FIVE-HYDROXYTRYPTAMINE (ANTAGONISTS) AND FELINE AORTIC EMBOLISM

by

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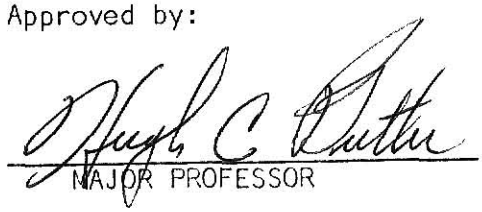

MAJOR PROFESSOR

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INTRODUCTION

Although abdominal aortic embolism, as it is observed in the cat, is only a secondary manifestation of cardiomyopathy and endocarditis, it results in a dramatic set of signs. The embolism usually lodges at the iliac bifurcation. The cats afflicted with this disease entity have either paresis or complete paralysis of the hind legs. Their femoral pulse is usually absent; however, it may be present in a weakened form. The hind legs are cold when compared to the front legs. The gastrocnemius muscle is often firm, while other muscles may have diminished tone. Cyanosis may be noted in the toes. All of these signs would indicate that circulation in the hind legs is deficient.

There is indirect evidence (Butler, 1971) that 5-hydroxytryptamine (5-HT) released from the clot obstructing the aortic blood flow may cause collapse of the collateral circulation to the hind limbs. Five-hydroxytryptamine is also known as serotonin, but will be referred to in this thesis by its longer but more chemically correct name or the abreviation thereof. Described herein are experiments with iatrogenic blood clots and 5-HT antagonists to determine the relationship of 5-hydroxytryptamine to the observed vascular collapse.

REVIEW OF THE LITERATURE

Feline aortic embolism made its debut in the literature in 1930 when Collet reported on a case in a 7-year-old castrated male. The cat exhibited all of the typical signs associated with aortic embolism. It was not until 25 years later when Holzworth et al (1955) reported on aortic embolism again. Ten cats were included in this report, all of them showing typical signs of aortic embolism. Liu et al (1970) stated that there had been 50 reported cases of aortic embolism at the time of their report. They added 47 more cases of aortic embolism, all of them exhibiting typical signs of the disease entity.

Imhoff (1961) reported some rather startling work that indicated the posterior paresis seen in these cats did not result from the mere mechanical occlusion of the aorta. In this work three groups of cats were used. In the first group of cats a ligature was placed at the iliac bifurcation. None of the five cats in this group exhibited signs of posterior paresis or paralysis when examined 24 hours following surgery. The aortograms revealed the presence of collateral circulation supplying blood to the hindlegs. These cats were followed for one month with the only change being an increase in the collateral circulation. In a second group of five cats ligatures were placed around aorta at the bifurcation of the iliac arteries, just caudal to the caudal mesenteric artery and on the circumflex iliac arteries as they emerged from the aorta. The results observed in these cats were the same as those in the first group of cats. The third group of cats consisted of three cats in which a clot was produced in the posterior aorta to simulate an aortic embolism. This was done by placing a permanent suture at the iliac bifurcation, ligating the circumflex iliac arteries and placing umbilical tape around the aorta just

caudal to the caudal mesenteric artery. Ten to twenty units of bovine thrombin was then injected into the cul-de-sac that had been formed in the aorta, thereby clotting the blood that had been trapped in the area. The umbilical tape was removed after five minutes. All of the cats in this group showed posterior paresis. Aortograms revealed that the collateral circulation that had developed in the first two groups of cats did not develop in this group of cats. The cats having shown no improvement in locomotion after ten days, were euthanatized. Imhoff concluded that "The clot apparently played some part in causing the paraplegia."

Schaub and Meyers (1974) substantiated Imhoff's conclusions. Their work consisted of two groups of cats which contained eight cats in each group. In Group One the ligatures were placed around the deep circumflex iliac arteries, around the aorta just caudal to the caudal mesenteric artery, and at the iliac bifurcation. No blood was present in the cul-de-sac. In Group Two a clot was created at the iliac bifurcation by the injection of 10-20 units of thromboplastin. Collateral circulation was evaluated with the use of aortograms. The presence or absence of paralysis was noted and the blood flow to the hindlegs was measured by the hydrogen desaturation method preoperatively, immediately postoperatively, and three days postoperatively. In Group One no paralysis was seen and the aortograms indicated collateral circulation to the hind limbs. The blood flow in Group One was measured at 20% of the presurgical flow immediately postoperatively; however, at three days postoperatively, 114% of the presurgical blood flow was present. The cats in Group Two all exhibited paralysis and a reduced collateral circulation, as seen on the aortograms. The immediate postsurgical blood flow in these cats was 30% of the presurgical value; however, three days postoperatively, the blood flow had dropped to 14% of that which was seen presurgically.

Butler (1971) attempted to more clearly define the relationship between aortic embolism and posterior paresis. There were five groups of cats in this experiment. The first group consisted of four cats which were used to duplicate Imhoff's creation of a bloodless cul-de-sac. In Group Two 19 cats had different components of blood injected into the cul-de-sac. Group Three contained three cats in which histamine was injected into the cul-de-sac. Group Four contained 19 cats in which 5-hydroxytryptamine was injected into the cul-de-sac. In Group Five four cats had hypotonic saline injected in the cul-de-sac. The cats in Group One were maintained for 28 days to show the development of collateral circulation through angiography. Silk suture was used to ligate the aorta just caudal to the caudal mesenteric artery, at the two circumflex iliac arteries, and just anterior to the iliac bifurcation. A bent needle was used to inject material into the cul-de-sac which had been formed by the ligatures. The needle was inserted at the point of the posterior ligature and the material was injected after the ligature had been tied tightly around the needle. As the needle was withdrawn, the ligature was tightened further. The cats were evaluated at the end of 48 hours for evidence of paralysis. No paralysis was seen in any of the cats in Group One. In the second group of cats studied by Butler, the following components of blood were injected into the cul-de-sac: 1) autologous serum; 2) autologous plasma; 3) whole white blood cells; 4) whole autologous red blood cells; 5) supernatant of autologous lysed red blood cells; and 6) autologous lysed white blood cells. Of these cats, only those cats which had the supernatant of autologous lysed red blood cells injected into the cul-de-sac showed any evidence of paralysis after 48 hours. Three of four cats exhibited posterior paralysis. All of the cats in which histamine was injected into the cul-de-sac walked well. The cats in Group Four were injected with varying amounts of

5-HT creatinine sulfate. Butler reported that in cats the blood levels of 5-HT varied from 0.68 mg/ml to 3.8 mg/ml of blood. Of those cats which were injected with 3 mgs of 5-HT, six out of eight cats showed evidence of paralysis. In the group of cats which were injected with 0.8 to 1 mg of 5-HT, 3 out of 4 showed paralysis. Of seven cats injected with amounts of 5-HT varying from 0.3 mg to 0.6 mg, four were paralyzed. All four of the cats in Group 5 walked well. Butler's work shows that conditions similar to those seen in aortic embolism can be created when 5-HT is injected into the cul-de-sac.

Michal (1970) showed platelets released ADP and 5-HT. It was also shown that the release of these substances stimulated the further aggregation of platelets. This was substantiated by Nemier et al (1972).

Hoak (1964) studied the composition of a thrombus with the use of electron microscopy. Long-chain saturated fatty acids were injected into the tail veins of mice. This resulted in the creation of thrombi throughout the body. The thrombi were examined with the light and electron microscopes and found to contain large aggregates of platelets. The platelets near the surface of the thrombi appeared to have lost their granules, while those platelets in the center of the thrombi still contained granules.

Somlyo and Somlyo (1970) in an extensive review of the literature on vascular smooth muscle discussed the action of 5-HT. Both excitatory and inhibitory action on vascular smooth muscle has been observed. Activity varies from species to species and also within the body of an individual. There is a great deal of work reported in this review that indicates that 5-HT has a vasoconstrictive effect on many vessels. It excites vascular smooth muscle by acting on the membrane potential of the cells. This is primarily a direct effect; however, there is some evidence of indirect action

of 5-HT associated with catecholamine release. There is also evidence of a synergistic interaction of adrenergic compounds with 5-HT. The vasoconstrictive potential of bradykinin is increased in the presence of 5-hydroxytryptamine. A normally vasodilatory level of bradykinin will result in intense venular constriction if 5-HT is present. This was demonstrated by Zweifach (1964) and Weiner and Burton (1967).

Page (1968) summarized work reported on 5-hydroxytryptamine to that time in the form of a book. He stated that endogenous 5-HT is chiefly found in platelets and in nervous tissue. Platelets both take up and release 5-hydroxytryptamine much like the nerve endings of the brain that take up and release catecholamine. Page reports platelets contain almost all of the blood 5-HT; however, small amounts can be extracted from red and white cells. The primary site for removal of circulating serotonin is in the lungs. The liver is the next most important site for removal of 5-HT. It is degrauated by acetylation to 5-hydroxyendoleacetic and 5-hydroxyendoleaceturic acid, both of which are excreted in the urine.

Although the platelet does not have the ability to produce 5-hydroxytryptamine, it does have a great affinity for the uptake of body stores of 5-HT. Thrombin releases most of the 5-HT from platelets in a short period of time. Intravascular aggregation of red blood cells can be created by the presence of 5-HT. Free 5-HT is quickly bound to tissues, especially the reticuloendothelial cells of the liver and spleen. Although 5-HT is found in the mast cells of some species, this is not true in the cat.

Page (1968) referred to 5-HT as an amphibaric compound because it has a variable effect on arterial blood pressure, i.e., when neurogenic vascular tone is low, 5-HT will increase the blood pressure and when the tone is high, 5-HT will lower the blood pressure. For the most part the function of

5-hydroxytryptamine is that of a vasoconstrictor; however, in certain instances and with certain vessels it can be a vasodilator.

Many studies have been done on various drugs to establish their specificity as an antagonist to 5-HT. Among these drugs were methysergide, cyproheptadine and doxepin.

Stone et al (1961) has shown that cyproheptadine possesses both anti-serotonin and anti-histaminic activity at strengths equal to some of the strongest antagonists for both 5-HT and histamine.

Krantz and Carr (1969) showed that methysergide is a strongly specific antagonist for 5-HT. This was substantiated by work done by Gorlitz and Frey (1973) who compared the blocking effects of antagonists of epinephrine and 5-HT on their mutual receptor sites. Isolated strips from rat fundus which is sensitive to 5-HT and isolated vas deferens which is fairly sensitive to norepinephrine were used to evaluate the drugs in an in vitro study. Five-HT or norepinephrine was added to the bath containing the tissues until maximal contraction was reached. At that point an antagonistic drug was added until its maximum reversal affect was reached. The two 5-HT antagonists studied were methysergide and cyproheptadine. The methysergide showed a high factor of safety between its effect on 5-HT and norepinephrine having a ratio of 1:1260. Cyproheptadine had a lower factor of safety since its ratio was 1:126. Therefore it was concluded that cyproheptadine is specific as a 5-HT antagonist only when the concentrations necessary to block the action of 5-HT are not unduly exceeded.

The Chas. Pfizer Company has provided this researcher with information on a drug known as doxepin. The company's information indicates that doxepin is potent as an antagonist to both 5-HT and histamine in vivo as well as in vitro. The drug also has a mild to slight anti-cholinergic activity with some peripheral vasodilitation. The recommended dosage in dogs is 1 mg/lb

body weight daily. The chemical formula for doxepin is 11 dimthylamino-propylidine-6 H-diebenz (b, e) oxepin hydrochloride.

Cognizant of the facts that 5-hydroxytryptamine was released by platelets and could cause vasoconstriction, researchers armed themselves with 5-HT antagonists and began perusing the relationship between clot formation and clinical signs seen in various disease conditions. Many methods have been used to create a simulated embolus. To mention a few, Imhoff (1961) injected bovine thrombine, Ozdemir et al (1974) merely allowed nature to take its natural course, Hageman et al (1973) injected Diatomaceous earth and reported that others had used glass beads mixed with blood.

The use of glass beads may have an interesting aspect. Venter and Kaplin (1974) showed that catecholamines when attached to glass beads would release very slowly over a long period of time. They also report that it was necessary for the beads to be in contact with the tissue before a biological response would be elicited. The beads used were 300 mm. in diameter. The small amount of catecholamine that was leached off the beads into the solution could not cause the biological effects observed, i.e., there must be contact with the beads and the tissue; thus the catecholamines were acting while covalently coupled to the glass beads.

Hageman et al (1973) showed in the dog that microembolism produced by the injection of Diatomaceous earth in the pulmonary artery would cause elevation of the perfusion pressure in the lungs. Further, it was found that this elevation in perfusion pressure was enhanced when 5-HT, bufotenine and d-amphetamine were infused. The infusion of other vasoconstrictors, such as norepinephrine, angiotensin-2 and histamine did not affect the perfusion pressure. The increased perfusion pressure produced by the Diatomaceous earth was reduced when aminophylline, methysergide or cyproheptadine were administered intravenously. Hageman felt the vasoconstriction observed

after microembolism was mediated by 5-hydroxytryptamine.

Hageman's observations were supported by Ozdemir et al (1974) in a study using mongrel dogs in which fresh blood clots were infused into the pulmonary artery. The blood clots were obtained by withdrawing 50 ml of blood, allowing it to clot and preserving it at 4° C for no longer than one hour. The clot was deiced by being pressed through a gauze filter. The blood was maintained at 4° C so that the 5-HT would not be degraded which occurs within a few minutes if the platelet clot is held at room temperature. Some of the dogs in this study had their lungs removed and then reimplanted in the chest to eliminate neural influence on the micro-circulation. The infusion of the clots into the right pulmonary artery produced severe changes in the left pulmonary vascular bed. The same changes were produced when 5-HT was infused into these animals. Two of the research groups in this study were pre-treated with 5-HT antagonists. Reserpine was used to reduce the 5-HT platelet content and in the other group massive dosages of heparin were used. The heparin acted directly as a 5-hydroxytryptamine antagonist. It also prevented platelet aggregation and rupture. In the animals that were pre-treated with the reserpine and the heparin, the changes grossly and microscopically were mild as compared to those that were not treated. The animals with 5-HT or blood clots showed interstitial edema, hemorrhage and decreased capillary flow in both the reimplanted lung and the intact lung. Thus, Ozdemir et al concluded that there was a humoral influence from 5-HT in pulmonary embolism.

Flamm et al (1972) induced vasospasm of the basilar artery of the cat by applying blood to the area or by mechanical manipulation. They were able to prevent the vasospasms seen with the application of whole blood by the use of parenteral administration of phenoxybenzamine at a dosage rate of 5 mg/kg or by topical application of phenoxybenzamine. They also found that phenoxybenzamine did not prevent or alleviate the spasms induced by

mechanical manipulation. Three milliliters of blood was obtained from a femoral arterial catheter and was applied directly to the basilar artery for a period of three minutes before the blood was flushed away. The spasm seen lasted for twenty minutes. A mean change of a minus 36% in the vessel diameter was noted. The phenoxybenzamine if administered one and one-half hours prior to the placement of the whole blood would prevent any spasm from developing. It was assumed by Flamm that this was the time necessary for the drug to become fixed to receptor sites on the vessel. The application of blood prior to the one and one-half hour time period resulted in a spasm that diminished in degree as the one and one-half hour time period approached. Flamm et al stated that "several workers have attempted to isolate the agent or agents in blood responsible for the production of spasm. The consensus favors either serotonin or other heat-stable compound of blood which is different from serotonin or angiotensin."

Nielsen and Owman (1971) showed in an in vitro study using the cat's middle cerebral artery that 5-HT in concentration of 8×10^{-7} moles would result in a vasoconstrictive response measuring between 250 to 350 dyn. This response could be prevented if 10^{-4} moles of methysergide were added to the solution.

Banna and Anderson (1968) did a series of experiments using cats and 5-hydroxytryptamine antagonists to study their effects on spinal neuronal activity. The drugs included were methysergide, 2 bromo-d-lysergic acid, diethylamide, d-lysergic acid, cinanserin, and cyproheptadine. Their work indicated that if 0.2 mg/kg or less of methsergide was used the response was dependent on the dose of 5-HT. It was found that the blood pressure response to methysergide in dosages above 0.2 mg/kg was quite marked; however, the effects varied from a pressor to a depressor response. Minimal cardiovascular changes were seen with the other drugs that were used. Methysergide

showed good antagonism toward the excitatory effects of 5-HT, but did not influence its depressor effects in the nervous system. Cyproheptadine at 1.2 mg/kg appeared to have a good antagonistic effect of the excitatory phase of 5-HT, but like methysergide was unable to block the depressant phase of 5-HT.

Allen et al (1974) conducted in vitro experiments on the canine basilar artery and the canine middle cerebral artery with the use of vasoactive agents. Among the agents tested were 5-HT, three different prostoglandins; epinephrine, norepinephrine, histamine, angiotensin, potassium chloride and bradykinin. The contraction of these arteries was measured when the above mentioned substances were added at physiologic levels. From the response seen, it was concluded that 5-HT was probably the agent in the blood responsible for cerebral arterial spasms following subarachnoid hemorrhage.

In additional work done by Allen et al (1974) it was found that the majority of the contractile activity of cerebral spinal fluid following subarachnoid hemorrhage was a result of 5-HT levels. Methysergide reversibly blocked the arteries response to 5-HT; however, methysergide caused vasoconstriction of its own; up to 58% of that caused by the addition of 5-HT. No further vasoconstriction could be obtained with the addition of 5-HT after the methysergide had been added to the chamber. Phenoxybenzamine irreversibly blocked the arteries response to 5-HT, serum and cerebral spinal fluid that had been collected from an area of subarachnoid hemorrhage.

An in vivo study was done by Allen et al (1974) which showed that the spasm produced by pure 5-HT and blood could be reversed with the use of phenoxybenzamine. The 5-hydroxytryptamine injected was at physiologic concentrations around a dog's cerebral artery. Allen states that 5-HT is found in the platelets and released over a period of several days as the clot lyses and the suspended platelets break down. The additional free 5-HT prolongs the spasm which is seen.

Nemir et al (1972) reported that prolonged ischemia would result in cellular destruction and increased capillary permeability. This, in turn, would cause a wide variety of materials with vasoactive properties to be released, i.e., 5-HT, histamine, prostoglandins, acetylcholine, adenosine derivatives, bradykinin, and other vasoactive peptides.

Nemir's work dealt with pulmonary embolism in the dog. He stated that the aggregation of platelets resulted in the release of granules which contained 5-HT, ADP and epinephrine. He found that in a state of circulatory stasis the concentration of these agents would increase rapidly due to the self-perpetuating cycle of platelet aggregation. Nemir found that the intravenous administration of 1 mg/kg of cyproheptadine HCl would ameliorate the hemodynamic response of 5-HT and autologous blood. Cyproheptadine also decreased the capacity of platelets to aggregate in an area of circulatory stasis. Nemir's observations that 5-hydroxytryptamine levels increase with circulatory stasis and that cyproheptadine decreases platelet aggregation are consistent with Page's (1968) report.

RESEARCH DESIGN - METHODOLOGY

DESIGN

A total of 33 clinically-normal, domestic shorthair cats were used in this experiment. Mature cats of both sexes were used. Their weights ranged from 1.4 kg to 4.2 kg. The cats were divided into six groups. With the exception of two cats (DB-1 and DB-2) in Group 6, the terminal aorta of each cat was occluded with four to five ligatures which formed a 1½ cm cul-de-sac.

In Group 1, ten cats (C-1-C-10) were used. A blood clot was formed in the cul-de-sac of each cat in Groups 1, 2, 3 and 4. Group 1 was used as a control. Cats in Groups 2 (Ms-1-Ms-8) were given varying amounts of methysergide[a], intravenously. Group 3 consisted of four cats (Cy-1-Cy-4), which were given cyproheptadine[b] intravenously. The cats (D-1-D-4) in Group 4 were given doxepin[c] intravenously. Groups 2, 3 and 4 were used to evaluate the effects of 5-HT antagonists on cats, in which a clot had been created in the cul-de-sac, thereby simulating an aortic embolus. Two cats (L-1-L-2) were contained in Group 5. The aorta was ligated but no blood clot was created in the cul-de-sac.

Five cats (DB-1-DB-5) were included in Group 6. A dialysis bag containing either clotted blood or saline with glass beads, was placed retroperitoneally in each of these cats. The aorta of some of these cats (DB-3-DB-5) was ligated, but were left unligated in the remainder (DB-1-DB-2). This group of cats was used to determine the mode in which the blood clots affected the sign observed.

All of the cats were observed for varying lengths of time to evaluate motor function in their hind legs. Representative members from each group

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[a]Methysergide Maleste - Sandoz Pharmaceuticals, Hanover, NJ.
[b]Cyproheptadine HCl - Merck, Sharp & Dohme, West Point, PA.
[c]Doxepin HCl - Chas. Pfizer & Co. Inc., New York, NY.

had quadriceps muscle, lumbar spinal cord and grossly abnormal tissues examined histopathologically.

METHODOLOGY

The operative procedure (Figure 5-21), which resulted in the formation of a cul-de-sac, was the same in all of the cats. The cats were given no pre-anesthetic medication, but were masked with oxygen and 5% halothane[d] until a plane of anesthesia was reached (Figure 1) wherein the cats could be intubated. Following intubation, a surgical level of anesthesia was maintained with oxygen and 2% halothane (Figure 2).

The abdomen, an area over the medial saphenous vein, and the area over the external jugular veins was clipped with a No. 40 clipper blade. The cats were placed in dorsal recumbency and restrained with leg ropes. The abdomen was prepared with a betadine soap and alcohol scrub. Sterile, surgical drapes were used. A midline incision was made in the abdomen starting 1½ cm posterior to the umbilicus and ending 1½ cm anterior to the pubis. The subcutaneous tissues were divided along the linea alba. The linea alba was incised along its exposed length. This allowed visualization of the abdominal viscera and peritoneum. The intestines were displaced anteriorly and held in position with the use of a gauze sponge and the side blade of a baby Balfour retractor. A gauze sponge and a side blade of the retractor were used to reflect the bladder to a caudal-lateral position; the colon was retracted laterally by hooking it under the central blade of the baby Balfour retractor. This exposed the dorsal peritoneum, which covers the terminal portion of the aorta. The caudal mesenteric artery was identified (Figure 6). An incision was made posterior to the caudal mesenteric artery in the dorsal peritoneum. The incision was made to expose the aorta, from the caudal mesenteric artery to the coccygeal artery (Figure 7). A 2-0

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[d] Halothane - Ayerst Laboratories, Inc., New York, NY.

silk ligature was placed just posterior to the caudal mesenteric artery and was tied tightly (Figure 9). The deep circumflex iliac arteries, which exit on either side of the aorta, were ligated with 2-0 silk (Figure 10). A 2-0 silk ligature was passed around the caudal portion of the aorta, at the iliac bifurcation. This ligature was not tightened at this time (Figure 11). If a dorsal lumbar artery was present, it was ligated at this time (Figure 12).

In Groups 1, 2, 3 and 4, two ml of whole blood were drawn from each cat's jugular vein. Small glass beads were added to this blood, until the volume measured $2\frac{1}{2}$ ml. The glass beads were used to insure clotting of the blood. A bent 21-gauge needle was placed on the syringe and the needle was filled with blood from the syringe (Figure 16). The needle was inserted into the aorta at the iliac bifurcation. The ligature, which had been pre-placed around the bifurcation, was tightened around the needle at its point of insertion (Figure 17). Pressure upon the ligature was maintained. Blood was injected into the aorta, creating a bleb. The ligature was held tightly while the needle was withdrawn and subsequently tied (Figure 19). Approximately 0.2 ml to 0.5 ml of blood was injected into the cul-de-sac. The linea alba was closed with 2-0 gut in a simple interrupted pattern. Three-0 nylon, in an interlocking pattern, was used to close the skin.

None of the cats in Group 1 were given any antiserotonin drugs, as this was to be the control group. Five of these cats (C-1-C-5) were observed for 24 hours, at which time, euthanasia was performed. No histopathology was done on the tissues of these cats, Of the remaining cats in this group, one (C-8) was observed for 52 hours before euthanasia was performed. Three cats (C-6, C-7 and C-9) were observed for 66 hours. One cat (C-10) was observed for 146 hours. Histopathological examination was performed upon the tissues from cats C-6-C-10.

The cats in Group 2 were given methysergide intravenously via the medial saphenous vein. This was done one-half hour prior to the creation of the clot in the cul-de-sac. The methysergide was dissolved in normal saline solution just prior to its injection. Two of the cats (Ms-1-Ms-2) in this group were given 0.2 mg/kg, four of the cats (Ms-3-Ms-6) were given 2.0 mg/kg, and two of the cats (Ms-7-Ms-8) were given 4.0 mg/kg. Cats Ms-1 and Ms-2 were observed for 147 hours before euthanasia was performed. Cats Ms-7 and Ms-8 were observed for 91 hours and 95 hours respectively. Tissues from all the cats of Group 2 were examined histopathologically.

The cats in Group 3 were given 1.0 mg/kg of cyproheptadine intravenously via the medial saphenous vein, 40 minutes to one hour prior to the creation of a clot in the cul-de-sac of each. The cyproheptadine was dissolved in normal saline solution just prior to its injection. All of these cats were observed for 71 hours to 75 hours before euthanasia was performed. Each cat in Group 3 had tissues examined histopathologically.

The cats in Group 4 were given injectable doxepin intravenously one hour prior to the creation of a clot in the terminal aorta of each. Three cats (D-1-D-3) were given 2.0 mg/kg. The cats were observed for 40 hours to 123 hours. Tissues from cats D-1, D-2 and D-3 were examined histopathologically. The heart rate on all of the cats in Groups 2, 3 and 4 were monitored by auscultation as the anti-serotonin drugs were administered and for 15 minutes post injection.

The aorta of the two cats (L-1-L-2) in Group 5 were ligated but no blood clot was created within the aorta; attempts were made to insure that no blood was present in the cul-de-sac of each. These cats were maintained for 118 hours before euthanasia was performed, and their tissues were examined by histopathology.

In two cats (DB-1-DB-2) of Group 6 the aorta was not ligated and a dialysis bag containing approximately 1 ml of autologous blood and glass beads was placed by the aorta of each cat. Their peritoneums were sutured over the dialysis bags with 2-0 gut in a simple interrupted pattern to insure that the dialysis bags would stay in place. The aortas in cats (DB-3-DB-5) were ligated in the same manner as those of the cats in Group 1. An effort was made to insure that no blood was present in any cul-de-sac of this group. A dialysis bag containing autologous blood and glass beads was placed by the aortas of cats DB-3 and DB-4. The peritoneum was sewn over the dialysis bags to insure that the bags would stay within the retroperitoneal area. Cat DB-5 had a dialysis bag containing glass beads and saline placed retroperitoneally next to its ligated aorta. From this group, cats DB-1 and DB-2 were euthanatized after 21 and 18 hours respectively. Cats DB-3 and DB-4 were euthanatized after 19 and 23 hours respectively. Cat DB-5 was observed over a 75-hour period before euthanasia was performed. Only cat DB-5 had tissues examined histopathologically.

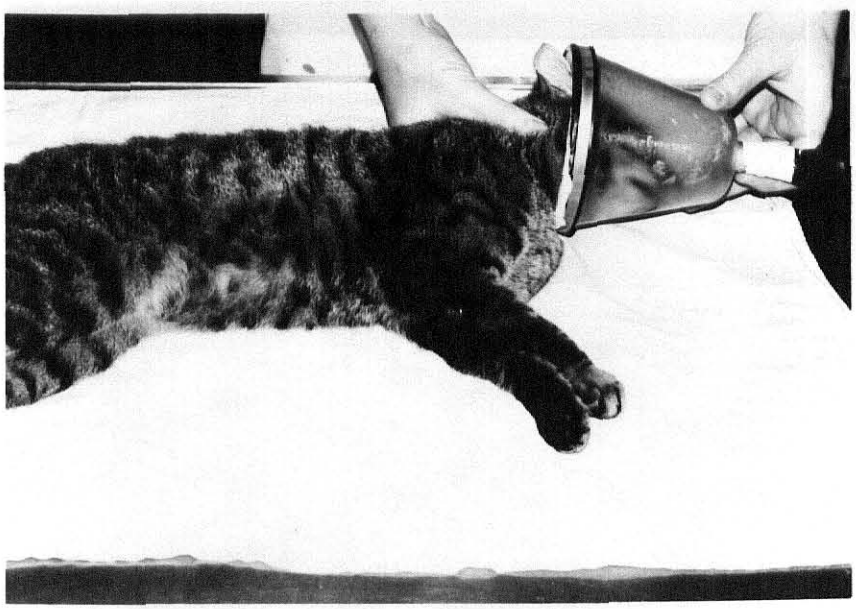

Figure 1: The cat is masked with 5% halothane and oxygen.

Figure 2: Surgical anesthesia is maintained with 2% halothane and oxygen delivered via endotracheal tube.

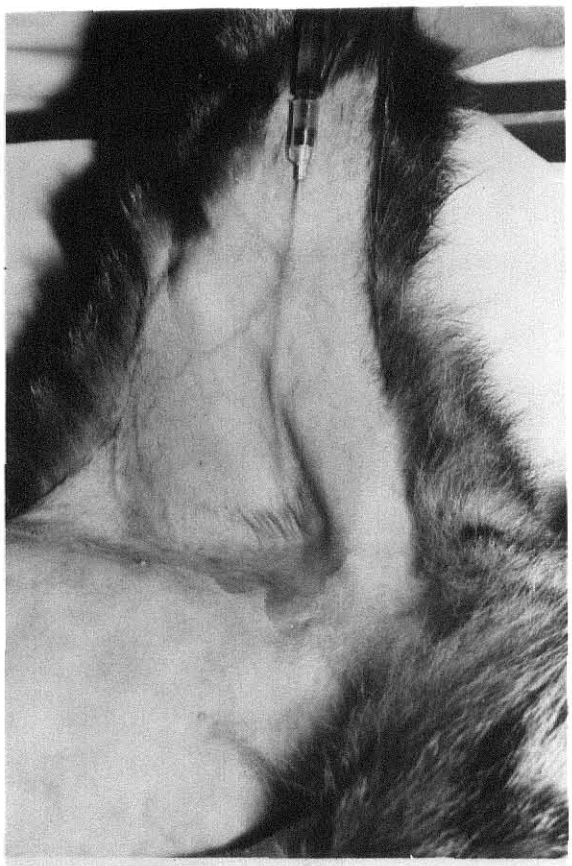

Figure 3: Five hydroxytryptamine antagonists are given via the medial saphenous vein.

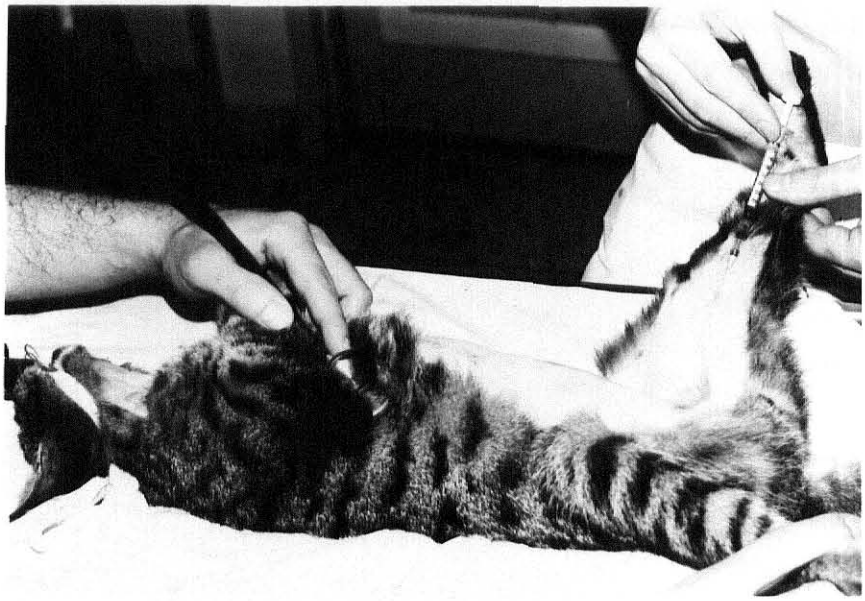

Figure 4: The heart rate is monitored while the drug is given.

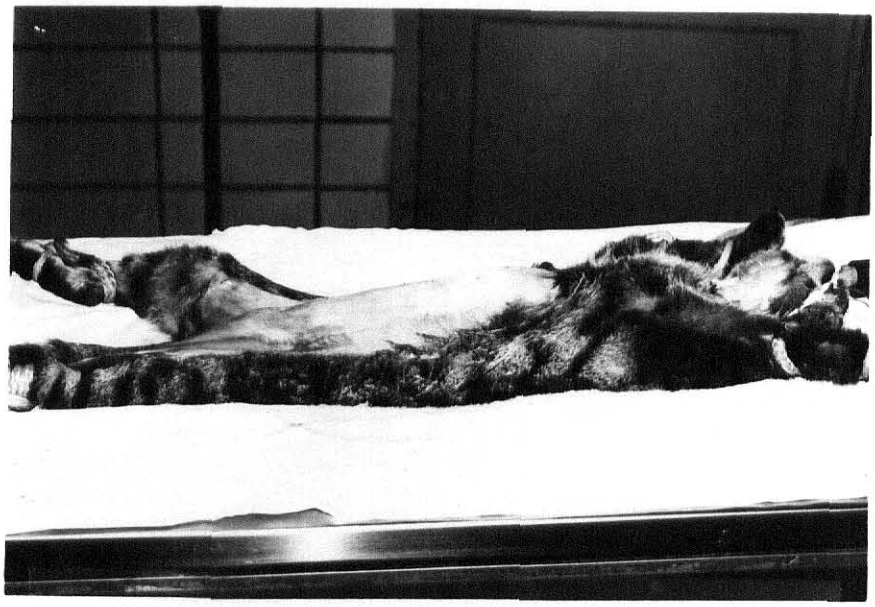

Figure 5: The cat is positioned in dorsal recumbency.

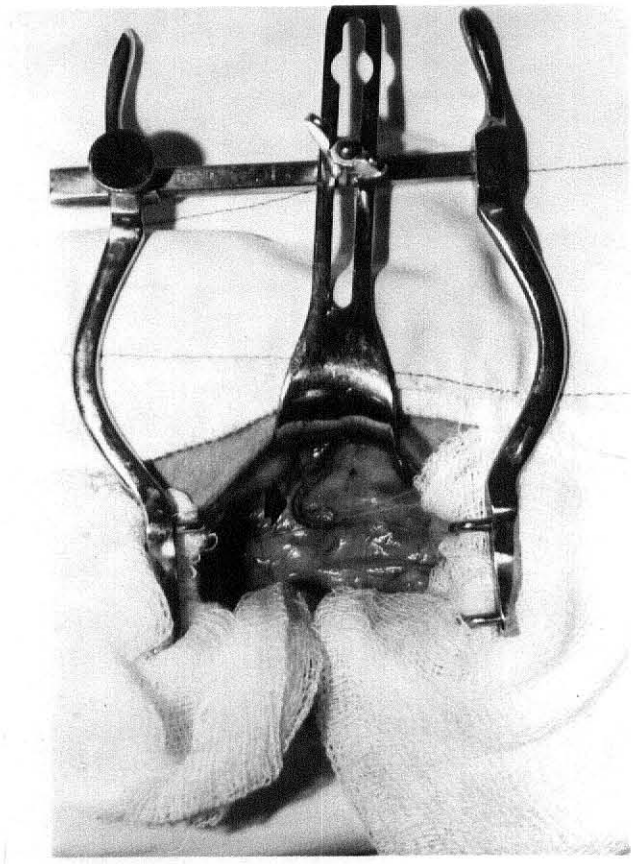

Figure 6: The intestines are reflected anteriorly; the bladder is reflected caudally and the colon is reflected laterally with a baby Balfour retractor. Note the caudal mesenteric artery in center of the surgical field.

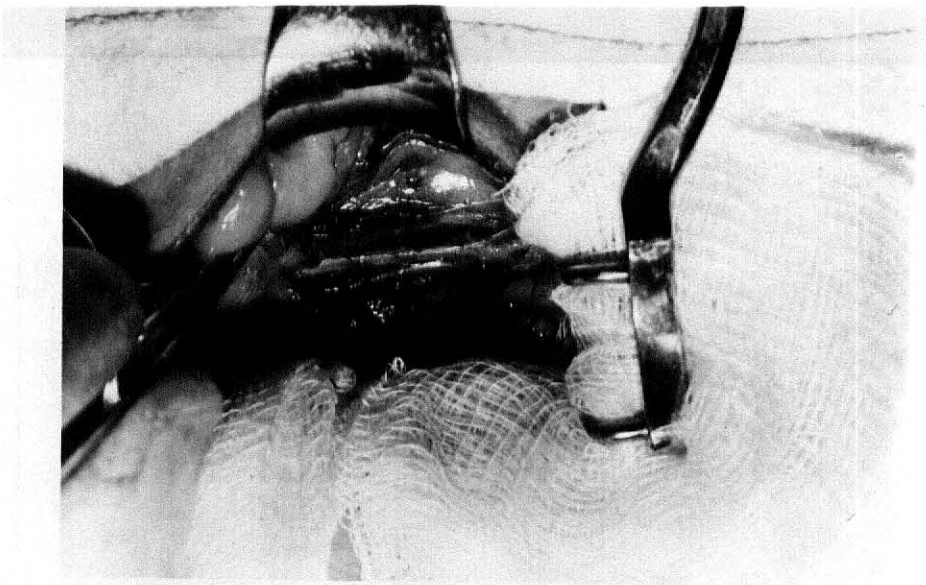

Figure 7: The terminal aorta is exposed isolating the caudal mesenteric artery, the two deep circumflex iliac arteries, the two external iliac arteries and the coccygeal artery.

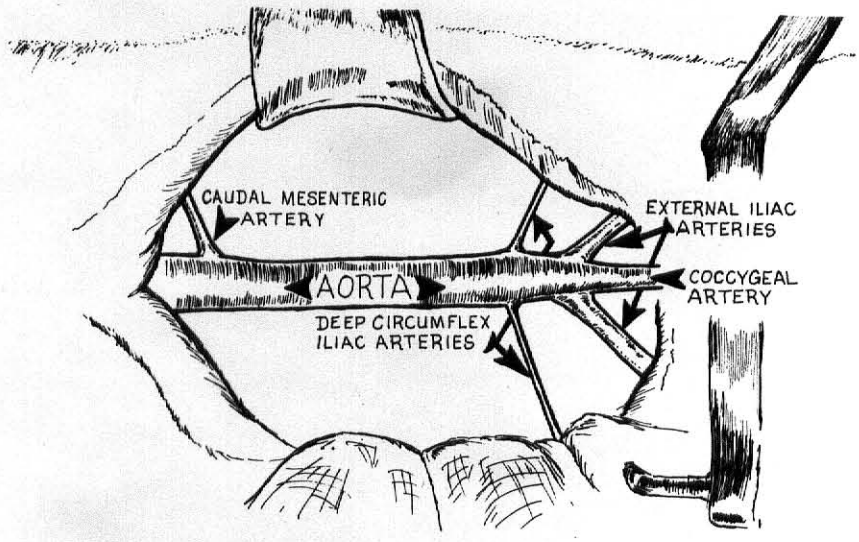

Figure 8: A schematic representation of surgical site and its pertinent vasculature.

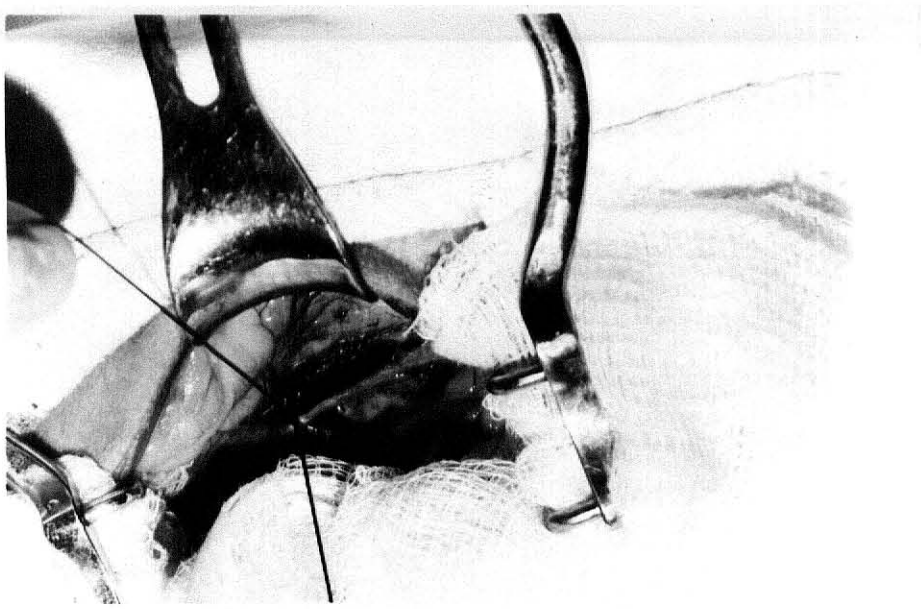

Figure 9: The aorta is ligated with 2-0 silk just posterior to the caudal mesenteric artery.

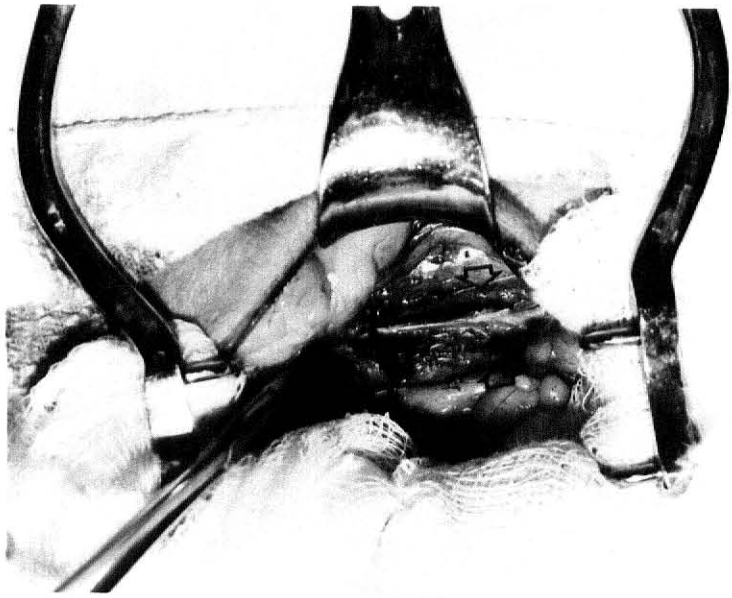

Figure 10: The two circumflex iliac arteries are ligated with 2-0 silk.

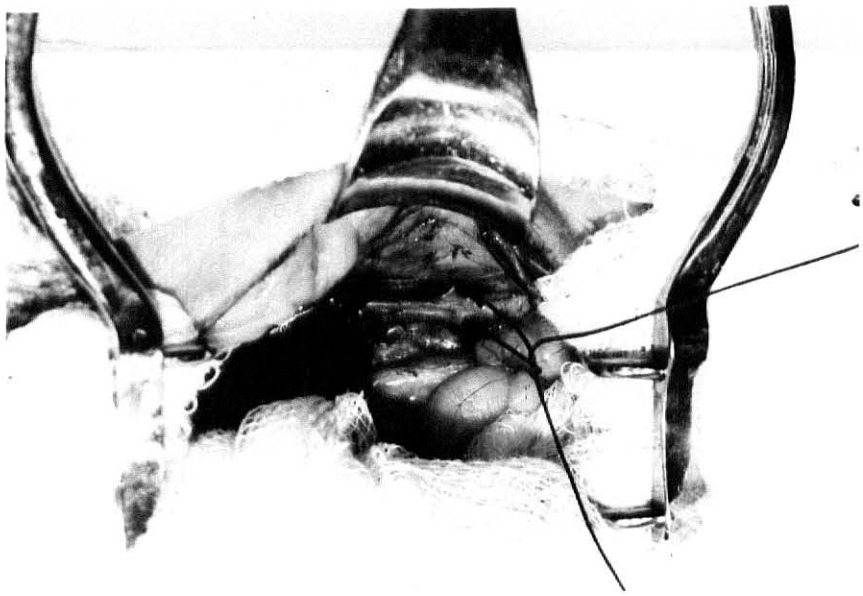

Figure 11: A loose ligature is placed around the aorta at the iliac bifurcation.

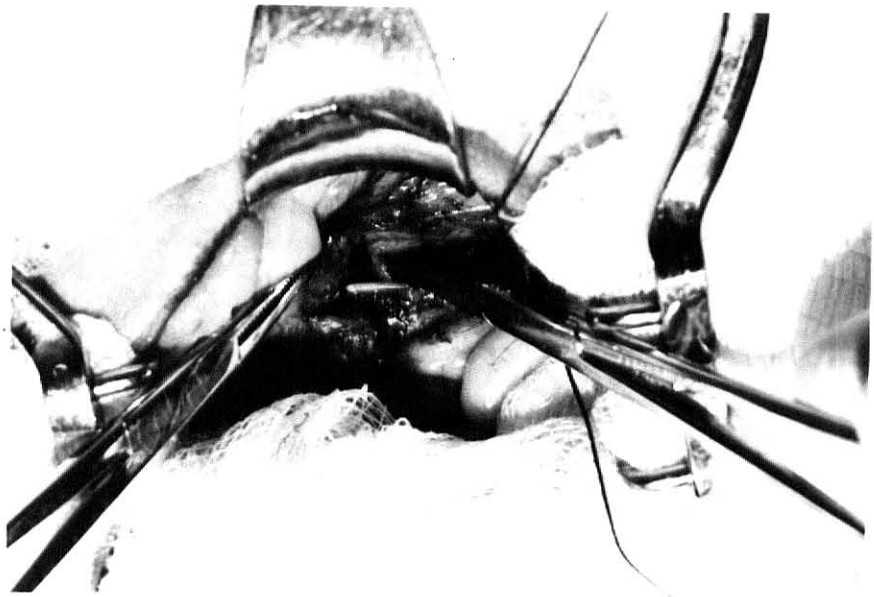

Figure 12: The lumbar artery, if present, is isolated and ligated with 2-0 silk.

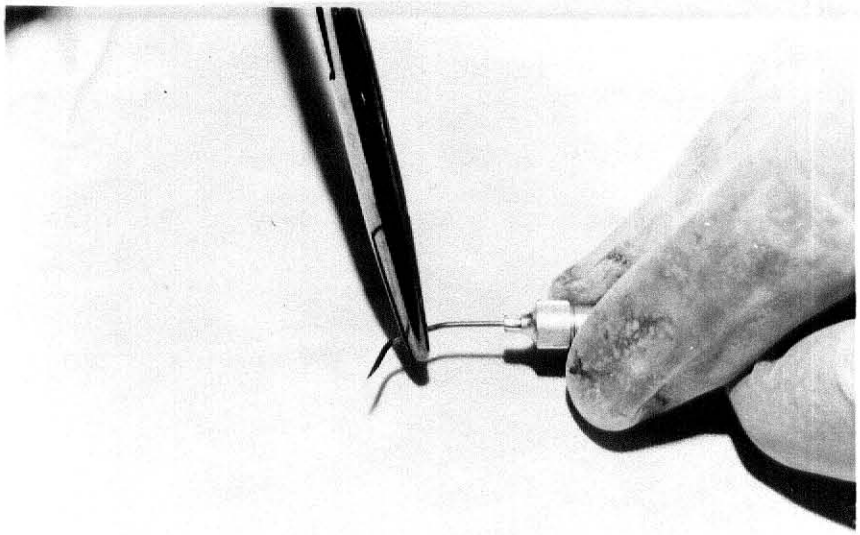

Figure 13: A 21-gauge needle is bent.

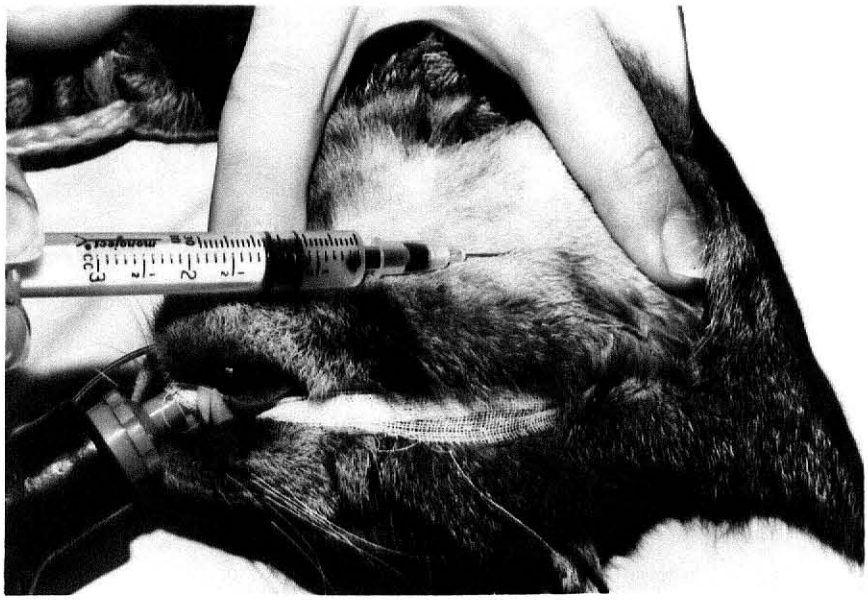

Figure 14: Two ml of blood are withdrawn from the cat's jugular vein.

Figure 15: Glass beads are poured into the blood until 2½ ml of volume is reached.

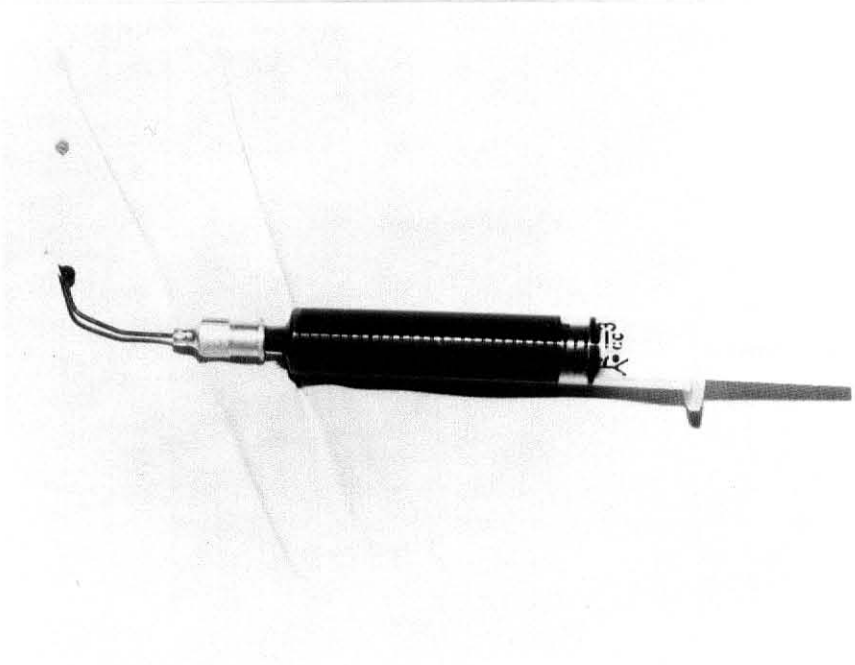

Figure 16: The bent needle is filled with blood from the syringe.

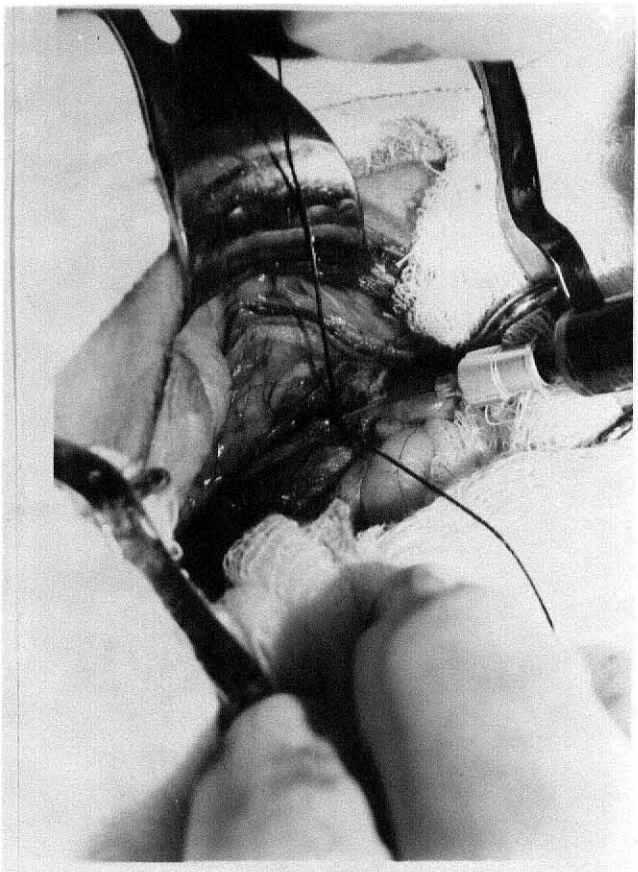

Figure 17: The needle is inserted into the aorta at the iliac bifurcation; the ligature is tightened around the needle.

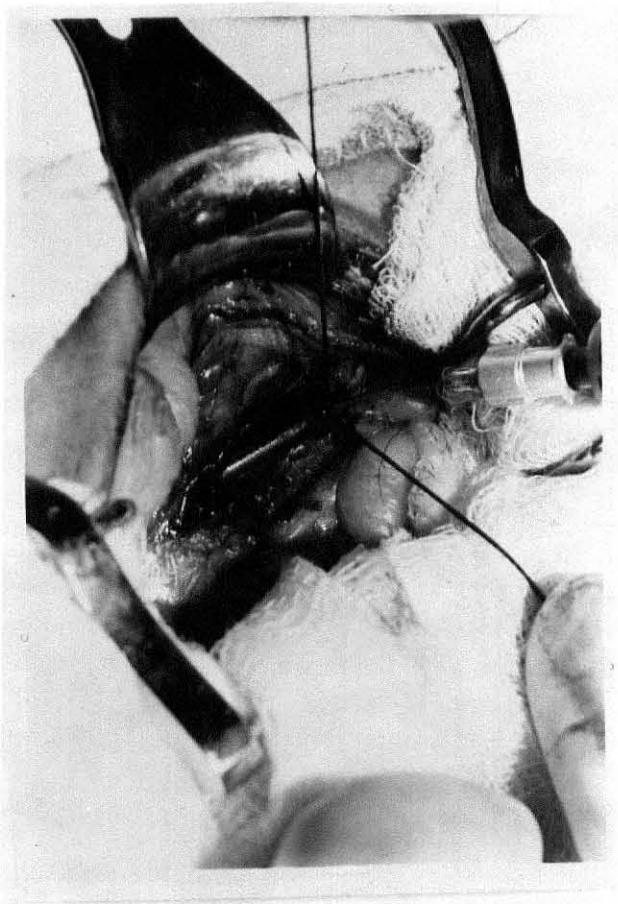

Figure 18: Blood is injected into the cul-de-sac.

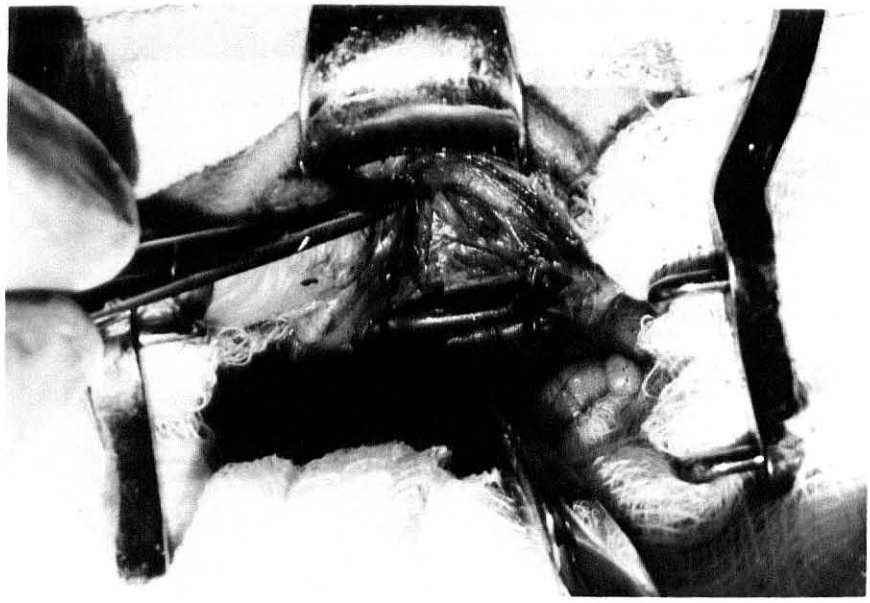

Figure 19: The needle is removed and the ligature is tied, thereby maintaining a bleb of blood in the aorta.

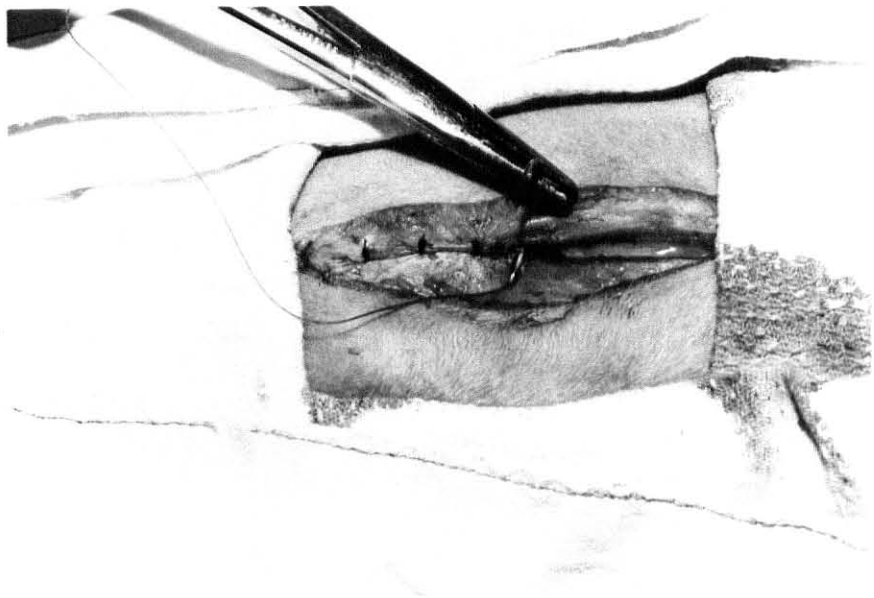

Figure 20: The linea alba is closed with 2-0 gut in a simple interrupted pattern.

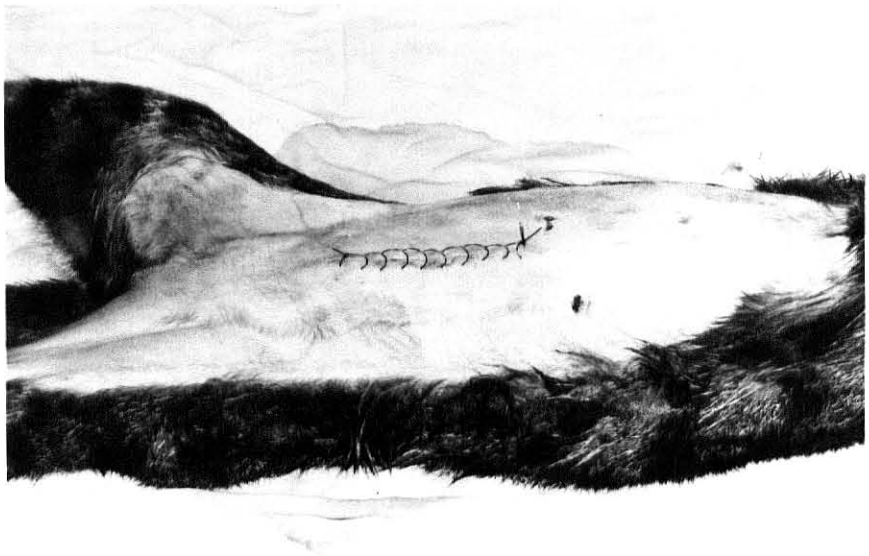

Figure 21: The skin is closed with 3-0 nylon in an interlocking pattern.

EXPERIMENTAL RESULTS

The locomotive function of all the cats was evaluated 24 hours following installation of the blood and glass beads in the cul-de-sac of the aorta. In those cats that were allowed to survive for longer than 24 hours, there was no appreciable change in the locomotive function of the back legs from the 24-hour examinations to the terminal examinations.

All of the cats in Group 1 showed signs of either paresis or complete paralysis of the hind legs. Cat C-5 exhibited the most normal function, and even at that, this cat knuckled over on the toes of each hind leg. Most of the cats could flex and extend the hip; however, function below the stifle in most cases was impaired. The cats would either shuffle their metatarsals along the ground or drag the dorsum of their toes on the ground. Cats C-1 and C-9 showed complete paralysis of the hind legs and were unable to flex or extend at the hips. Postmortem examination revealed a blood clot in the cul-de-sac in all of the cats. Table 1 summarizes the signs observed in the cats in Group 1.

Histopathologic examination of Cats C-6 through C-10 revealed that in all cases except one (C-8) pathology was present in quadriceps muscles. Hyaline degeneration and/or fragmentation of muscle fibers was the most consistently observed lesion. In Cats C-6 and C-10 these lesions were in focal areas throughout the examined muscle, while in cats C-7 and C-9 the lesions were more generalized. In Cat C-10 a focal infiltration of macrophages and round cells was observed in the muscle. For the most part, the lumbar spinal cords of these cats contained no abnormalities. Cat C-8 had some Wallerian degeneration at the L_6 level. A few dark, shrunken neurons were observed at the L_4 level in Cats C-9 and C-10. One cat, C-9, also had a severe hemorrhage cystitis. Table 7 summarizes the results of the histopathologic examination performed on the cats in Group 1.

All of the cats in Group 2, except for Cat Ms-6, exhibited signs similar to the cats of Group 1. Cat Ms-6, which was given 2.0 mg/kg of methysergide intravenously, walked without difficulty. When the methysergide was given at a dosage of 0.2 mg/kg no change in cardiac rate was noted; however, when 2.0 mg/kg was used, a decrease in the cardiac rate of 20 to 30 beats/minute was noted. When the methysergide was given at 4.0 mg/kg a bradycardia was detected. The heart rate dropped from between 120-130 beats/minute to 40-50 beats/minute. In all cases the heart rate returned to normal within 15 minutes. The results in Group 2 is summarized in Table 2. A blood clot was present in the cul-de-sac in all of these cats except Cat Ms-3. Hemolyzed blood was found in the cul-de-sac of this cat.

As in Group 1, the primary lesions observed in the muscles of the cats in Group 2 were hyaline degeneration and fragmentation of muscle fiber. Cellular infiltration of the muscle was observed in two cats (Ms-2 and Ms-3). In one of the cats (Ms-7) the muscle examined showed marked atrophy. Only Cat Ms-5 had abnormalities present in the lumbar spinal cord; i.e., a few dark, shrunken neurons were noted at the L_4 level. No abnormalities were noted in the lumbar cord in any other cat. Cat Ms-1 was sloughing the skin on its hind legs (Figure 24). Cats Ms-1 and Ms-6 had severe cystitis grossly and microscopically. Table 8 summarized the resulted of the histopathologic examination performed on the cats in Group 2.

The cats in Group 3 were all given 1.0 mg/kg of cyproheptadine. In this group of cats, Cat Cy-1 and Cy-4 walked well throughout their observation period. Cats Cy-2 and Cy-3 showed signs similar to those seen in Group 1. The heart rate of these cats was unchanged by the administration of cyproheptadine. Post mortem examination of Cat Cy-1 revealed that there was no organized clot in the cul-de-sac of the aorta; however, there was hemolyzed blood present. Cats Cy-2, Cy-3 and Cy-4 had small clots and hemolyzed blood in their cul-de-sacs. Table 3 summarizes the results observed in Group 3.

Two of the cats in Group 3, Cy-2 and Cy-4, had no histologic abnormalities present in the muscle or the spinal cord. Cat Cy-1 had only small areas of hyaline degeneration, muscle fiber fragmentation and cellular infiltration in the muscle. Also some focal Wallerian degeneration was noted in the dorsal white column of this cat at the L_3-L_4 level. The rest of the lumbar cord was normal. Cat Cy-3 exhibited the most marked histologic changes in this group. Generalized hyaline degeneration and muscle fiber fragmentation along with cellular infiltration and hemorrhage was seen in the muscle of Cat Cy-3. An area of perivascular cuffing was noted at the L_3-L_4 level of this cat. The rest of the lumbar spinal cord was normal. The femoral nerve was present on the muscle sections of this cat. It showed marked degeneration. Table 9 summarizes the histopathologic examination performed on this cat.

The cats in Group 4 were given doxepin prior to creation of the blood clot in the cul-de-sac. Of the three cats in the group that were given 2 mg/kg of doxepin, Cat D-1 exhibited minimal abnormality in gait; i.e., the cat knuckled on the toes of the left hind leg; however, the right hind leg was used normally. Cats D-2 and D-3 exhibited signs similar to those seen in the cats in Group 1. Following the administration of doxepin, Cat D-2's heart rate increased from 130 beats/minute to 208 beats/minute for a three-minute period. Cats D-1 and D-3 had no appreciable change in their heart rate. Cat D-4, which was given 5 mg/kg of doxepin, at first had an increase in its heart rate, but within five minutes this had become a severe bradycardia. The cat died eight minutes after the drug was given. Cats D-1, D-2 and D-3 all had blood clots in their cul-de-sacs at the time of necropsy. Table 4 summarizes the signs observed in the cats in Group 4.

Histopathology of Cat D-1 revealed no abnormalities in the muscle. In Cat D-2 the changes observed in the muscle were limited to a small area of fiber fragmentation and cellular infiltration. Also in this cat, some of the

fibers were noted to have many sarcolemma nuclei. Cat D-3 had hyaline degeneration present throughout the muscle sample. This cat, which died of shock, also had congestion of the kidneys and foci of necrosis in the liver. No abnormalities were noted in the spinal cord of any of these cats. Table 10 summarizes the histopathologic examination performed on these cats.

Of the cats in Group 5, Cat L-1 walked well. Cat L-2 showed signs of paresis. Neither cat had gross evidence of blood within the cul-de-sac at the time of postmortem. Table 5 summarizes the results of Group 5.

A dialysis bag was used in Group 6 to help determine the mode in which the blood clot affected the signs observed. When a dialysis bag containing whole blood and glass beads was placed close to an unligated aorta, as in DB-1 and DB-2, the cats walked well. When the aorta was ligated, as was the case with DB-3 and DB-4 and the blood and glass beads were placed within the dialysis bag, then signs similar to those seen in Group 1 were noted. Cat DB-5 which had saline and glass beads in the dialysis bag that was placed close to the cul-de-sac also showed signs similar to those seen in Group 1.

Table 2 summarizes the results of the histopathologic examination performed on Cats L-1, L-2 and DB-5. Cat L-1 had no abnormalities in either the muscle or the spinal cord. Cat L-2 had generalized hyaline degeneration and fiber fragmentation in the msucle along with shrinkage of some muscle bundles and focal atrophy of muscle. This cat also had Wallerian degeneration of the ventral white horn of the lumbar spinal cord at the L_3-L_4 level. Hyaline degeneration and macrophage infiltration were the only abnormalities noted in the muscle of Cat DB-5. This cat also had a few dark neurons in the ventral horn at L_3-L_4 level.

Of all the cats included in this study, those that exhibited moderate to severe hindleg disfunction also often had a firmness in the gastrocnemius muscle and at necropsy the muscles of the hind leg usually were pale (Figure 23).

Figures 25 through 36 are typical of the histology observed in the cats examined. It was also noted that several of the cats in various groups died of shock prior to their planned termination.

TABLE 1: UNMEDICATED CATS WITH BLOOD AND GLASS BEADS INSTILLED INTO CUL-DE-SAC

Cat	Survival Time (hours)	Signs Observed
C-1	24	Complete paralysis. No perception of deep pain in toes.
C-2	24	Able to flex and extend hips. Deep pain perception present. Drags dorsum of toes on ground.
C-3	24	Able to flex and extend right hip slightly. Complete paralysis of left leg. Drags dorsum of toes on ground. No deep pain perception in toes.
C-4	24	Slight flexion and extension of hips. No deep pain perception in toes or anus.
C-5	24	Able to flex and extend hip and stifle. Knuckles over on toes.
C-6	66	Able to flex and extend hip. Shuffling gait--metatarsal flat on ground. No deep pain perception in toes.
C-7	66	Able to flex and extend hip. Drags dorsum of toes on ground. No deep pain perception in toes.
C-8	52	Able to flex and extend at hips and stifle. Shuffling gait--metatarsals almost flat on ground. No deep pain perception in toes.
C-9*	66	Complete paralysis. No deep pain perception.
C-10	146	Able to flex and extend hips. Drags dorsum of toes on ground.

*Died of Shock

TABLE 2: CATS WITH BLOOD AND GLASS BEADS INSTILLED INTO CUL-DE-SAC FOLLOWING INTRAVENOUS METHYSERGIDE

Cat	Drug Dosage (mg/kg)	Survival Time (hrs)	Signs Observed
Ms-1	0.2	148	Able to flex and extend hip slightly. No deep pain perception in toes. Drags self around with front legs. Hypersensitive over rest of hind legs. Sloughing skin.
Ms-2	0.2	147	Complete paralysis. Marked cyanosis of pads. Firm quadriceps muscles. No deep pain perception in toes.
Ms-3	2.0	71	Able to flex and extend hip. Drags dorsum of toes on ground.
Ms-4*	2.0	75	Complete paralysis. Extreme rigidity. No deep pain perception of toes.
Ms-5	2.0	71	Knuckles on toes. Normal function otherwise.
Ms-6	2.0	72	Walked well.
Ms-7	4.0	95	Able to flex and extend hip slightly. Drags dorsum of toes on ground.
Ms-8	4.0	91	Able to flex and extend hips. Drags dorsum of toes on ground.

*Died of Shock

TABLE 3: CATS WITH BLOOD AND GLASS BEADS INSTILLED INTO THE CUL-DE-SAC FOLLOWING INTRAVENOUS CYPROHEPTADINE

Cat	Drug Dosage (mg/kg)	Survival Time (hrs)	Signs Observed
Cy-1	1.0	75	Walked well.
Cy-2	1.0	74	Knuckles on toes. Able to flex and extend hip and stifle.
Cy-3	1.0	71	Able to flex and extend hip and stifle. Drags dorsum of toes on ground. No deep pain perception in toes.
Cy-4	1.0	74	Walked well.

TABLE 4: CATS WITH BLOOD AND GLASS BEADS INSTALLED INTO THE CUL-DE-SAC FOLLOWING INTRAVENOUS DOXEPIN

Cat	Drug Dosage (mg/kg)	Survival Time (hrs)	Function of Hind Legs
D-1	2.0	96	Walked well on right hind leg. Knuckled on left hind leg, Otherwise normal.
D-2	2.0	123	Able to flex and extend hip and stifle. Knuckles on toes.
D-3*	2.0	40	Able to flex and extend hip. Drags dorsum of toes on ground.
D-4**	5.0	< 1	Undetermined.

*Died of Shock

**Died 8 minutes after the drug was given.

TABLE 5: LIGATION OF AORTA WITH NO BLOOD IN CUL-DE-SAC

Cat	Survival Time (hrs)	Signs Observed
L-1	118	Walked well.
L-2	117	Able to flex and extend hip. Shuffled metatarsals flat on ground.

TABLE 6: EXPERIMENTS WITH DIALYSIS BAGS

Cat	Substance in Dialysis Bag	Survival Time (hrs)	Status of Aorta	Signs Observed
DB-1	Whole Blood & Glass Beads	21	Unligated	Walked well.
DB-2	Whole Blood & Glass Beads	18	Unligated	Walked well.
DB-3	Whole Blood & Glass Beads	19	Ligated Cul-de-sac bloodless	Right metatarsals flat on ground. Drags dorsum of left toe on ground. No deep pain perception.
DB-4*	Whole Blood & Glass Beads	23	Ligated Cul-de-sac bloodless	Able to flex and extend at Drags dorsum of toes on gro
DB-5**	Saline and Glass Beads	75	Ligated Cul-de-sac bloodless	Able to flex & extend hip a stifle. Shuffles metatarsals along ground.

*Died of shock

**Extremely small for mature cat (1.5 kg).

TABLE 7: HISTOPATHOLOGY OF UNMEDICATED CATS

Cat	Quadriceps Muscles	Lumbar Spinal Cord	Special Comments
C-6	Focal hyaline degeneration	Normal	
C-7	Hyaline degeneration Fiber fragmentation	Normal	
C-8	Normal	Wallerian degeneration in the dorsal while column.	
C-9	Hyaline degeneration Fiber fragmentation	At L_4, a few dark, shrunken neurons.	Severe hemorrhagi cystitis
C-10	Focal hyaline degeneration Focal liver degeneration Focal macrophage and round cell infiltration	At L_4, a few dark, shrunken neurons.	

TABLE 8: HISTOPATHOLOGY OF CATS GIVEN METHYSERGIDE

Cat	Quadriceps Muscles	Lumbar Spinal Cord	Special Comments
Ms-1	Hyaline degeneration Fiber fragmentation Shrinkage of muscle bundles.	Normal	Severe hemorrhagic cystitis.
Ms-2	Hyaline degeneration Fiber fragmentation Mononuclear infiltration Severe edema Vasculitis	Normal	
Ms-3	Hyaline degeneration Fiber fragmentation Macrophage infiltration		
Ms-4	Hyaline degeneration	Normal	
Ms-5	Single focus of hyaline degeneration Few foci of fiber fragmentation.	At L_4, few dark, shrunken neurons.	
Ms-6	Few foci of hyaline degeneration. Few foci of fiber fragmentation.	Normal	Severe purulent cystitis.
Ms-7	Atrophy of whole muscle.	Normal	
Ms-8	Hyaline degeneration Fiber fragmentation	Normal	

TABLE 9: HISTOPATHOLOGY OF CATS GIVEN CYPROHEPTADINE

Cat	Quadriceps Muscle	Lumbar Spinal Cord	Special Comments
Cy-1	Small area of hyaline degeneration. Small area of fiber fragmentation. Small area of macrophage and PMN infiltration.	L_3-L_4 focal Wallerian degeneration in dorsal white column.	
Cy-2	Normal	Normal	
Cy-3	Hyaline degeneration Fiber fragmentation Macrophage and PMN infiltration. Massive hemorrhage	L_3-L_4 perivascular cuffing.	Degeneration of the femoral nerve.
Cy-4	Normal	Normal	

TABLE 10: HISTOPATHOLOGY OF CATS GIVEN DOXEPIN

Cat	Quadriceps Muscle	Lumbar Spinal Cord	Special Comments
D-1	Normal	Normal	
D-2	Small area of fiber fragmentation. Small area of macrophage and round cell infiltration. Few fibers with many sarcolemma nuclei.	Normal	Sarcosporidia in muscle.
D-3	Hyaline degeneration	Normal	Congested kidney. Foci of necrosis in liver.

TABLE 11: HISTOPATHOLOGY OF CATS WITHOUT BLOOD CLOT IN THE CUL-DE-SAC

Cat	Quadriceps Muscle	Lumbar Spinal Cord
L-1	Normal	Normal
L-2	Hyaline degeneration Fiber fragmentation Shrinkage of muscle bundles. Focal atrophy of muscle.	L_3-L_4 Wallerian degeneration of the ventral white horn.
DB-5	Hyaline degeneration Macrophage infiltration	L_3-L_4 few dark neurons in ventral horn.

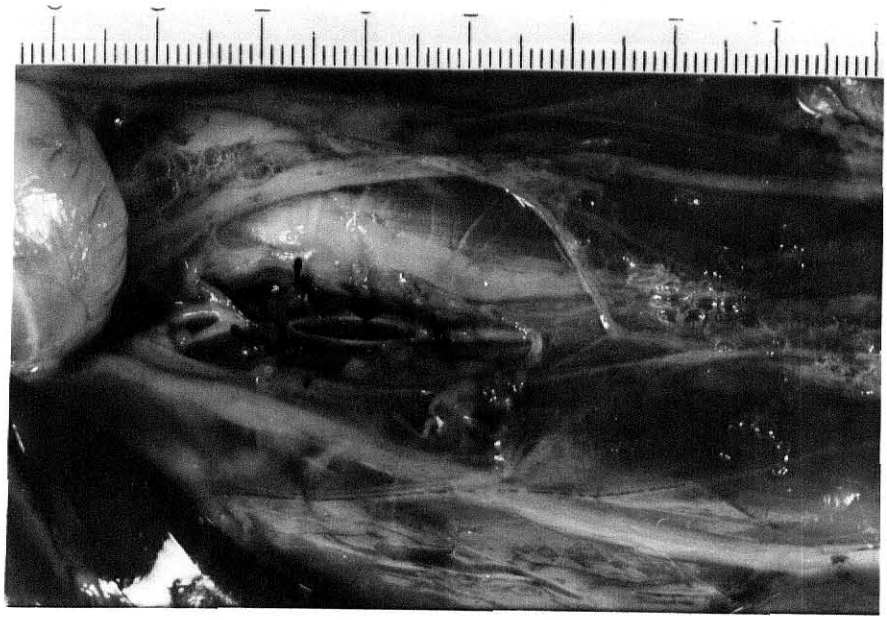

Figure 22: Generally, postmortem examination revealed the presence of a clot in the cul-de-sac like the one shown here. The scale is measured in centimeters.

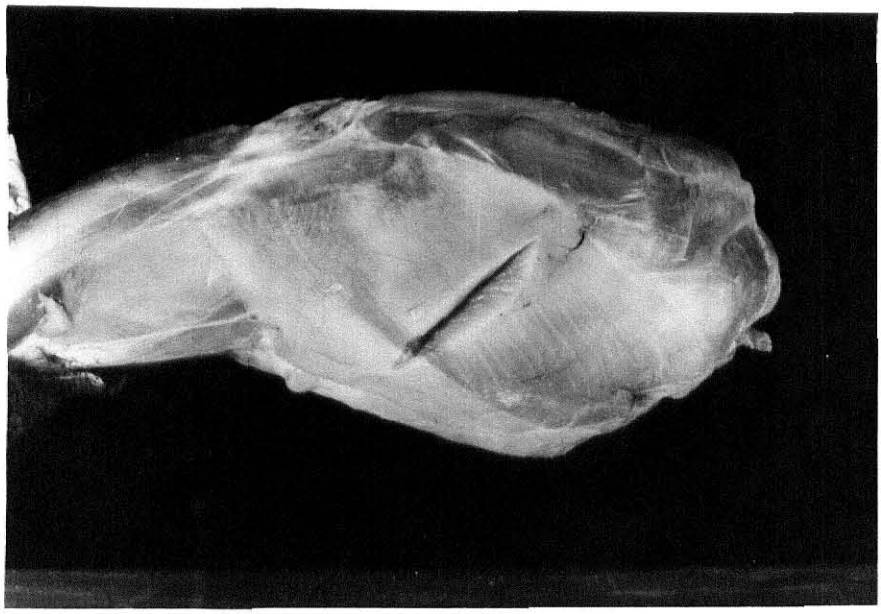

Figure 23: Many of the cats had grossly pale hindleg muscles.

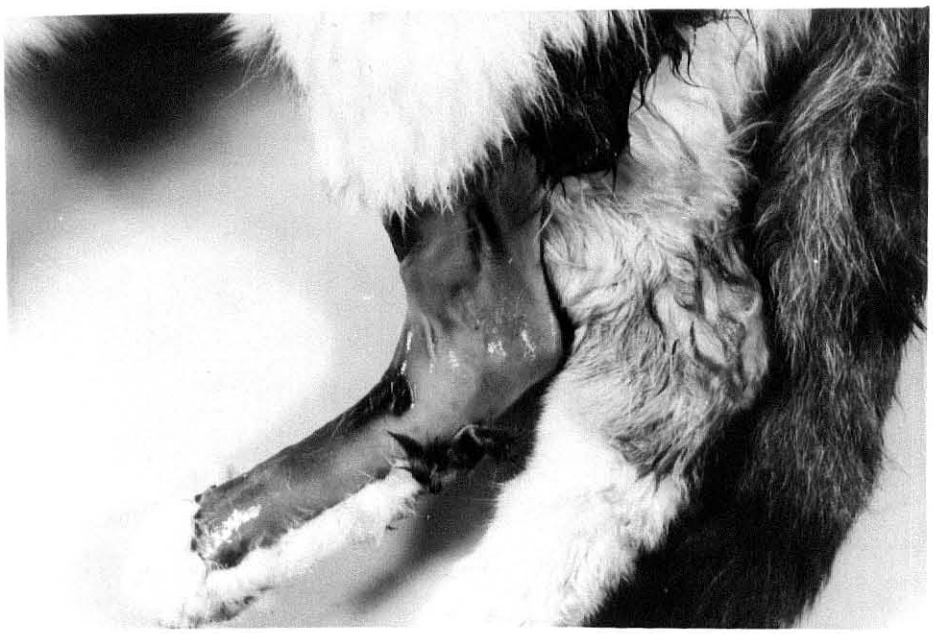

Figure 24: The skin is sloughing from the hind legs of cat Ms-1.

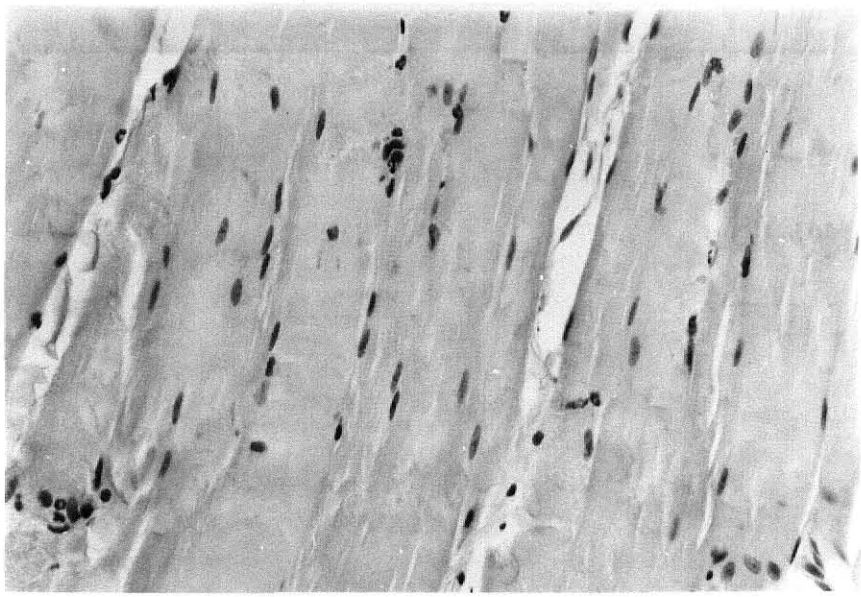

Figure 25: Normal muscle taken from cat Cy-2.
(Longitudinal section, H & E stain, 250X)

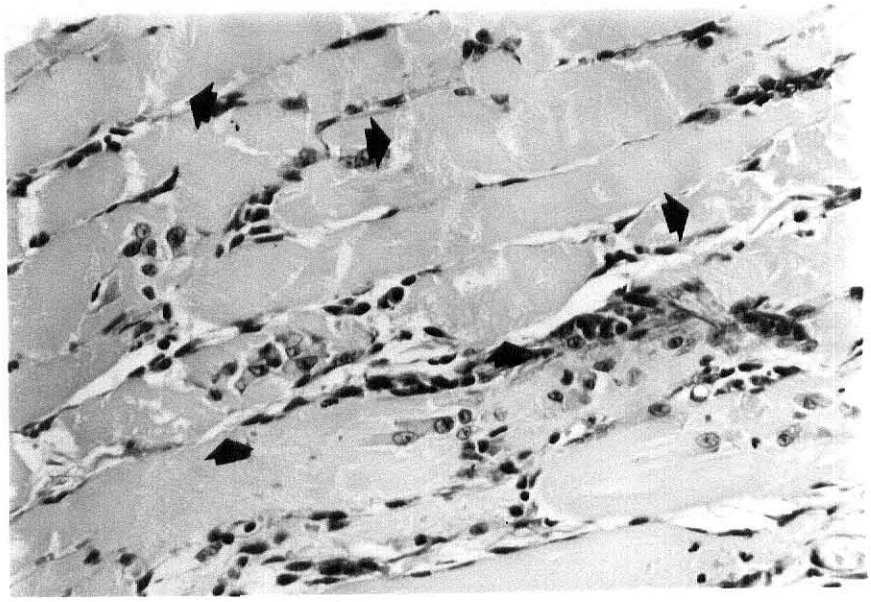

Figure 26: Muscle taken from cat Ms-3 showing hyaline degeneration and fiber fragmentation (arrows).
(Longitudinal section, H & E stain, 250X)

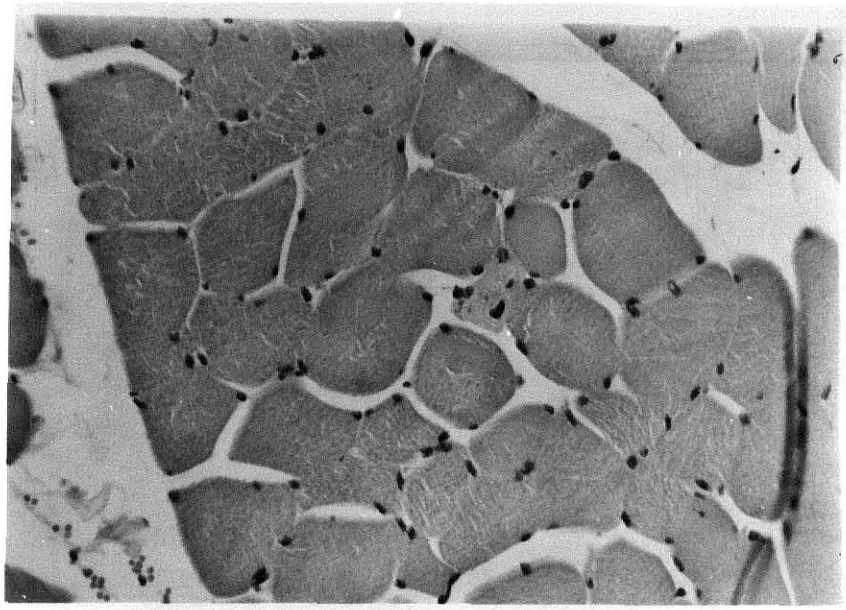

Figure 27: Normal muscle taken from cat Cy-2. (Cross section, H & E stain, 250X)

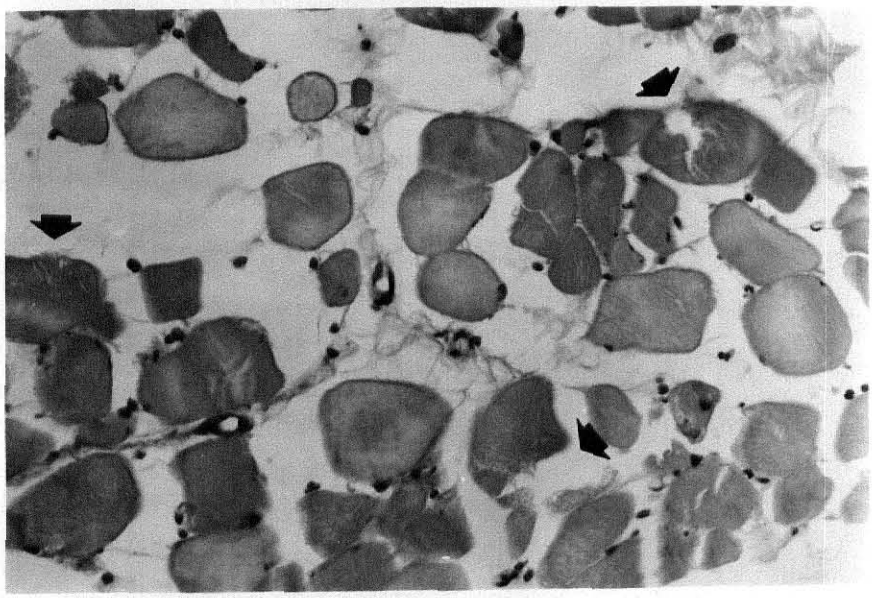

Figure 28: Muscle taken from cat C-7 showing hyaline degeneration, fiber fragmentation (arrows) and fiber separation. (Cross section, H & E stain, 250X)

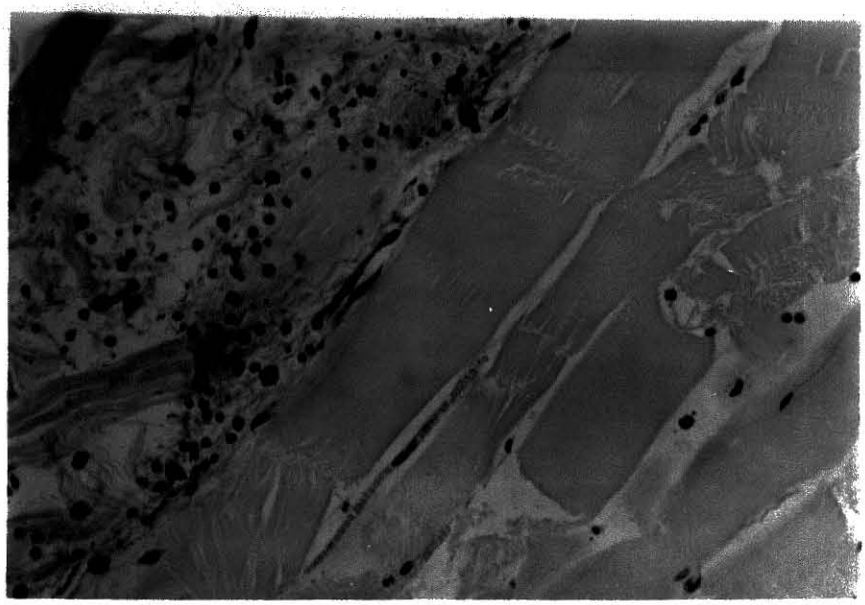

Figure 29: Muscle taken from cat Cy-3 showing hyaline degeneration, fiber fragmentation and PMN infiltration. (Longitudinal section, H & E stain, 250X)

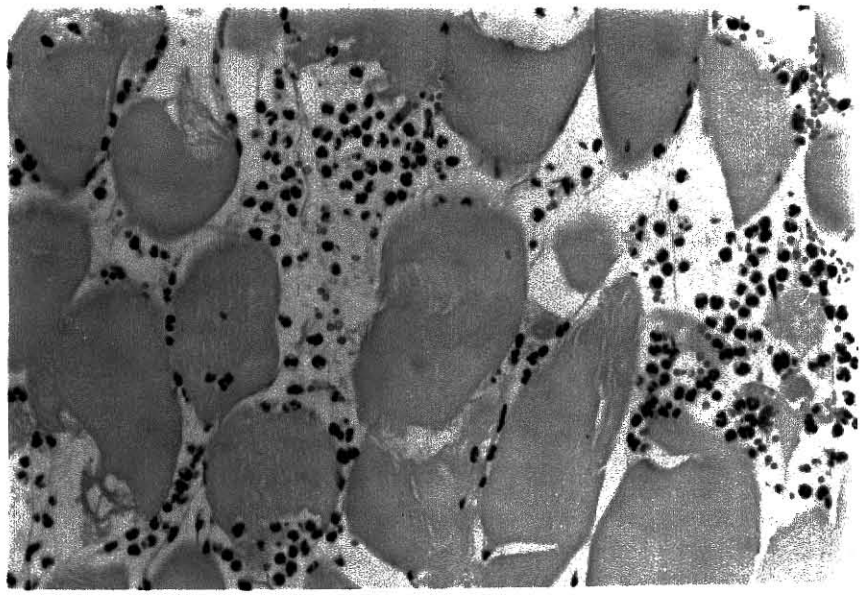

Figure 30: Muscle taken from cat Ms-2 showing hyaline degeneration, fiber fragmentation and PMN infiltration. (Cross section, H & E stain, 250X)

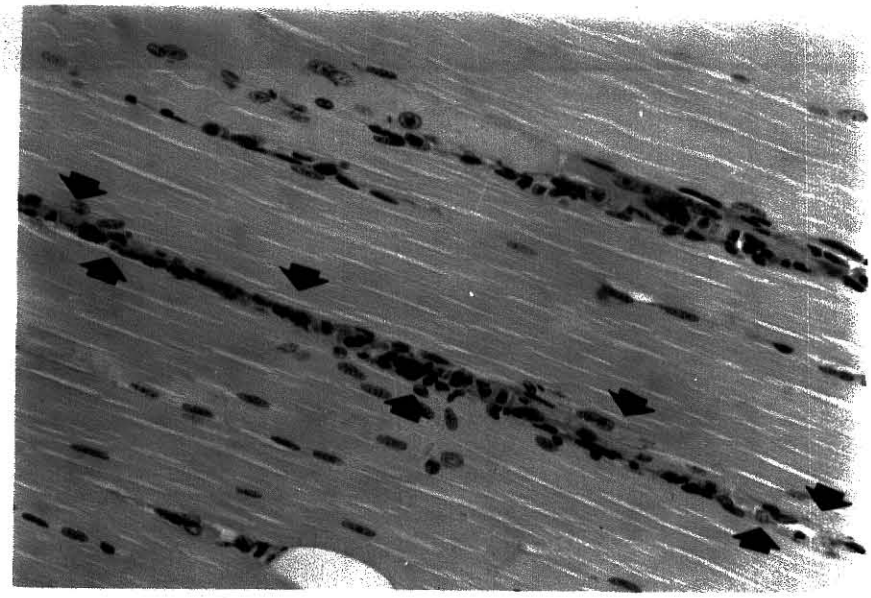

Figure 31: Muscle taken from cat D-2 showing fibers with many sarcolemma nuclei (between arrows). (Longitudinal section, H & E stain, 250X)

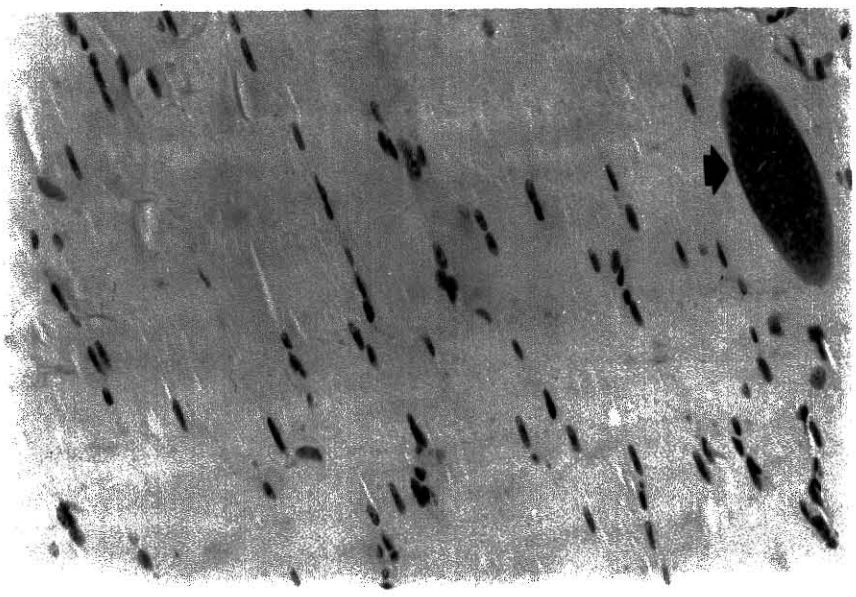

Figure 32: An incidental finding of sarcosporidia (arrow) was observed in the muscle of cat D-2. (Longitudinal section, H & E stain, 250X)

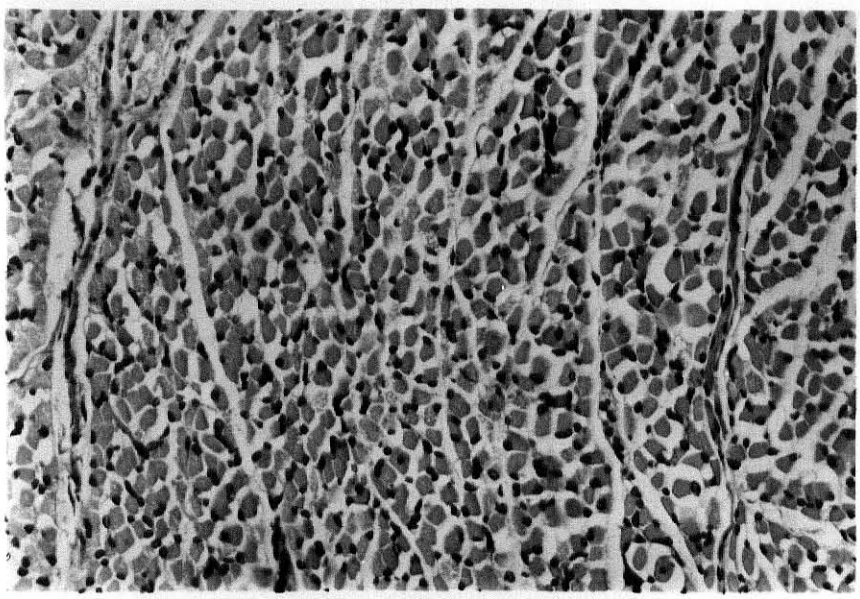

Figure 33: Muscle atrophy observed in cat Ms-7. (Cross section, H & E stain, 250X)

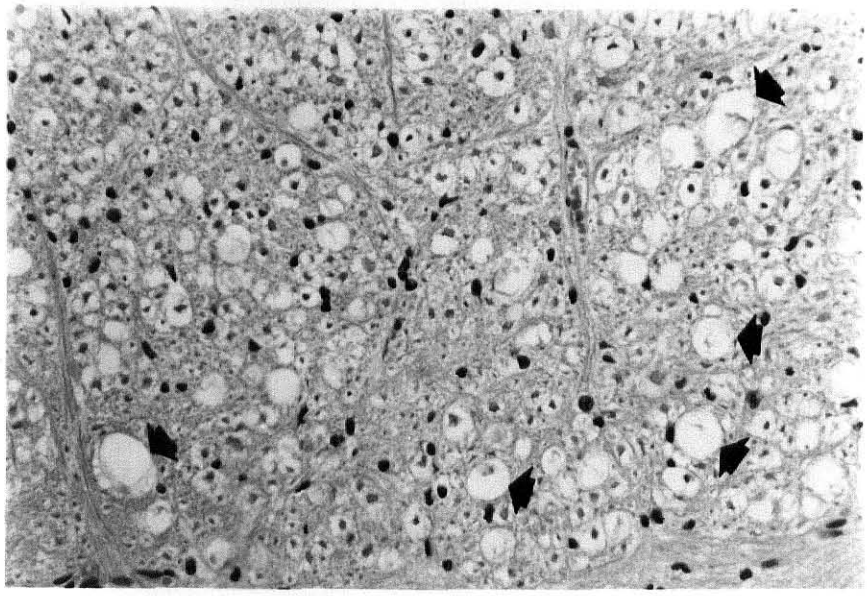

Figure 34: Typical example of Wallerian degeneration noted in the lumbar spinal cord of cats C-8, Cy-1 and L-2. Note the swollen myelin sheath with shrunken or absent axoplasm (arrows). (Cross section, H & E stain, 250X)

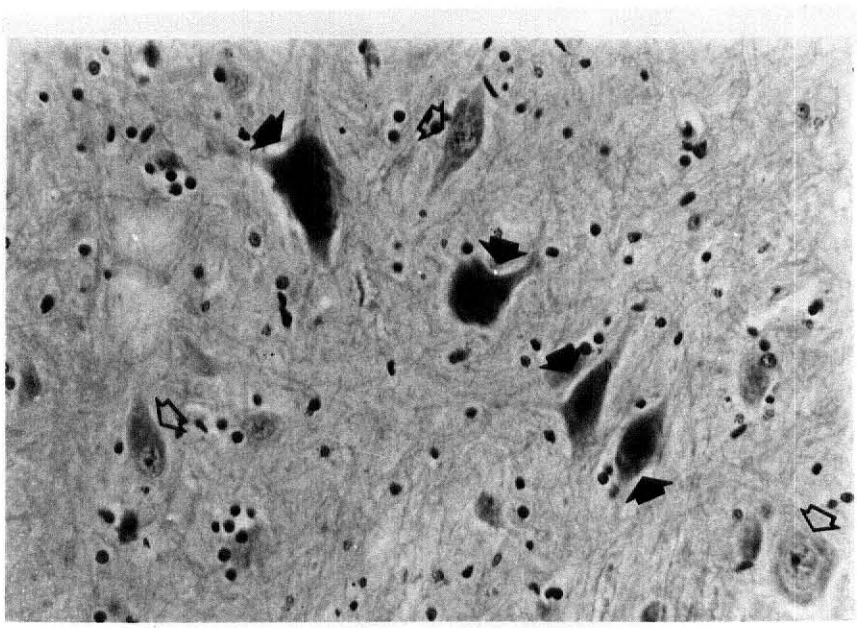

Figure 35: Dark staining neurons (solid arrows) observed in the spinal cords of several cats. Normal neurons (hollow arrows) are present in the same section. (Cross section, H & E stain, 250X)

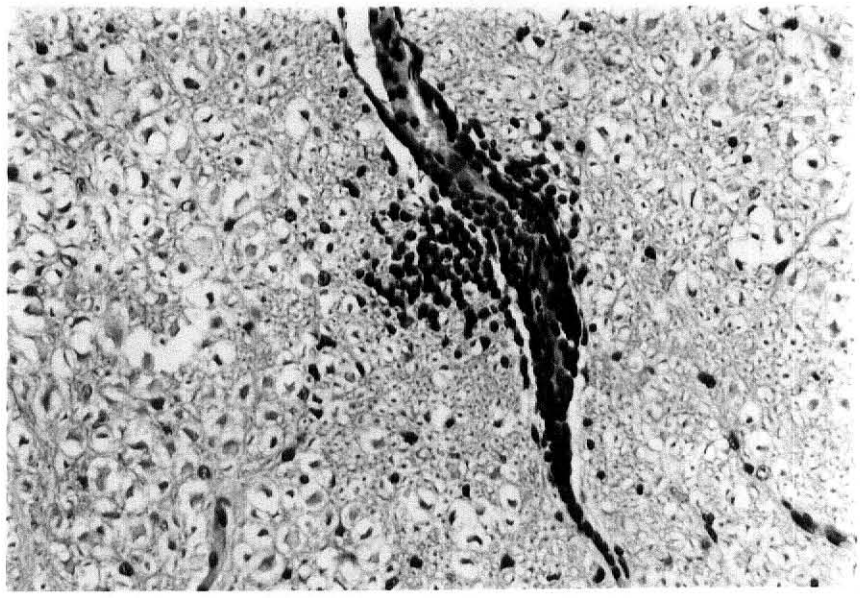

Figure 36: Cat Cy-3 had perivascular cuffing of some of the vessels in the lumbar spinal cord. (Cross section, H & E stain, 250X)

DISCUSSION

There are many reports in the literature concerned with the release of 5-hydroxytryptamine from a blood clot and the resulting pathophysiology. Some of these deal with pulmonic embolism (Hageman et al, 1973 and Ozdemir et al, 1974) and some are concerned with cerebral hematomas (Flamm et al, 1972 and Allen et al, 1974). The primary activity of 5-HT in these situations is that of vasoconstriction of both the primary vessel and collateral circulation. Although this evidence is not direct proof that 5-HT is the causative agent of the signs seen in feline aortic embolism, it does lend some support to this hypothesis.

Imhoff (1961), Schaub and Meyers (1974) have shown that a clot in the terminal aorta will cause paresis or paralysis. Butler (1971) has shown that 5-HT injected into a cul-de-sac will create a syndrome similar to that seen with the disease entity. Although this is not direct evidence that 5-HT is the substance responsible for the paresis or paralysis, it does lend more weight to that argument.

Certainly the fact that 5-HT is released by the platelets in a clot has been well documented (Page, 1968 and Michal, 1970). This results in further release of 5-HT and more aggregation of platelets, i.e., a positive feedback system. Since the 5-HT levels are high in pulmonary embolism where blood flow is static, one might assume that this is true in the hind legs of a cat with aortic embolism. To date no one has reported any measurements of blood 5-HT levels in cats with embolisms.

The results observed in the cats in Group 1 indicate that mixing glass beads with autologous blood is an effective way to reproduce the symptoms seen in feline aortic embolism. Since paresis or paralysis was created in

ten out of ten cats, Group 1 is a good base for evaluating the effectiveness of a 5-hydroxytryptamine antagonist. The locomotive defects observed are consistent with those reported by Imhoff and Schaub and Meyers who used bovine thrombin to create the clot and Butler, who injected blood components and 5-HT into an aortic cul-de-sac created in cats.

Methysergide was used the most extensively in this investigation because it was the only drug reported to be a specific antagonist to 5-HT. In an effort to prove or disprove the hypothesis that 5-HT is the agent responsible for the signs observed in this disease entity, such a specificity is necessary. The work of Allen et al (1974) and Banna and Anderson (1968) indicates that methysergide has vasoconstructive properties. Allen reported that methysergide caused arterial constriction to 58% of the level observed when 5-HT was applied. Banna and Anderson reported that the administration of methysergide could result in either a pressor or a depressor effect on the blood pressure in different cats. Thus, the side effects of methysergide may negate its value as a specific antagonist. The results observed in Group 2 are hard to interpret since both the drug and the agent under investigation can cause vasoconstriction.

It appears that in this investigation the most effective dosage of methysergide is 2.0 mg/kg. The signs observed in the cats given 0.2 mg/kg were equal to the most severe signs observed in Group 1. Banna and Anderson (1968) noted that at 0.2 mg/kg or less, the antagonistic effectiveness of methysergide was dependent on the level of 5-HT present. Assuming that the levels of 5-HT are high due to the stasis of blood in the hind legs, this dosage of methysergide may not be adequate. When 4.0 mg/kg is given, the heart rate is markedly altered. This might affect the distribution of the drug to its receptor sites, thus diminishing its effectiveness. The change in rate noted when 2.0 mg/kg is given is minimal, which would allow good distribution of the drug.

Cyproheptadine and doxepin are antagonists to both 5-HT and histamine. Butler (1971) has shown that histamine will not reproduce the sign observed in this syndrome. Thus, the effects observed in this investigation by the administration of cyproheptadine or doxepin were probably manifestation of the drugs antagonistic properties for 5-hydroxytryptamine. The facts that Cat Cy-1 had only hemolyzed blood in its cul-de-sac and Cats Cy-2, Cy-3 and Cy-4 had only small clots in their cul-de-sacs were probably a result of cyproheptadine's ability to interfere with platelet aggragation (Page, 1968 and Nemir et al, 1972). Whether the beneficial affect of cyproheptadine was due to inhibition of platelet aggregation and diminished 5-HT release or direct 5-HT antagonism or a combination of both, cannot be determined from the data obtained in this experiment. Since Butler was able to create paralysis with hemolyzed blood injected into the cul-de-sac, 5-HT antagonism is the most likely route of action.

The glass beads used to create the blood clot in the cul-de-sac may affect the efficiency of the drugs used. Venter and Kaplin (1974) showed that catecholamines were released very slowly from glass beads to which they were covalently coupled. If the same is true of 5-HT and other compounds released by platelets, then one might expect these substances to be continuously released even beyond the point when all the 5-hydroxytryptamine antagonist was metabolized. However, no cats were observed to have worsening of signs.

When Groups 1, 2, 3 and 4 are compared on the basis of any sign of paresis or paralysis versus normal locomotion, all ten cats in Group 1 exhibited paresis or paralysis; Group 2 had one of eight with normal locomotion; Group 3 had two of four with normal locomotion and none of the cats in Group 4 exhibited a totally normal gait. If you eliminate the cats given 0.2 mg/kg and 4.0 mg/kg of methysergide from Group 2 for the previously

stated reasons, then the comparison in Group 2 becomes three with paresis or paralysis and one with normal locomotion. The numbers involved in Groups 2, 3 and 4 are too small for individual statistical comparison; however, when compared by the chi-square method on a control (Group 1) versus drug (Groups 2, 3 and 4) basis, the treated groups had significantly greater numbers of cats with normal locomotion ($p=0.0741$). If Group 4 is eliminated from the comparison, then the beneficial effect of treatment is highly significant ($p=0.0101$).

Although the administration of cyproheptadine has not been shown to be statistically better than no treatment, the trend suggests that this procedure is beneficial. If four cats were added to Group 3 and only one of these cats walked normally, then this group would become statistically highly significant ($p=0.0101$).

Groups 5 and 6 both contain cats (L-2 and DB-5)[8] that should have walked well, but did not. If one combines all of the cats reported on by Butler, Imhoff, Schaub and Meyers with their aortas ligated but with no blood or blood components present in the cul-de-sac, the total is 26, all of which walked normally. It is possible that in the case of DB-5 the cat's size inhibited the development of effective colateral circulation. This cat was small for a mature cat, weighing only 1.5 kg. No weights are reported by other workers. With Cat L-2, size cannot be considered as a factor as the cat was normal in that respect. Using the 26 previously reported cats as a control group, one might assume that biological variance is responsible for the results observed in Cats L-2 and DB-5.

According to Jubb and Kennedy (1963) the pathology observed in muscle due to ischemia will be dependent on the degree of colateral circulation present. Since the gastrocnemius muscle has only a single nutrient vessel, it was not used for the histologic evaluation of muscle damage. The path-

ology observed in these cats is consistent with the pathology seen due to ischemia and not denervation. The varying degrees of hyaline degeneration, fiber fragmentation and cellular infiltration are dependent on the duration and severity of the ischemia. Muscle is more resistent to ischemic necrosis than some other tissues, such as skin (Jubb and Kennedy, 1963).

Of the abnormalities seen in the lumbar spinal cord, only the Wallerian degeneration can be attributed to ischemia of tissues. The dark, shrunken neurons may be due merely to the autolysis process, or it may be an antemortum change (Jubb and Kennedy, 1963). There must be injury to the spinal column before Wallerian degeneration will occur. It is interesting to note that Cat L-2 was one of the three cats in which this was observed and that one of the other cats (Cy-1) had no abnormalities in its gait. Only focal areas of degeneration were seen in Cat Cy-1.

The cystitis that was seen can be attributed to manipulation and a diminished blood supply to the bladder since the umbilical and the urogenital arteries arise posterior to the site where the aorta was ligated (Miller, 1964). A full bladder was expressed to give the best visualization of the surgical site. The trauma associated with bladder evacuation, coupled with a diminished blood supply, might lead to cystitis.

Group 5 was to act as histologic control for Group 1. Since only one of the two cats in this group was able to walk normally, this Group's value as a control Group is questionable.

In Group 6, the observations indicate that merely having a blood clot in the area of the terminal aorta, as was the case in DB-1 and DB-2, will not create paralysis, i.e., the major vessel to the legs must be occluded. If one assumes that a substance is released by the blood clot, then Cats CB-3 and DB-4 would indicate that, at least in part, this substance moves passively to the areas it affects. Certainly the numbers involved in this group prevent one from making a concrete judgment.

CONCLUSION

The injection of autologous blood mixed with glass beads into a cul-de-sac that has been formed in the terminal abdominal aorta is an effective method of reproducing the clinical manifestations observed in feline aortic embolism. All of the cats which only had blood and glass beads injected into the aorta exhibited either paresis or paralysis of the hind legs.

When considering the three 5-hydroxytryptamine antagonists (methysergide, cyproheptadine and doxepin) on drug groups versus control group basis, there is a high probability that the drugs were of benefit in protecting some of the cats used in this study from paresis or paralysis. Of the three drugs used, cyproheptadine has the most promise of being beneficial because two out of four cats in this group walked normally and the drug had negligible effects on the heart rate. Both methysergide and doxepin had marked effect on the cardiac rate when used at high levels. A lack of sufficient numbers prevents one from drawing the firm conclusion that 5-HT is directly involved in the pathogenesis of the paresis or paralysis seen in feline aortic embolism.

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FIVE-HYDROXYTRYPTAMINE ANTAGONISTS AND FELINE AORTIC EMBOLISM

by

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Clinically, feline aortic embolism is manifested by paresis or paralysis of the hind legs. Mere occlusion of the terminal aorta will not cause these signs to develop; i.e. a clot must be present. The clinical condition can be duplicated if the aorta is ligated just posterior to the caudal mesenteric artery, at the deep circumflex iliac and abdominal arteries and at the external iliac bifurcation, thus forming a cul-de-sac into which autologous blood mixed with glass beads is injected. All of the cats in which only this was done had either paresis or paralysis of the hind legs.

Histopathology was done on representative cats from each group included in the investigation. The pathologic changes seen in the quadriceps muscle are consistent with those observed in ischemic conditions. The changes included hyaline degeneration, fiber fragmentation and cellular infiltration. The degree and severity of change seen is dependent on the degree of ischemia. Other than a small amount of Wallerian degeneration observed in three cats, no significant abnormalities were observed in the lumbar spinal cord.

In Groups 2, 3 and 4, cats were administered different 5-hydroxytryptamine antagonists intravenously prior to the creation of a blood clot in cul-de-sac. The antagonists used were methysergide, cyproheptadine and doxepin. Of the cats in which 1.0 mg/kg of cyproheptadine was administered, two out of four had a normal gait. Although none of the three cats given doxepin had a totally normal gait, the only abnormality noted in one of the cats was knuckling on the toes of one foot. The results obtained indicate that 5-hydroxytryptamine antagonists have promise as an effective agent for preventing paresis or paralysis in feline aortic embolism. The active role of 5-HT in the production of paresis or paralysis in feline aortic embolism is more clearly implicated by the work done in this investigation.