Evaluation of carbon filament induced connective tissue formation, as a means of controlled strangulation of the canine portal vein

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Evaluation of carbon filament induced
connective tissue formation, as a means of controlled
strangulation of the canine portal vein

by

Robert D. Barstad

A Thesis Submitted to the
Graduate Faculty in Partial Fulfillment of the
Requirements for the Degree of

MASTER OF SCIENCE

Department: Veterinary Clinical Sciences
Major: Veterinary Clinical Science

Signatures have been redacted for privacy

Iowa State University
Ames, Iowa
1985

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I. INTRODUCTION

Over the past twenty years, many veterinarians have faced the challenge of surgical ligation or partial attenuation of portasystemic venous shunts in dogs and cats. These congenital or acquired anomalous venous pathways allow nutrient and waste-laden blood returning from the small bowel, colon, spleen, and pancreas to bypass the liver and enter directly into the systemic venous circulation. Without portal venous blood, the liver is unable to perform the necessary metabolic and detoxifying functions that are essential to support life. The long term physiologic ramifications for the animal are staggering. Without some form of intervention, the prognosis for survival is at best guarded. Treatment of portasystemic shunt cases by medical therapy alone has been of little value (15,47). Due to the fact that shunts are a structural problem, surgical intervention has become the accepted mode of therapy, by itself or in combination with medical management. The surgical success rate with these extremely complex cases has improved through the years to only an acceptable level.

Potential life threatening complications associated with surgery arise from a number of areas. From anesthetic complications, such as intraoperative renal shutdown, to bleeding disorders secondary to liver dysfunction. The primary complication is portal hypertension secondary to actual attenuation or total ligation of the venous shunt.

The procedure for surgical correction of a single extrahepatic shunt has been reviewed in the recent literature (8,11,12,15). Even though guidelines for safe attenuation exist, an occasional animal still
succumbs to unexplained postoperative portal hypertension. It is probable that other psychological changes accompanying rapid surgical attenuation or ligation may play as much of a role in the development of postoperative shock, as does portal hypertension. It is for these animals that a method of controlled closure of a venous structure would be most advantageous.

The portal vein of the dog and cat may be completely obstructed if the procedure is performed slowly over several weeks time (11). It seems reasonable to assume that complete obstruction of venous shunts could be accomplished without causing signs of portal hypertension if several weeks time were allowed for occlusion. Slow ligation would allow the liver parenchyma to adapt to the rerouted blood flow. It would also allow portal collateral circulation to accommodate any developing portal hypertension.

Flexible carbon fiber has been used in human and veterinary medicine for the repair of damaged or severed ligaments and tendons (48). It demonstrates a high level of biocompatibility with tissues, as it induces the formation of mature collagenous fibrous tissue along its skeletal framework (21). Collagen deposition along the fibers has been reported to reach fifteen times the volume of the original carbon implant (21,25). Two criteria must be met for the collagen to form along the carbon fiber.

1. The fibers must be implanted where a source of vascularized connective tissue and mesenchymal cells are readily available.

2. The fibers must be implanted under a working load, i.e., tension (37).
It was my hypothesis that carbon fiber tow, when wrapped around a venous structure under tension, would produce a fibrous connective tissue reaction that would occlude the vessel in several weeks. Intraluminal portal pressures would provide constant static tension on the encircling carbons strands by forcing the vein wall out to meet the implant. This slow strangulation would allow the hepatic vasculature and the portal system to adapt to the steadily increasing portal pressure and avoid death due to acute portal hypertension.
II. LITERATURE REVIEW

A. Evolution of Carbon as a Biomaterial

The element carbon is unique in chemistry because of the vast number of compounds it forms. It serves as the basic building block in an estimated 1 million organic compounds, which are found widely distributed in nature. In the solid form, it is found in large amounts as coal and in smaller amounts as graphite, the pure crystalline form. It also surrounds us in the atmosphere as gaseous carbon dioxide. All plant and animal life is composed of complex organic compounds containing carbon in combination with hydrogen, nitrogen, oxygen, and other elements.

The biocompatibility of elemental carbon has been recognized for centuries. Primitive societies used burnt bamboo splinters to perform tattooing as part of their cultural heritage (7).

During the first half of the 20th century carbon was primarily used in industry. One of its main uses was for the formation of carbon black used in car tire production. During the second world war, advances in biomaterials research began to accelerate. Due to the need for refractory materials in the nuclear energy and aerospace fields new physical carbon forms were developed. These new, almost pure forms of carbon exhibited very low chemical reactivity (7). In 1967, a National Aeronautics Space Administration project was responsible for the recognition of carbon as a bio-compatible substance which was suitable for surgical implantation (7).

Two forms of carbon were initially investigated in the medical
field, vitreous or glassy carbon, and high purity carbon yarn or filaments (7). Vitreous carbon has been used for the development of structural prostheses and tissue-to-prosthesis interfaces. Our only interest in this form is that its chemical composition is the same as that of carbon fiber. Vitreous carbon has been found to be extremely inert when implanted into human and canine tissues (7,43). The purity and consequently the chemical inertness and strength of all carbons considered for implantation is a function of the pyrolysis temperature in the final processing step. The higher the temperature, the purer the end products of heat decomposition (7). Early in the investigation of these two forms of carbon, wide variations in the purity of the final product existed between manufacturers (7). Processing has now been standardized, resulting in implantable products that are nearly 100% pure (41).

1. **Carbon fiber**

Carbon fibers are available in multifilament, twist free bundles called "tows" (Fig. 1). Each individual filament is from 6-10 um in diameter (Fig. 1). Multiple longitudinal grooves run the length of each filament (Fig. 1). Tows are commercially available containing 1,000 (1K) to 10,000 (10 K), filaments (Fig. 1) with the 10,000 filament tow being most widely utilized in clinical applications (3,4,17,23,27,34,51,52,54).

Commercially available carbon fiber tow is subjected to a process called sizing, in which an epoxy resin is applied to the outer layer of the filaments. This allows the tows to be handled without fraying or falling apart when they are woven into fabrics by textile machinery.
These sizing resins are highly toxic to tissues (11,23,51). Tows to be implanted must either be purchased unsized, (3,17) or the epoxy resin must be completely removed by rinsing with chemical solvents (4,23,26,27,34,51,54). Methyl-ethyl-ketone (3,4,27,48,54) and acetone (3,26) have been used for this purpose. With the sizing removed, handling of the filaments becomes difficult. Medical grade carbon fiber tow is produced without epoxy sizing, but traces of silicone oil lubricant applied during manufacturing may contaminate the surface (3). Rinsing with cold petroleum ether will remove this residue (3).

2. **Polylactic acid and poly(ε-caprolactone)**

Polylactic acid coated carbon fibers have been examined by several investigators (2,36). Polylactic acid coating of the carbon tows allows for easier handling, much like the epoxy resins. Polylactic acid undergoes gradual hydrolysis to lactic acid, and is then metabolized to carbon dioxide and water (2,36). Degradation may take several weeks to occur, but it is felt that the polylactic acid does not interfere with tissue response to the carbon (2,16,36).

Poly(ε-caprolactone) is a hydrolyzable polymer that has also been used as a sizing agent for carbon fiber (41). It is compatible with tissues, and degradation rate is directly related to the thickness of the coating layer. Random hydrolysis of ester linkages results in breakdown products of ε-caprolactone and ε-hydroxycaproic acid, which are water soluble and excreted in urine, feces, and expired water (37).
Figure 1. Carbon fiber

A. 3K, Carbon fiber tow, 4.0x
B. 3K, Carbon fiber tow, (frayed) 8.0x
C. Individual carbon filaments, 1300x
D. Individual carbon filaments, 5800x
3. **Tissue response**

The primary use of carbon fiber in human and veterinary surgery has been in the area of tendon and ligament repair \( (4,17,19,23,26,28,31,32,33,48,51,53,54) \). Its use has also been applied to the repair of abdominal incisional hernias in sheep, \( (30) \) and in correction of deviation of the bovine penis \( (34) \). Human and animal tissues react to the carbon scaffold in a unique manner. Abundant fibrous connective tissue and eventually mature collagen forms along the template of carbon fiber tow. Fibrous tissue may gradually increase until six to fifteen times the volume of the original implant is reached \( (21,25) \). This process has been called neotendon formation \( (18) \).

Two important criteria must be met for this reaction to occur. First, the carbon must be implanted under a working load \( (21,38) \). When carbon fiber tow was chopped and inserted into muscle, intermuscular compartments, subcutaneously, around the calcaneal tendon, and into cortical bone in rats, rabbits and sheep, there was little or variable amounts of fibrous connective tissue formed \( (38) \). When carbon fiber was implanted both freely coiled and under a working load, it was found that only the fibers held under tension produced acceptable amounts of fibrous connective tissue \( (21,38) \).

Secondly, the carbon fibers must be implanted near a pool of vascularized connective tissue where mesenchymal cells or more specifically fibroblasts, are readily available \( (21,38) \). In New Zealand white rabbits that had carbon fiber achilles tendon replacement, removal of the posterior tibial nerve from the neuromuscular bundle resulted in
greatly diminished fibrous connective tissue production (21). In intact rabbits, carbon induced "Neotendon" rapidly developed from young fibroblastic tissue outgrowths of the loose mesenchymal tissue of the perineurium and adventitia of the blood vessels in the adjacent neurovascular bundle (21).

At the cellular level, the reaction to carbon was described by Forster et al. as inert foreign body fibrosis (21). Inert foreign body fibrosis is a diffuse spread of fibrous tissue between or around the inert implant or foreign body, coupled with an absence of tissue necrosis and the presence of simple macrophage or foreign body giant cell reaction (21). It was concluded by Forster et al. that, "The long term presence of single multinucleated cells coating the carbon filaments widely separated by masses of quiet fibrous tissue," was a "clinically acceptable situation" (21). Several investigators, concluded that implanted carbon fibers were well-tolerated and produced little evidence of causing an adverse inflammatory reaction (25,27). A slight eosinophilic response, noted by Mobini et al., was attributed to incomplete removal of polymer sizing from the carbon fibers (34). Goodship et al. examined filamentous carbon in fibroblast cell cultures and in vivo and determined that fibroblasts align themselves and grow along the grooves on the individual carbon filaments (23) (Fig. 1). This physical scaffold encourages fibrous connective tissue to grow in an organized fashion similar to that of a natural tendon, unlike randomly formed scar tissue (23,34). As the neotendon develops, the carbon filaments undergo gradual mechanical failure. This structural
breakdown allows an increasing load to be placed on the neotendon and stimulates the newly formed fibrous tissue to become oriented in the direction of the stress (21,23,27). Individual fragments of carbon may be engulfed by macrophages and removed to regional lymph nodes (25,27). Clinically, this does not present any problems (25,27). Tayton et al. investigated the carcinogenic potential of implanted carbon in Wistar rats and concluded that it was safe to use as an implantable biomaterial (49).

4. **Summary**

With proper preparation carbon fiber tow is an acceptable substance for surgical implantation. When implanted under tension, close to a source of vascularized mesenchymal cells, it produces abundant fibrous connective tissue on its skeletal framework. This connective tissue response may reach as much as 15 times the volume of the original implant.

B. **Anomalous Venous Systems in Small Animals**

1. **Pathophysiology and clinicopathology**

The majority of animals presented with portacaval vascular anomalies exhibit some form of hepatoencephalopathy (93%), gastrointestinal disturbance (75%), or other clinical sign related to liver insufficiency (80%) (12).

The exact mechanism of neurologic irritation is not known, but it is felt by most authors that ammonia plays a primary role (12). Plasma amino acid ratios, short chain fatty acids, and mercaptans may play an
important role (46). Byproducts of protein metabolism, amino acids and urea, are processed in the colon. Urease producing bacteria hydrolyze intestinal urea, and other bacteria deaminate amino acids to form ammonia. In the normal dog, ammonia is absorbed from the colon and is delivered to the liver by the portal venous system. In the liver, it is converted through the Krebs cycle into blood urea which is excreted by the kidneys. Dogs with diminished portal blood supply to the liver usually show increased concentrations of resting blood ammonia, and decreased concentrations of blood urea nitrogen (46,50). The degree of hyperammonemia does not always correlate with the severity of the clinical signs (12,46).

Ammonia tolerance tests have been used as the primary biochemical diagnostic aid for the diagnosis of portasystemic shunts (46). A resting blood ammonia level is established, then 100 mg/kg ammonium chloride is administered orally. Thirty minutes post administration a second ammonia sample is obtained (5). A threefold increase indicates an inability of the liver to process the ammonia, and primary liver disease or portasystemic shunting is suspected.

The gastrointestinal disturbances seen are primarily attributed to the nausea and bowel hypermotility that accompany high levels of toxic substances circulating in the blood stream. Overgrowth of intestinal anaerobes may also contribute to bowel abnormalities (46,50). Approximately one third of the animals presented with portasystemic shunts have a leukocytosis (12). This elevated white blood cell count is probably due to intestinal bacteria circulating in the blood stream.
that normally should have been filtered by the liver (12).

The clinical signs related to liver insufficiency are usually subtle when compared with the neurologic or gastrointestinal abnormalities. Weight loss may be noted over a period of time with most animals. Decreased liver metabolism of proteins results in an ongoing state of catabolism (46, 50). Hypoproteinemia is common in animals with liver insufficiency due to decreased hepatic production of albumin. Protein may also be lost in ascitic fluid if portal hypertension exists.

Bleeding disorders may be seen due to decreased production of necessary clotting factors in the liver. Anemia may result secondary to blood loss, destruction of red cells, or decreased production (12). Red cells may also appear microcytic due to the toxic effects of ammonia in the alteration of heme synthesis (12).

Polydipsia and polyuria when seen, result from primary hepatocellular dysfunction. Serum glutamic pyruvic transaminase, (SGPT), may increase to two times normal (12, 46). This reflects hepatocyte degeneration secondary to nutritional derangements or endotoxemia (46). Serum alkaline phosphatase, (SAP), is variably elevated, and may rise secondary to gastrointestinal disturbances (46, 50).

2. **Anatomy and surgical considerations**

In normal animals, the liver receives twenty to twenty-five per cent of its blood flow from the hepatic artery, and the portal vein provides the remaining seventy-five to eighty per cent (12). The venous and arterial blood mixes in the sinusoids for hepatocellular processing. With anomalous venous systems, blood from the small bowel, colon, spleen,
and pancreas follows the path of least resistance into the inferior vena cava and the systemic circulation (12).

Five major types of macrovascular, anomalous venous systems have been recognized in the dog and cat (18,46). Macrovascular patterns account for ninety-eight per cent of all clinical cases seen. They are 1. persistent ductus venosus, (left or right), 2. portacaval shunt, 3. portal azygos shunt, 4. mesenteric or splenorenal collaterals, and 5. portal azygos shunt with discontinuation of the prerenal postcava (18,46). Structural variations may occur within each of these groups (12). Approximately twenty-eight per cent of all clinical cases present with intrahepatic patent ductus venosus (12). Demanding surgical techniques to repair intrahepatic shunts have been developed (12,13). The remaining seventy per cent are single (50%), or multiple (20%), extrahepatic shunts (12). Multiple extrahepatic shunts are usually formed secondary to primary liver disease, and are not good candidates for surgical repair (12). Single extrahepatic shunts are by far the most amenable to surgical repair. Several authors have reviewed the surgical techniques (8,10,11,47).

Attenuation or ligation of a shunt is performed only when portal pressures are measured by a water manometer or strain gauge transducer (11). Communication between the measuring device and the portal system is accomplished via an eighteen or twenty gauge catheter inserted into a sacrificial branch of a jejunal vein. The base of the manometer should be centered at the level of the right atrium (8,11,12). The level of the right atrium may be found by measuring one half the distance from
the sternum to the dorsal spinous process at the third intercostal space (28). It may be approximated by resting the base of the manometer in the inguinal canal of an animal in dorsal recumbency (11). After a midline celiotomy, and experienced surgeon can often identify the shunt where it enters the inferior vena cava, cranial to the renal veins (12). If the shunt can not be identified grossly, positive contrast portal venography should be performed. Once the shunt has been located, it should be dissected from the surrounding mesentery. Suture material is placed around the shunt and is slowly tightened until a portal pressure of twenty to twenty-three centimeters of water, or sixteen to eighteen millimeters of mercury is attained (12). If the pressure does not rise above twenty-three centimeters of water with the anomalous vessel completely occluded the vessel is permanently ligated (11,12). Sudden elevation of portal venous pressure above twenty-five centimeters of water may result in compromised splanchnic perfusion, severe portal hypertension, ascites and death (12). Some dogs cannot survive even acute, moderate portal hypertension (12). Following attenuation or ligation of anomalous vessels, several patients have died even though the measured portal pressure did not exceed accepted limits (11).

Conversely, E.M. Breznock has stated, "The portal vein of the dog and cat may be completely obstructed if the procedure is performed slowly over several weeks time before complete obstruction is realized" (12).

3. **Summary**

Animals with anomalous venous systems, (portasystemic shunts), are unusually presented with clinical of neurologic dysfunction, gastro-
intestinal disturbance, or other signs of hepatic insufficiency.

The ammonia tolerance test serves as a useful diagnostic aid. Total proteins, albumin, blood urea nitrogen (BUN), serum glutamic pyruvic transaminase (SGPT), and serum alkaline phosphatase (SAP), and complete blood counts serve as useful clinicopathologic tools in monitoring portal blood supply to the liver.

One half of all the cases, (single extrahepatic shunts), lend themselves to reasonable surgical repair. Unexplained, sudden death still occurs with partial attenuation or complete ligation of some shunts, even when guidelines for safety are followed.
III. MATERIALS AND METHODS

A. Animals

Fifteen preconditioned young adult dogs (estimated to be approximately 1-5 years old) were obtained from the Laboratory Animal Resources department of the College of Veterinary Medicine, Iowa State University. All dogs had been vaccinated with Norden laboratory's Vanguard DA2PL and Fromme laboratory's Rabvac. All dogs were wormed with Strongid T and were confined at the Laboratory Animal Resources farm for three weeks prior to the initiation of the project. There were 9 females and 6 males all weighing between 10 and 20 kilograms. They were maintained on pelleted dry food containing 24.5% protein, and 6.2% fat throughout the study. Each animal was evaluated by physical examination, complete blood count, heartworm test, and serum chemistries including glucose, urea nitrogen, alkaline phosphatase, pyruvic glutamic transaminase, and ammonia tolerance tests. If the dogs were found to be normal by all parameters measured, they were entered in the study.

B. Experimental Design

Each animal was randomly assigned to one of three groups. The first group of six dogs received 3K, polycaprolactone coated, double wrapped carbon sutures around their portal veins. Double wrapped 2-0 chromic catgut was substituted in place of carbon in the second group of six dogs. The third group of three dogs served as control animals. The surgical dissection around the portal vein was performed, the sutures were placed and then removed prior to closure of the abdomen.
All animals were induced with thiopental and maintained on halothane gas anesthetic. Lactated ringers was administered through a cephalic catheter at the rate of 10ml/kg for the first hour and 5 ml/kg for the second hour. A ventral midline incision was made from the xiphoid cartilage to a point 4 cm caudal to the umbilicus. Saline moistened lap sponges and a balfour retractor were placed to aid in visualization. A segment of the jejunum was isolated and an 18 gauge intravenous catheter was inserted into the jejunal vein. It was fixed firmly in place with 4-0 nylon sutures around the shaft and the hub of the catheter. The catheter was then attached to a 36 inch, heparanized saline charged extension set and water manometer fitted with a three way stopcock at the base. A baseline portal pressure was measured and recorded with the base of the manometer leveled at the height of the right atrium. The level of the right atrium was determined by measuring one half the distance from the sternum to the dorsal spinous process at the 3rd intercostal space (28). Careful measurements of the table, dog, and manometer base height were recorded to insure that identical measurements could be repeated at a later date with each animal. The portal vein was isolated by gentle retraction of the duodenum to the left side of the abdomen. All implants were placed around the portal vein approximately one inch cranial to the point of entry of the last identifiable jejunal vein. Using a Halstead hemostat, the mesentery was gently separated away from the portal vein to allow implant passage. The catgut or carbon strands were then double wrapped around the vein and gently tightened until a 1 to 4 cm of water increase was attained on
the water manometer. Four throw knots were used consistently with both carbon and catgut. After knotting, the portal pressure was again checked and recorded. At this point, the carbon sutures were cut free from the control dogs and the portal pressure was again measured. The tip of the left lateral liver lobe was removed by the guillotine technique using 2-0 chromic catgut.

The abdomen was then temporarily closed with the jejunal catheter in place and the extension set protruding through the incision and the animal was transported to radiology. Two view portal venography was then performed under stringent conditions to insure reproducibility at a later date. A Harvard infusion pump was used to deliver 50% Hypaque at a rate of 12 cc's in 18 seconds. Using a rapid film changer, exposures were taken at 12, 14, 16, and 18 seconds in both lateral and ventral-dorsal recumbancy.

The dog was returned to surgery where the jejunal catheter was removed and the jejunal vein ligated. The abdominal incision was closed with simple interrupted 0 prolene sutures in the abdominal wall, and simple interrupted 2-0 catgut subcutaneous and subcuticular sutures. A continuous Ford interlocking of 4-0 nylon was used for skin closure. The surgical procedures performed were carefully monitored to insure consistency in all details.

Rectal temperature, appetite, attitude, and stool consistency were monitored for the first week post implantation. Appetite, attitude, stool consistency, and weekly body weights were monitored thereafter.

At the eight week point, all animals were returned to surgery.
Jejunal catheters were placed as previously described and portal pressures measured. Portal venography was repeated using the same criteria as before. The dogs were euthanatized and photographs of the implant site were taken. The implant site, liver, kidney, small bowel, pancreas, and regional lymph nodes were saved for histopathology.
IV. RESULTS AND DISCUSSION

A. Portal Venography

The attempt to document lumen closure by the use of portal venography proved to be a highly inaccurate technique. Even though every attempt was made to standardize the procedure, results were erratic suggesting the introduction of numerous variables. Fifteen of the 30 radiographic views taken showed sizeable increases in lumen diameter at the implant site. However, findings on post mortem exam and from the histologic reactions seen, it is highly unlikely that lumen enlargement actually occurred.

Two radiologists independently measured dye columns at the level of the implant sites. Four exposures (12, 14, 16, and 18 seconds) were taken of each view, (lateral and ventral-dorsal) immediately post implantation and at the 8 week sacrifice. The mean of the four post implant measurements was compared with the mean of the four measurements for that respective view at the 8 week sacrifice. Both radiologist's results were kept separate and the two sets of data were compared.

To discuss the actual results of the measurements would be futile. Enough variability existed between the individual radiologists' measurements that a discussion of the reasons for error would be more appropriate.

The first area identified as a problem was the radiographic phenomena of contrast streaming. As Hypaque dye mixes with the portal blood, the dilutional effect in addition to the movement of the blood and dye past the implant site may cause a lack of positive contrast that is
very unpredictable. Turbulence created by the decreased lumen diameter at the implant site may also cause distortion of the contrast column. Without the ability to clearly delineate the vein wall accurate measurements are impossible. I had attempted to circumvent this problem by using large amounts of contrast agent (12cc) administered over a sustained length of time (18 seconds). A Harvard infusion pump was used for this purpose and also to maintain a constant pressure on injection.

The second problem encountered as a variable between the two radiologists was the identification of the exact implant location. With several animals the implant site was narrowed for a variable distance. Each radiologist had to choose the spot that they felt was the narrowest portion of the lumen, and in some cases this varied.

Several other problem areas beyond the control of the radiologists were identified. The positioning of the portal vein within the animal was altered at the time of surgical implantation of the catgut or carbon. The viscera was shifted to the left side of the abdominal cavity by retraction of the descending duodenum. Physical dissection was performed around the portal vein with a Halstead hemostat and implants were placed and positioned. The viscera was replaced, the abdomen closed, and the first set of portal venograms was performed. Upon recovery, the abdominal viscera certainly returned to a more natural position moving with it the portal vein. This movement, although slight could make major differences when results are measured in fractions of millimeters.

At the time of implantation, it was noticed that the portal veins remained indented on all three of the control dogs after the implants
had been removed. This constricted area was visible on portal venography immediately following the surgery in two of the three dogs. I attribute this sustained artifact to contraction of the small amount of smooth muscle found within the portal vein wall. Portal vein diameters increased in all three of the control dogs over the course of the study. I suspect that the smooth muscle under the carbon and catgut implants may also have responded in the same manner. With relaxation of the smooth muscle the vein wall would expand out to meet the resistance of the encircling implant.

Eleven of the 15 animals gained weight during the course of the study. This increase in abdominal fat could have altered radiographic measurements by changing the position of the portal vein or increasing the distance between the portal vein and the film cassette. A magnifying effect occurs as objects are moved away from the film and towards the beam.

Other physiological factors that could cause variability in the size of the portal vein, are the phase of respiration at time of exposure, marked changes in systolic or central venous pressures, and the hydration state of the animal.

It is my feeling that the number of variables, both human and physiological, are too numerous to overcome. As medicine has been described as an inexact science, so in this instance is radiology.
B. Clinical Pathology

Clinical pathological monitoring was performed at three times during the study. Prior to implantation, at the midpoint, (4 weeks) and prior to sacrifice, (8 weeks) the following parameters were monitored. White blood cell counts, including PCV, Hb, total protein, and fibrinogen. Serum chemistries included glucose, albumin, urea nitrogen, (BUN) alkaline phosphatase, and glutamic pyruvic transaminase. Ammonia tolerance tests were also performed.

All parameters measured over the eight weeks of the study were found to be normal, with only two exceptions. Two dogs (5334 Catgut and 5378 Control) exhibited a moderate leukocytosis with a left shift at the midpoint of the study. They did not exhibit any clinical signs suggestive of the cause of the white cell response. Their appetites and attitudes remained normal, but both had a mild elevation of their rectal temperature, 103.0 and 102.8, respectively. The rectal temperatures returned to normal in 48 hours and the leukocytosis and left shift were gone when the final blood counts were run before sacrifice. No evidence of infection was found at post mortem exam.

If reasonable occlusion of the portal vein would have been achieved, marked changes in the blood picture would have been expected.

Portal Pressures (Table 1)

Resting portal pressures were measured prior to manipulation of the portal vein, after the implant was in place, and at the 8 week point prior to the second series of portal venograms. Table 1 is a summary of the recorded measurements. The "Difference" column on the far right side
Table 1. Summary of portal pressures in centimeters of water

<table>
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<tr>
<th>Dog #</th>
<th>Implant Type</th>
<th>Resting Portal Pressure</th>
<th>Post Implant Portal Pressure</th>
<th>8 Wk Portal Pressure</th>
<th>Diff.</th>
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<tr>
<td>5309</td>
<td>Carbon</td>
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<td>14.0</td>
<td>11.0</td>
<td>-3.0</td>
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<tr>
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<td>Carbon</td>
<td>10.6</td>
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of the table is the measured change in portal pressure from the time of implantation until sacrifice.

A negative value in this column would indicate a decrease in the portal pressure between the time of implantation and sacrifice. This would be expected if little fibrous connective tissue was formed and the portal vein remained patent. The portal system has a remarkable ability to adapt to moderate increases in portal pressures.

A positive value in this column indicates a rise in the portal pressure that we expected to see if the portal vein was slowly being occluded. Five of the twelve implant dogs exhibited a rise in pressure, but so did two of the three control dogs. This would suggest that other factors played an important role in determining the measured pressures. Since the same measurement techniques were used for all readings, the variability must lie within the dog's own physiologic processes.

Variability in hydration state, systolic blood pressure, central venous pressure, and position of the abdominal viscera could all play a role in the measurement of portal pressures from one day to the next. The variability in the results of this study indicate that setting exact guidelines for maximum portal pressures may lead to error on the part of the surgeon.

Dog 5337 serves as one example. Baseline portal pressures were measured and checked twice. A reading of 10.6 cm/water was obtained for the reading both times. After dissection around the portal vein and the placement of a double wrapped carbon ligature that indented the wall of the vein approximately 25%, the portal pressure was rechecked two times.
and was found to be 9.6 cm/water. The catheter was flushed and the manometer recharged to insure that no errors were made, and again the pressure was 9.6 cm/water. With the portal vein partially occluded by the ligature it is hard to understand how the post implant pressure could be lower.

Dog 5235 also exhibited peculiar portal pressure responses when the double wrapped 2-0 chromic catgut suture was applied. The catgut was tightened around the vein until approximately 50% of the portal vein lumen was occluded. The manometric pressure elevated only by 2.2 cm/water to 10.2 cm/water. The suture was loosened and the catheter and manometer were flushed to insure a patent system. Upon reapplication of the catgut, identical tension was required to reach a pressure of 10.2 cm/water.

These two dogs illustrate that portal system pressures do not always respond in a consistent manner to closure of the portal vein.

D. Post Mortem Exam

On post mortem exam, 13 of the 15 dogs showed a remarkable amount of external capsular fibrosis of the left lateral lobes of the liver. This is the area where the liver biopsy specimen was removed at the time of implant placement. The external capsule was traumatized as the assistant retracted the liver lobe caudal to the incision for ease of removal of the biopsy specimen. Histologic examination confirmed that no damage occurred below the level of the external capsule.

The implant sites appeared very quiet, with no signs of inflammation or undesired fibrosis in the surrounding tissues. Only one catgut
implant dog had a small adhesion of the omentum to the implant site and also to the left lateral liver lobe where the tip had been removed.

The catgut and carbon sutures were easily visualized. Little if any fibrous connective tissue formation could be seen. Each portal vein was opened through the implant site to check for signs of internal lumen narrowing. The indentations seen in the portal vein under each implant varied, depending on the amount of tension that had been applied to the carbon or catgut at the time of placement. Additional narrowing of the lumen due to fibrous connective tissue formation was not noted. The intimal surfaces were smooth and no signs of thrombus formation were seen.

E. Histopathology

Histopathologic examination was performed on sections of the implant sites, liver, duodenum, jejunum, regional lymph nodes, pancreas, and kidneys. All cellular or structural abnormalities were graded on a zero to four plus scale. A zero indicated a total lack of the cellular response or structural change being examined. A one plus rating indicated the presence of a cellular component in low numbers or a minimal amount of structural change. Two plus indicated a moderate number of the specific cellular components or a moderate amount of structural change. Three plus indicated a marked amount of a specific cellular components or a marked amount of structural change. The four plus rating indicated the maximum cellular or structural response seen in the specimens being evaluated. A mean grade was obtained for the cellular response or structural change for the dogs in each group (i.e.,
carbon, catgut, and controls).

1. **Pancreas**

   No significant lesions were seen in the pancreatic tissue. With its close proximity to the implant site on the portal vein, the quiescent appearance of this sensitive glandular structure suggested a lack of inflammation in the soft tissues adjacent to the carbon or catgut.

2. **Duodenum and jejunum**

   No significant lesions were seen in the small bowel sections. If significant portal hypertension had occurred, we would have expected to find hemosiderin laden macrophages, and/or congestion in the lamina propria.

3. **Kidneys**

   The kidneys were evaluated for the presence of peripelvic or interstitial lymphocytes and plasma cells, dilated glomerular capillaries, mineralized casts, prominence of the juxtaglomerular apparatus, and old dog inclusion bodies. Mineralized casts, considered to be normal background lesions, were seen in 13 of the 15 dogs. All other cellular or structural changes in the renal tissue were insignificant.

4. **Liver**

   Hepatic tissue was evaluated for dilatation of the portal veins, centrilobular vacuolar degeneration, cholestasis, old dog inclusion bodies, portal vein polymorphonuclear lymphocytes, dilated lymphatics,
lipogranulomas, surface fibrosis and eosinophils, and hemosiderin deposits. Centrilobular, vacuolar hepatocellular degeneration of mild to moderate severity was noted in all animals, on both the pre-implant biopsy and the sections taken at sacrifice. Vacuolation within hepatocytes was diffuse throughout the cytoplasm suggesting intracellular accumulation of glycogen or water. The grading system indicated that as a group there was a decrease in centrilobular hepatocyte vacuolation over the eight weeks of the study. If impairment of blood flow through the portal vein had been achieved, we would not have expected to see an improvement in the histologic character of the liver parenchyma (18). No other significant changes were seen in the liver sections.

5. **Lymph nodes (Table 2)**

Regional lymph nodes were evaluated for the presence of primary and secondary follicles, plasma cells, hemosiderin, eosinophils, sinus histiocytes, red blood cells in the sinuses, and erythrophagocytosis. Lymphatic tissue adjacent to both carbon and catgut showed a tendency towards the formation of secondary follicles (Fig. 2). Secondary follicles develop within the lymph node during periods of increased functional activity (24).

Plasma cells were noted in higher numbers among the catgut implant group (x=2.17) when compared with the carbon (x=1.33) and control (x=1.0) groups. The increased presence of plasma cells is indicative of an increased ability to produce antibodies. This seems reasonable as the protein component of the catgut should be recognized by the body and defenses as a foreign antigen. Equally interesting is the apparent lack
Figure 2. Histopathology

A. Lymph node, secondary follicles, 75x

B. Splendore-Hoepli Phenomenon, 475x

C. Fibroblast alignment along carbon filaments, 195x

D. Fibroblast alignment along carbon filaments, 485x
Table 2. Summary of histologic responses in regional lymph nodes to carbon and catgut implants

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\(^a\)Eosinophils.

\(^b\)Sinus Histiocytes.
of increased plasma cell numbers in response to poly(ε-caprolactone) coated carbon. It is probable that carbon has less antigenic potential than chromic catgut.

Eosinophil populations were elevated with both the carbon (x=1.5) and catgut (x=2.17) groups when compared with the control group (x=0). Eosinophil presence has been associated with a T-lymphocyte response, and an activation of cell mediated immunity within the lymph node (56). This reaction was most pronounced within the lymph nodes associated with the catgut group.

Sinus histiocytes were noted to have increased minimally within the lymphoid tissue of the carbon (x=1.0) and catgut (x=.83) group when compared with the control group (x=.33). Recently, sinus histiocytes have been reclassified as a previously undescribed stage of B-lymphocytes (44). Most often B-lymphocytes play a primary role in the production of antibodies (35). No other significant changes were seen within the lymph nodes.

The reader should be reminded that the grading system used, provided a subjective quantitation of the cellular components present. This grading system was only used to establish trends or tendencies, not to collect hard data for statistical analysis. It should also be noted that the mesenteric lymph nodes harvested may also receive lymphatic drainage from areas beyond the implant site. Local or generalized infections, parasitic infestations or generalized allergic reactions could influence the cellular changes within these lymph nodes.
6. **Implant site (Table 3)**

Sections of the implant site were prepared by cutting the tissue at right angles to the longitudinal axes of the implants. The amount of fibrosis associated with the catgut group (x=3.17) actually exceeded that of the carbon group (x=2.00).

The cellular reaction to the carbon filaments was a granulomatous response. Granulomas are characterized by the infiltration, maturation and aggregation of young mononuclear phagocytes. The reaction to the carbon filaments is consistent with classification as a "low turnover" granuloma. Low turnover granulomas show little evidence of macrophage death, immigration, or mitosis (9,56). They are produced in response to poorly degradable chemically inactive, nontoxic substances (1).

Almost all of the cellular activity seen in association with the carbon occurred within the implant itself (Fig. 3). Individual carbon filaments had been forced apart by the newly formed fibrous connective tissue, increasing the diameter of the fiber from within. Macrophages, multinucleated giant cells, endothelial cells in new capillaries, and fibroblasts were the predominant cell types seen interspersed between the separated carbon filaments (Fig. 3). It is thought that multinucleated giant cells are produced by the fusion of macrophages as a result of simultaneous attempts to ingest the same particle of foreign material (56). Blood-filled capillaries were noted with regular frequency in the new connective tissue between carbon filaments.

Fibroblasts were found in moderate numbers and did appear to align themselves along the individual carbon filaments as seen in longitudinal...
Table 3. Summary of histologic responses for carbon and catgut implants

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*Multi Nucleated Giant Cells.

*Polymorphonuclear Leucocytes.*
Figure 3. Carbon implant

A. Carbon implant, dog 5465, 30x
B. Carbon implant, dog 5465, 75x
C. Carbon implant, dog 5465, 195x
D. Carbon implant, dog 5465, 475x
section (Fig. 2). Total fibrous connective tissue formation within the carbon tow was estimated to produce only a one fold increase in the diameter of the original implant. This amount of fibrous connective tissue was far less than had been reported in tendon repair research. No neutrophil or eosinophil response to the carbon was noted. Dense rings of an intensely eosinophilic substance were seen surrounding several carbon filaments cut in cross section (Fig. 2). This condition has been described as the Spendore-Hoepli Phenomenon, named for the men who first described it (29). It is believed to be an in vivo antigen-antibody precipitate, which has been seen in association with fungi, helminths, gram negative bacteria, and silk sutures (29).

The cellular reaction to catgut occurred at the periphery of the fiber with no invasion into the suture material. The fibrous connective tissue formation, secondary to the degradation of chromic catgut was much more dramatic than we had expected. The production of mature fibrous connective tissue secondary to catgut implantation consistently exceeded that of the carbon group.

The inflammatory reaction to catgut could also be described as a granulomatous response. Macrophages could be seen attached to small portions of the peripheral catgut, and multinucleated giant cells were not prevalent. Polymorphonuclear leucocytes in small numbers were seen in association with the catgut sutures.
Figure 4. Catgut implant

A. Catgut implant, dog 5466, 30x
B. Catgut implant, dog 5466, 75x
C. Catgut implant, dog 5466, 195x
D. Catgut implant, dog 5466, 475x
V. SUMMARY AND CONCLUSIONS

The induction of fibrous connective tissue on or within the skeletal framework of carbon fiber tow occurred in insufficient quantities to achieve the goal of this study. Fibrous connective tissue produced at a rate of 8-15 times the implant, as has been reported, would have been adequate to a least partially occlude the portal vein. The studies performed by Ralis and Forster emphasized the need for a source of vascularized connective tissue and mesenchymal cells, and that a functional working load be placed on the carbon filaments. The cellular components were present on histopathology in both carbon and catgut groups, and appeared to be readily available. The question that we could not answer at the onset of study was if the lumenal pressures of the portal vein pushing outward on the encircling implant would provide a sufficient working load. The results are clear. The low pressures found within the portal system are insufficient to place a functional amount of tension on the carbon fiber. Without adequate tension, a minimal amount of fibrous connective tissue was formed. The response to the carbon by the body's cellular defense system was classified as a low turnover granuloma. This was considered to be a biocompatible situation as low turnover granulomas form in response to inert foreign material.

Chromic catgut also produced an inflammatory granulomatous response forming more mature fibrous connective tissue than the carbon filaments. The volume formed was judged to be insufficient to adequately provide closure of a venous structure. Although the collagen formation was
minimal, catgut may serve as a useful adjunct to nonabsorbable suture materials such as silk or nylon when partial attenuation of a portasystemic shunt is attempted. Using the nonabsorbable suture material to partially attenuate the vessel and achieve acceptable pressures, a second ligature of chromic catgut could be applied directly over it. Any reduction in lumen size would be at best minimal, but helpful. Increasing the volume of the catgut encircling the shunt may aid in forming more fibrous connective tissue.

Poly(ε-caprolactone) worked well as a sizing material. The carbon filaments were easy to handle and no evidence of adverse tissue reactions was obtained.

The use of portal venography as an instrument to document lumen closure, proved to be a highly inaccurate technique. As discussed previously, a number of variables such as change in position of the portal vein, make accurate documentation of the lumen closure impossible. Contrast streaming occurred consistently making lumen diameter measurements vary greatly. The ability of the normal portal system to adapt to increased fluid volumes, such as rapidly injected Hypaque, made consistent distention of the portal vein difficult. It is my feeling that the use of portal venography should be used only as a diagnostic aid to locate anomalous venous structures and never as a measurement device. Much has been written about the use of portal pressures as a guideline for attenuation of portasystemic shunts. A maximum portal pressure of 23 centimeters of water is considered safe when attenuation or ligation of a shunt is attempted. I would caution
any surgeon performing this task to adhere loosely to these guidelines. With the variable pressure measurements I encountered in this study, it would seem easy to push too close to the high end of the guidelines, only to find that your measured pressure was inaccurate and actually higher than you thought. Upon recovery of the animal, portal hypertension might occur. In application of the catgut, or carbon fiber tows in this experiment, we found that portal pressures did not always respond consistently when multiple measurements were made. The measured pressures in some animals responded sluggishly as ligatures were tightened around the portal vein. In other animals, the slightest decrease in lumen diameter seemed to stimulate a marked pressure response. In this study, we used sacrificable mesenteric veins to make our measurements. Perhaps direct catheterization of the portal vein would provide pressures that are less subject to error.
VI. BIBLIOGRAPHY


VII. ACKNOWLEDGMENTS

Trust in the Lord with all your heart, do not rely on your own insight, in all your ways acknowledge Him, and He will make straight your paths (Proverbs 3:5&6).

This project was funded in part by a research grant provided by the Iowa State University, Department of Biomedical Engineering. Supplemental funding was provided by the Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Iowa State University.

Carbon fiber preparation, photomicrograph reproduction, and photographic verification of all facets of the project were funded by Eklund Medical Products of Des Moines, Iowa. I wish to thank Mr. Lorne Scharneberg and Dr. Eklund specifically, for their help with this research.

My special thanks to my wife Jan, whose encouragement and sacrifices have made this work possible.

I wish to convey my gratitude to Drs. Ron Grier, David Merkley, Darrell Trampel, and Ray Greer, for their input and support throughout this entire project.

I also would like to thank Drs. Liz Riedesel, Barb Selcer, and Sandra McNeel for their assistance with the radiographic portion of this study.

Last but not least, I would like to thank the many senior veterinary students and recent graduates of Iowa State University who tirelessly aided in all facets of this project.
VIII. APPENDIX: INFORMATION ON THE USE OF ANIMALS IN RESEARCH AND TEACHING

This research was conducted according to the rules and regulations of the Animal Welfare Act.