particles became smaller and were bounced about by the laser pulses. In other words, once a stone is reduced in size, it is much more difficult to "keep up" with these fragments as the pulse frequency is increased. Energy is therefore lost to the environment, and in the recoil of the small particles from the blast effect of the laser, making the process more inefficient.

The plateau in fragmentation efficacy discussed previously plus this interaction occurring at higher pulse energies might have important clinical implications. These results suggest that increasing the pulse energy and pulse frequency beyond a certain point may eventually lead to a paradoxical decrease in the fragmentation efficacy instead of an increase as one would think. The remaining "wasted" energy may represent a potential for tissue injury. This issue needs to be addressed in an animal study investigating the safety of this laser for intracorporeal lithotripsy.

The multi-YAG laser was able to successfully photofragment the hardest urinary stones. Fragmentation of COM stones was achieved at all levels of pulse energy and pulse frequency. Not unexpectantly, a significantly greater amount of energy was required compared to the UA stones. Also, the plateau in fragmentation efficacy did not occur until 0.9 J in the COM group compared to 0.6 J with the UA stones indicating there is a higher threshold for fragmentation efficacy with harder density calculi.

The number of milligrams of stone fragmented per pulse of laser energy cannot be easily measured during multi-YAG laser lithotripsy but this value can be calculated as described earlier. This is an important parameter to analyze. It is an indirect measure of the time or rate of fragmentation and this differs from the fragmentation efficacy which measures the effectiveness of fragmentation with respect to total energy.

As one would expect, our results showed a linear relationship between the rate of fragmentation and the pulse energy (Figure 2.5 and 2.6). This relationship was statistically significant for both the UA and COM stones. If the total energy required to fragment a certain weight of stone is equal for two different pulse energy settings, then the number of pulses required will be less for the higher pulse energy. This translates into a greater mass of stone being fragmented per pulse of laser energy discharged. Therefore, these results reveal that the effectiveness of stone ablation with respect to the rate of fragmentation, is improved as one increases the pulse energy.

Conversely, the pulse frequency did not significantly affect the rate of fragmentation. Instead an interaction between the pulse energy and pulse frequency was again seen. Because the rate of fragmentation is calculated from the raw data already presented, these results are not unexpected. Like the fragmentation efficacy results, a point is reached where an increase in both the pulse energy and the pulse frequency is associated with a

decrease in the rate of fragmentation such that fewer milligrams of stone are fragmented per pulse of laser energy. Again, if one considers that it is much more difficult to focus all the laser's energy on the stone particles at higher pulse frequencies and energies because of increased stone movement, then it makes sense that as more pulses of laser energy are lost to the environment, a fewer number of milligrams would be fragmented.

Our observations with cystine stones are interesting. The charring on the stone surface and the burning smell emitted suggests that the thermal-mediated mechanism for fragmentation may be the principal mode of ablation for this type of stone. It is possible that the laser energy is primarily absorbed by the organic crystalline structure of the stone in addition to the water within the crystal, and this leads to a greater amount of heat being liberated and retained within the stone. More fiber damage and charring of the laser fiber also occurred with the fragmentation of cystine stones. It is likely that as the stone surface became carbonated, some of this residue was deposited on the laser fiber. Since this residue would then absorb laser energy, the laser fiber itself would be ablated or melted away.

2.5 Conclusions

In summary, these *in vitro* findings reveal that the new multi-YAG laser has very acceptable stone ablating properties for all variety of urinary stones, including COM and cystine. For softer stones, effective fragmentation occurs between 0.6 J to 1.5 J and between 0.9 J and 1.5 J for harder, difficult to treat calculi. The fragmentation efficacy (J/mg) does not improve beyond 0.6 J for soft calculi or past 0.9 J for hard stones. However, the rate of fragmentation (mg/pulse) does show a positive linear relationship with pulse energy for both soft and hard stones. Based on these experimental results, we

believe that the multi-YAG laser might be an excellent tool for fragmenting urinary calculi, especially for the difficult to treat COM stones. Animal studies are now required to investigate the safety of this laser for intracorporeal lithotripsy.

Table 2.1: Mean weights and standard errors for uric acid stones (n= 60) fragmented by the multi-YAG laser in an *in vitro* experimental setting. No significant difference between means by two-way ANOVA (p 0.98 for frequency and p= 0.89 for energy).

	0.3 J (mg ± SE)	0.6 J (mg ± SE)	0.9 J (mg ± SE)	1.2 J (mg ± SE)	1.5 J (mg ± SE)	Mean (mg ± SE)
5 Hz	103.90 (23.94)	89.70 (14.99)	142.00 (46.32)	97.58 (19.99)	106.38 (32.12)	107.91 (12.46)
10 Hz	104.80 (28.44)	98.90 (22.40)	107.35 (25.84)	118.28 (48.22)	112.95 (38.79)	108.46 (13.63)
15 Hz	124.42 (44.14)	91.58 (19.02)	101.12 (20.92)	118.80 (46.56)	90.48 (19.34)	105.28 (13.32)
Mean (mg ± SE)	111.04 (17.63)	93.39 (10.02)	116.82 (23.03)	111.55 (21.30)	103.27 (16.51)	107.22 (7.46)

Table 2.2: Mean weights and standard errors for calcium oxalate monohydrate stones (n= 60) fragmented by the multi-YAG laser in an *in vitro* experimental setting. No significant difference between means by two-way ANOVA (p= 0.86 for frequency and p= 0.99 for energy).

	0.3 J (mg ± SE)	0.6 J (mg ± SE)	0.9 J (mg ± SE)	1.2 J (mg ± SE)	1.5 J (mg ± SE)	Mean (mg ± SE)
5 Hz	127.98 (33.21)	133.88 (42.93)	147.75 (43.07)	141.08 (36.71)	131.85 (33.08)	136.50 (15.21)
10 Hz	149.80 (44.94)	153.08 (50.51)	140.00 (46.92)	134.90 (45.89)	149.15 (46.85)	145.38 (18.76)
15 Hz	141.65 (46.50)	137.58 (45.59)	136.58 (42.18)	111.92 (36.59)	131.72 (35.61)	131.89 (16.68)
Mean (mg ± SE)	139.81 (22.08)	141.51 (24.39)	141.44 (23.08)	129.30 (21.21)	[illegible].58 (20.50)	137.93 (9.64)

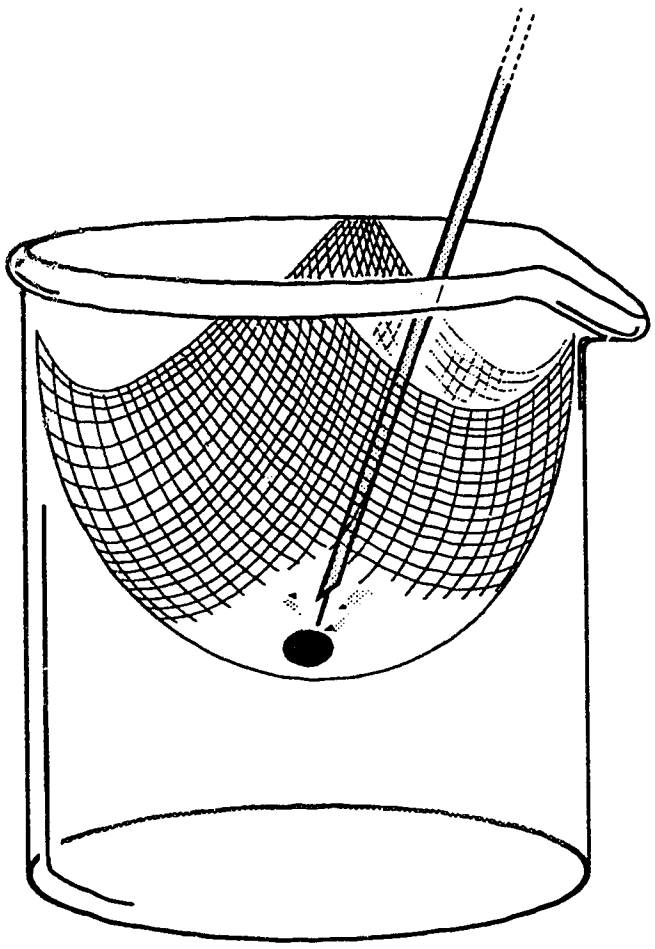


Figure 2.1: Controlled *in vitro* experimental set up. Each urinary calculus was placed on a screen with 1.5 mm spacing, immersed in 0.9% saline, and fragmented under ureteroscopic control with the multi-YAG laser until all particles fell through the sieve.

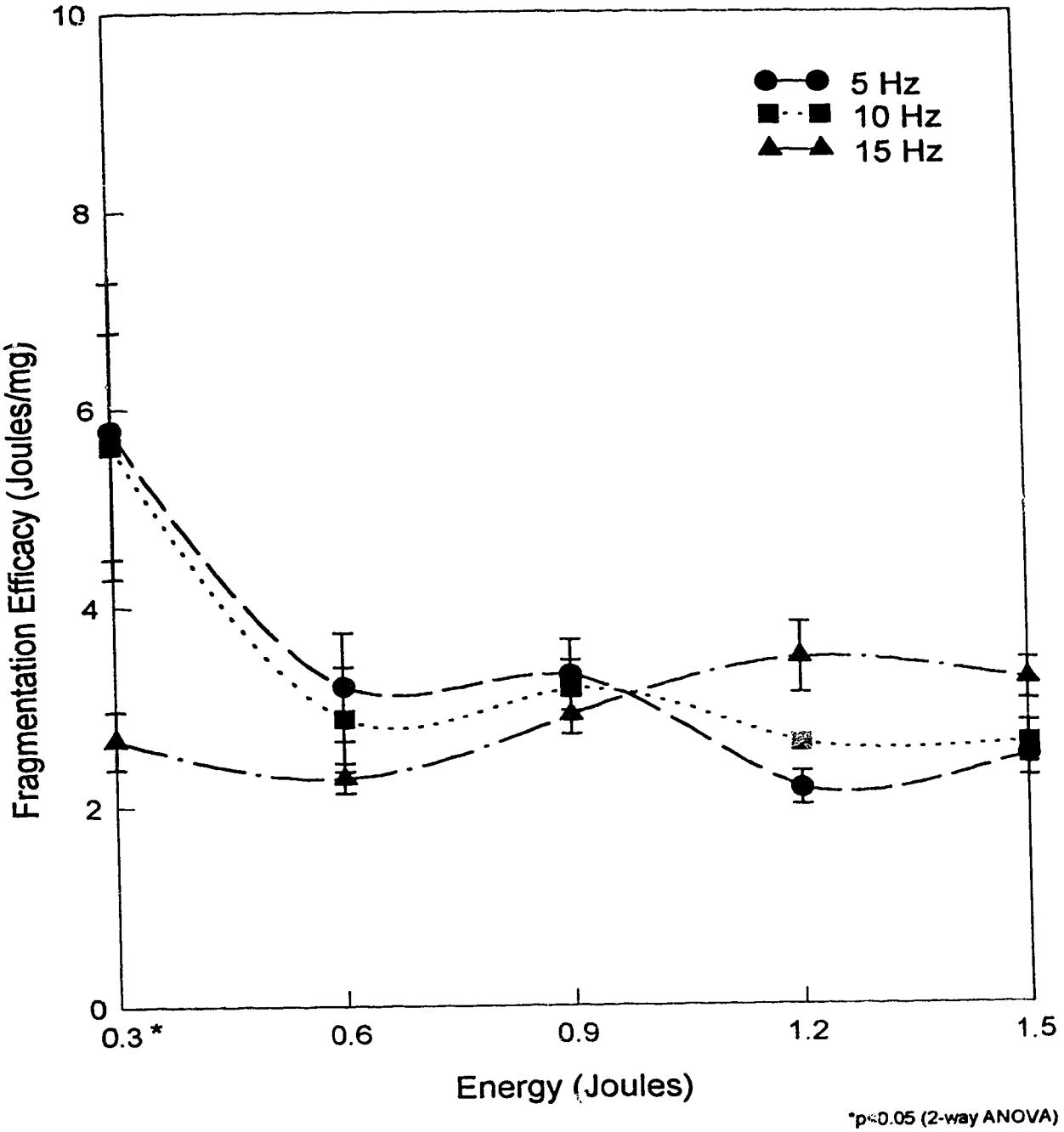


Figure 2.2: *In vitro* fragmentation of UA calculi with the multi-YAG laser. Mean fragmentation efficacies for changes in pulse frequency plotted as a function of pulse energy. Pulse frequency did not significantly effect fragmentation efficacy ($p= 0.4069$) but pulse energy did ($p= 0.0013$). Significantly more energy was required for fragmentation at 0.3 J/pulse compared to other energy levels ($p<0.05$).

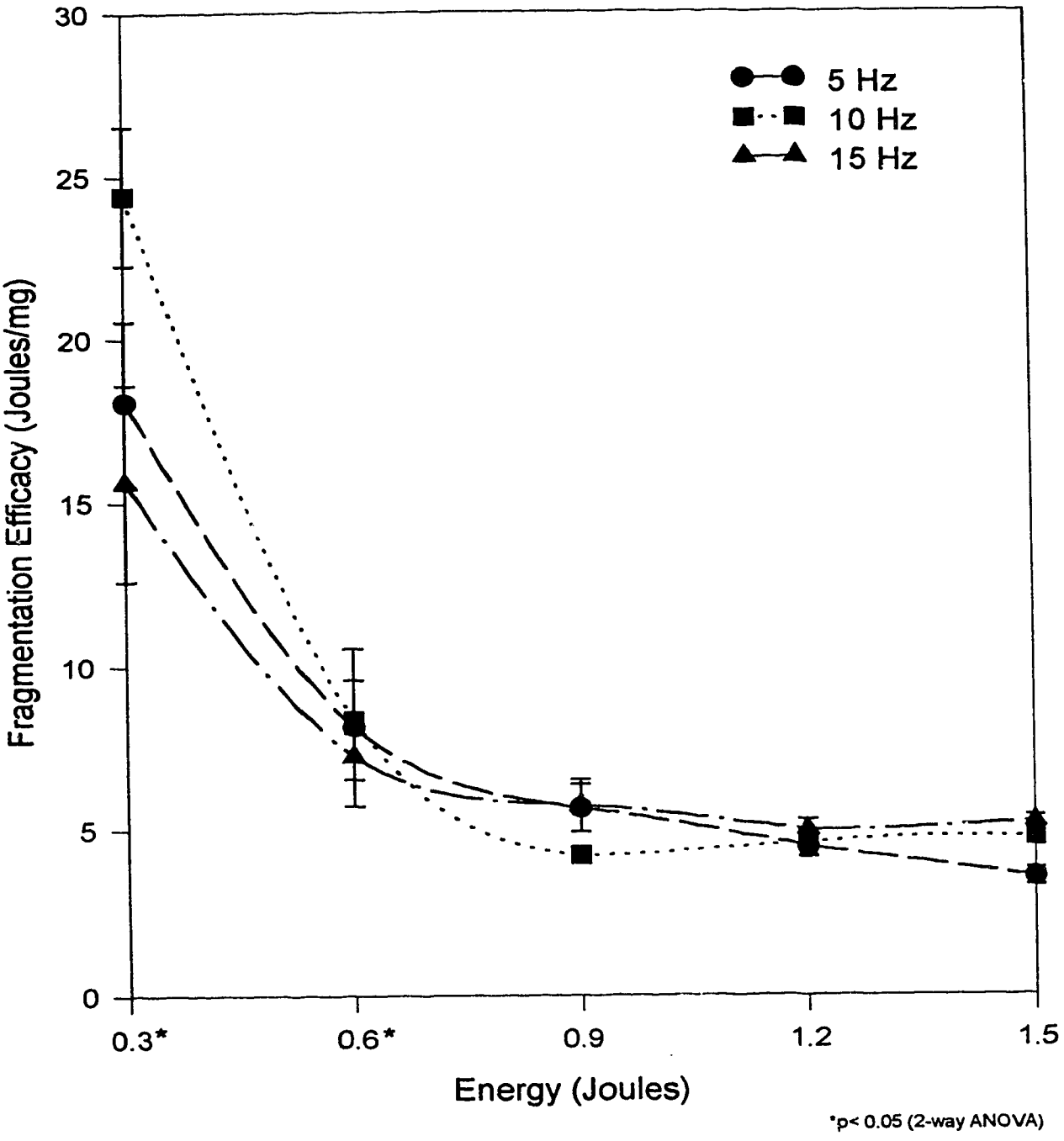


Figure 2.3: *In vitro* fragmentation of COM calculi with the multi-YAG laser. Mean fragmentation efficacies for changes in pulse frequency plotted as a function of pulse energy. Pulse frequency did not significantly effect fragmentation efficacy ($p= 0.2560$) but pulse energy did ($p= 0.0001$). Significantly more energy was required for fragmentation at 0.3 and 0.6 J/pulse compared to other energy levels ($p<0.05$).

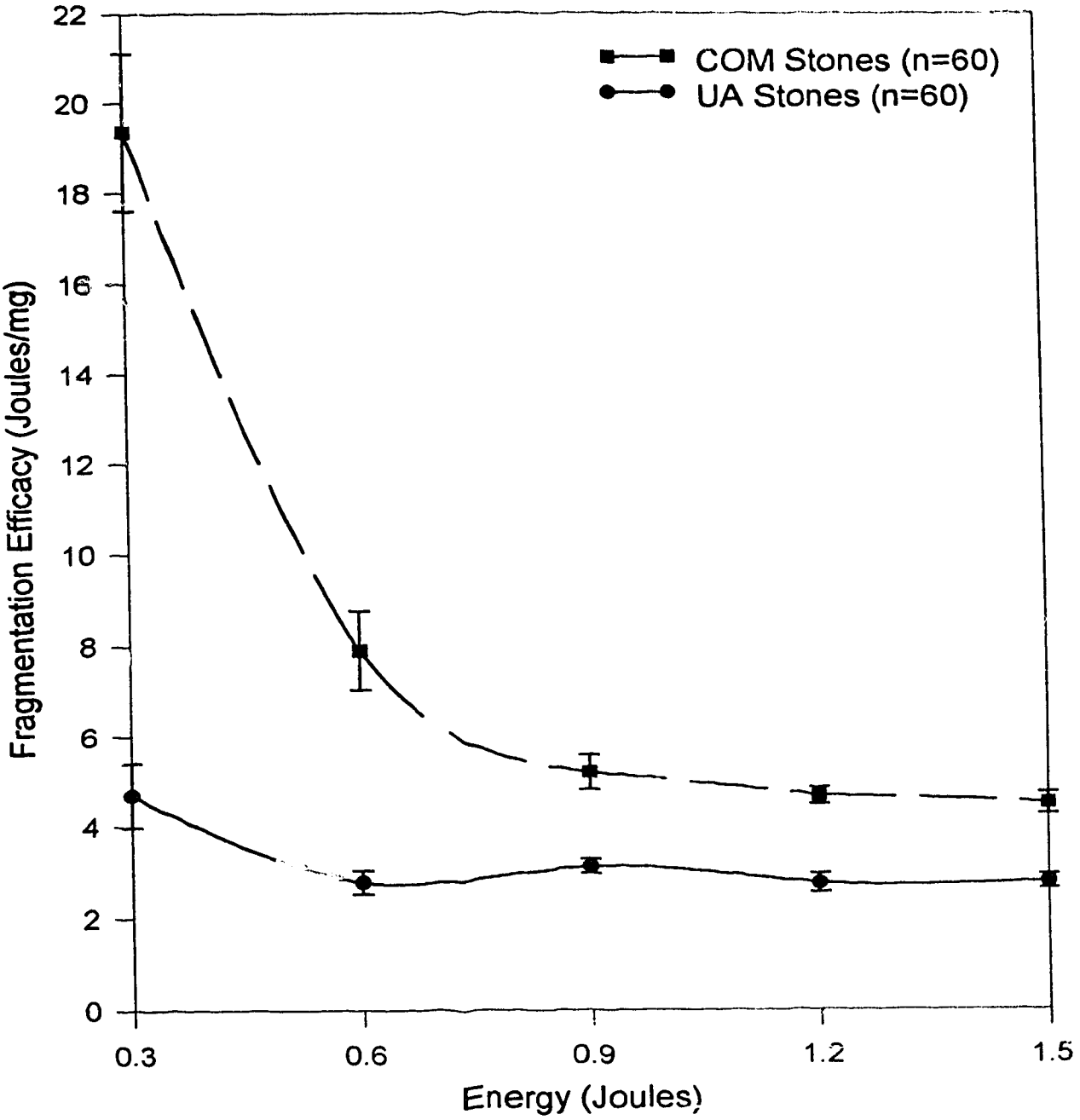


Figure 2.4: Comparison of the mean fragmentation efficacies for UA and COM calculi plotted as a function of the pulse energy. Compared to UA stones, COM calculi required a significantly greater amount of energy to fragment the same unit mass of stone ($p = 0.0001$).

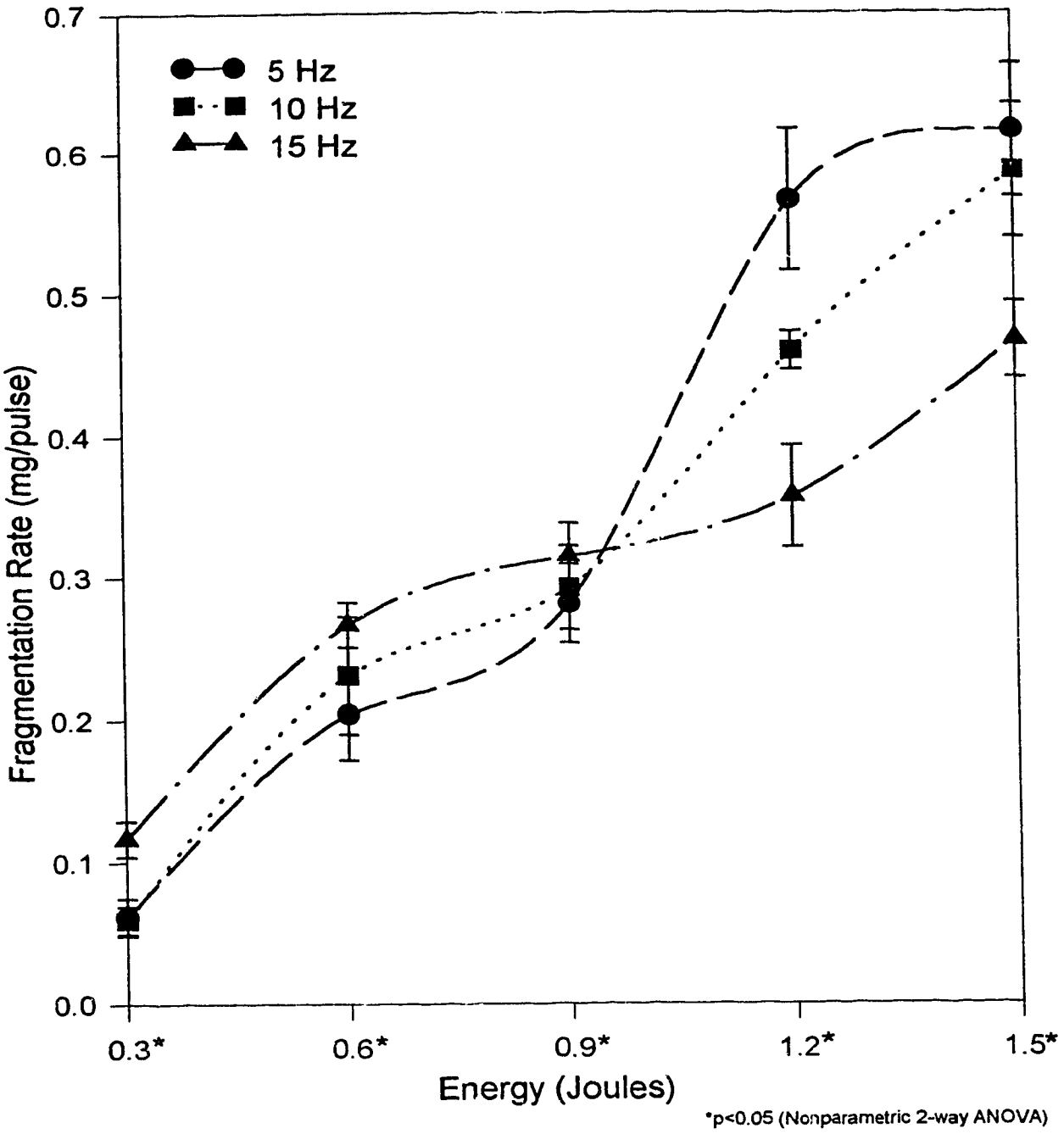


Figure 2.5: *In vitro* fragmentation of UA calculi with the multi-YAG laser. Mean fragmentation rates for changes in pulse frequency plotted as a function of pulse energy. Pulse energy significantly affected the fragmentation rate ($p=0.0001$) while the pulse frequency did not ($p=0.7158$). A significant linear relationship between the pulse energy and the fragmentation rate was also demonstrated.

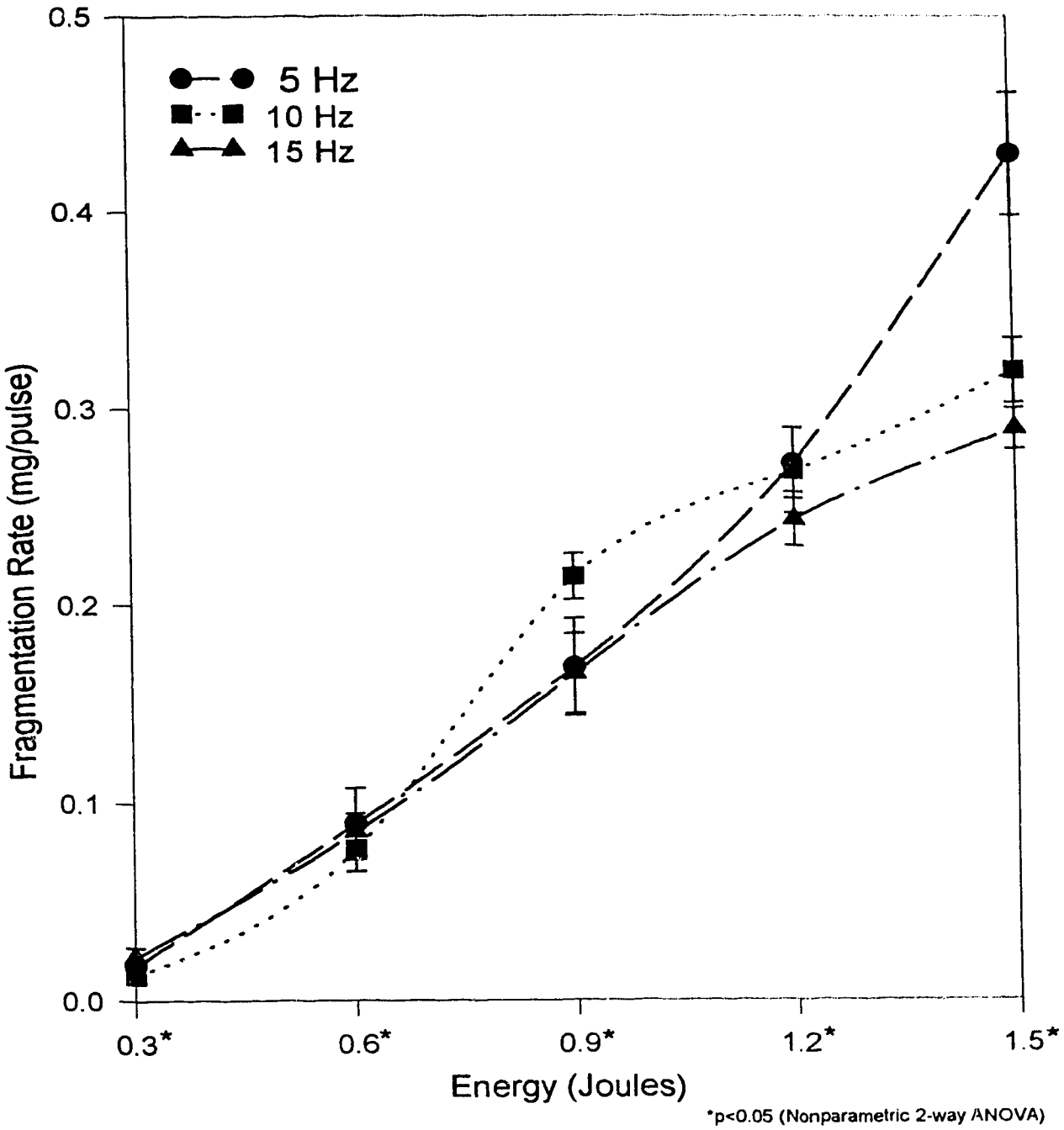


Figure 2.6: *In vitro* fragmentation of COM calculi with the multi-YAG laser. Mean fragmentation rates for changes in pulse frequency plotted as a function of pulse energy. Pulse energy significantly affected the fragmentation rate ($p=0.0001$) while the pulse frequency did not ($p=0.1233$). A significant linear relationship between the pulse energy and the fragmentation rate was also demonstrated.

Chapter 3

Assessment of Multi-YAG Laser Lithotripsy in an *Ex Vivo* Pig Model

3.1 Introduction

The first phase of this project investigated the stone-fragmenting properties of the multi-YAG laser in a controlled *in vitro* setting. These studies demonstrated that this laser was able to effectively ablate a variety of human urinary calculi, including hard density COM and cystine stones, using the 1440 nm wavelength in a pulsed mode. Therefore, the multi-YAG laser might be an important clinical tool for the practicing urologist.

Prior to initiating human clinical trials however, the safety of this laser needs to be studied. The potential for injury with multi-YAG laser lithotripsy is related to its 1440 nm wavelength. As described previously, this wavelength is well absorbed by water and therefore can produce tissue ablation when the laser energy is applied directly to tissue. Whether significant damage occurs when the energy is applied to a stone within the urinary tract is not yet known. We believe that the multi-YAG laser causes stone fragmentation mainly through a thermally-mediated effect such that water within the stone is vaporized leading to stone ablation. Continuous wave lasers fragment stones in this way (Watson, 1985) but are not useful clinically because this thermal energy is retained within the stone and then transmitted to surrounding tissue causing trauma (Tanahashi, 1981). This should not occur with the multi-YAG laser because its pulse duration of 650 µsec is sufficiently short enough that the thermal energy supplied to the stone should be spatially confined to the stone itself. Tissue damage is most likely to occur from scattered energy and/or laser energy that misses the stone completely and is absorbed primarily by the tissue. If all the laser energy can be directed onto the calculus, we believe that clinically

significant tissue damage should not occur. Whether this can be performed in reality needs further investigation.

A preliminary *ex vivo* animal model would be an ideal starting point to study this method of photofragmentation prior to initiating an *in vivo* study. Therefore, the objectives of this study were to develop an *ex vivo* animal model to study the tissue effects of intraureteral lithotripsy with the multi-YAG laser and to determine whether this treatment is associated with significant acute tissue injury.

3.2 Materials and Methods

3.2.1 Preparation of *Ex Vivo* Model

The renal unit of an adult pig was used as an animal model because its size and anatomy are similar to that of the human. A total of 54 kidneys with intact ureters were harvested from swine at a local slaughterhouse. The specimens were removed within twenty minutes of the animals' death and were kept hypothermic to prevent cellular autolysis. To prepare the *ex vivo* model, each ureter was first dilated to 8F using a Teflon dilator (Cook Urological Inc., Spencer, IN). Nine French grasping forceps (Karl Storz Endoscopy-America, Inc., Culver City, CA) were passed retrograde up the ureter, advanced out through the kidney, and an human urinary calculus was grasped and deposited in the upper third of the ureter. A constricting ligature of 3-0 silk was placed proximal to the stone to simulate an impacted calculus and also to localize the area of treatment. The entire renal unit was suspended within a glass beaker and two 33 gauge thermocouples (Omega Engineering Inc., Stamford, CT) were placed in the peri-ureteral tissue at the level of the calculus to measure the temperature changes during laser treatment. The specimen was then secured in a heat-conductive gelatin (Sigma Gelatin Type A: From Porcine Skin, G-

2500; 30 per cent solution, Sigma Chemical Company, St. Louis, MO) in order to stabilize the model. In this way, we were able to perform multi-YAG laser lithotripsy through an ureteroscope (Figure 3.1).

3.2.2 Human Calculi

All stones were from humans and were supplied by Louis C. Herring and Company (Orlando, Florida) and by local urologists. Stones were analyzed by an integrated crystallographic assessment at Louis C. Herring and Company. All stones were first weighed to the nearest 0.1 mg and measured to the nearest 0.01 mm. Stones selected for lithotripsy were at least 4.50 mm or greater in width.

3.2.3 Treatment Groups

Two ureters acted as non-operative controls and received no stones or treatment. Another four ureters had stones implanted but underwent ureteroscopy only and served as treatment controls. Three ureters were treated as "worst-case scenarios". In these cases, the laser fiber was placed against the ureteral wall and intentionally discharged for one to two seconds at 0.3, 0.6, and 0.9 Joules (J). The pulse frequency was kept constant at 10 Hz.

Multi-YAG laser lithotripsy with the Zeiss OPMILAS 144/60 Plus Surgical Laser System (Carl Zeiss, Inc., Princeton, NJ) was performed on the stones in the remaining 45 renal units. A 400 μm laser fiber (3M Specialty optical fiber, FT-400-LMT. Auriga Fibre Optics Ltd., Thornhill, ON, Canada) was used for fragmentation and the procedure was done under direct visualization with a 7.5F Storz semi-rigid ureteroscope (Karl Storz Endoscopy- America, Inc., Culver City, CA) while irrigating with 0.9 per cent saline solution. The fiber tip was kept in contact with the stone and the end-point of treatment was complete fragmentation such that residual stone particles could be easily flushed from

the ureter. Temperature changes associated with laser lithotripsy treatment were measured in degrees Celsius with an Omega OM-202 Temperature Logger (Omega Engineering, Inc., Stamford, CT).

3.2.4 Experimental Method

An experimental design was devised using pulse energy (0.3 J, 0.6 J, 0.9 J, 1.5 J) and pulse frequency (5 Hz, 10 Hz, 15 Hz) as study variables. Our previous *in vitro* investigations have shown that the multi-YAG laser is capable of fragmenting different varieties of human urinary calculi. However, below 0.6 J, fragmentation of calcium oxalate monohydrate (COM) stones is inefficient and would be clinically impractical. Therefore, 18 uric acid (UA) calculi were studied at 0.3 and 0.6 J and 24 COM or calcium phosphate dihydrate (CPD/brushite) stones were treated at 0.6 J and above. These different stones were first sorted into three weight groups and then assigned to the various experimental levels in a block randomized fashion. Therefore, using this design, three repetitions were performed at each level for a total of 42 lithotripsy treatments. Finally, three cystine stones were treated using the same experimental protocol but were not considered for statistical analysis. Two were fragmented at 0.6 J and 10 Hz and the third was treated at 0.9 J and 15 Hz.

3.2.5 Histologic Analysis

Following treatment, the involved segment of ureter was fixed in ten per cent buffered formalin solution and then paraffin processed, embedded, cut, and stained with routine hematoxylin and eosin. Each specimen was examined histologically and graded according to a predetermined tissue injury scale. Grade 0 was defined as intact or denuded mucosa with no necrosis and grade 1 consisted of mucosal necrosis involving up to one third of the ureteral wall (Plate 3.1). Grade 2 injury was necrosis involving up to two thirds of the ureteral wall (Plate 3.2) and grade 3 changes consisted of transmural necrosis of the ureter

(Plate 3.3). Grade 0 and 1 were classified as low grade tissue damage and grade 2 and 3 injuries were categorized as a higher degree of trauma. Histologic analysis was performed by a pathologist who was blinded to the treatment group of each specimen.

3.2.6 Statistical Analysis

For each experimental level, the mean change in temperature associated with lithotripsy was calculated. These means were compared with a two-way analysis of variance to determine if there was difference in the temperature generated during laser lithotripsy as the pulse energy and pulse frequency were varied. Simple descriptive analysis was made of the tissue changes that occurred in the control ureters, in the ureters containing cystine stones, and in the "intentionally-ablated" ureters. Finally, logistic regression analysis was performed on the injury scores from the remaining 42 ureters treated with multi-YAG laser lithotripsy. Pulse frequency, pulse energy, total energy required for fragmentation, and the stone weight were all compared to determine if any of these variables could predict high grade ureteral tissue injury as an outcome. All statistical analyses were performed using SAS/STAT software, version 6.09 (SAS Institute Inc., Cary, NC).

3.3 Results

3.3.1 Subjective Observations

This *ex vivo* animal model functioned very well. Although there was obviously no blood supply in the tissues, the model seemed to simulate the anatomic aspects of an *in vivo* setting quite satisfactorily. However, the technique of ureteroscopy was probably somewhat easier compared to the *in vivo* or clinical setting. The ureter remained relatively straight with very little buckling once dilated, the ureter remained patent without mucosal folds inhibiting visualization.

Like our *in vitro* studies, fragmentation was most effective when the laser fiber was gently abutted against the stone and fired. The laser could only be discharged for short bursts as visualization was quickly limited by the spray of stone dust emitted from the calculus. With continuous irrigation this dust cleared quickly and fragmentation was not delayed significantly but could be resumed within one to two seconds. The amount of debris ejected from the stone appeared to become greater as the pulse energy and pulse frequency were increased.

As described previously for our *in vitro* fragmentation, the stones were fragmented by drilling into the surface of the stone with the fiber. Multiple drill holes were joined and eventually the stone fragmented along cleavage planes. Like our *in vitro* findings, we noted greater stone movement at the higher pulse frequencies and this made ablation of smaller residual particles more difficult. It was during these situations where small pin-point burns in the mucosa and ureteral wall were occasionally noted following treatment.

3.3.2 Temperature Changes Associated with Laser Lithotripsy

The mean change in temperature associated with laser lithotripsy was calculated for each experimental level (Figure 3.2). Both the pulse energy ($p= 0.771$) and the pulse frequency ($p= 0.152$) did not significantly affect these changes. When a pulse frequency of 15 Hz was used for fragmentation, the mean temperature change was higher when compared to the other pulse frequencies, but this difference did not attain statistical significance ($\alpha= 0.05$).

3.3.3 Tissue Effects of the Control and "Intentionally-Ablated" Ureters

When examined histologically, the non-operative controls and the treatment controls all had tissue injury scores of grade 0. Conversely, each of the "intentionally-ablated" ureters sustained grade 2 and 3 tissue injury irrespective of the pulse energy used (Table 3.1).

3.3.4 Tissue Effects of Multi-YAG Laser Lithotripsy

Recall that grade 0 and grade 1 histological changes were classified as low grade injury and grade 2 and grade 3 changes were considered to be moderate and high grade injury respectively (Plates 3.1, 3.2, and 3.3). Overall, 37/42 (88.1%) of the ureters treated with multi-YAG laser lithotripsy sustained low grade injuries and 5/42 (11.9%) had higher grade changes. Of the low grade injuries, 8/37 (21.6%) were grade 0 and 29/37 (78.4%) were grade 1. In the high grade injury ureters, 4/5 had grade 2 trauma and 1/5 had a grade 3 injury. It should be noted that these high grade injuries were all found to be microscopically focal in nature. When the two stone groups are considered, 16/18 (88.9%) of the ureters which had UA stones fragmented had low grade injury, and 2/18 (11.1%) had higher grade tissue effects. For the COM stones, 21/24 (87.5%) of the ureters had low grade changes and 3/24 (12.5%) were found to have high grade injuries. Of the three cystine stones that underwent laser lithotripsy, two of the ureters received diffuse grade 1 injury and the third sustained focal grade 2 damage (Table 3.1). Using logistic regression analysis, pulse frequency, pulse energy, total energy required for fragmentation, and the stone weight were not found to be statistically significant predictors of injury (Table 3.2).

3.4 Discussion

Prior to initiating and recommending multi-YAG laser lithotripsy therapy for human patients, animal studies are mandatory to demonstrate the laser's safety. We have chosen an *ex vivo* animal model to begin these investigations. We sought to study the effects of pulse energy and pulse frequency on tissue injury as it pertains to laser lithotripsy with the multi-YAG laser in the 1440 nm pulsed mode. Because there have been no similar studies using this laser, we wanted to test this instrument over a relatively wide range of parameters but centered around the maximal efficacy ascertained from *in vitro* fragmentation. A total of twelve experimental levels were established to study pulse energy from 0.3 to 1.5 J and pulse frequency from 5 to 15 Hz. Using this experimental design, a large sample size is required to achieve significant results. This *ex vivo* system is relatively inexpensive and has allowed us to study such a population in a controlled experimental setting. A similar design using this many numbers would be difficult to perform in an *in vivo* setting since the costs and time requirements would be prohibitive.

Other lasers used for lithotripsy have also been studied with an *ex vivo* model. Watson and colleagues (Watson, 1983) tested the safety of the Q-switched and non Q-switched Nd:YAG lasers by focusing the beams directly onto pig urothelium. Also, Strunge et al. studied the effects of fragmenting intraureteral calculi with the Q-switched Alexandrite laser (Strunge, 1991). Calcium oxalate stones were placed in eight *ex vivo* pig ureters and then fragmented by placing the fiber tip blindly on the calculi. Like Strunge et al., our model consisted of placing a stone in an *ex vivo* pig ureter and performing lithotripsy in order that the total effect of this treatment could be evaluated. However, we chose to fragment the calculi completely and to do the lithotripsy under direct visualization since we believe that this more accurately simulates an *in vivo* or clinical setting. Assuming that this model can be validated in an *in vivo* study, it could then be applied to the investigation

of other laser systems or the study of non-laser intracorporeal fragmenting devices. Comparison between different modalities could be better accomplished using such a standardized approach.

Preparation of this model by implanting a human urinary stone in the ureter or performing ureteroscopy by itself was not associated with significant trauma since all our non-operative controls and treatment controls had a tissue injury scores of grade 0 on histologic analysis. Any further degree of trauma detected in the treatment ureters can therefore be assumed secondary to the laser.

There are some disadvantages to using an *ex vivo* model and these have to be taken into account during the analysis of the experimental data. First, because of the lack of blood supply, we are unable to assess the inflammatory response that occurs with treatment. This is obviously important since severe intramural inflammation may be a cause of late stricture formation. However, because we are only interested in assessing the acute tissue effects of our treatment, the lack of this information should not be substantial.

In addition, the 1440 nm pulsed multi-YAG laser interacts with tissue through a thermally-mediated mechanism. It could therefore be argued that by using a hypothermic *ex vivo* model, the amount of injury associated with treatment could be underestimated. This may be one of the sources of error in the experiment, but we do not believe it to be significant enough to influence the outcome data. The maximum mean temperature change associated with lithotripsy occurred at a pulse frequency of 15 Hz and a pulse energy of 0.6 J and was 21.67 °C (Figure 3.2). Even if we were to add this temperature change onto a normal body temperature of 37 °C, this would bring the maximum temperature change to 59.4 degrees. This is just beginning to approach the temperature range where protein denaturation and coagulation occurs. In an *in vivo* system this temperature rise

would be buffered by the room temperature saline irrigant and the normal blood flow in the tissues. Both would act as a heat sink to transfer thermal energy away from the treatment area.

In an attempt to quantify the amount of ureteral injury associated with multi-YAG laser lithotripsy, a tissue injury scale was devised which included four categories of injury. Histologically, injury was defined as the presence of necrosis within the cross section of the ureteral wall and the grade of injury was measured by the level of necrosis within the wall. The degree of inflammation or hemorrhage was not estimated due to the lack of an intact blood supply in this *ex vivo* model. At the present time, this grading scale has not been verified with chronic *in vivo* studies. We are therefore assuming that a positive correlation exists between an increasing grade of tissue injury and the potential for long-term complication. Since grade 0 and grade 1 injuries only involve the superficial layers of the ureter, normal tissue healing should occur without scarring. These injuries have therefore been classified as a low grade of injury. Conversely, grade 2 and grade 3 changes consist of damage into the muscular layers of the ureter and represent an increased level of trauma. These grades were categorized as high grade tissue injury because they at least appear to have the potential to cause late complications. However, all of the grade 2 and 3 injuries were focal in nature. In actuality they may be no more traumatic than a perforation secondary to a ureteral catheter or guide wire. Therefore, these estimations of tissue injury are likely on the conservative side.

To our knowledge, this type of injury grading scale has not been previously used to quantify ureteral injury in an *ex vivo* animal model. Watson and associates (Watson, 1987a) developed a similar system in an *in vivo* pig model. In order to assess the safety of the pulsed dye laser for fragmenting ureteral calculi, ureteral cross sections were histologically analyzed following laser induced shock wave lithotripsy and graded

according to four levels of microscopic injury. These levels were defined based on the degree of inflammation present in the wall of a treated ureter. This grading system also was not validated through chronic studies.

As new diagnostic and therapeutic modalities become available for the intracorporeal management of ureteral pathology, animal studies will be required to assess the safety of these new techniques. A standardized tissue injury scale would be desirable to objectively measure tissue effects. The grading scheme described here appears to correlate with the level of thermal energy since pulse frequency was associated with higher grades of tissue injury (vide infra). If the system is shown to be consistent in an *in vivo* setting, it could easily be adapted to the study of other intracorporeal devices which have the potential to damage tissues with a thermally-mediated mechanism.

Of the 42 ureters containing UA, COM, or CPD stones, 37/42 (88.1%) had low grade tissue injury and 5/42 (11.9%) had high grade damage following multi-YAG laser lithotripsy. As noted previously, the five ureters with high grade injury all received focal damage only. In fact, even the low grade injuries were focal with no evidence of diffuse or circumferential trauma,. The focality of the tissue injury is consistent with our subjective impression that ureteral wall damage seemed to occur when attempting to fragment the smaller residual particles of stones. In actual clinical practice, instead of trying to ablate these smaller fragments with the laser, one would be more likely be extract them with grasping forceps or allow them to pass spontaneously These particles were fragmented with the laser in order to standardize the lithotripsy treatment for the different experimental levels. Therefore, these results suggest that fragmentation of ureteral calculi with the multi-YAG laser can be performed safely without consistently causing high grade tissue injury since the frequency of higher grade tissue injury may be slightly over-

estimated in this study. In addition, if higher levels of injury do occur, they appear to be focal in nature with only a small area of damage resulting.

Logistic regression analysis did not reveal any significant predictors of tissue injury associated with multi-YAG laser lithotripsy. However, compared with the variables of pulse energy, total energy required for fragmentation, and the stone weight, the pulse frequency began to approach statistical significance ($p=0.1491$) suggesting an association between higher grade tissue injury and increasing pulse frequency. If the tissue injury data is examined with respect to the pulse frequency (Figure 3.4), it reveals that of the five ureters with high grade injuries, four occurred at a pulse frequency of 15 Hz. Only one ureter sustained this higher degree of trauma when a pulse frequency of 10 Hz or less was used. This level of statistical significance implies that there is a fifteen per cent chance that these results are due to chance alone, but an 85 per cent likelihood that the result is genuine. Because the outcome variable is high grade ureteral tissue injury, and one that should be avoided at all costs, we believe that this p-value should be considered to be clinically significant. Therefore, we feel that this evidence is strong enough to state that multi-YAG lithotripsy should only be performed with the low pulse frequencies such that all discharged energy is directed at the calculus. Pulse frequencies greater than 15 Hz may be associated with a clinically significant increase in high grade tissue injury. The previous *in vitro* studies have shown no increase in fragmentation efficacy with increasing frequency, therefore using a value of 5-10 Hz would be appropriate. These studies also revealed that the efficacy of fragmentation decreased as the pulse frequency was increased at the higher pulse energy settings. This paradoxical decrease in efficacy was felt to be related to the size of the particles being fragmented by the laser. As the residual stone particles became smaller, it was more difficult to keep the laser focused on the stone fragments and some of the laser energy was being lost to the surrounding environment. This may explain the reason for the increased thermal injury that occurred with an

increased pulsed frequency. This is again consistent with our subjective observations where gross injury to the ureter seemed to occur when smaller fragments were being treated.

Thermal injury associated with increased pulse frequency may also be related to the pulse duration of the multi-YAG laser. Anderson and Parrish showed that the shorter the pulse duration of a laser, the more the laser energy will be confined to a progressively smaller target area (Anderson, 1983) and this is linked to the thermal relaxation time of the target chromophore. The thermal relaxation time is defined as the time required for heat generated from absorbed laser light by the chromophore to cool to one half of the original value immediately after the laser pulse (Nelson, 1991). Therefore, if a laser pulse is in the same order or shorter than the thermal relaxation time of the target chromophore, the spatial confinement of heat will be maximized. The pulse duration of the multi-YAG laser is 650 μsec. Compared to the nanosecond range of the Q-switched Nd:YAG laser or the microsecond domain of the pulsed dye lasers, the multi-YAG laser will thus have less spatial selectivity with respect to its thermal energy and relatively more of this energy will diffuse away from the original target area. According to our findings, this may become more important as the frequency is increased and/or smaller particles of stone are fragmented. As the pulse frequency is increased from 5 Hz to 15 Hz, the time between laser pulses decreases three and a half times from 250 μsec to 70 μsec. Although 70 μsec should still be longer than the thermal relaxation time of the stone, and therefore allow enough time for cooling, there may be enough diffusion of thermal energy to cause a higher incidence of thermal tissue damage. Also, when smaller stone fragments are treated, there is less stone mass to absorb this thermal energy and this may also account for increased diffusion of energy to the tissues causing injury.

Cystine stones were easily fragmented with the multi-YAG laser. However, even though the grade of tissue injury that occurred in these ureters was quantitatively similar to the other stones, two of the ureters sustained diffuse, circumferential tissue damage as opposed to the focal injury seen previously. Also, as these stones were fragmented, charring of the stone surface was noted, especially at 15 Hz, and there was often a burning odor that could be detected during treatment. It is possible that because of the organic nature of cystine stones, they may absorb more heat and act as thermal conductors. Ablation of these calculi appears to be associated with increased heat dissipation resulting in a more diffuse thermal injury. Although these are rare stones, further investigation into this phenomenon is warranted.

The fact that high grade tissue injury was sustained in all the ureters that were treated as worse-case scenarios is not unexpected. The multi-YAG laser is designed to be a surgical cutting laser when used in the 1440 nm wavelength mode. We believe that the multi-YAG laser fragments stones mainly through a thermally-mediated mechanism as water in the stone absorbs the 1440 nm laser light. Therefore, these interactions which bring about effective stone fragmentation are the same interactions that could cause tissue ablation if caution is not employed. These findings emphasize that photofragmentation of calculi with the multi-YAG laser must be performed with the fiber in contact with the stone at all times and with adequate visualization. When these principles are not followed, the risk of tissue damage is quite high.

Despite the risk for tissue injury with the multi-YAG laser, there are some important advantages with this system which justify further study in an *in vivo* setting. First, the multi-YAG laser is able to fragment all types of urinary calculi. This means that even those stones which have been historically the most difficult to treat, should not pose a problem during lithotripsy. This eliminates the need to diagnose the stone type prior to

treatment. Finally, the dual wavelength capability of the multi-YAG laser make this a multi-purpose laser available for many medical applications. In this day of fiscal restraint, this is an important factor to consider when choosing new equipment for surgical therapy.

3.5 Conclusions

The acute effects of multi-YAG laser lithotripsy were studied in an *ex vivo* animal model. Moderate to high grade tissue injury is possible if the laser fiber is intentionally discharged against the ureteral wall. Therefore, one would expect the same grade of tissue injury in the clinical situation if there is inadvertent exposure to the ureter with the laser. However, if care is taken to fragment calculi with adequate visualization and with the fiber in contact with the stone, lithotripsy with the multi-YAG laser can be performed safely with only low grade tissue changes resulting. The pulse energy used for fragmentation did not correlate with increased injury but an increase in pulse frequency did appear to cause higher grade injury to the ureter. Although these results are encouraging, further study is mandatory to assess both the acute and chronic effects of multi-YAG laser lithotripsy in an *in vivo* setting. We believe that our findings justify further assessment of this laser in live animals.

Table 3.1: Acute tissue effects in the non-operative controls, treatment controls, and "intentionally-ablated" treatment groups.

Treatment Groups	Energy (J)	Frequency (Hz)	Tissue Injury (grade)
Non-operative controls (n=2)	N/A	N/A	0
Ureteroscopy only (n=4)	N/A	N/A	0
IA-1	0.3	10	2
IA-2	0.6	10	2
IA-3	0.9	10	3

Abbreviations: IA, Intentionally-ablated

Table 3.2: Results of logistic regression analysis testing for possible predictors of moderate to high grade tissue injury using the multi-YAG laser for ureteral lithotripsy.

Variable	p-Value
Pulse Frequency	0.1491
Pulse Energy	0.4739
Total Energy Required for Fragmentation	0.8238
Stone Weight	0.6442

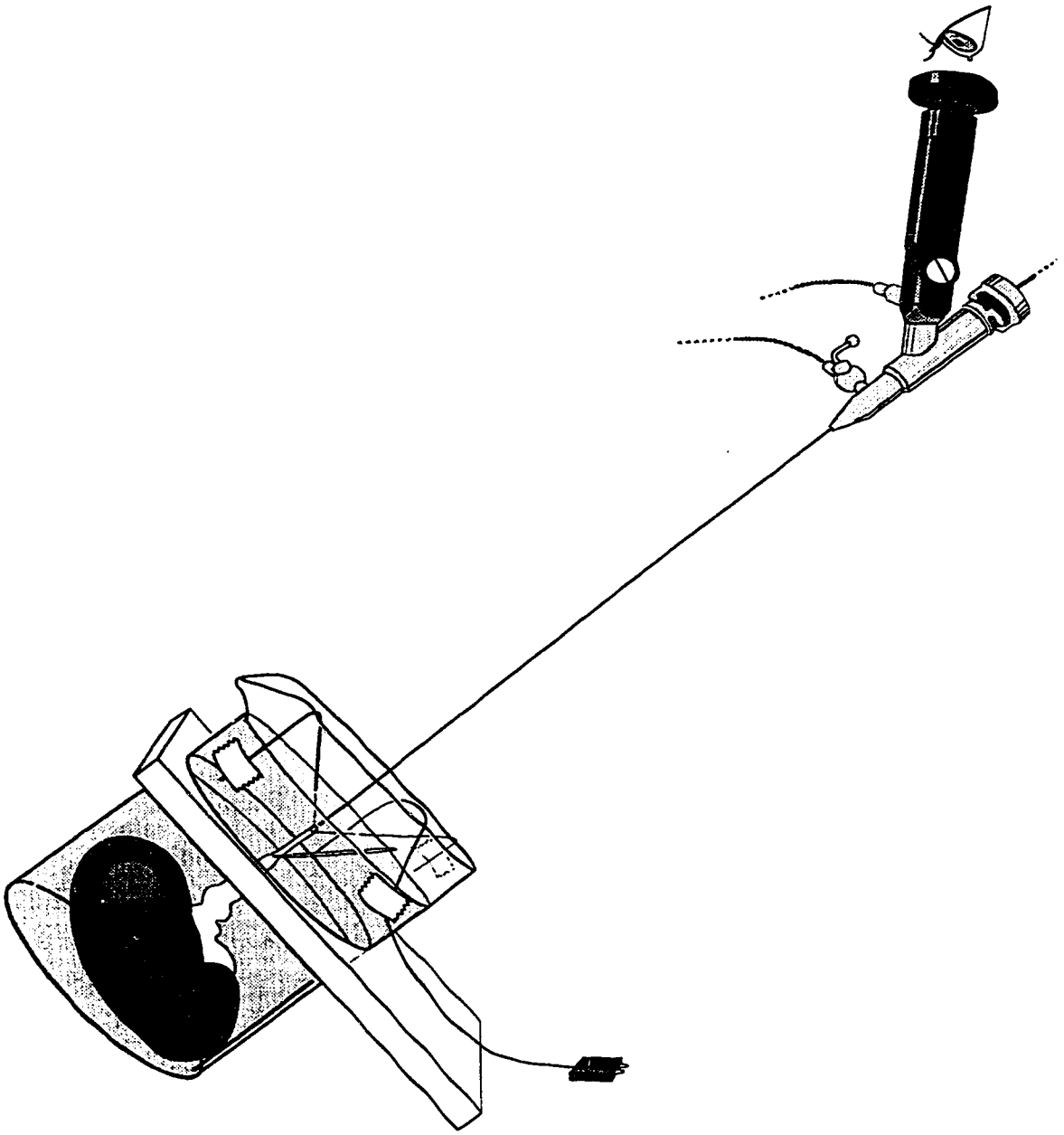


Figure 3.1: *Ex vivo* pig model for multi-YAG laser lithotripsy. Following implantation of a urinary calculus within the proximal ureter and placement of thermocouples in the peri-ureteral tissue, the renal unit was suspended in heat-conductive gelatin. Once set, laser lithotripsy was performed under direct visualization through an ureteroscope.

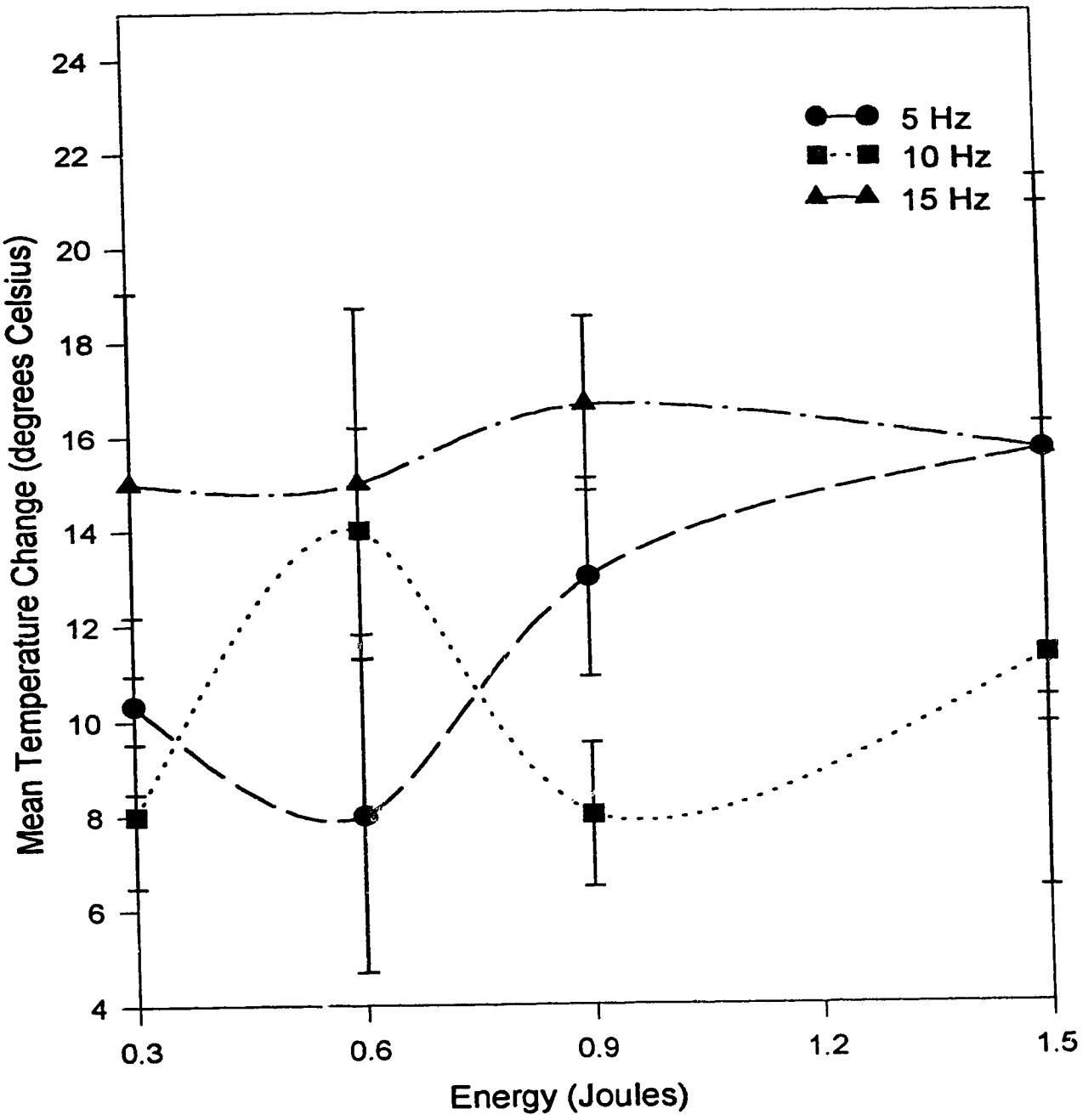


Figure 3.2: Mean temperature change associated with multi-YAG laser lithotripsy for pulse frequency as a function of pulse energy. These changes were not significantly different ($p = 0.152$ for pulse frequency and $p = 0.771$ for pulse energy).

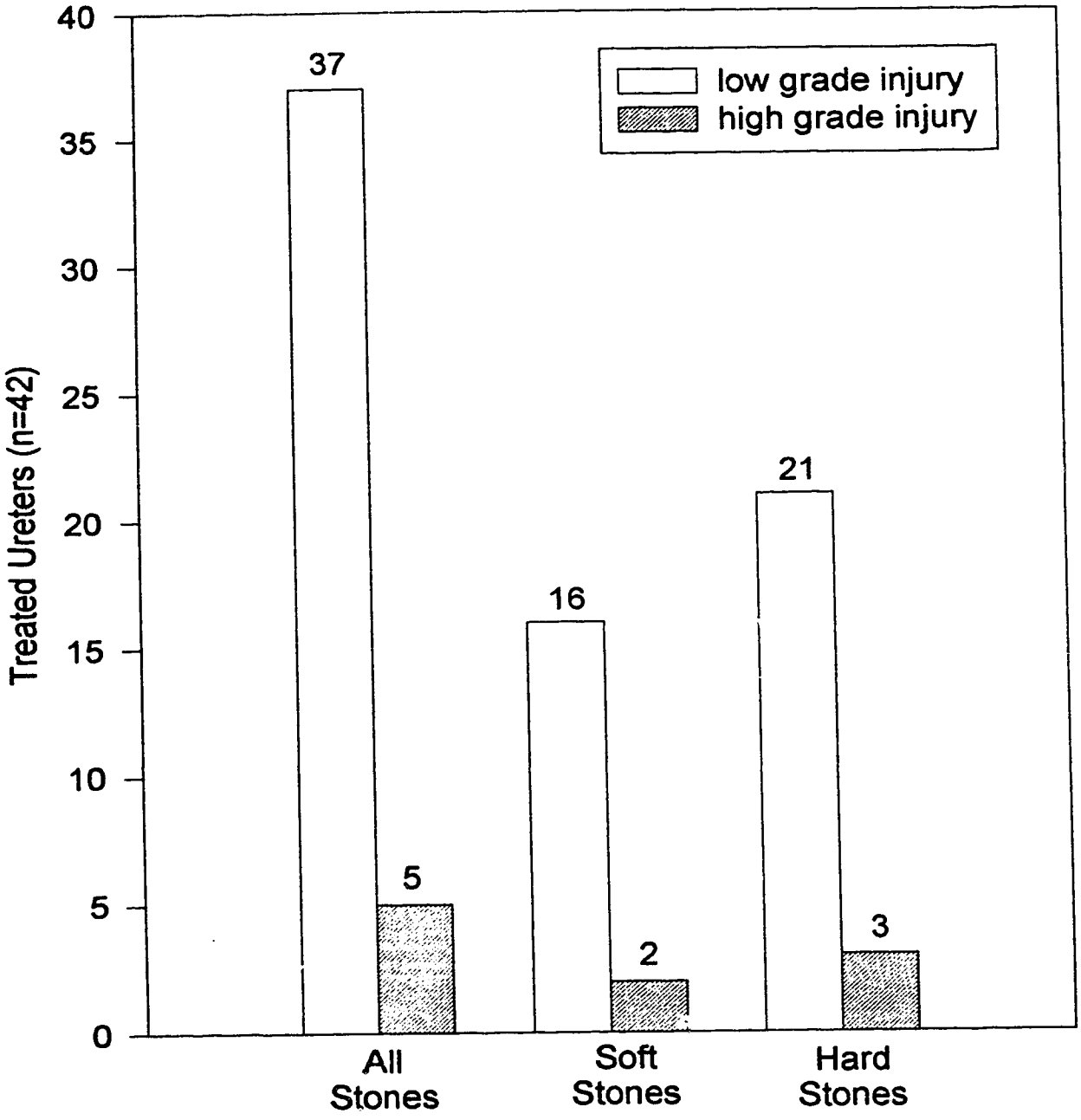


Figure 3.3: Acute ureteral tissue injury associated with multi-YAG laser lithotripsy performed in an *ex vivo* model.

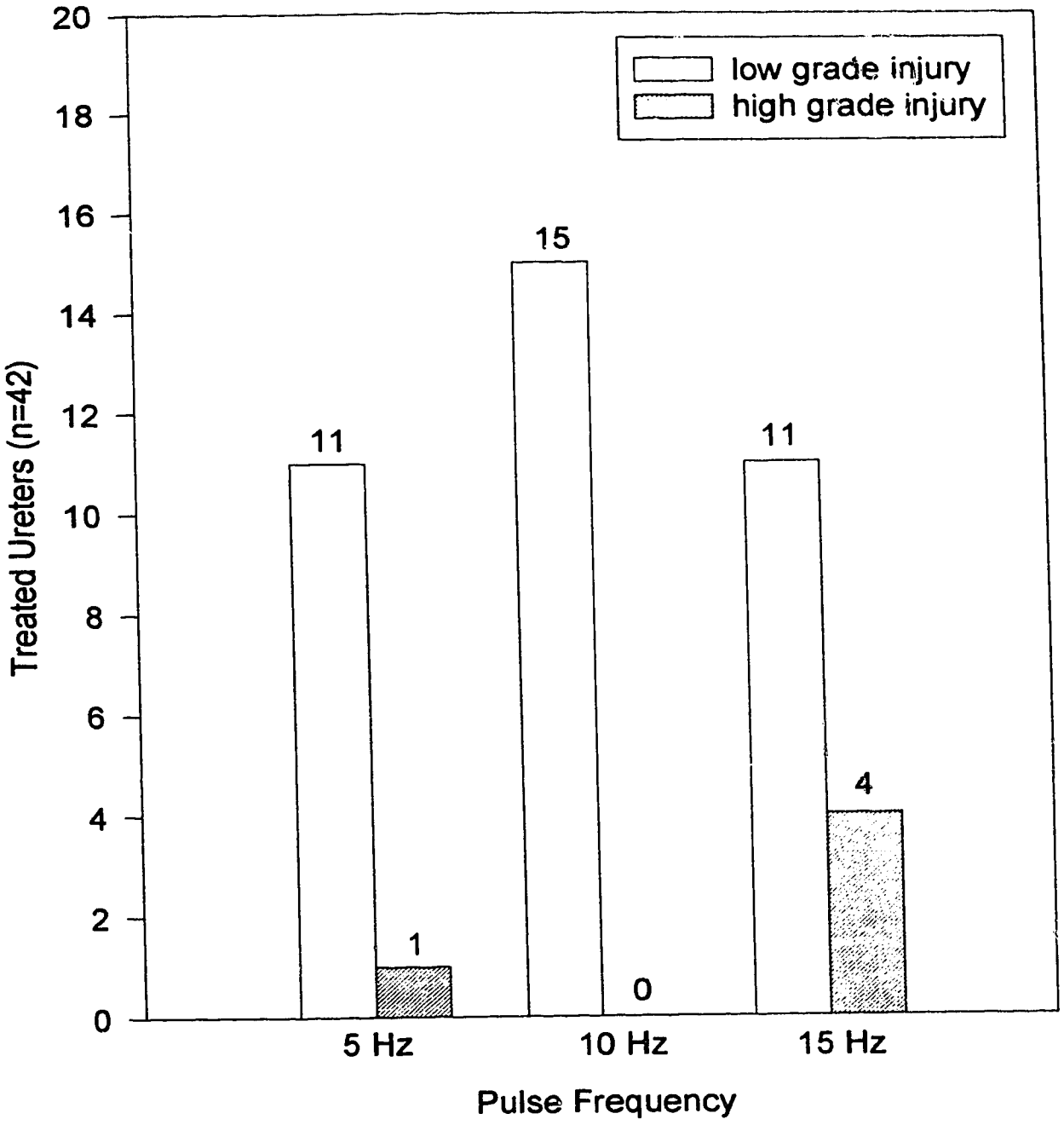


Figure 3.4: Acute ureteral tissue injury associated with multi-YAG laser lithotripsy plotted as a function of the pulse frequency.

A

B

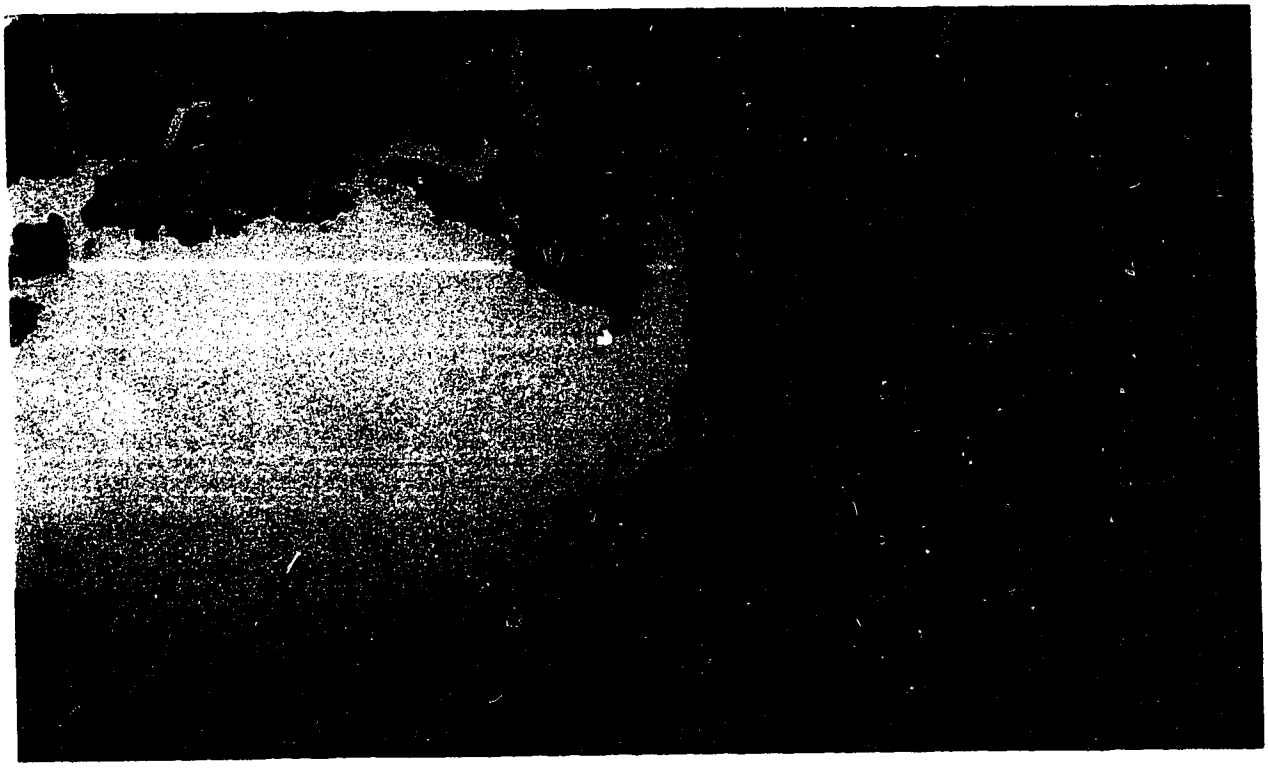


Plate 3.2: *A*, Grade 2 tissue injury in an *ex vivo* ureteral segment (necrosis involving up to two thirds of ureteral wall); magnification 4x. *B*, Grade 2 injury; magnification 10x. Grade 2 injury was classified as moderate grade trauma.

Plate 3.3 Grade 3 tissue injury in an *ex vivo* ureteral segment (transmural necrosis or perforation of the ureteral wall); magnification 10x. Grade 3 injury was classified as high grade trauma.

Chapter 4

Assessment of Multi-YAG Laser Lithotripsy in an *In Vivo* Pig Model

4.1 Introduction

Using an *ex vivo* pig model, we have demonstrated that multi-YAG laser lithotripsy can be performed safely within the ureter when the laser fiber is kept in direct contact with the stone during treatment. Although we felt our *ex vivo* model functioned well, there was no blood supply and the *ex vivo* ureter itself was simple to ureteroscope because it remained in a fixed position. Therefore, the acute inflammatory response of this treatment plus the effect of adding the technical response of *in vivo* ureteroscopy need to be evaluated. Also, the long-term effects of multi-YAG laser lithotripsy cannot be evaluated in the *ex vivo* setting and these need to be assessed before considering its use in humans. Therefore, the objectives of this segment of the study were to investigate the acute and chronic tissue effects of multi-YAG laser lithotripsy in an *in vivo* animal model.

4.2 Materials and Methods

4.2.1 Animal Species

Nine female pigs (domestic swine) weighing between 30 and 40 kg were used for this study. All animals were cared for at the Health Sciences Laboratory Animal Services facilities at the University of Alberta. The study protocol was approved by the University of Alberta's Health Sciences Animal Welfare Committee, and we adhered to the guidelines established by the Canadian Council on Animal Care for the care and use of experimental animals (Olfert, 1993).

4.2.2 Experimental Method (Operative Procedure)

Each animal was initially sedated with a pre-medication of intramuscular butorphanol (Torbugesic, Ayers Laboratories, Montreal, PQ, Canada, 0.2 mg/kg), ketamine hydrochloride (Ketalean, MTC, Cambridge, ON, Canada, 11 mg/kg), xylazine (Rompun, Haver Pharmaceuticals, Etobicoke, ON, Canada, 2.2 mg/kg), and glycopyrolate (Robinul, Sabex, Montreal, PQ, Canada, 0.01 mg/kg). The animal was placed supine on the operating table and inhalational isoflurane (AErrane, Anaquest, Mississauga, ON, Canada, 1.5-2.5%), was administered for maintenance anesthesia. The upper and lower limbs were secured to the table and then the abdomen and perineum were prepared with iodine solution (Betadine, Purdue Federick, Toronto, ON, Canada) and covered with sterile towels and drapes. Cefazolin (Ancef, Smith Kline Beecham, Oakville, ON, Canada) 250 mg intramuscular was given pre-operatively for antibiotic prophylaxis. This dose was repeated at eight hours intervals and continued for 24 hours post-operatively.

A lower midline skin incision was made with a scalpel and deepened into the peritoneal cavity. A 7.5F Storz semi-rigid ureteroscope (Karl Storz Endoscopy- America, Inc., Culver City, CA) was then used to inspect the bladder and identify the ureteral orifices. A 0.028" floppy guidewire was positioned up one of the ureters and the ureteroscope then removed. After placing a self-retaining retractor in the incision and packing off the large and small intestines, the appropriate kidney and upper ureter were exposed and the ureter was isolated with a penrose drain. Two stay sutures of 4-0 chromic were placed on the medial and lateral margins of the renal pelvis and a pyelotomy was performed using a #12 scalpel and Pott's dissecting scissors. The previously placed ureteral guide wire was grasped and its proximal end was brought out through the pyelotomy incision. The ureter distal to the pyelotomy was then progressively dilated over the guidewire from 8F to 14F using Teflon ureteral dilators (Cook Urological Inc., Spencer, IN). A human urinary calculus was placed into the ureter and impacted approximately 1.5 to 2.0 cm distal to the

pyelotomy site. Stone width ranged from 3.0 mm to 4.1 mm and were provided by Louis C. Herring and Company (Orlando, FL). Stones were composed of a mixture of calcium oxalate monohydrate and calcium oxalate dihydrate. Analysis was performed by an integrated crystallographic assessment at Louis C. Herring and Company. All the calculi were given 432,000 rads of gamma irradiation preoperatively to effect sterilization. To prevent proximal migration of the calculus, and also to isolate the laser treatment area, a moistened umbilical tape was used to gently constrict the ureter proximal to the stone. In three of the cases, a 33-gauge thermocouple (Omega Engineering, Inc., Stamford, CT) was placed in the ureteral wall adjacent to the calculus to measure the changes in temperature associated with laser photofragmentation.

Ureteroscopy without dilatation of the ureteral orifice was performed on the affected ureter until the stone was visualized. Under direct visualization, laser lithotripsy was performed with the Zeiss OPMILAS 144/60 Plus Surgical Laser System (Carl Zeiss, Inc., Princeton, NJ) and a 400 μm laser fiber (3M Specialty Optical Fiber FT-400-LMT, Auriga Fibre Optics, Thornhill, ON, Canada). All the procedures were carried out by the same operator (TW). Our previous *ex vivo* studies revealed that a pulse frequency of 15 Hz was associated with a greater incidence of ureteral injury during lithotripsy. Therefore, the pulse frequency during this study was maintained at the lowest setting of 5 Hz. Pulse energies of 0.9 to 1.2 J were used for fragmentation. Typically, 1.2 J was used initially to break the calculus into two to three pieces and 0.9 J was used subsequently to ablate these smaller particles. The pulse duration in this laser system is constant at 650 μsec. All photofragmentation was performed under direct visualization and treatment was considered complete when all residual stone particles could be flushed from the ureter with the ureteroscope. The ureter was irrigated continuously with 0.9 per cent normal saline during laser treatment.

Following fragmentation, the ureteroscope and constricting umbilical tape were removed and the area of treatment was marked by placing a 4-0 silk suture in the adventitia of the ureter. A 6 or 7F ureteral stent (Mentor, Goleta, CA) was then placed in the ureter. The pyelotomy was closed over the stent with a running and locking 4-0 chromic suture. The operative site was not drained. The animal's bladder was inspected with a 17F cystoscope to confirm appropriate placement of the stent and the bladder was then emptied. The fascial and muscular layer of the abdominal wall were closed with running 2-0 polyglycolic acid suture. The subcutaneous tissue was reapproximated with running 3-0 polyglycolic acid suture and the skin was closed with a running subcuticular stitch also using 3-0 polyglycolic acid. The animal was recovered from its anesthetic and was then transported to the housing unit for post-operative care and monitoring. Pain was controlled with intramuscular butorphanol (Torbugesic, Ayers Laboratories, Montreal, PQ, Canada, 0.4 mg/kg). This was administered as necessary for approximately 24 hours post-operatively. Food and water was started twelve hours post-operatively.

4.2.3 Treatment Groups

The acute effects of multi-YAG lithotripsy on ureteral tissue were studied in three animals (H-8, H-9, H-10). Laser lithotripsy was performed in five out of a total of six ureters. One of the animals had a stone impacted in the contralateral ureter and underwent ureteroscopy only. This specimen acted as an operative control to assess the amount of inflammation sustained from the pyelotomy, placement of a calculus, and ureteroscopy. In each case the animals were recovered for 24 hours and then euthanized with pentobarbitol (Euthanyl, MTC, Cambridge, ON, Canada, 20 cc). The remaining six animals (H-2, H-3, H-4, H-5, H-6, H-7) were recovered for at least six weeks in order to assess the chronic effects of therapy. Prior to euthanasia with pentobarbitol (Euthanyl, MTC, Cambridge, ON, Canada, 20 cc), these chronic animals had their ureteral stents removed and underwent retrograde pyelography under inhalation isoflurane anesthesia (Aerrane,

Anaquest, Mississauga, ON, Canada, 1.5-2.5%) to assess the status of the involved ureteral lumen.

4.2.4 Histologic Analysis

At the time of euthanasia, necropsy was performed and the involved ureteral segment and a segment of the corresponding distal ureter were harvested. The distal ureteral segment served as a treatment control to assess the effect of ureteroscopy by itself on the ureteral wall. Specimens were placed in 10 per cent buffered formalin solution and then paraffin processed, embedded, cut, and stained with routine hematoxylin and eosin for histological analysis. The proximal ureteral segment was sectioned both proximal to the marking suture and distal to it. In this way, the individual effects of the pyelotomy incision and the laser treatment could be assessed. Each specimen was examined histologically and graded according to a predetermined tissue injury scale. Analysis was performed by one pathologist who was blinded to the treatment group of each specimen. For the acute ureters, grade 0 was defined as intact or denuded mucosa with no necrosis or inflammation present. Grade 1 consisted of mucosal necrosis and/or inflammation involving up to one third of the ureteral wall only. Grade 2 injury was necrosis and/or inflammation involving two thirds of the ureteral wall and grade 3 changes consisted as transmural necrosis and/or severe transmural inflammation of the ureter. For the chronic specimens, the numeric scale was the same but the ureters were graded according to the presence of fibrosis instead of necrosis. Re-epithelialization, squamous metaplasia, mucinous metaplasia, and the presence or absence of a patent ureteral lumen were also evaluated. Like our previous ex vivo investigations, grade 0 and 1 were classified as low grade tissue damage and grade 2 and 3 injuries were categorized as a moderate and high degree of trauma respectively.

4.3 Results

4.3.1 Subjective Observations

All calculi were successfully fragmented with the multi-YAG laser in this *in vivo* experimental setting. Like our previous *in vitro* and *ex vivo* investigations, fragmentation was accomplished by placing the fiber against the stone and firing the laser. Short bursts of laser discharge were used because visualization became limited as a result of the debris emitted from the stone surface. This cleared rapidly as a result of the continuous irrigation allowing resumption of lithotripsy treatment.

Laser lithotripsy was technically more difficult to perform in this *in vivo* model compared to our *ex vivo* experience. The ureteral caliber in these 30-40 kg animals was noticeably narrower than the ureters obtained from the slaughterhouse animals making ureteroscopy more challenging. The tortuosity and redundancy of the porcine ureter also added to the technical demand of the procedure. Time spent doing the lithotripsy treatment ranged from fifteen to fifty minutes. The rate limiting factor was not the laser treatment itself but efforts to maintain the stone in direct visualization to avoid discharging the laser into the ureteral wall. Any mucosal tears or abrasions that were sustained during the process of implanting the calculus or performing ureteroscopy, decreased the visibility of the stone and made the overall procedure much longer. When the stone was in good position it was very easy to place the laser fiber on the stone and perform the lithotripsy in a controlled fashion. At a frequency of 5 Hz there was very little movement or propulsion of the stone within the ureter. However, the procedure became increasingly difficult as the stone broke apart and the smaller pieces fragmented.

Our first chronic animal sustained significant intra-operative complications secondary to the urteroscopy and laser lithotripsy. There were multiple focal and partially

circumferential burns in the ureteral wall and the ureter was grossly perforated with the ureteroscope. This animal was therefore considered to be a "worst-case scenario".

In general, all of the chronic animals had an unremarkable post-operative recovery with no evidence of sepsis, renal colic, or urine leak. One animal (H-5) experienced respiratory difficulties intra-operatively and post-operatively with symptoms and signs of pulmonary congestion. The animal responded to intramuscular furosemide and subsequently recovered without difficulty. We do not attribute this complication to be a result of the multi-YAG lithotripsy. Another animal (H-2) lost her stent on the second post-operative day but had no acute complication as a result of this. H-3 also lost her stent at some point since it was absent at the time of retrograde pyelography.

4.3.2 Temperature Change Associated with *In Vivo* Laser Lithotripsy

The minimum, maximum, and mean change in temperature associated with multi-YAG laser lithotripsy is shown in Table 4.1. The mean change in temperature for all three treatments was 20.67 °C and the highest temperature measured during any of the treatments was 43 °C.

4.3.3 Acute Tissue Effects of *In Vivo* Laser Lithotripsy

The acute gross and histologic findings that occurred as a result of laser lithotripsy are summarized in Table 4.2 and Figure 4.1 respectively. Of the five ureters that had laser lithotripsy, 3/5 sustained low grade tissue injury and the remaining two had higher grade changes. The treatment control distal ureters in these animals revealed low grade histologic changes in 4/5 specimens and high grade damage in only one ureter. Finally, the operative control ureter had grade 0 or low grade tissue injury at the site of the impacted stone and grade 0 damage at the distal portion of the ureter.

4.3.4 Chronic Tissue Effects of *In Vivo* Laser Lithotripsy

The radiologic and gross anatomic findings are shown in Table 4.3 and the histologic results of multi-YAG laser lithotripsy are represented graphically in Figure 4.2. The retrograde pyelograms were normal in 3/6 animals (Plate 4.1) and considered obstructed or partially obstructed in the remaining animals. Two of three obstructed pyelograms occurred in the animals whose stents were lost post-operatively (H-2 and H-3). For H-2, only one film was obtained because of technical difficulties. Contrast was present up to the proximal ureter but no definite narrowing was seen (Plate 4.2*A*) Because of this equivocal result we considered this to be obstructed. In the case of H-3, contrast was seen to the level of the ureteropelvic junction on the first film (Plate 4.2*B*). However, because the proximal ureter was extremely tortuous, more contrast was injected. On the second film, contrast was visible in the renal collecting system. The initial hold up of contrast may simply be a result of the tortuosity of the ureter. However, we cannot be sure of this and have therefore read the result as at least a partial obstruction. The third animal, H-5, also had free flow of contrast up a somewhat tortuous ureter with only a small amount of contrast entering the renal pelvis (Plate 4.3). Once again, we have diagnosed this as a partial obstruction.

Five animals had low grade injury at the site of laser lithotripsy with no microscopic evidence of a stricture. One animal had grade 3, or full-thickness fibrosis, with narrowing of the lumen. However, this result represented only one tissue section out of nine sections taken from that site. The other sections showed grade 0 or 1 injury with a widely patent ureteral lumen. There was also abundant foreign body giant cell reaction in the adventitia of the ureter in this one section suggesting the presence of suture material. Therefore, this one section most likely represents a cut through the distal margin of the pyelotomy as it approached the laser treatment site. All the distal ureteric margins were found to have low

grade tissue injury scores of 0 and 1. One animal (H-3) was felt to have narrowing of the distal ureter microscopically, however, there was no evidence of this on the retrograde pyelogram. In none of the acute or chronic animals were splinters from the laser fiber detected on histological analysis.

4.4 Discussion

Our investigations using the multi-YAG laser for intra-ureteral lithotripsy in an *ex vivo* animal model demonstrated that this form of therapy could be done safely when the laser energy was directed and kept in contact with the stone. An elevated pulse frequency and briefly discharging the laser against the ureteral wall were associated with an increased incidence of high grade tissue injury. Although the pulse frequency was not a statistically significant predictor of high grade injury, we felt that this was a clinically significant finding and therefore performed multi-YAG laser lithotripsy in this study with a pulse frequency of 5 Hz. Pulse energies of 0.9 to 1.2 J were used because it was shown previously that these would be the most efficacious energy levels for fragmenting calcium oxalate stones while at the same time not increasing the incidence of tissue injury.

As noted above, a number of factors made laser lithotripsy technically difficult in this *in vivo* pig model. The ureteral caliber, the tortuosity and redundancy of the porcine ureter, and any complicating flaps of mucosa all contributed to the complexity of the procedure. Although some or all of these factors can be encountered in human clinical practice, we feel that ureteroscopy in this model was overall more difficult to perform than in the adult human patient. In addition, all stones were fragmented to particles less than approximately one millimeter so they could easily flush from the ureter. Fragmenting these smaller particles was not only time-consuming, but it was also difficult to perform

laser stone ablation because of targeting and increased stone movement. All these factors may have contributed to some of the tissue injury sustained during treatment. In actual clinical practice, fragments less than two millimeters would be better managed by manual extraction with grasping forceps. Employing this treatment strategy, there would likely be less tissue injury.

Despite these concerns, the results from both the acute and chronic animals are encouraging. The number of subjects in our study samples are small so that statistical analysis of the data is not possible. However, important information can be obtained from descriptive analysis of the specimens. Six ureters were evaluated for acute tissue injury. Histologic analysis of the laser site and the distal ureter (treatment controls) showed that low and high grade injury were present for both groups. Higher grade tissue injury was not limited exclusively to the laser treated segments. Since the distal ureteral segments assessed the tissue effects of ureteroscopy, this shows that ureteroscopy itself contributes to the overall tissue injury. Therefore, this confounds the results somewhat because we cannot assume that the histologic injuries at the lithotripsy site are solely the result of the laser energy. Some of these high grade changes may be secondary to the endoscopic instrumentation. These results are in contradistinction to our *ex vivo* results where we found that ureteroscopy was not associated with significant trauma. In this regard, our *ex vivo* model may therefore be a more sensitive and appropriate measure for the acute tissue effects of the laser by itself since the acute inflammatory effects of ureteroscopy are eliminated. These findings also remind us that ureteroscopy is not entirely benign and is itself capable of high grade tissue injury. When Watson et al. investigated the safety of the pulsed dye laser for lithotripsy in a pig model (Watson, 1987a), they found that significant inflammation and trauma was sustained at the distal ureter secondary to dilatation and ureteroscopy. They stressed in their study that to help avoid this type of injury, the safest course was to use instruments with the smallest possible diameter.

The chronic effects of multi-YAG laser lithotripsy were evaluated by retrograde pyelography and histology. According to retrograde pyelography, three of the animals had some obstruction of their involved ureter. In the first two cases (H-2 and H-3), these animals' ureters were without ureteral stents during their recovery so that stricture formation in this situation would not be totally unexpected. A pyelotomy in a 6 to 8F ureter might be able to cause significant scarring and a stricture if left unstented. However, the histology of the laser treatment and the pyelotomy segments in these animals showed grade 0 injury. In both cases, an intact mucosa or evidence of re-epithelialization was present and no significant fibrosis was seen. Since these results do not correlate with the radiologic results, it suggests that our decision to call these animals obstructed radiologically may have been overestimated. The third animal with partial obstruction on retrograde pyelography (H-5) had full-thickness fibrosis (grade 3 injury) and microscopic evidence of a stricture. However, when this histologic result was reviewed, these changes were only present in one out the nine 4 μm sections. It also appeared that this section was taken through the pyelotomy site because there was significant foreign body giant cell reaction present in the adventitia suggestive of suture material. Therefore, the site of the obstruction or stenosis most likely occurred at the pyelotomy site and not at the site of laser lithotripsy. We found that performing retrograde pyelograms in the *in vivo* setting was technically difficult and as discussed above, it may be associated with falsely positive results. One way of overcoming this problem would have been to repeat the studies *ex vivo* following necropsy.

The three remaining animals had normal retrograde pyelograms and all had corresponding low grade injury on histology with no evidence of stricture formation. Therefore, these results suggest that even though multi-YAG laser lithotripsy is capable of causing focal areas of high grade tissue damage, these injuries do not appear to have a deleterious effect

on the ureter in the long-term. Also, our suppositions in the previous chapter that the high grade, focal lesions seen in some of the *ex vivo* ureters might not be associated with long-term complications appear to be correct. These focal injuries are probably comparable to the damage sustained in clinical practice when a guidewire is perforated through the ureter. These are most often considered to be minor degrees of trauma and respond well to ureteral stenting, or sometimes no stenting at all.

This study was not specifically designed to validate our tissue injury grading scales, but some important observations can still be made. An ideal acute tissue injury scale would not only describe the acute effects of injury but would also have some important prognostic significance. Our grade 2 and 3 injuries were classified as high grade. Because these injuries involved the muscular layers of the ureter, we believed that they might be associated with long-term complications such as fibrous stricture formation. However, these acute changes did not appear to correlate with long-term complication in our chronic animals. Because of the difficulty we experienced performing lithotripsy in some of our chronic animals, we presume that some of the ureters sustained grade 2 or 3 injury acutely. Despite this, only one ureter had evidence of high grade histological damage chronically and this was exclusively on one tissue section adjacent to the pyelotomy site. Additional sections from this ureter and the remaining ureters all had low grade tissue changes with patent and re-epithelialized lumens. In order to predict long-term outcome, a more sensitive scale is likely needed. Since all our acute injuries were found to be focal in nature, our chronic results seem to suggest that high grade injuries which are focal may heal without deleterious long-term sequelae. Dense fibrosis and stricture formation probably require a more diffuse injury. Perhaps a quantitative scale taking into account the surface area and depth of acute damage might better predict this type of outcome. A chronic *in vivo* study investigating low grade, high grade, focal, and diffuse injury from the multi-YAG laser would have to be performed to answer this question. However, this

would be difficult to design because it would not be possible to know what the acute injury was exactly.

4.5 Conclusions

An *in vivo* experimental pig model was used to study the acute and chronic tissue effects of multi-YAG laser lithotripsy. Both low grade and high grade acute tissue injuries were sustained during treatment, but some of this trauma was the result of ureteroscopy. These injuries have consistently been focal in nature and did not lead to stenosis or stricture formation of the ureter at the site of the lithotripsy treatment. These results show that multi-YAG laser lithotripsy can be performed successfully and safely in an *in vivo* setting.

Table 4.1: Minimum and maximum recorded temperatures and temperature changes associated with *in vivo* multi-YAG laser lithotripsy in three animals.

Animal	Minimum Temperatue (°C)	Maximum Temperature (°C)	Temperature Change (°C)
H-6	15	40	25
H-7	21	39	18
H-9	24	43	19
Mean	20.0	40.7	20.7

Table 4.2: Summary of the acute *in vivo* effects of multi-YAG laser lithotripsy within the ureter.

Animal	Renal Unit	Gross Findings	Histology (Injury Score)
H-8	Right *	Mild hydroureteronephrosis to site of stone.	0
	Left	Hydroureter to pyelotomy site.	0
H-9	Right	Normal	1
	Left	Mild hydroureteronephrosis to pyelotomy site.	1
H-10	Right	Normal	2
	Left	Hydroureter to site of laser treatment.	3

* Acted as an operative control (stone placed in ureter but no laser lithotripsy performed).

Table 4.3: Summary of the chronic *in vivo* effects of multi-YAG laser lithotripsy within the ureter.

Animal	Retrograde Pyelogram Results	Gross Anatomic Results	Histology (Injury Score)	
H-2 (Worst-case scenario)	Possible obstruction at the ureteropelvic junction.	Hydroureteronephrosis to pyelotomy/laser site.	Laser site- Pyelotomy site-	0 0
H-3	Significant ureteral tortuosity with partial obstruction.	Hydroureteronephrosis to pyelotomy/laser site.	Laser site- Pyelotomy site-	0 0
H-4	Normal	Normal	Laser site- Pyelotomy site-	0 1
H-5	Partial obstruction.	Pyelotomy/laser site adherent to lower pole of kidney with distension to this level.	Laser site- Pyelotomy site-	2-3 0
H-6	Normal	Normal	Laser site- Pyelotomy site-	0-1 0-1
H-7	Normal	Slight hydroureteronephrosis to pyelotomy/laser site.	Laser site- Pyelotomy site-	0 0

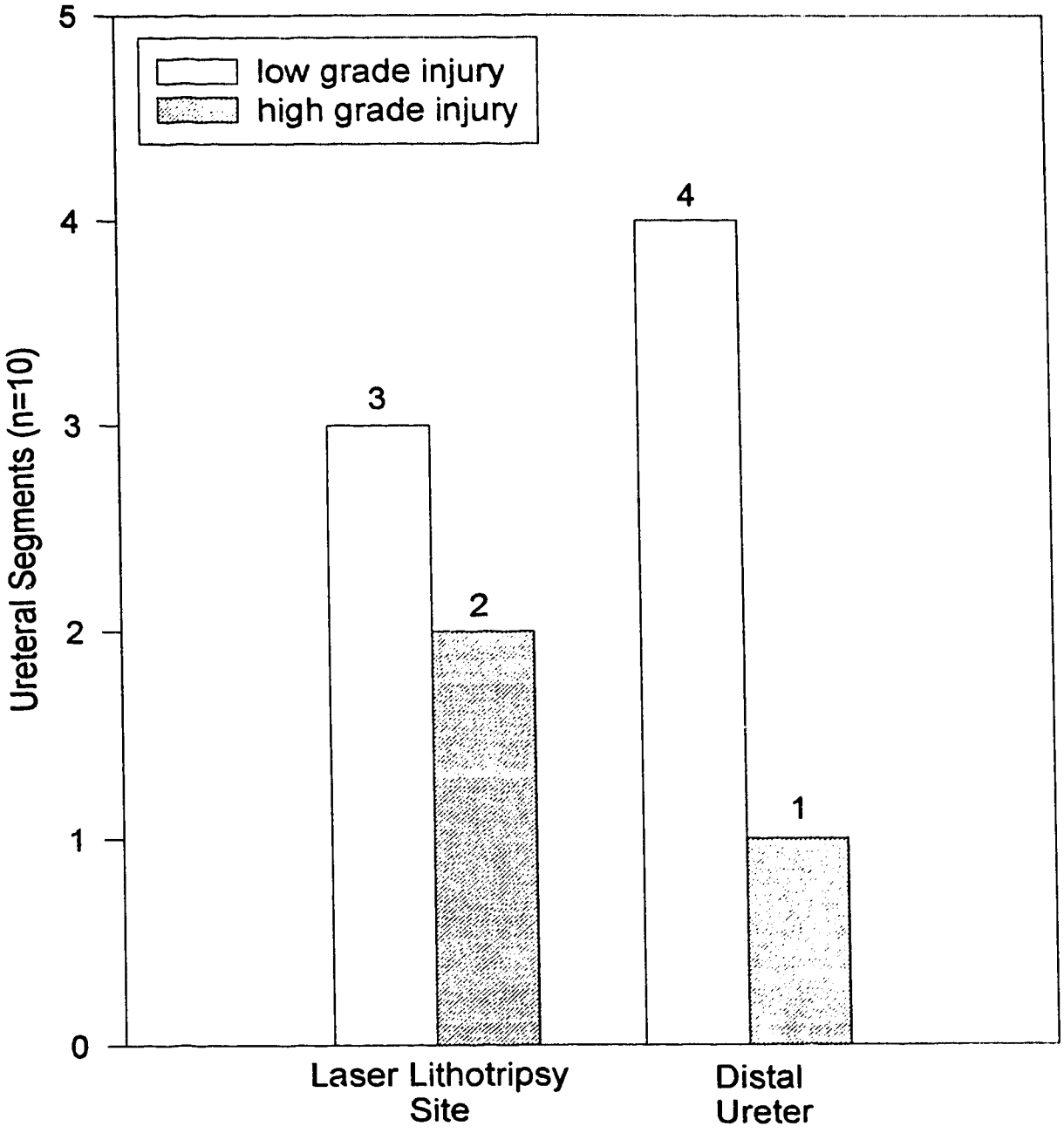


Figure 4.1: Comparison of the acute tissue injury associated with multi-YAG laser lithotripsy and ureteroscopy (distal ureter/treatment controls) in an *in vivo* pig model. Both are capable of producing high grade tissue trauma.

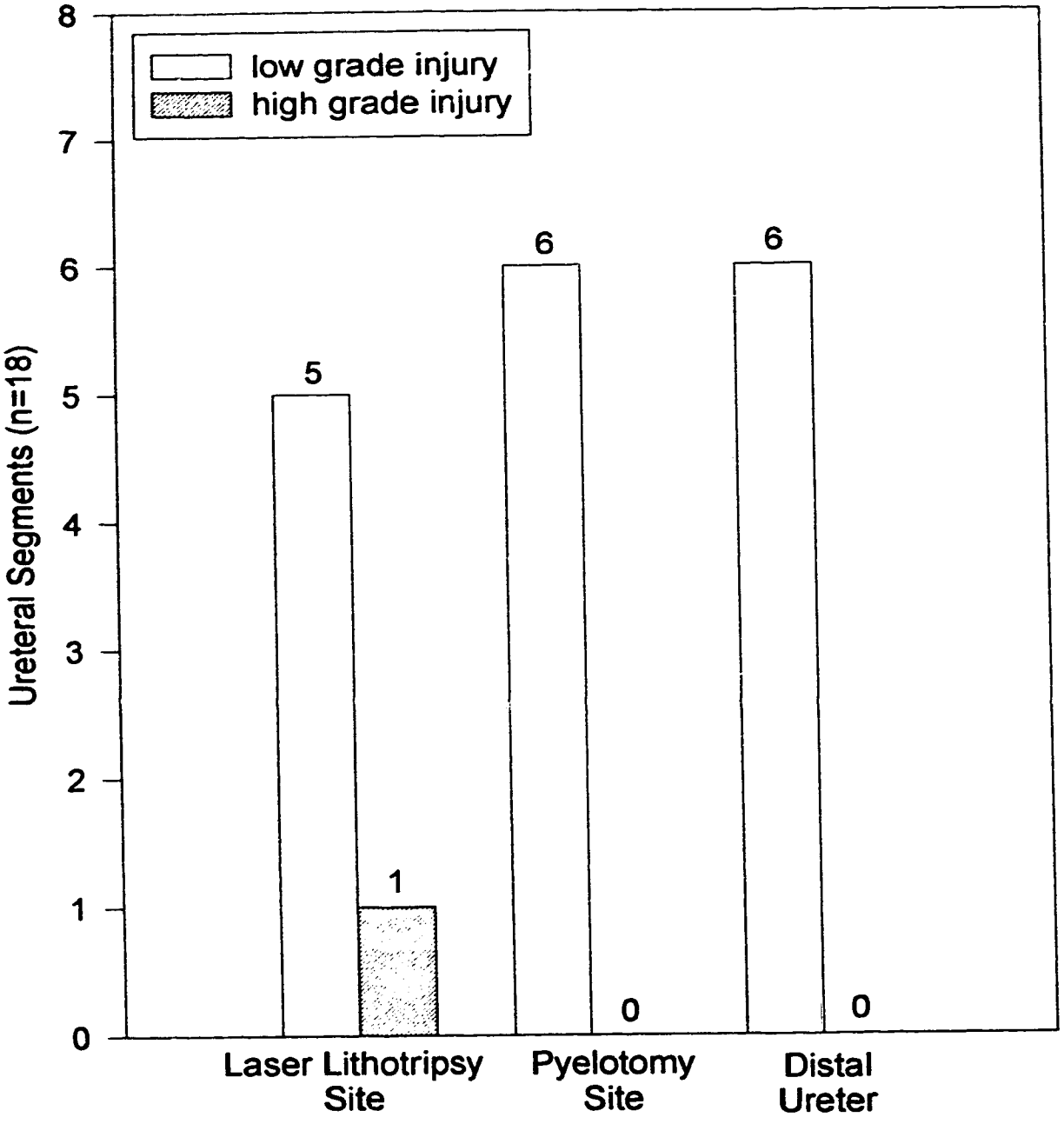


Figure 4.2: Comparison of the chronic ureteral tissue injury associated with multi-YAG laser lithotripsy, preparation of the experimental model (pyelotomy site/operative control), and ureteroscopy (distal ureter/treatment control).

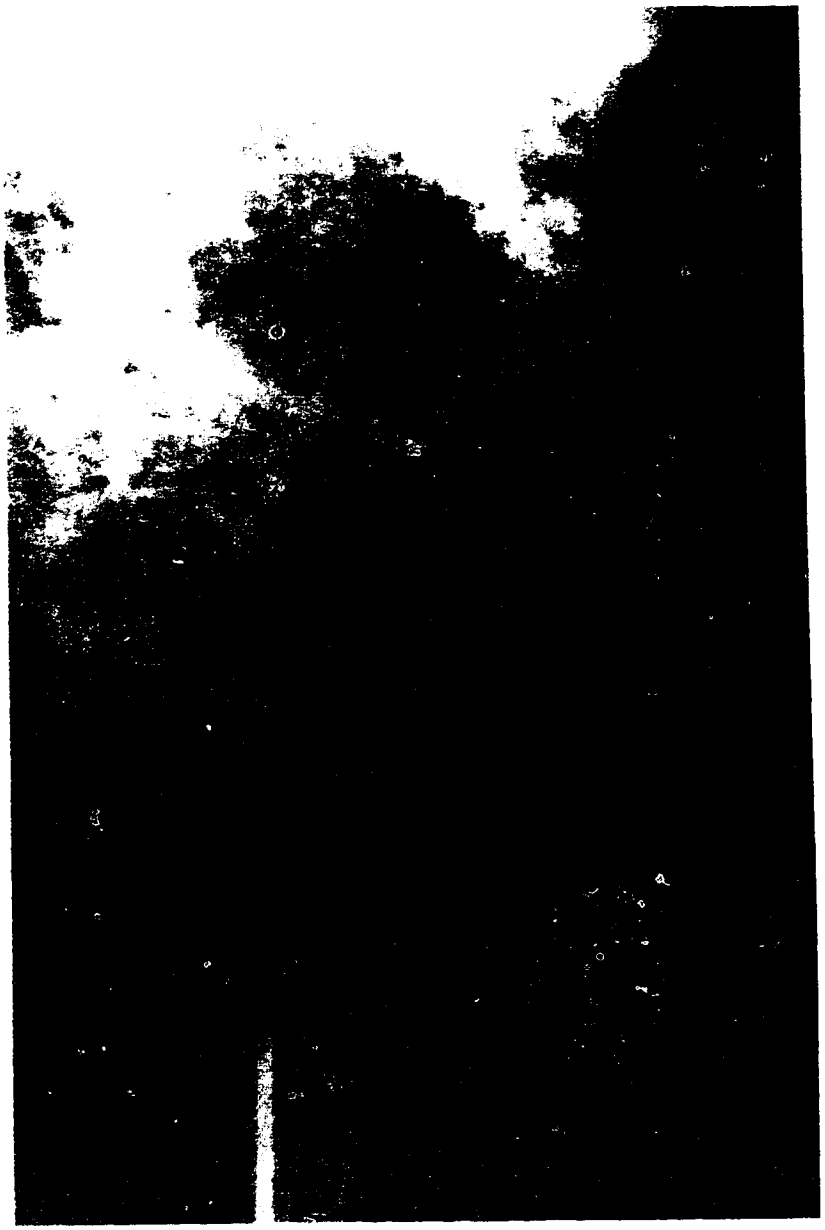


Plate 4.1: Normal retrograde pyelogram study performed on animal H-6 at seven weeks post-operatively.

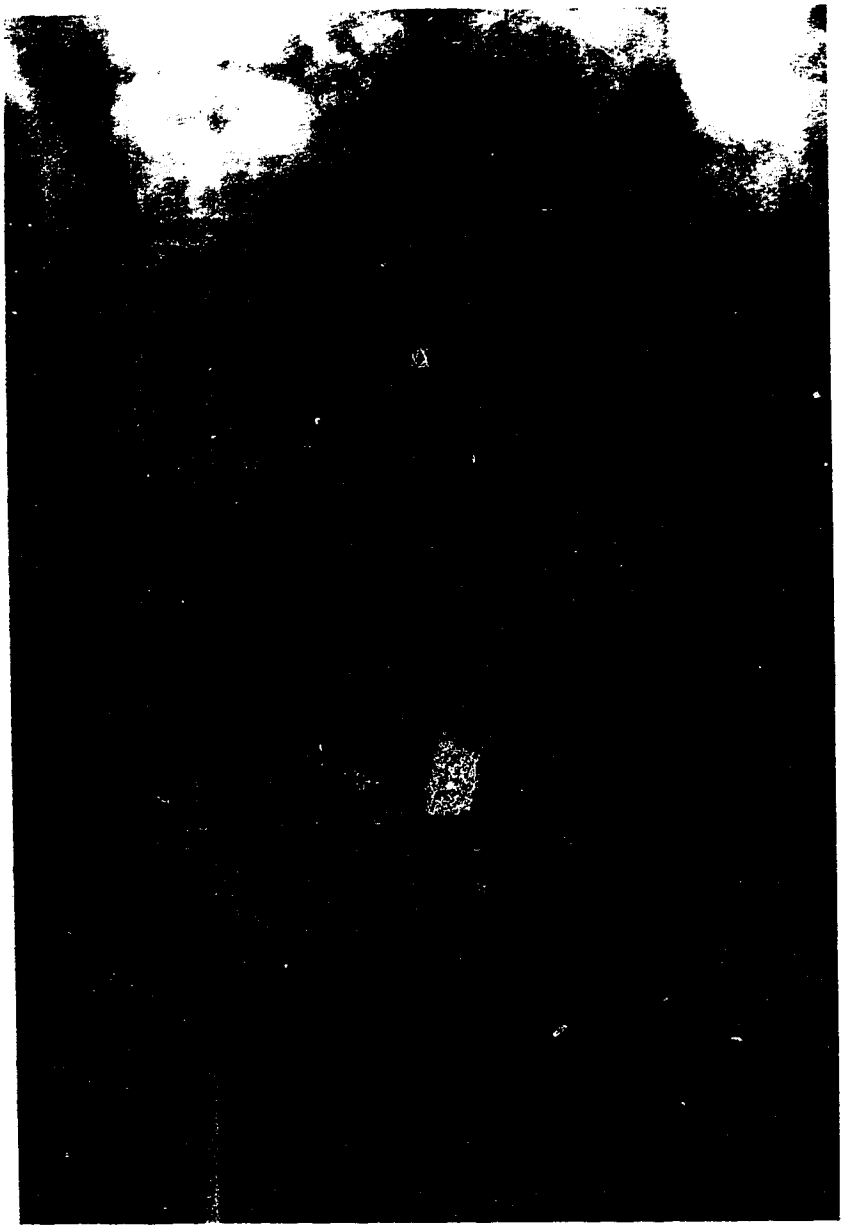


Plate 4.2: Retrograde pyelogram performed on animal H-2 at seven weeks post-operatively demonstrating a possible obstruction at the ureteropelvic junction.

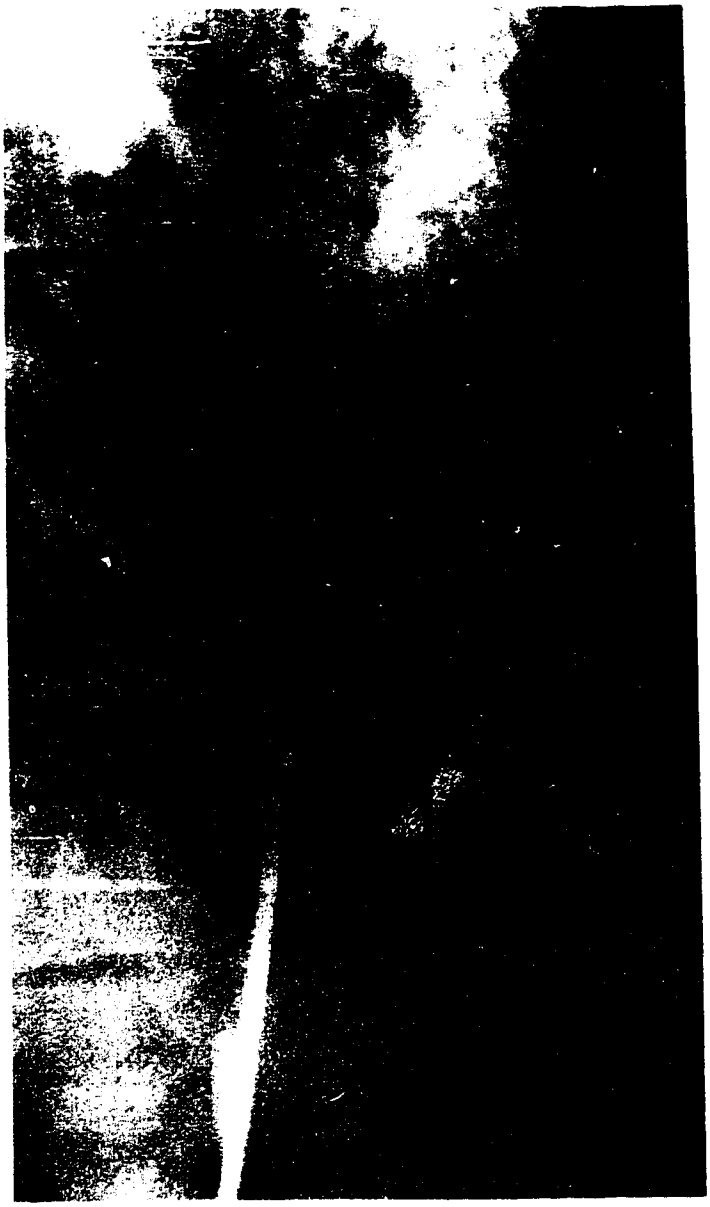


Plate 4.3: Retrograde pyelogram performed on animal H-3 at seven weeks post-operatively demonstrating a possible obstruction at the ureteropelvic junction.

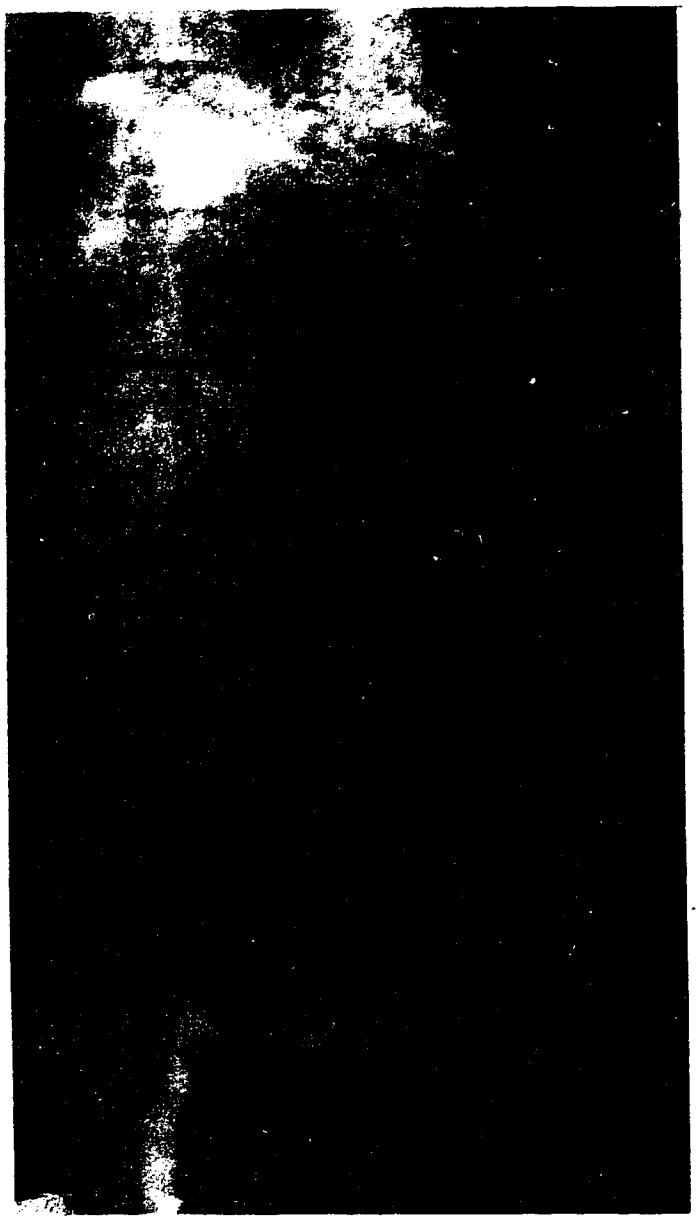


Plate 4.4: Retrograde pyelogram performed on animal H-5 at seven weeks post-operatively. Possible ureteric obstruction at the ureteropelvic junction.

Chapter 5
General Discussion and Conclusions

5.1 General Discussion

This three-part project was undertaken to assess the usefulness of the 1440 nm Nd:YAG laser for fragmenting urinary calculi. We were specifically interested in how effective and efficacious this laser would be at fragmentation and whether stone ablation could be performed within the urinary tract without causing significant injury. The most popular laser used for intracorporeal lithotripsy is the pulsed coumarin dye laser. Although this laser has a wide margin of safety, one of its drawbacks is that hard density stones, such as COM, are often recalcitrant to fragmentation. This laser has also been criticized for its lack of cost effectiveness (Segura, 1990a). It has been designed solely for laser lithotripsy, and is thus a single-purpose laser. Conversely, the multi-YAG laser is very powerful so that even the hardest stones are susceptible to fragmentation. Also, because the multi-YAG laser has two wavelengths, it allows one to cut and coagulate so that it can be used for multiple medical applications. Therefore, if it could be shown in an experimental setting that the multi-YAG laser is effective and safe for intra-corporeal lithotripsy, then human studies could eventually be performed. Ultimately, this would define another application for the multi-YAG laser, making it an even more useful tool in medicine.

This project was designed to follow a step-wise progression so that each part of the overall project used information gained from the previous experiments. The preliminary studies by Moore et al. (Moore, 1990) revealed that the multi-YAG laser could fragment synthetic calculi, but it was not known whether authentic human stones could be ablated with the same level of effectiveness. Our *in vitro* studies were therefore designed to assess how well the multi-YAG laser could fragment human stones. Assuming that human

stones could be fragmented, these investigations would also reveal the optimal parameters for stone ablation. Using these parameters, stone ablation would then be studied in an *ex vivo* animal model to assess the acute tissue changes associated with multi-YAG laser lithotripsy. Based on the wavelength characteristics of the multi-YAG laser, some tissue injury from the laser would not be totally unexpected. Therefore, it would be desirable to also know the optimal parameters for stone ablation as they relate to acute tissue injury so that this injury could be minimized. An *ex vivo* animal model would allow a large population to be studied so that these assessments could be performed and conclusions made based on statistical analysis. Finally, once the parameters for efficacious and safe fragmentation were established, an *in vivo* study could be performed to assess both the acute and chronic effects of multi-YAG laser lithotripsy. All this information could then be used to safely study multi-YAG laser lithotripsy in the clinical setting.

Our *in vitro* studies revealed that the multi-YAG laser was able to fragment all varieties of human urinary calculi. Most importantly, high density stones like COM and cystine could also be fragmented with relative ease. The parameters studied were the fragmentation efficacy (J/mg) and the fragmentation rate (mg/pulse). The most efficacious fragmentation occurred at a pulse energy between 0.6 J to 1.5 J for softer stones, and between 0.9 J and 1.5 J for harder calculi (Figures 2.2 and 2.3). In both situations, a plateau in the fragmentation efficacy was demonstrated. Efficacy did not improve beyond 0.6 J/pulse for soft calculi or beyond 0.9 J/pulse for hard stones. Conversely, the rate of fragmentation had a positive linear relationship with pulse energy for both soft and hard calculi so that the best rate of fragmentation occurred at a pulse energy of 1.5 J. Finally, the pulse frequency did not significantly affect the fragmentation efficacy or the fragmentation rate. To the busy clinical urologist interested in using laser energy for lithotripsy, the more important of these two parameters would probably be the fragmentation rate since most would want a tool that could fragment a symptomatic urinary stone in the least possible

time. Therefore, according to our *in vitro* investigations, and without considering the safety of the laser, the optimal parameters for fragmenting any variety of urinary stone with the multi-YAG laser would be a pulse energy of 1.5 J used with any pulse frequency between 5 and 15 Hz.

The *ex vivo* animal studies were meant to investigate the acute tissue injury associated with multi-YAG laser lithotripsy and to establish optimal parameters that would minimize these acute changes. Using the same range of laser settings, it was found that lithotripsy could be performed safely as long as the fiber was kept in contact with the stone at all times during laser discharge. If the laser was inadvertently fired against the ureteral wall, high grade tissue injury occurred. Statistically, the pulse energy, pulse frequency, total energy required for fragmentation, or the weight of the stone did not significantly predict the level of tissue injury. However, the pulse frequency approached statistical significance since high grade tissue injury was associated with a higher pulse frequency. Because of the potentially serious nature of causing high grade tissue injury, this was felt to be a clinically significant result. Therefore, when the *in vitro* and *ex vivo* results are taken together, the optimal parameters for multi-YAG laser lithotripsy would be a pulse energy of 0.9 to 1.5 J at a pulse frequency of 5 Hz. Using these settings should theoretically maximize the effectiveness and rate of fragmentation while minimizing the acute tissue injury associated with the treatment.

These parameters were then studied in an *in vivo* pig model to assess whether the same level of acute injury occurred in the live setting and if this type of injury was acceptable with respect to chronic tissue injury and healing. Similar to the *ex vivo* results, both low grade and high grade acute tissue injury were seen in the ureteral segments. However, it was felt that a significant proportion of the injury may have been due to the inflammatory effects of ureteroscopy and not by the laser. Chronically, no strictures or consistent full-

thickness fibrosis occurred at the laser site. Therefore, we believe that these completed experimental results, taken together, reveal that the multi-YAG laser is very efficacious and effective at fragmenting human urinary calculi; also, intracorporeal fragmentation can be performed safely with an acceptable level of acute tissue injury that does not appear to cause long-term complication.

There is a lack of standardization in the literature with respect to the definitions and units of measurement used in laser lithotripsy studies. Consequently, one can only indirectly compare our results with those of other laser systems currently in clinical use. The multi-YAG laser appears to closely resemble the holmium:YAG laser both in physics and possibly in the mechanism of fragmenting urinary calculi. This laser is also a multiple purpose, multi-wavelength, near-infrared laser which is able to emit pulsed light at a wavelength of 2100 nm. Like the multi-YAG laser, this wavelength is strongly absorbed by water. There is limited data specifically related to lithotripsy with the Ho:YAG laser, but Watson has suggested that with a pulse duration of 250 μsec, energies of 0.5 to 1.0 J are required for efficient fragmentation (Watson, 1994). He has found that this laser is able to fragment all types of urinary stones including COM and cystine and suggests that a typical regimen is to use 1 J pulses at 5 Hz with a 320 μm fiber. Our results compare favorably with this in that we also found effective fragmentation over these same pulse energies with a fixed pulse duration of 650 μsec.

As noted previously, the pulsed coumarin dye laser is the most popular clinical laser used for lithotripsy in North America. The majority of *in vitro* studies investigating the efficacy of this laser have used pulse energies ranging from 30 to 140 mJ (0.03 to 0.14 J) with a pulse duration of 1 μsec (Nelson, 1994; Wu, 1993; Atala, 1990; Bhatta, 1989; Watson, 1987b). These energies are considerably lower than what we have used to achieve effective fragmentation. Stone fragmentation would be very tedious and inefficient with

the multi-YAG laser at these same pulse energies. This difference is likely related to the pulse duration. Despite lower pulse energies, the pulsed dye laser is able to produce higher peak powers as a result of its shorter pulse length. These high peak powers lead to plasma formation and subsequent liberation of acoustic shock waves which bring about stone fragmentation. The Q-switched Nd:YAG laser operates in the nanosecond-domain and also is capable of efficient fragmentation at pulse energies of 35 to 120 mJ (0.035 to .12 J) because of plasma-mediated shock wave energy (Hofmann, 1988). However, in clinical use, both these lasers are not consistently able to fragment the hard density calculi (Begun, 1994). The multi-YAG and the Ho:YAG lasers are on average more powerful and therefore appear to be better suited to fragmenting the harder, difficult to treat stones, like COM or cystine.

Concerning acute tissue injury that can occur with laser lithotripsy, our *ex vivo* and *in vivo* results again appear to be very similar to published results using the Ho:YAG laser. For example, Piergiovanni and associates evaluated the risk of perforation within a pig bladder and ureter for the Ho:YAG laser (Piergiovanni, 1994). The laser was discharged at an energy setting of 0.5 J and a frequency of 5 Hz. They were able to perforate the ureter with 20 J of energy and the bladder with 40 J. When the laser fiber was placed along the axis of the ureter and discharged, the acute tissue effects were equivalent to our results in the "intentionally-ablated" ureters. There was complete necrosis of the epithelium and partial necrosis of the lamina propria and muscular layer. Watson has noted that although the holmium laser is able to fragment all types of urinary stones, any misdirection of the laser fiber can cause significant ureteric tissue injury (Watson, 1994). He has therefore suggested that the Ho:YAG laser is more safely used in a large, dilated ureter where visualization is excellent. These results show that the multi-YAG laser has very similar tissue properties compared to the Ho:YAG laser since we also found that high grade ureteric injury will occur if the ureteral wall is contacted with the laser fiber. We also

agree with Watson's conclusion that visualization must be excellent. Otherwise, the risk for significant tissue injury is too great.

The acute effects of laser lithotripsy with the pulsed coumarin dye laser has been studied in an *in vivo* pig model (Watson, 1987a). Watson et al. showed that pulsed dye laser lithotripsy was associated with only mild degrees of inflammation in the ureteral walls of all their subjects. In a worst case setting, the laser fiber was placed against the ureteral wall and between five and twenty pulses of 25 to 30 mJ laser energy was delivered to one point on the urothelium. Histologic analysis of the acute effects showed a small patch of acute inflammation and hemorrhage to the lamina propria and superficial muscle. The pulsed dye laser was also able to perforate the ureteral wall at these energies, but only after 50 to 100 pulses with the fiber pushed firmly against the ureteral wall. These perforations were not sufficiently large enough to permit extravasation of radiographic contrast material.

These results indicate that the pulsed dye laser seems to have a wider margin of safety in comparison to the multi-YAG or Ho:YAG lasers. The most important reason for this is that the multi-YAG laser is significantly more powerful than the pulsed dye laser and uses higher pulse energies. The pulsed dye lasers which are used clinically typically use pulse energies of 30 to 140 mJ. Conversely, the multi-YAG laser's minimum energy setting is 0.3 J, or 300 mJ; more than twice that of the pulsed dye laser's maximum energy setting. As noted before, unlike the pulsed dye laser, these higher energies allow the multi-YAG laser to fragment any urinary stone including COM and cystine. Another reason for the difference in tissue effects seen between these two lasers is related to the wavelengths of light emitted from them. Although the 504 nm light emitted from the pulsed dye laser is minimally absorbed by tissue and hemoglobin, there is greater absorption of the light by the pigments contained within the urinary stones. Therefore, the majority of laser energy

interacts with the calculus. This helps to make the pulsed dye laser relatively tissue-safe compared to the multi-YAG laser whose wavelength of 1440 nm is significantly absorbed by tissue. However, this characteristic essentially limits the pulsed dye laser to be a single purpose medical laser since it does not have the capability to ablate or coagulate tissue. Finally, the 1 μsec pulse duration of the pulsed dye laser probably also plays a role in its overall safety. As discussed previously, a shorter pulse width means that any thermal energy produced by the pulsed dye laser will be more spatially confined to the target area and less thermal energy should diffuse to the surrounding tissues.

To our knowledge, there have been no *in vivo* studies to date investigating the chronic tissue effects of Ho:YAG laser lithotripsy. However, since the acute effects of this laser on pig urothelium appears to be almost equivalent to our acute histology, we would expect very similar chronic results with the holmium laser as with the multi-YAG laser. Concerning the pulsed dye laser, in the same *in vivo* study described above by Watson and colleagues, two ureters were studied four weeks after laser energy was delivered directly to the urothelium (Watson, 1987a). In both cases, no chronic changes could be detected in the ureteral walls.

The overall clinical results of laser lithotripsy were reviewed in detail at the outset of this thesis. Of particular interest are the preliminary results reported for the Ho:YAG laser (Watson, 1993; Denstedt, 1994; Matsuoka, 1995,]. Because this laser is related in many respects to the multi-YAG laser, these initial clinical results suggest that the multi-YAG laser will be useful clinically. More importantly though, we believe that our completed experimental results are the best evidence that the multi-YAG laser will be effective clinically. We therefore feel that our results justify further study of the multi-YAG laser in the form of a phase I clinical trial.

Based on the outcomes from this animal study, the methodology of such a trial can now be designed. Because we have shown that multi-YAG lithotripsy can be performed safely within the ureter, this implies that it should also be useful in the bladder, renal pelvis, or renal calyces where the collecting system is more spacious and the risk of inadvertent contact with the urothelium reduced. Therefore, included in this trial would be patients with ureteric, renal, or bladder calculi. According to our *ex vivo* results, diffuse circumferential ureteral injury occurred with the photofragmentation of cystine calculi. Therefore, the only absolute exclusion criteria at this time would be patients with known cystine stones or those where adequate visualization cannot be obtained.

It was our subjective observation in both the *ex vivo* and *in vivo* experiments that a large proportion of the ureteral tissue injury occurred when the smaller stone particles were being pursued and fragmented. During these situations it was often difficult to keep the laser fiber in constant contact with the stone particle so that laser energy was delivered directly to the urothelium. Consequently, we believe that the multi-YAG laser should only be used to break the calculus into pieces which can then be manually extracted. Manual extraction with forceps or other devices would not be considered a failure of laser lithotripsy but an adjunct to the overall procedure.

Finally, we would use the same protocol for laser therapy in the human setting that was followed in our *in vivo* pig model. Because the pulse energy was shown not to be a significant factor leading to high grade tissue injury, lithotripsy would begin at 0.9 J. If adequate fragmentation did not occur at this rate, the energy could be progressively increased to a level of 1.5 J. Our results showed no significant benefit in the efficacy of fragmentation by increasing the pulse frequency. However, because there did appear to be a higher incidence of high grade tissue injury with a higher pulse frequency, we would always maintain the pulse frequency at low repetitions of 5-10 Hz depending on the stone

size. At times we found ureteroscopy within the porcine ureter to be technically challenging because of the small ureteral caliber, the tortuosity and redundancy of the ureter, and also as a result of mucosal flaps which were often raised by inserting the stone into the ureter. Since some of these factors may not necessarily be present in the adult human, we would hope that this will improve the overall safety of the multi-YAG laser from what was demonstrated in the current study.

In conclusion, we believe that these experimental results demonstrate that the multi-YAG laser can effectively fragment human urinary calculi in a clinically efficient and rapid manner. We also believe our *ex vivo* and *in vivo* animal studies reveal that this laser is safe to use for intracorporeal lithotripsy. Further study is now required in a human clinical setting.

Bibliography

Alken, P, Hutschenreiter, G, Günther, R, and Marberger, M. Percutaneous Stone Manipulation. *J Urol* 125:463, 1981.

Anderson, RR and Parrish, JA. Selective Photothermolysis: Precise Microsurgery by Selective Absorption of Pulsed Radiation. *Science* 220:524-527, 1983.

Andersson, L and Sylven, M. Small Renal Caliceal Calculi as a Cause of Pain. *J Urol* 130:752, 1983.

Assimos, DG, Boyce, WH, Harrison, LH, McCullough, DL, Kroovand, RL, and Sweat, KR. The Role of Open Stone Surgery Since Extracorporeal Shock Wave Lithotripsy. *J Urol* 142:263-267, 1989.

Atala, A, Pumphrey, JA, and Steinbock, GS. Pulsed-Dye Laser Fragmentation of Urinary and Biliary Calculi In Vitro. *J Endourol* 4:175-182, 1990.

Baba, S, Asanuma, H, and Tazaki, H. Pulsed Dye Laser Lithotripsy for Ureteral Stone Fragmentation. *Keio J Med* 42:209-211, 1993.

Begun, FP. Modes of Intracorporeal Lithotripsy: Ultrasound Versus Electrohydraulic Lithotripsy Versus Laser Lithotripsy. *Sem Urol* 12: 39-50, 1994.

Ben-Bassat, M, Ben-Bassat, M, and Kaplan, I. A Study of the Ultrastructural Features of the Cut Margin of Skin and Mucous Membrane Specimens Excised by Carbon Dioxide Laser. *J Surg Res* 21:77-84, 1976.

Benizri, E, Wodey, J, Amiel, J, and Toubol, J. Comparison of Two Pulsed Lasers for Lithotripsy of Ureteral Calculi: Report on 154 Patients. *J Urol* 150:1803-1805, 1993.

Benson, RC, Farrow, GM, Kinsey, JH, Cortese, DA, Zincke, H, and Utz, DC. Detection and Localization of *in situ* Carcinoma of the Bladder with Hematoporphyrin Derivative. *Mayo Clin Proc* 57:548, 1982.

Bhatta, KM and Nishioka, NS. Effect of Pulse Duration on Microsecond-Domain Laser Lithotripsy. *Lasers Surg Med* 9: 454-457, 1989.

Bhatta, N, Isaacson, K, Bhatta, KM, Anderson, and RR, Schiff, I. Comparative Study of Different Laser Systems. *Fertil Steril* 61:581-591, 1994.

Boline, GB and Belis, JA. Lasertripsy of Upper Urinary Tract Calculi after Unsuccessful Extracorporeal Lithotripsy or Ureteroscopy: Comparison with Primary Lithotripsy. *J Endourol* 7:473-476, 1993.

Bolton, DM, Peters, JS, and Costello, AJ. Experience with the Pulsed Dye Laser in Management of Ureteric Calculi. *Aust N Z J Surg* 62:788-790, 1992.

Boyce, WH. Organic Matrix of Human Urinary Concretions. *Am J Med* 45:673-683, 1968.

Boyce, WH, Garvey, FK, Strawcutter, HE. Incidence of Urinary Calculi Among Patients in General Hospitals, 1948 to 1952. JAMA 161:1437, 1956.

Boyle, ET Jr., Segura, JW, Patterson, DE. The Role of Open Surgery in Stone Disease. *J Urol* 141:243A (Suppl 4, pt 2).

Brannen, GE, Bush, WH, and Lewis, GP. Caliceal Calculi. *J Urol* 135:1142, 1986.

Bridges, WB. Laser Oscillation in Singly Ionized Argon in the Visible Spectrum. *Appl Phys Lett* 4:128-130, 1964.

Cass, AS. Extracorporeal Chock Wave Lithotripsy for Ureteral Calculi. *J Urol* 147:1495-1498, 1992.

Chaussy, C, Schmiedt, E, Jocham, D, Brendel, W, Forssmann, B, and Walther, V. First Clinical Experience with Extracorporeally Induced Destruction of Kidney Stones by Shock Waves. *J Urol* 127:417-420, 1982.

Chaussy, CG. ESWL: Past, Present, and Future. *J Endourol* 2:97, 1988Clayman, RV, Surya, V, Miller,RP, Castaneda-Zuniga, WR. Percutaneous Nephrolithotomy: An Approach to Branched and Staghorn Renal Calculi. *JAMA* 250:73, 1983.

Clayman, RV, Elbers, J, Miller, RP. Percutaneous Nephrostomy: Assessment of Renal Damage Associated with Semi-Rigid (24F) and Balloon (36F) Dilation. *J Urol* 138:203, 1987.

Clayman, RV and Bagley, DH. Ureteronephroscopy. In Gillenwater, JY, Grayhack, JT, Howards, SS, and Duckett, JW (eds): Adult and Pediatric Urology, Mosby Year Book, St. Louis, 1991.

Coe, FL and Kavalach, AG. Hypercalciuria and Hyperuricosuria in Patients with Calcium Nephrolithiasis. *N Eng J Med* 291:1344-1350, 1974.

Coe, FL, Parks, JH, and Asplin, JR. The Pathogenesis and Treatment of Kidney Stones. *N Eng J Med* 327:1141-1152, 1992.

Coe, FL and Raisen, L. Allopurinol Treatment of Uric Acid Disorders in Calcium Stone Formers. *Lancet* 1:129-134, 1973.

Coptcoat, MJ, Ison, KT, Watson, G, and Wickham, JEA. Lasertripsy for Ureteric Stones in 120 Cases: Lessons Learned. *Br J Urol* 61:487-489, 1988.

Denstedt, JD, Eberwein, PM, and Singh, RR. The Swiss Lithoclast: A New Device for Intracorporeal Lithotripsy. *J Urol* 148:1088-1090, 1992.

Denstedt, JD, Sales, JL, Razvi, HA, and Eberwein, PM. The Holmium Laser: A New Multipurpose Laser for Urology. *Can J Urol* 1:A38, 1994.

Denstedt, JD, Razvi, HA, Rowe, E, Grignon, DJ, and Eberwein, PM. Investigation of the Tissue Effects of a New Device for Intracorporeal Lithotripsy. The Swiss Lithoclast. *J Urol* 153:535-537, 1995.

DeVita, MV and Zabetakis, PM. Laboratory Investigation of Renal Stone Disease. *Clin Lab Med* 13:225-234, 1993.

Dorros, G and Seeley, D. Understanding Lasers. A Basic Manual for Medical Practitioners Including Extensive Bibliography of Medical Applications. Futura Publishing Company, Inc., Mount Kisco, New York, 1991.

Drach, GW. Urinary Lithiasis: Etiology, Diagnosis, and Medical management. In Walsh, PC, Retik, AB, Stamey, TA, and Vaughn, ED Jr. (eds): Cambell's Urology, Sixth Edition. WB Saunders Company, Philadelphia, 1992.

Drach, GW, Dretler, S, Fair, W, Finlayson, B, Gillenwater, J, Griffith, D, Lingeman, J, and Newman, D. Report of the United States Cooperative Study of Extracorporeal Shock Wave Lithotripsy. *J Urol* 135:1127-1133, 1986.

Dretler, S, Watson, G, Murray, S, and Parrish, J. Laser Fragmentation of Ureteral Calculi: Clinical Experience. *Lasers Med Surg* 6:191, 1986.

Dretler, SP, Watson, G, Parrish, JA, and Murray, S. Pulsed Dye Laser Fragmentation of Ureteral Calculi: Initial Clinical Experience. *J Urol* 137:386-389, 1987a.

Dretler, SP. Laser Photofragmentation of Ureteral Calculi: Analysis of 75 Cases. *J Endourol* 1:9-14, 1987b.

Dretler, SP. Laser Lithotripsy: A Review of 20 Years of Research and Clinical Applications. *Lasers Surg Med* 8:341-356,1988a.

Dretler, SP. Stone Fragility. A New Therapeutic Distinction. *J Urol* 139:1124-1127, 1988b.

Dretler, SP. Techniques of Laser Lithotripsy. *J Endourol* 2:123-129, 1988c.

Dretler, SP. An Evaluation of Ureteral Laser Lithotripsy: 225 Consecutive Patients. *J Urol* 143:267-272, 1990.

Dretler, SP and Bhatta, KM. Clinical Experience with High Power (140 MJ), Large Fiber (320 Micron) Pulsed Dye Laser Lithotripsy. *J Urol* 146:1228-1231, 1991.

Ekelund, L, Lindstedt, E, Lundquist, SB. Studies on Renal Damage from Percutaneous Nephrolitholapaxy. J Urol 135:682, 1986.

Evans, RM, Reddy, PK, Hulbert, JC, and Hernandez-Graulau, JM. Treatment of Ureteral Calculi Measuring 1 cm or Greater in Their Largest Dimension, Using the Pulsed Dye Laser or Extracorporeal Shock-Wave Lithotripsy. *Sem Urol* 10:199-201, 1992.

Fair, HD. In vitro Destruction of Urinary Calculi by Laser-Induced Stress Waves. *Med Instru* 12:100-105, 1978.

Finlayson, B, Vermeulen, CW, and Stewart, EJ. Stone Matrix and Mucoprotein from Urine. *J Urol* 86:355-363, 1961.

Finlayson, B and Reid F. The Expectation of Free and Fixed Particles in Urinary Stone Disease. *Invest Urol* 15:442-448, 1978.

Foye, WO, Hong, HS, Kim, CM, and Prien, EL, Sr. Degree of Sulfation in Mucopolysaccharide Sulfates in Normal and Stone Forming Urines. *Invest Urol* 14:33-37, 1976.

Frangos, DN and Rous, SN. Incidence and Economic Factors in Urolithiasis. In Rous, SN (ed): Stone Disease. Diagnosis and Management. Grune and Stratton, Inc., Orlando, 1987.

Fugelso, P and Neal, PM. Endoscopic Laser Lithotripsy: Safe, Effective Therapy for Ureteral Calculi. *J Urol* 145:949-951, 1991.

Fuller, TA. Laser Physics. In Smith Jr., JA (ed): Lasers in Urologic Surgery. Chicago, Year Book Medical Publishers, 1985.

Gautier, JR, Rossignol, G, Caissel, J, and Quintens, H. Pulsed Dye Laser in the Treatment of 325 Calculi of the Urinary Tract. *Eur Urol* 18:6-9, 1990.

Guesic, JE, Marcos, HM, and Van Uitert, LG. Laser Oscillations in Nd-Doped Yttrium Aluminum, Yttrium Gallium and Gadolinium Garnets. *App Phys Lett* 4:182-184, 1964.

Goldman, IL, Resnick, MI, and Buck, AC. In Wickham, JEA and Buck, AC (eds): Renal Tract Stone. Metabolic Basis and Clinical Practice. Churchill Livingstone, London, 1990.

Gordon, JP, Zeiger HJ, and Townes, CH. The Maser- New Type of Microwave Amplifier, Frequency Standard, and Spectrometer. *Phys Rev* 99:1264-1274, 1955.

Govier, FE, Gibbons, RP, Correa, RJ, Brannen, GE, Weissman, RM, and Pritchett, TR. Pulsed Dye Laser Fragmentation of Ureteral Calculi: A Review of the First 50 Cases Performed at Virginia Mason Medical Center. *J Urol* 143:685-686, 1990.

Graff, J, Diederechs, W, Schulze, H. Long-term Follow Up in 1003 Extracorporeal Shock Wave Lithotripsy Patients. *J Urol* 140:479, 1988.

Grasso, M and Bagley, DH. Flexible Ureteroscopic Lithotripsy Using Pulsed-Dye Laser. *J Endourol* 4:155-160, 1990.

Grasso, M, Shalaby, M, El Akkad, M, and Bagley, DH. Techniques in Endoscopic Lithotripsy Using Pulsed Dye Laser. *Urol* 37:138-144, 1991.

Grasso, M and Bagley, DH. Endoscopic Pulsed-Dye Laser Lithotripsy: 159 Consecutive Cases. *J Endourol* 8:25-27, 1994.

Hecht, J. The Laser Guidebook. McGraw-Hill Book Company. New York. 1986.

Hiatt, RA, Dabs, LG, Friedman, GD. Frequency of Urolithiasis in a Pre-Paid Medical Care Program. *Am J Epid* 115:255, 1982.

Higoshihara, E, Horie, S, Takeuchi, T, et al. Laser Ureterolithotripsy with Combined Rigid and Flexible Ureterorenoscopy. *J Urol* 143:273-274, 1990.

Hofmann, R., Hartung, R., Geissdörfer, K., Ascherl, R., Erhardt, W.,Schmidt-Kloiber, H., and Rescher, E. Laser Induced Shock Wave Lithotripsy- Biologic Effects of Nanosecond Pulses. *J Urol* 140:1077-1079, 1988.

Hofmann, R and Hartung, R. Laser Lithotripsy of Ureteral Calculi. *Urol Res* 18 (Suppl 1):S49-S55, 1990.

Hosking, DH, Erickson, SB, Van Den Berg, CJ, Wilson, DM, and Smith, LH. The Stone Clinic Effect in Patients with Idiopathic Calcium Urolithiasis. *J Urol* 130:1115-1118, 1983.

Howard, JE, Thomas, Jr., WC, Barker, LM, Smith, SH, and Wadkins, CL. The Recognition and Isolation from Urine and Serum of a Peptide Inhibitor ro Calcification. *J Hopkins Med J* 120:119-136, 1967.

Howard, SS, Merill, E, Harris, S. Ultrasonic Lithotripsy: Laboratory Evaluations. *Invest Urol* 11:273, 1974.

Huffman, JL. Ureteroscopy. In Walsh, PC, Retik, AB, Stamey, TA, and Vaughn, ED Jr. (eds): Cambell's Urology, Sixth Edition. WB Saunders Company, Philadelphia, 1992.

Jacques, SL. Laser-Tissue Interactions. Photochemical, Photothermal, and Photomechanical. *Surg Clin N Am* 72:531-558, 1992.

Javan, A, Bennett, WR, Jr, Herriott, DR. Population Inversion and Continuous Optical Maser Oscillation in a gas Discharge Containing a HeNe Mixture. *Phys Rev Let* 6:106-110. 1961.

Jenkins, AD. Calculus Formation. In Gillenwater, JY, Grayhack, JT, Howards, SS, and Duckett, JW (eds): Adult and Pediatric Urology, Mosby Year Book, St. Louis, 1991.

Johnson, CM, Wilson, DM, O'Fallon, WM, Malek, RS, and Kurland, LT. Renal Stone Edidemiology: A 25-Year Study in Rochester, Minnesota. *Kid Int* 16:624-631, 1979.

Kaude, JV, Williams, CM, Millner, MR. Renal Morphology and Function Immediately after Extracorporeal Shock-Wave Lithotripsy. *Am J Roent* 145:305-313, 1985.

Kaye, KW and Clayman, RV. Tamponade Nephrostomy Catheter for Percutaneous Nephrostolithotomy. *Urol* 27:441, 1986.

Knapp, PM, Kulb, TB, Lingeman, JE. Extracorporeal Shock Wave Lithotripsy-Induced Perirenal Hematomas. *J Urol* 139:700, 1988.

Kriegmair, M, Baumgartner, R, Knuechel, R, et al. Fluorescence Photodetection of Neoplastic Urothelial Lesions Following Intravesical Instillation of 5-Aminolevulinic Acid. *Urol* 44:836-841, 1994.

Lang, EK. Percutaneous Nephrostolithotomy and Lithotripsy: A Multi-Institutional Survey of Complications. *Radiology* 162:25, 1987.

Leahy, AL, Ryan, PC, McEnte, GM, Nelson, AC, and Fitzpatrick, JM. Renal Injury and Recovery in Partial Ureteric Obstruction. *J Urol* 142, 199-205, 1989.

Lee, WJ, Smith, AD, Cubelli, V. Complications of Percutaneous Nephrolithotomy. *Am J Radiol* 148:177, 1987.

Liedl, B, Jocham, D, Lunz, C. Five Year Follow-Up of Urinary Stone Patients Treated With Extracorporeal Shock Wave Lithotripsy. *J Endourol* 2:157, 1988.

Lingeman, JE, Newman, D, Mertz, JHO, Mosbaugh, PG, Steele, RE, Kahnoski, RJ, Coury, TA, and Woods, JR. Extracorporeal Shock Wave Lithotripsy: The Methodist Hospital of Indiana Experience. *J Urol* 135:1134-1137, 1986.

Lingeman, JE and Kalb, TB. Hypertension following Extracorporeal Shock Wave Lithotripsy. *J Urol* 137:154A, 1987.

Lingeman, JE, Woods, J, Toth, PD. Role of Lithotripsy and its Side Effects. *J Urol* 141:793, 1989a.

Lingeman, JE, Smith, LH, Woods, JR, and Newman, DM. Bioeffects and Long-term Results of ESWL. In Moster, MB (ed): Urinary Calculi: ESWL, Endourology, and Medical Therapy. Lea and Febiger, Philadelphia, 1989b.

Lingeman, JE, Smith, LH, Woods, JR, and Newman, DM. Percutaneous Procedures. In Moster, MB (ed): Urinary Calculi: ESWL, Endourology, and Medical Therapy. Lea and Febiger, Philadelphia, 1989c.

Lingeman, JE, Siegel, YI, Steele, B, Nyhuis, AW, and Woods, JR: Management of Lower Pole Nephrolithiasis: A Critical Analysis. *J Urol* 151:663-667, 1994.

Loughlin, KR and Sharpe, JF, Jr. Preliminary Experience with the Pulsed Dye Laser for Treatment of Urolithiasis. *Lasers Surg Med* 11:1-4, 1991.

MacDermott, JP, Grove, J, and Clark, PB. Laser Lithotripsy with the Candela MDL-2000 LaserTripter. *Br J Urol* 71:512-515, 1993.

Maes, KE, Cummings, R, Moora, PA, and Sherk, HH. Short-term Clinical Results of Knee Arthroscopy Utilizing the 1.44 μm Nd:YAG Laser. In Katzir, A and Anderson, R (eds): Laser Surgery: Advanced Characterisation, Therapeutics and Systems IV. Proceedings of the Society for Photo-Optical Instrumentation Engineers. Volume 2128: 170-172, 1994.

Maiman, TH. Stimulated Optical Radiation in Ruby. *Nature* 187:493-494, 1960.

Mandel, NS and Mandel GS. Physiochemistry of Urinary Stone Formation. In Pak, CYC (ed): Renal Stone Disease. Pathogenesis, Prevention, and Treatment. Martinus Nijhoff Publishing, Boston, 1987.

Mandel, NS and Mandel GS. Urinary Tract Stone Disease in the United State Veteran Population. II. Geographical Analysis of Variations in Composition. *J Urol* 142:1513-1515, 1989.

Marberger, M, Hofbauer, J, Turk, C, Hobarth, K, and Albrecht, W. Management of Ureteric Stones. *Eur Urol* 25:265-272, 1994.

Martiniuk, R, Bauer, JA, McKean, JDS, Tulip, J, and Mielke, BW. New Low-Wavelength Nd:YAG Laser at 1.44 μm: Effect on Brain. *J Neurosurg* 70:249-256, 1989.

Matsuoka, K, Iida, S, Nakanami, M, Koga, H, Shimada, A, Mihara, T, and Noda, S. Holmium: Yttrium-Aluminum-Garnet Laser for Endoscopic Lithotripsy. Urol 45:947-952, 1995.

Mattioli, S, Cremona, M, Benaim, G, and Ferrario, A. Lithotripsy with a Q-Switched Alexandrite Laser System. *Eur Urol* 19:233-235, 1991.

Mayo, ME, Krieger, JN, Rudd, TG. Effect of Percutaneous Nephrostolithotomy on Renal Function. *J Urol* 133:167, 1985.

McCullough, DL. Extracorporeal Shock Wave Lithotripsy. In Walsh, PC, Retik, AB, Stamey, TA, and Vaughn, ED Jr. (eds): Cambell's Urology, Sixth Edition. WB Saunders Company, Philadelphia, 1992.

Mee, SL and Thuroff, JW. Small Caliceal Stones: Is Extracorporeal Shock Wave Lithotripsy Justified? *J Urol* 139:908, 1988.

Meyer, JL. Nucleation Kinetics in the Calcium Oxalate-Sokium Urate Monohydrate System. *Invest Urol* 19:197-201, 1981.

Moore, RB, Tulip, J, McPhee, M.S. Calculi Photofragmentation with a New Long Wavelength Nd:YAG Laser. (Abstract) Canadian Urological Association Meeting, Vancouver, British Columbia, June, 1990.

Morse, RM and Resnick, MI. Ureteral Calculi: Natural History and Treatment in an Era of Advanced Technology. *J Urol* 145:263-265, 1991.

Mulvaney, WP and Beck, CW. The Laser Beam in Urology. *J Urol* 99:112-115, 1968.

Nelson, JB and Graham, JB. Lithotripsy of Urinary Calculi by Tunable Pulsed Dye Lasers: A Randomized In Vitro Study. *J Urol* 151: 656-659, 1994.

Nelson, JS and Berns, MW. Laser-Tissue Ineractions. In Laser Non-Surgical Medicine. New Challenges for an Old Application. Lancaser, Penn. Technomic Publishing Company Inc., 1991.

Nicely, ER, Maggio, MI, and Kuhn, EJ. The Use of a Cystoscopically Placed Cobra Catheter for Directed Irrigation of Lower Pole Caliceal Stones During Extracorporeal Shock Wave Lithotripsy. J Urol 148:1036-1039, 1992.

Nishioka, NS, Teng, P, Deutsch, TF, and Anderson, RR. Mechanism of Laser-Induced Fragmentation of Urinary and Biliary Calculi. *Lasers Life Sci* 1:231-245, 1987.

Nishioka, NS, Kelsey, PB, Kibbi, AG, Delmonico, F, Parrish, JA, and Anderson, RR. Laser Lithotripsy: Animal Studies of Safety and Efficacy. *Lasers Surg Med* 8: 357-362, 1988.

Nishioka, NS, Domankevitz, Y, and Venugopalan, V. Tissue Ablation with a Pulsed Nd:YAG Laser Operating at 1.44 μm and a Pulsed Holmium Laser Operating at 2.12 μm. Technical Bulletin. Carl Zeiss, Inc. February, 1992.

Olfert, ED, Cross, BM, and McWilliam, AA (eds). Canadian Council on Animal Care. Guide to the Use of Experimental Animals, 2nd edition Volume 1. Ottawa, Bradda Printing Services, Inc., 1993.

Pak, CY, Kaplan, R, Bone, H. A Simple Test for the Diagnosis of Absorptive, Resorptive, and Renal Hypercalciurias. *N Engl J Med* 292:497-500, 1975.

Pak, CYC, Britton, F, Peterson, R, Ward, D, Northcutt, C, Breslau, NA, McGuire, J, Sakhaee, K, Bush, S, Nicar, M, Norman, DA, and Peters, P. Ambulatory Evaluation of Nephrolithiasis. Classification, Clinical Presentation and Diagnostic Criteria. *Am J Med* 69:19-30, 1980.

Pak, CYC, Skurla, C, and Harvey, J. Graphic Display of Urinary Risk Factors for Renal Stone Formation. *J Urol* 134:867-870, 1985.

Pak, CYC and Resnick, MI. Introduction. In Resnick, MI and Pak, CYC (eds): Urolithiasis. A Medical and Surgical Regerence. WB Saunders Company, Philadelphia, 1990.

Patel, CK. Selective Excitation Through Vibrational Energy Transfer and Optical Maser Action in N_2-CO_2. *Phys Rev Lett* 13:617-619, 1964.

Patterson, DE, Segura, JW, Leroy, AJ. The Etiology and Treatment of Delayed Bleeding Following Percutaneous Lithotripsy. *J Urol* 133:447, 1985.

Pensel, J, Frank, F, Rothenberger, K, Hofstetter, A, Unsold, E. In Kaplan, I (ed): Laser Surgery IV. Proceedings of Fourth International Symposium on Laser Surgery. Jerusalem, Academic Press, 1981, pp 4-6.

Piergiovanni, M, Desgrandchamps, F, Cochand-Priollet, B, Janssen, T, Colomer, S, Teillac, P, and Le Duc, A. Ureteral and Bladder Lesions after Ballistic, Ultrasonic, Electrohydraulic, or Laser Lithotripsy. *J Endourol* 8:293-299, 1994.

Polanyi, TG. Physics of Surgery with Lasers. *Clin Chest Med* 6:179-202, 1985.

Preminger, GM, Harvey, JA, and Pak, CYC. Comparative Effecacy of "Specific" Potassium Citrate Therapy Versus Conservative Management in Nephrolithiasis of Mild to Moderate Severity. *J Urol* 134:658-661, 1985a.

Preminger, GM, Peterson, R, Peters, PC, and Pak, CYC. The Current Role of Medical Treatment of Nephrolithiasis: The Impact of Improved Techniques of Stone Removal. *J Urol* 134:6-10, 1985b.

Preminger, GM. Is There a Need for Medical Evaluation and Treatment of Nephrolithiasis in the "Age of Lithotripsy"? Sem Urol 12:51-64, 1994.

Prien, EL. Studies in Urolithiasis: II. Relationships between Pathogenesis, Structure, and Composition and Calculi. *J Urol* 61:821-836, 1949.

Prince, CL and Scardino, PL. A Statistical Analysis of Ureteral Calculi. *J Urol* 83:561, 1960.

Psihramis, KE. Laser Lithotripsy of the Difficult Ureteral Calculus: Results in 122 Patients. *J Urol* 147:1010-1012, 1992.

Raney, AM. Electrohydraulic Ureterolithotripsy. Preliminary Report. *Urol* 12:284-285, 1978.

Reddy, PK, Hulbert, JC, Lange, PH. Percutaneous Removal of Renal and Ureteral Calculi: Experience with 400 Cases. *J Urol* 134:662, 1985.

Resnick, MI and Spirnak, JP. Chemolysis. In Resnick, MI and Pak, CYC (eds): Urolithiasis. A Medical and Surgical Regerence. WB Saunders Company, Philadelphia, 1990.

Riehle, RA, Fair, WD, and Vaughn, D. Extracorporeal Shock Wave Lithotripsy for Upper Urinary Tract Calculi. *JAMA* 255:2043, 1986.

Riuz, H and Saltzman, B. Aspirin Induced Bilateral Renal Hemorrhage after Extracorporeal Shock Wave Lithotripsy: Implications and Conclusions. *J Urol* 143:791, 1990.

Robertson, WG, Hambleton, J, and Hodgkinson, A. Peptide Inhibitors of Calcium Phosphate Precipitation in the Urine of Normal and Stone-Forming Men. *Clin Chim Acta* 25:247, 1969.

Robertson, WG. Epidemiology of Urinary Stone Disease. *Urol Res* 18(Suppl):S3-S8, 1990.

Rubin, JI, Arger, PH, Pollack, HM. Kidney Changes after Extracorporeal Shock Wave Lithotripsy: CT Evaluation. *Radiology* 162:21-24, 1987.

Schawlow, AL and Townes, CH. Infrared and Optical Masers. *Phys Rev* 112:1940-1949, 1958.

Schmidt, A and Eisenberger, F. Lasertripsy of Ureteral Calculi Using Pulsed Dye Laser with Automatic Shut-Off after Tissue Contact. *J Endourol* 7:201-204, 1993.

Schoborg, TW. Efficacy of Electrohydraulic and Laser Lithotripsy in the Ureter. *J Endoruol* Vol 3:361-365, 1989.

Schulze, H, Haupt, G, Piergiovanni, M, Wisard, M, von Niederhausern, W, and Senge, T. The Swiss Lithoclast: A New Device for Endoscopic Stone Disintegration. *J Urol* 149:15-18, 1993.

Scott, R. The Epidemiology of Urolithiasis. In Wickham, JEA and Buck, AC (eds): Renal Tract Stone. Metabolic Basis and Clinical Practice. Churchill Livingstone, London, 1990.

Seftel, A, and Resnick, MI. Metabolic Evaluation of Urolithiasis. *Urol Clin N Am* 17:159-169, 1990.

Segura, JW. Editorial Comments. In Dretler, SP. An Evaluation of Ureteral Laser Lithotripsy: 225 Consecutive Patients. *J Urol* 143:267-272, 1990a.

Segura, JW. Role of Percuaneous Procedures in the Management of Renal Calculi. *Urol Clin N Am* 17:207-216, 1990b.

Segura, JW. Surgical Management of Urinary Calculi. *Sem Neph* 10:53-63, 1990c.

Segura, JW. Percutaneous Management. In Walsh, PC, Retik, AB, Stamey, TA, and Vaughn, ED Jr. (eds): Cambell's Urology, Sixth Edition. WB Saunders Company, Philadelphia, 1992.

Sierakowski, R, Finlayson, B, Landes, RR. The Frequency of Urolithiasis in Hospital Discharge Diagnoses in the United States. *Invest Urol* 15:438-441, 1987.

Smith, FGW (ed). CRC Handbook of Marine Science, Vol. I. CRC Press, Cleveland, 1974.

Sorokin, PP and Lankard, JR. Stimulated Emission Observed from an Organic Dye, Cholo-aluminum Phthalocyanine. *IBM J Res Dev* 10:162-163, 1966.

Spirnak, JP and Resnick, MI. Extracorporeal Shock Wave Lithotripsy. In Resnick, MI and Pak, CYC (eds): Urolithiasis. A Medical and Surgical Regerence. WB Saunders Company, Philadelphia, 1990a.

Spirnak, JP and Resnick, MI. Percutaneous Management. In Resnick, MI and Pak, CYC (eds): Urolithiasis. A Medical and Surgical Regerence. WB Saunders Company, Philadelphia, 1990b.

Spirnak, JP and Resnick, MI. Ureteroscopy. In Resnick, MI and Pak, CYC (eds): Urolithiasis. A Medical and Surgical Regerence. WB Saunders Company, Philadelphia, 1990c.

Stacholy, J and Goldberg, EP. Microstructural Matrix-Crystal Interactions in Calcium Oxalate Monohydrate Kidney Stones. *Scan Elect Micro* 2:781-787, 1985.

Stanley, KE and Winfield, HN. Management of Staghorn Calculi: Percutaneous Nephrolithotripsy Versus Extracorporeal Shock-Wave Lithotripsy. *Sem Urol* 12:15-25, 1994.

Stein, BS. Laser-Tissue Interaction. In Smith, Jr., JA, Stein, BS, and Benson, RC (eds.): Lasers in Urologic Surgery. St. Louis, Mosby-Year Book, Inc., 1994.

Strunge, C, Brinkmann, R, Flemming, G, and Engelhardt, R. Interspersion of Fragmented Fiber's Splinters Into Tissue During Pulsed Alexandrite Laser Lithotripsy. *Lasers Surg Med* 11:183-187, 1991.

Tanahashi, Y, Numata, I, Kambe, K, Harada, K, Chiba, Y, Toyota, S, Oridasa, S, Miyakawa, T, Tahira, K, and Fukatsu, T. Transurethral Disintegration of Urinary Calculi by the Use of the Laser Beam. In Kaplan, I (ed): Laser Surgery IV. Proceedings of Fourth International Symposium on Laser Surgery. Jerusalem, Academic Press, 1981, pp 30-33.

Tulip, J. Long-Wavelength Nd:YAG Laser. U.S. Patent 5,048,023, September, 1992.

Ueno, A, Kawamura, T, Ogawa, A, and Takayasu, H. Relation of Spontaneous Passage of Calculi to Size. *Urol* 10:544-546, 1977.

Van Assendelft, OW. Spectrophotometry of Haemoglobin Derivatives. Royal Vangorcum Ltd., The Netherlands, 1970.

Vandeursen, H, Pittomvils, G, Boving, R, and Baert, L. High Energy Pulsed Dye Laser Lithotripsy: Management of Ureteral Calcium Oxalate Monohydrate Calculi. *J Urol* 145:1146-1150, 1991.

Watson, GM, Wickham, JEA, Mills, TN, Brown, SG, Swain, P, and Salmon, PR. Laser Fragmentation of Renal Calculi. *Br J Urol* 55:613-616, 1983.

Watson, GM. Laser Fragmentation of Urinary Calculi. In Smith Jr., JA (ed): Lasers in Urologic Surgery. Chicago, Year Book Medical Publishers, 1985.

Watson, GM and Wickham, JEA. Initial Experience with a Pulsed Dye Laser for Ureteric Calculi. *Lancet* 2:1357-1358, 1986.

Watson, G., Murray, S., Dretler, S.P., and Parrish, J.A. An Assessment of the Pulsed Dye Laser for Fragmenting Calculi in the Pig Ureter. *J Urol* 138: 199-202, 1987a.

Watson, G, Murray, S, Dretler, SP, and Parrish, JA. The Pulsed Dye Laser for Fragmenting Urinary Calculi. *J Urol* 138:195-198, 1987b.

Watson, GM and Wickham, JEA. The Development of a Laser and a Miniturised Ureteroscope System for Ureteric Stone Management. *World J Urol* 7:147-150, 1989.

Watson, G and Smith, N. A Comparison of the Pulsed Dye and Holmium Lasers for Stone Fragmentation: In Vitro Studies and Clinical Experience. In Katzir, A and Anderson, R (eds): Laser Surgery: Advanced Characterisation, Therapeutics and Systems IV. Proceedings of the Society for Photo-Optical Instrumentation Engineers. Volume 1879: 139-142, 1993.

Watson, GM. Principles of Laser Stone Destruction. In Smith, Jr., JA, Stein, BS, and Benson, RC (eds.): Lasers in Urologic Surgery. St. Louis, Mosby-Year Book, Inc., 1994.

Webb, DR and Fitzpatrick, JM. Percutaneous Nephrolithotripsy: A Functional and Morphogical Study. J Urol 134:587, 1985.

Williams, CM, Kaude, JV, Newman, RL. ESWL: Long-Term Complications. *Am J Roent* 150:311, 1988.

Williams, RC. Long Term Survey of 538 Patients with Upper Tract Urinary Stones. *Br J Urol* 35:416-422, 1963.

Wilson, WT and Preminger, GM. Extracorporeal Shock Wave Lithotripsy. An Update. *Urol Clin N Am* 17:231-242, 1990.

Winburn, DC. What Every Engineer Should Know About Lasers. Marcel Dekker, Inc., New York. 1987.

Winfield, HN, Clayman, RV, Chaussy, CG. Monotherapy of Staghorn Renal Calculi: A Comparative Study Between Percutaneous Nephrolithotomy and Extracorporeal Shock Wave Lithotripsy. *J Urol* 139:895-899, 1988.

Wong, SK, Mathieu, P, and Pace, P. Eye-Safe Nd:YAG Laser. *Appl Phys Lett* 57: 650-652, 1990.

Wu, TT, Hsu, TH, Chen, MT, and Chang, LS. Efficacy of *In Vitro* Stone Fragmentation by Extracorporeal, Electrohydraulic, and Pulsed-Dye Laser Lithotripsy. *J Endourol* 7:391-393, 1993.

Young, HH, and McKay, RW. Congenital Valvular Obstruction of the Prostatic Uretthra. *Surg Gynecol Obstet* 48:409, 1929.

Zerbib, M, Flam, T, Belas, M, Debre, B, and Steg, A. Clinical Experience with a New Pulsed Dye Laser for Ureteral Stone Lithotripsy. *J Urol* 143:483-482, 1990.

Zhong, P and Preminger, GM. Differing Modes of Shock-Wave Generation. *Sem Urol* 12:2-14, 1994.