THE EFFECTS OF SEROTONIN
ON THE VASA NERVORUM

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ABSTRACT

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By

Barry D. Stringfield

A number of works, extending as far back as the Seventeenth Century, have been written establishing the essential nature of the vasa nervorum in the maintenance of normal function of peripheral nerves. Serotonin has been proved to be an important neurotransmitter and an important vasoactive autacoid with effects that differ from vascular bed to vascular bed. Prior to this work, no study has delineated the effect that 5-HT has on the vasculature of peripheral nerves. Therefore, this investigation was designed to determine both the effect that 5-HT has on the vasa nervorum and if UML 491 (methysergide maleate) could reverse these effects. Also, this thesis discusses the effect that 5-HT has on the function of the peripheral nerves.

The quartz rod transilluminator was utilized in _in vivo_ observations of vascular beds in peripheral nerves of the cat, rabbit and Sprague-Dawley rat. The cat was used for the majority of the experiments. Photomicrographs were made to supplement the recorded data. A total of 25 vascular beds and 219 vessels were used in recording data. They were monitored for character of flow, vessel size, and

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number of white emboli. A total of 73 administrations of 5-HT were made with a major emphasis on I.A. injections and topical applications. In some experiments, the serotonin antagonist UML 491 was administered following 5-HT applications or injections. Significant results were obtained from these experiments.

Both I.A. (0.4µg to 900µg) and topical (1µg to 86µg) administrations of 5-HT induced sludging of blood, slowing of flow, stasis, dilation, constriction and increased numbers of white emboli. UML 491 caused a return to the control state in vessels which demonstrated sludging, stasis and slowing of flow.

In accordance with previous studies, a discussion was made on the mechanisms by which the above findings occurred, and the role that 5-HT plays in the peripheral nervous system.

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ON THE VASA NERVORUM

By

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INTRODUCTION

Although the true significance of the vasa nervorum has not been realized by the scientific community until recent times, many investigators, dating back as early as 1627, have recognized the possible importance of these structures. This thesis will outline the progression of studies dealing with the anatomical nature and functional importance of the vasculature of the nerve. The arguments and studies surrounding the controversy concerning the relative importance of the vasa nervorum in maintaining normal nerve function will be discussed. In addition, this thesis will present investigations pertaining to the controversy between the relative importance of the peripheral nerve regional arterial supply and the longitudinal vascular plexus in sustaining normal vasa nervorum blood flow.

Although serotonin has been known to be a significant central nervous system neurotransmitter and an important vasoactive autacoid, no role has been delegated to it with regard to the peripheral nerves and few investigations have studied its effect on the vasa nervorum. Thus, two purposes of this study are to determine serotonin's effects on the vasculature of the nerve, and to ascertain if the function of the nerve can be altered in any way.

CHAPTER I

LITERATURE REVIEW

Man's awareness that the vasa nervorum exist and that they may be of importance to the nerve, has its ancient beginnings in Aristotle. As cited by Blunt (1957), Van der Speighel (1627) wrote of the vasa nervorum in man: "It is ridiculous to think, as Aristotle stated in his account of the body, that there should be any mucous-like, whitish and gelatinous substance around the nerves, from which the nerves themselves spring and from which they are nourished, when their nourishment may be obtained from these vessels." Ruysch (1701) and Von Haller (1752) were also cognizant of both the presence and the potential significance of the vasa nervorum.

Prior to the 1900's, it was generally believed that the vasa nervorum had little to do with the normal function of peripheral nerves. Instead, it was thought that the only essential requirement for maintaining neuron function was the anatomical and physiological continuity of its axon and cell body. Consequently, the majority of physiologic experiments in which peripheral nerves were involved, were conducted with complete disregard for their vascularity.

A. Anatomical Studies

Von Haller, in 1756, was probably the first researcher to consider the vasa nervorum in any detail. Isenflamm and Doerffler, in 1768, constructed the first work devoted entirely to the vasa nervorum. They were able to demonstrate and then describe a network of vessels around the nerves by the injection of colored wax into the vasa nervorum.

Hyrtl's two papers (1859, 1864) systematically pointed out that

1. each nerve receives nutrient arteries;
2. supplying arteries give no branches to adjacent muscles;
3. each supplying artery divides into a descending and an ascending branch which anastomoses with corresponding branches of adjacent arteries. A longitudinal anastomosis is consequently formed, establishing a collateral circulation.

Quénu and Lejars (1890, 1894) further determined that a nerve is not supplied by one artery, but receives a number of vessels which are constant in origin. They also reported that each subcutaneous nerve has an accompanying artery.

Tonkow and Bartholdy, working independently, obtained remarkably similar results, in 1897, which are generally accepted today. They obtained the following information:

1. All nerves receive blood vessels which traverse the epineurium to enter the interfascicular connective tissue (perineurium).

2. Blood vessels which supply nerves are derived from the nearest available source, i.e. a main arterial trunk alongside a nerve, or less frequently, one of its muscular branches which passes close to the nerve.

3. When an artery crosses a nerve, it supplies that nerve. (An exception occurs when a fascial plane intervenes, e.g. the transverse cervical artery crosses the brachial plexus but does not supply it.)

4. Contrary to what Quénu and Lejars maintained, the origins of the nutrient arteries are not constant.

5. The number of nutrient arteries in any one nerve is variable and the number of vessels supplying a nerve increases distally. Bartholdy attributes the increases in the number of supplying vessels to the nerve's entering into regions of greater vascularity as it passes distally.

6. After the nutrient artery divides into a descending and an ascending branch in the epineurium, the branches give rise to smaller branches (finer arterioles) laterally, and these branches anastomose to form a rectangular arteriolar meshwork which lies in the perineurium. The rectangular meshwork of arterioles then gives rise to capillaries which are known to extend into the endoneurium. Tonkow and Bartholdy also remarked that a continuous longitudinal plexus is established through the anastomosis of adjacent nutrient arteries.

Until recently, the structure and topography of the capillary bed in the peripheral nerve *in vivo* had not been investigated. Lang (1962) identified a capillary plexus in the outermost layer of the epineurium (conjunctiva nervorum). Lindström (1963) further describes fine glomerulus-like capillary formations in this layer.

With the use of a modified Leitz intravital microscope, Lundborg and Brånemark (1968) described arterioles, venules and capillaries in the connective tissue of the deeper layers of the epineurium, and in the perineurium, which they designated extra-fascicular vascular plexuses (Figure 1). They also delineated an intrafascicular, endoneurial vascular bed which was thought to be in communication with the extra-fascicular plexus. Under higher resolution, they were able to visualize vessels in both plexuses which run parallel, perpendicular, and sometimes obliquely to the longitudinal axis of the nerve (Figures 2-3).

The means by which nerves maintain their blood supply, and whether or not the longitudinal vascular plexus predominates in importance over the regional blood supply, has challenged investigators as far back as 1878, the date of Mayer's paper on the effects on the nerve's excitability after devascularization. Further discussion on this subject will be covered in the next two sections.

B. Experimental Studies

Perhaps the best was to evaluate the role of the vasa nervorum is by correlating the function of the nerve with different grades of flow. Until recently, the lack of precise methods for qualitative or quantitative evaluation

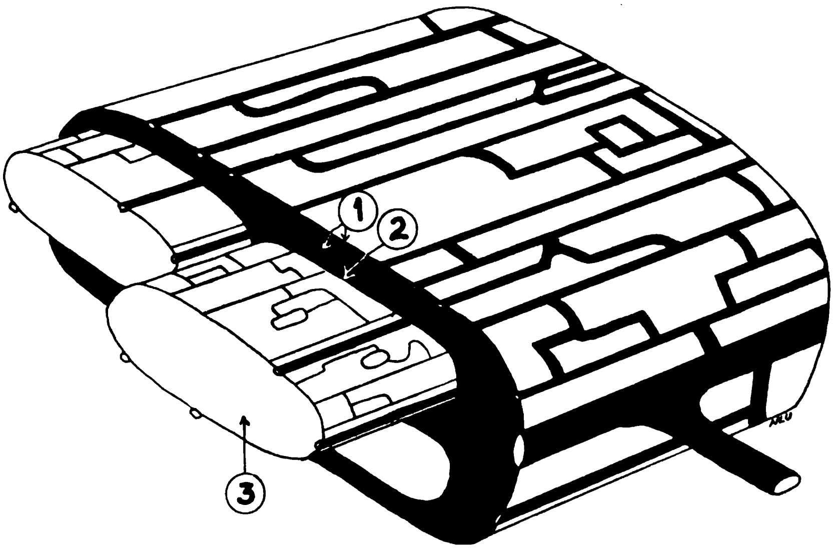

Figure 1. Schematic representation of a nerve trunk.

The two exposed fascicles demonstrate the intrafascicular, the perineural and the epineural microvascular systems. (1) Epineurium, (2) Perineurium, (3) Endoneurium. Note the great number of anastomoses between the epineural vessels. The epineural vessels are in communication with the perineural vascular plexus (consisting of arterioles, capillaries and venules), and thereby with the intrafascicular capillary bed. Modified from Lundborg and Branemark (1968).

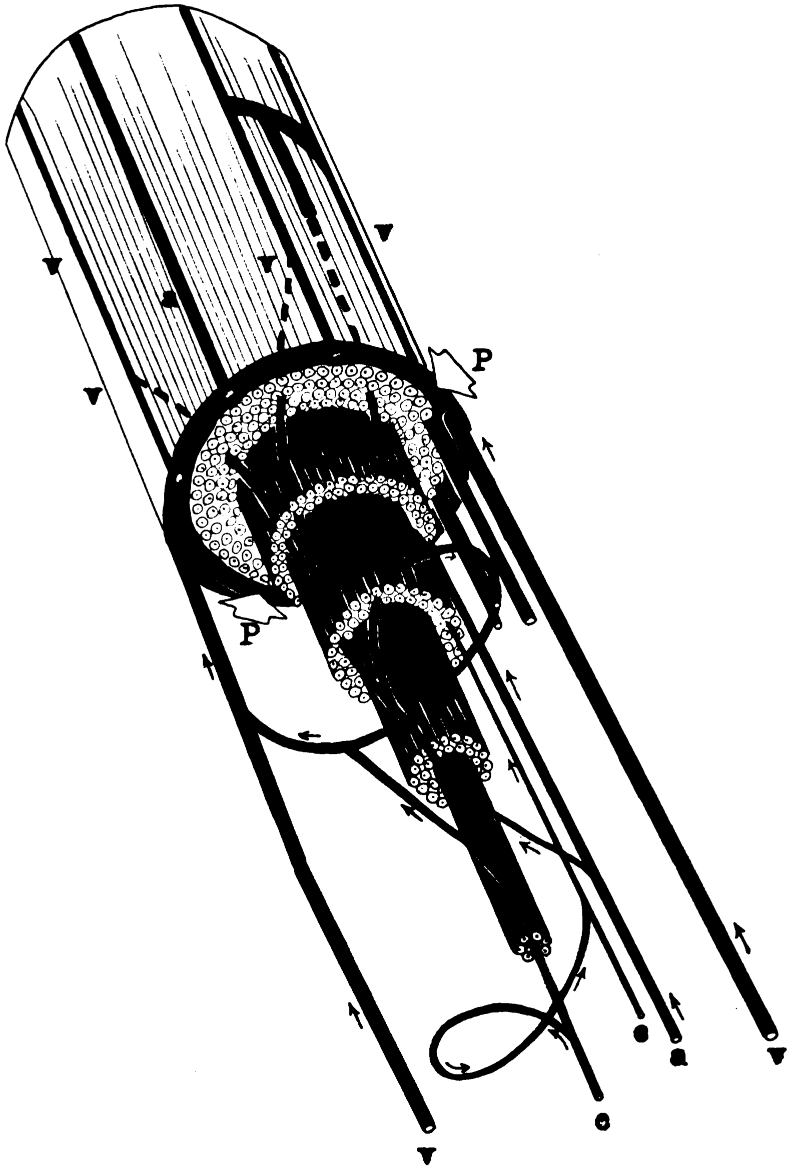

Figure 2. Diagram of the intrafascicular microvascular architecture as viewed by vital microscopic studies.

(p) Perineurium, (a) Arteriole, (v) Venule, (c) Capillary. Note the capillary loops, sometimes arranged in planes perpendicular to the longitudinal axis of the nerve. Arrows denote direction of blood flow. Modified from Lundborg and Branemark (1968).

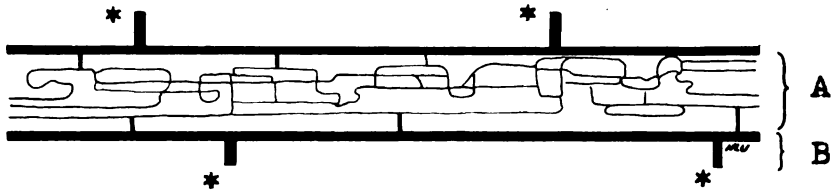

Figure 3. Diagramatic representation of the microvascular architecture of a peripheral nerve, delineating the relationship between intrinsic and extrinsic systems.

(A) Intrinsic system, i.e. the fascicular vascular bed, continuous throughout the entire length of the nerve and mainly consisting of capillaries. (B) Extrinsic system, i.e. the nutrient arteries at *, and the epineural vessels. These two systems communicate with each other by anastomoses along the entire length of the nerves. Modified from Lundborg and Branemark (1968).

of the nerve's circulation has resulted in artificial means for producing ischemia in the nerve. The two different methods selected have been compression and devascularization (Lundborg and Brånemark, 1968).

1. Compression Studies

Using direct pressure on their own nerves, Bastien and Vulpian (1855) and Waller (1862) performed the earliest reported compression experiments. They reported a late involvement of pain sense. Mitchell (1872) reported, "When the pressure has been removed, rapid recovery of sensibility and motion ensue, unless the pressure has been severe and long continued." Waller (1862) provided a good example of delayed recovery when he found that after compressing his own left radial nerve for forty-five minutes, loss of motion and sensibility incurred and signs of recovery were not evident until after eleven days.

Erb (1876), Lüderiwitz (1881), and Dejerine and Bernheim (1899) found that after applying localized pressure to a nerve, motor loss occurred with no apparent abnormality in sensory function. The Medical Research Council of Britain in 1920 stated that with partial or transient damage to a nerve, e.g. a piece of shrapnel passing close to the nerve, "the axis cylinders are damaged but Wallerian degeneration does not take place; they temporarily lose their normal conductivity but retain trophic power over the distal segment of the nerve, [and] ...in a simple case the function of the nerve is restored within a few days or weeks."

Ramon y Cajal (1928) described thinning of axis cylinders compressed by mildly tight ligature. He also reported that after sectioning the nerve distal to the ligature, the distal segment was able to resume its full diameter and full capacity for regeneration. Hassin (1940) made reference to a paper by von Büngner (1891) in which thinning of the myelin sheath was described both proximally and distally to the ligature.

Lewis et al. (1931) studied the effects of pressure on the peripheral nerves in man after applying a sphygmomanometer cuff to the upper arm. Their results revealed that impairment produced by the cuff affected touch before pain and pain before motion. When pressures below systolic pressure were used, no effect was obtained. The selective character of the impairment was explained by a greater sensitivity of the smaller nerve fibers to anoxia. The paralysis was also found to begin distally and progressed in a centripetal (or proximal) manner. Lewis et al. thought that this last finding was due to the fact that the fibers innervating the more distal structures were located along the outside of the nerve. They placed a second cuff below the first and found that removal of the first cuff was followed by recovery from paralysis even though the second cuff was applied, like the first, at a pressure above systolic pressure. However, after a latent period, the paralysis did return. Following this last experiment, the authors concluded that the paralysis they observed was due to ischemia of the compressed part and not to peripheral stasis. They also observed that the nerve immediately below the cuff was less excitable than the lower portion. They subsequently expressed the opinion that pressure on the nerve influenced its electrical conduction capacities only by means of local ischemia.

Weir Mitchell (1872), a well-known physician in the latter part of the Nineteenth Century, similarly wrote of impairment of nerve function due to pressure, but used different terminology. In his experiments, he attempted to measure "the amount of pressure needed to arrest the passage of nerve force" by applying a chamois leather bag containing "quick silver" (mercury) to the sciatic nerves in rabbits. He found that a gradual loss in the conduction capacity of the nerve over a period of ten to twelve minutes occurred after applying 20 inches (50.8 cm) of mercury. His theory was that the loss of conduction capacity was due to a mechanical interruption of nerve force; this was opposed to the explanation given by Lewis et al. that ischemia is the cause of such an impairment.

Intrigued by a phenomenon which was first described by Erb (1876) as a maintenance of faradic excitability by a nerve peripheral to a lesion which blocks conduction from above, Denny-Brown and Brenner (1944a) designed an experiment to study the effect of direct pressure on the nerve. In one set of experiments, they clamped brass plates on the sciatic nerves of cats at pressures varying from 170 to 430 grams. In order to observe the long term effects of direct pressure, they applied lower pressures of 7 to 44 grams for periods up to eight weeks. The more severe pressures were applied for two hours and induced transient paralysis lasting from nine to eighteen days; no gross abnormality of sensation was observed. These changes were accompanied by an intermittent loss of myelin at the nodes of Ranvier and axonal swelling which was strictly limited to the site of previous ischemia. Recovery of motor conduction was early, but restitution of the myelin took up to six months. Continuous compression of nerves at pressures above 9 grams induced complete motor paralysis and total sensory loss within five to eight days. The compression also produced congestion, edema and degeneration on both sides of nerves which were still conducting touch and pain sensations; all fibers appeared demyelinated to about the same degree. They concluded that the reason for selectivity of fibers involved in a block of conduction lies in the functional properties of the block rather than in the size of the fibers affected. In addition, the compressed segment of nerve was found to be preserved in a state of ischemia necrosis with myelin, Schwann cells, and axis-cylinders intact. The effect of pressure on the nerve was attributed entirely to ischemia.

Durward (1948) reported that in many of the past experiments dealing with the effect of local pressure on the nerve function, it is difficult to determine if it was ischemia, or the action of the pressure itself on the nerve fibers, which caused the observed effects. However, Durward ascertained that Lewis, Pickering and Rothschild's (1931) experiments and the later set of studies by Denny-Brown and

Brenner (1944b) demonstrated valid proof that the altered state of the nerve which they observed after applying pressure was due to ischemia.

In the hope of imitating the carpal tunnel syndrome and related disorders, Weisl et al. (1964) compressed the sciatic nerve in rats by placing a piece of polythene tubing slit lengthwise over the nerve. This experimental procedure only slightly reduced the cross-sectional area of the nerve, but resulted in: (1) swellings found distally and proximally to the tubing; (2) an increase in axonal size; (3) a delay in electrical conduction; and (4) an increase in the tortuosity and number of vasa nervorum in the swollen areas of the nerve. Their findings are similar to those found in the carpal tunnel type syndromes. Weisl et al. theorized that the swelling was probably due to a partial obstruction of the vasa nervorum followed by a transudation of fluid. They remarked that it is not difficult to believe, as Dustin did in 1917, that edema fluid can be invaded by fibroblasts, producing the fibrous type lesion originally described by Marie and Foix (1913). If it is accepted that nerve constriction produces edema followed by fibrosis, then prompt surgical relief of the compression becomes mandatory if irreversible damage is to be prevented.

In the more recent study by Lundborg and Brånemark (1968), blood flow in the rabbit's tibial nerve, altered by applying a tourniquet to the rabbit's leg, was checked with a Leitz intravital microscope and found to have completely

stopped. The tourniquet was released after ischemic periods varying from one half to twelve hours, and in every case flow in the epineural and intraneural vascular bed reappeared immediately or within thirty seconds. Strikingly few signs of injury were seen: intravascular granulocytosis was seen in a few cases; a few venules were blocked; and only occasional perivascular mast cells were seen. Recovery occurred within a few seconds to ten minutes.

2. Devascularization Experiments

Mayer (1878) was the first reported investigator to study nerves subject to devascularization. He found that the devascularized facial nerve *in situ* in the rabbit lost its excitability in fifteen to thirty minutes. Fröhlich and Tait (1904), in a similar experiment on the sciatic nerve of the rabbit, obtained comparable results. Pointing out the difficulty there is in avoiding trauma to the nerve when devascularizing it, Koch (1926) stressed that the above experiments require confirmation. In 1905, Okada produced degeneration of the sciatic nerve by ligating the inferior gluteal artery of rabbits, implicating that the regional arterial supply dominates. Fifteen years later, Torraca removed the epithelial sheath of the sciatic nerve in the dog and surrounded it with a rubber covering. He observed only a transitory paralysis in the affected limb and full movement soon returned. Torraca attributed this to the ability of the longitudinal vascular plexus to make up for regional arterial supply deficiencies.

Koch (1926) investigated the extent to which different regional vessels supplied the sciatic nerve in the rabbit, and concluded the following: (1) the longitudinal blood supply may compensate for, but does not dominate over, the regional blood supply; and (2) the distal part of the nerve appears to have a richer blood supply than the proximal part.

Realizing the far-reaching implications of Okada's work, Adams (1943) decided to repeat his experiments, eliminating trauma to the nerves as much as possible. Unlike Okada, Adams did not observe any significant degeneration of the sciatic nerves. He attributed Okada's findings to trauma incurred by the nerves during the course of the experiments. Adams's viewpoint of the role of the regional arterial supply versus the longitudinal vascular plexus can be summarized in one of his concluding statements:

> Although ligation of a source of supply may produce a temporary local diminution of blood supply it is difficult to conceive how the effect could be other than a transient one, especially in view of the facility with which the longitudinal pathway is capable of enlarging and thus compensating for the local loss.

Bulbring and Whitteridge (1941) performed a study dealing with the physiological state of the nerve after intra-arterial and intravenous injections of adrenaline. The intra-arterial injections were made through a cannula inserted in an iliac artery. Upon injecting varying dosages of adrenaline intra-arterially, they found increases of the $\alpha\beta$ spike averaging 100%, with submaximal electrical

stimulation. Bulbring and Whitteridge stated that this phenomenon was due to a lowering of the threshold. They also described a lowering in threshold, after adrenaline injections, in the δ spike with submaximal stimuli given at rates up to 40 per second. Clamping the aorta for one minute produced changes in the δ spike which were similar to the adrenaline experiments, but it did not affect the spike. The same dose of adrenaline injected intravenously, as that injected intra-arterially, produced one half the effect on the nerve action potential. Bulbring and Whitteridge suggested that there was a good possibility that adrenaline produced an increase in the K/Ca ratio in the nerve, and that this was responsible for the shift in the threshold.

Bentley and Schlapp (1943) found that after removing the regional arterial blood supply in rabbit sciatic nerves and the nerve's external popliteal continuation -- but leaving the longitudinal vascular plexuses intact -- there was no significant change in the action potential after electrical stimulation. They next severed all nutrient arteries to the nerves except the uppermost supplying vessels. After three to four hours, stimulation of the external popliteal nerve revealed an action potential which was 80% of the original. At this time, the blood supply to the upper part of the nerve was cut off. Subsequently, the action potential diminished to near zero in half an hour. Bentley and Schlapp concluded that the anatomical arrangement of the blood supply provides a wide margin of safety and a

small though definite blood supply is essential for the maintenance of conduction in the nerve.

One of Bentley and Schlapp's concluding remarks -- that the circulatory requirements of the nerve are small -- was refuted by Bacsich and Wyburn (1945). At the suggestion of the Peripheral Nerve Sub-Committee of the Medical Research Council, Bacsich and Wyburn set out to find more substantial evidence defining the role of the longitudinal plexus in its relationship to the regional arterial supply of nerves. They studied two groups of rabbits: one in which the sciatic nerve was crushed but the regional arterial supply was left intact, and another group of animals in which the sciatic nerves were both crushed and separated from their regional vascular supply. They next observed the epineural vasculature for a period of two weeks and found no difference in the two groups. Bacsich and Wyburn stated that if there had been diminution of blood flow due to deprivation of the regional arterial blood supply, then there would have been a consistent difference in the vascularity between the two groups. They expressed that a nerve deprived of its blood supply would have no change in epineural vasculature after it was crushed; however, their experiments demonstrated change in both groups of animals.

Guided by the concept that ischemia of peripheral nerves may lead to peripheral neuropathy, Roberts, Jarvis and Key (1943) and Roberts (1948) performed a number of experiments which help to evaluate the role of the vasa

nervorum in its relationship to peripheral neuropathy. Roberts and co-workers (1943) carried out a series of acute experiments and later, in 1948, Roberts confirmed their results with similar experiments. The sciatic nerve was exposed in dogs and cats, and kept at 37°C. One of the following procedures was performed and in each case 2% Chicago Blue dye was injected into the aorta:

1. Aviation and submarine air embolism suggested to Roberts the possibility that embolism may produce ischemia in the vasa nervorum. He injected lycopodium spores and powdered graphite into the inferior gluteal artery, supplying the sciatic nerve, in the dog. In addition, rats were placed in a pressure chamber which simulated an altitude of 12,000 feet. When the rats were removed, the vasa nervorum were found to be filled with air bubbles. In both the dogs and the rats, a patchy distribution of dyes was found deposited in the vasa nervorum, indicating ischemia.

2. Ligation of a segmental nutrient artery produced, like the emboli experiment, a patchy distribution of dye.

3. Stripping the perineurium again produced a patchy and variable distribution of dye.

4. Stretching the nerve longitudinally demonstrated the obliteration of the vasa nervorum, like a rubber tube is obliterated with stretching, with a complete absence of dye in the stretched portion of the nerve.

5. In the nerves which were constricted by a rope tourniquet placed around the middle of the right thigh, the

dye was uninjected for several centimeters above and below the constriction, indicating partial ischemia. These tourniquet experiments illustrated that the vasa nervorum can be obliterated by compression of the limb with indirect pressure on the nerve.

6. Constriction of the nerve with ligatures placed between two nutrient arteries obliterated the blood supply between the ligatures. Consequently, this procedure eliminated both the regional and longitudinal blood supplies.

In the first series of experiments, the animals were sacrificed, but in a second series of experiments, the animals were allowed to survive from one to seven weeks, to observe the effect of the above procedures on nerve function. Ligation of only a single nutrient artery was not followed by any clinically discernible evidence of dysfunction of the sciatic nerve. But, when all nutrient arteries located between the knee and hip joints supplying the sciatic nerve were ligated, muscle weakness was noted to occur. Stripping the epineurium from the sciatic nerve between the knee and hip joints was followed by muscle weakness as well as a partial and sometimes complete loss of sensibility to pin pricks, pinching, or heat, and by "tophic ulcers" located on the dorsal surfaces of the dog's feet. After injecting graphite or lycopodium spores into a nutrient artery of the sciatic nerve, all of the seven dogs that were studied showed evidence of nerve impairment as soon as they recovered from anesthesia. Like the preceding experiments, loss of

muscle strength, accompanied by loss of sensibility to painful pricking, pinching, or heat, occurred in these animals. Additionally, findings of hypo-active patellar reflexes and absent "ankle jerk" reflexes were observed. Because of experimental difficulties, survival studies on stretching and compression of the nerve were not completed. Histological examination, in the three experiments completed, revealed degeneration of the sciatic nerve in each.

Durward (1948) noted that although Adams (1943) did not observe a significant number of nerves which had undergone degeneration, he did find degenerative changes in a small minority of his animals. Durward suggested that some other factor might be involved in causing the few degenerations that occurred. He also remarked that extensive operations are carried out on peripheral nerves, with little or no regard for their vascular supply and are commonly successful. This event, experienced by surgeons, has led them to act, perhaps unknowingly, on the assumption that the pattern of the blood supply in nerves is peculiar and is so disposed to allow extensive mobilization without essential devascularization.

Durward found that after severing all nutrient and epineural vasculature of rabbit sciatic nerves, from the buttock to the knee, degeneration was produced in all nerves involved. However, he found that mere ligation of the nutrient vessels supplying the nerves did not result in degeneration. From his findings Durward concluded that

Adams's discordant results can readily be explained by the occurrence of damage to epineural vessels during manipulation, or perhaps more likely by thrombosis within the ligated vessels spreading to the epineural vessels or beyond, and so involving the longitudinal vascular plexus. This speculative suggestion is supported by Sunderland (1945) who believes that if a nerve is roughly and carelessly stripped from its bed, not only will the superficial vascular system on the surface of the nerve be disturbed, but embarrassment of the intraneural circulation is liable to take place.

As well as having a regional vascular supply and a longitudinal vascular plexus, the vasculature of the nerve -- as depicted earlier -- has been described as having an extrinsic system (vasa nutritia and epineural vessels) and an intrinsic system (fascicular vascular plexuses). Prior to Lundborg and Brånemark's studies, in 1968, the relationship of these two newly described entities had not been investigated. These investigators set out to determine the effect that various combinations of partial or complete elimination of one or both of these systems had on the microvascular bed of the tibial nerve in the rabbit. The following experiments were conducted, and these results were obtained:

1. All the nutrient arteries to the nerve between the knee and ankle were cut, thus excluding the extrinsic system. This did not produce any reduction of the intraneural flow.

2. With microdiathermy all recognizable longitudinal

epineural vessels just distal and proximal to the observed area of the nerve in which nutrient arteries were cut, were then coagulated under the dissecting microscope. No demonstrable impairment of the intraneural flow resulted.

3. Both elimination of the extrinsic system, by cutting the regional arterial vessels, and elimination of the intrinsic system distal to the observation site, by cutting the nerve distal to the observation site, were done. This procedure produced only slight impairment in the intraneural microcirculation.

4. When a similar procedure was done, identical to the preceding experiment with the exception that the nerve was cut proximally to the observation site, the same result was obtained.

5. In the next experiment, the intrinsic system was removed by cutting the nerve proximally and distally to the observation site. However, two nutrient vessels were left attached. This procedure resulted in only slight impairment of the intraneural plexus. It was consequently concluded that the remaining nutrient arteries could, by themselves, sufficiently supply the intraneural vascular bed. Even when one of the supplying arteries was detached, a flow of blood was still observed in the microvascular system.

6. The epineurium of the tibial nerve was stripped from knee to ankle and the nerve was divided in the middle of the dissected segment. Next, the intraneural circulation was studied at the edge of the site of the division. Some

venules were occluded and showed no flow, but circulation in most of the arterioles, capillaries and venules was not impaired at all, or only slightly impaired. Similarly, capillary loops arranged perpendicular to the longitudinal axis of the nerve were found to have intact circulation.

Lundborg and Brånemark's studies thus show that the nerve can tolerate some degree of trauma. Because the intrinsic and extrinsic systems have been shown to supplement one another in the most efficient way, the two investigators remarked that the vasa nervorum have resistance to moderate degrees of trauma, e.g. moderate surgical mobilization of a nerve.

3. Other Experimental Studies

The Effects of Injury on Peripheral Nerve Function, Vasculature and Ionic Constituents

After Lundborg and Brånemark's (1968) conclusion that the nerve is capable of withstanding moderate amounts of trauma, there arises the question of how much trauma is needed to cause significant damage to the nerve. Frazier and Sibert (1920) studied 500 cases of post-World War I injuries which involved a bullet or piece of shrapnel that had passed close to the nerve without actually lacerating it or tearing its sheath. They reported that 45% had complete motor loss, 15% had complete sensory loss, and degeneration did not occur in any.

Woodhall and Davis's 1950 investigation of the effect of various kinds of injury involving peripheral nerves in

189 young men who had suffered wounds during combat revealed both structural and quantitative alterations of the intrinsic blood vessels. The changes included: (1) acute thrombosis; (2) intimal proliferation; (3) an increase in the number of arteries in the epineurium and interfascicular space; (4) histologic evidence that ischemia due to occlusion of the major longitudinal vessels and lateral nutrient vessels leads to an excessive deposition of collagen in the distal segment of the nerve. They found that the distal nerve segment demonstrated these changes more strikingly, and concluded that the changes were probably a direct sequel to interference with the longitudinal vascular plexus. Woodhall and Davis suggested that changes compatible with those described in ischemia may occur when nerve segments are extensively mobilized, particularly the distal segment, and nutrient vessels are severed. Also, they remarked that the neurosurgical problem involved in peripheral nerve mobilization cannot be disregarded, especially when a comparison with acute lesions in experimental animals is made.

Since it has been shown that certain types of injuries can lead to alterations of the nerve vasculature, a concern arises regarding what changes occur in the environment immediately surrounding the axons, and what effects these changes have on nerve function. Seneviratne et al. (1972a, 1968) observed transient phases of increased excitability of the median nerve during the early ischemic and post-ischemic periods, after a sphygmomanometer cuff was applied to cats' extremities. The studies of Porter and Wharton (1949) support these results.

Seneviratne et al. proposed that the excitability changes result from an alteration of potassium ion equilibrium. In the 1972a set of experiments, they induced hyperkalemia in the cat and obtained, as in the ischemia experiments, a transient phase of hyperexcitability. These investigators argued that the excitability changes occurring during the post-ischemic period are due to hypoxia causing an increased efflux of K^+ from the axon; the increased extracellular accumulation of K^+ produces the depolarization of the axon. Seneviratne et al. remarked that this initially leads to an increase in the excitability of the fiber, with further K^+ increases, causing additional depolarization and conduction block. They believed that the comparable hyperkalemia results were due to an increase in K^+ concentration in the endoneural and periaxonal space.

In two other experiments, Seneviratne et al. combined hyperkalemia with limb ischemia, and also induced hypernatremia. Only the first experiment revealed changes in nerve excitability. They noted an increased rate of depolarization which was greater than that seen in their previous experiments, dealing with hyperkalemia and ischemia separately. They also noticed a significantly increased post-ischemic recovery time. This observation was described as the result of increased periaxonal K^+ concentration coupled with anoxia.

Investigations Concerning Vasa Nervorum Permeability

Another phenomenon which has been associated with altered nerve function is altered vascular permeability. With the use of albumin labeled with fluorescent marker, Olsson (1966) showed that increases in vasa nervorum permeability occurred after nerve crush. His findings lend no support to Weiss's theory (1943 a and b, 1945) that endoneural edema following constriction of the peripheral nerve occurs as a result of obstruction of the centrifugal flow of fluid in the endoneurium. Nonetheless, Olsson's results do uphold the theory proposed by Denny-Brown and Brenner (1944 a and b), and commented on later by Weisl et al. (1964), that edema in compressed nerves is derived from neural blood vessels.

Welch and Davson (1972) point out that like the central nervous system, the peripheral nervous system also has a blood-nerve barrier that is important in maintenance of normal function. They remark that many investigators in the past have unjustifiably given more attention to penetration of materials through the sheath surrounding the nerve than to the permeability of the vasa nervorum. In their studies, they administered radioactive sodium, chloride, potassium and thiourea to rabbits via the marginal ear vein. They assayed the levels of these materials in the plasma and sciatic nerves of the animals and found that although the uptake by the nerve varied markedly from animal to animal, it was very much slower than the uptake by muscle. They

thought that this finding was evidence in support of a blood-nerve barrier. Welch and Davson were also able to determine that sodium and chloride were ten times more permeable in the nerve than in the brain, and potassium was three times more permeable in the peripheral nerve.

Seneviratne (1972b) used a fluorescent, evans blue-albumin tracer in rats in which a state of diabetes was induced with injections of alloxan. He found an increased permeability of endoneural capillaries and, in some areas of the nerve, an increased permeability of the perineural sheath. Seneviratne's results signify that there is a gross impairment of the integrity of the blood-nerve barrier in the alloxan-diabetic rat. He suggested that in areas where the perineurium was intact, the osmotic effect of the accumulation of edema-forming proteinaceous fluids could inhibit capillary filtration and formation of endoneural fluid. Also, Seneviratne remarked that the net effect is tissue hypoxia and cellular damage with subsequent segmental demyelination.

C. Serotonin

Although it was not isolated until recent times, serotonin (5-hydroxytryptamine, 5-HT) has been known by investigators for about a century. The vasoconstrictor properties of blood which is allowed to clot has been attributed to this substance for many nears. 5-Hydroxytryptamine has been given a variety of names, such as vasotonin (Goodman and Gilman, 1971).

Rapport, Green and Page (1948) isolated the constrictor substance from beef serum, identified its indole nucleus, and coined the name serotonin. With ultraviolet absorption spectrophotography, Rapport (1949) determined that this crystalline beef serum isolate is composed of equimolar parts of creatinine, sulfuric acid and serotonin. He identified serotonin as the active moiety and deduced its empirical formula, $C_{10}H_{14}O_2N_2$. Rapport's work prompted Hamlin and Fischer (1951) to synthesize serotonin. Their synthesized compound possessed the same vasoconstrictor properties as its biologically derived counterpart. The introduction of synthetic 5-HT made large quantities of serotonin available and touched off an explosion of studies. Approximately 4,000 papers were quoted in Erspamer's recent book (1966) (Goodman and Gilman, 1971).

Erspamer and Asero (1952) obtained some of the serotonin which Rapport had isolated, and found that it was the same substance with which they had been working since the 1930's, but under the name enteramine. Erspamer first recognized that the vasoactive material was present in enterochromaffin cells of the stomach and intestine. Erspamer suggested in the late 1940's that this substance was an alkylamine, hence the name enteramine (Goodman and Gilman, 1971). However, Page (1968) stated that Erspamer and his associates did not isolate or structurally identify their material.

About 90% of the 5-HT present in the body -- approximately 10 mg in humans -- is localized in the enterochromaffin

cells of the small intestine and stomach (Erspamer, 1966). Much of the rest is in platelets and brain tissue. Smaller amounts of 5-HT can be found in lung, liver, spleen and adrenal medulla (Page, 1968).

Serotonin is synthesized from 2% of the dietary intake of tryptophan (Hagen and Cohen, 1966). Synthesis occurs primarily in the enterochromaffin cells of the G.I. tract and in the brain (Page, 1968). The major sites of 5-HT degradation are the lung (the most active site of serotonin breakdown) and the liver (Thomas and Vane, 1967).

1. Vascular Smooth Muscle Effects

Serotonin is known to be a smooth muscle stimulant in that it causes contraction of isolated veins and arteries, as well as uterus, intestine, bronchiolar muscle, and denervated nictitating membrane (Page, 1968). In citing Daniel's work in 1964, Goodman and Gilman (1971) contend that 5-HT fails to invoke smooth muscle contraction in the absence of calcium ions. Wooley and Gommi (1965) proposed that serotonin might act by forming a ternary complex with Ca^{++} and membrane lipid, acting as a carrier to transport Ca^{++} to the interior of the muscle cells. They called the lipid member of the complex the receptor site. Since the calcium requirement is not unique for 5-HT action, but is common to the actions of a number of spasmogens and secretogogues, Goodman and Gilman (1971) suggest that serotonin promotes inward calcium movement by increasing membrane permeability.

5-HT's effect on the vasculature is not as simple as it may first appear. Serotonin investigations have shown that the drug causes variable changes in the dimensions of vessels, which depend on neurogenic vascular tone, vascular bed, and dosage of 5-HT.

Page (1952) injected serotonin intravenously in normal dogs and observed a short phase of decreased blood pressure and bradycardia immediately after the injection; this was followed by a pressor phase which was sustained several minutes. The initial depressor phase was attributed to a left ventricular chemoreceptor-mediated reflex response (Bezold-Jarisch reflex), and is known to be inhibited by atropine or vagal section (Page and McCubbin, 1953a). The mechanism of the second phase will be commented on later.

Conversely, Page (1952) found that a prolonged depressor phase followed I.V. injections of 5-HT in most of the cats he studied, and in some dogs. Page (1952) and Page and McCubbin (1953a and 1956) produced similar depressor responses in neurogenically-induced hypertensive dogs subjected to I.V. injections of 5-HT. They concluded that one of the most important factors determining the vascular response to serotonin is the degree of pre-existing neurogenic tone.

After producing neurogenic hypotension in dogs with the administration of ganglionic blocking agents, Page (1952) found that a pressor response occurred subsequent to I.V. injections of 5-HT. Page and McCubbin (1953a) consequently derived the term "amphibaric" to describe 5-HT's actions on blood pressure. Page (1952) proposed that the observed increased vascular tone after I.V. injections of 5-HT may be due to an increased vascular smooth muscle receptor sensitivity to norepinephrine.

Page and McCubbin (1956) showed that I.V. injections of antihistamines could produce a blockade in 5-HT's ability to induce a depressor response. Their finding indicates that histamine release is one mechanism, and most likely the predominant one, involved in the prolonged depressor response following 5-HT injections. Feldberg and Smith's studies (1953) provide support for Page and McCubbin's finding in demonstrating that serotonin causes release of histamine from the skin of cats, dogs, and rats, cat gastrocnemius muscle, and rat diaphragmatic muscle tissue.

A study by McCubbin, Kaneko and Page (1960) suggests that this depressor response may at least in part be due to inhibition of synaptic transmission in the central nervous system. McCubbin et al. injected 5-HT into the lateral ventricles of dogs in which the vagus nerves were cut, and increased blood pressure was produced by carotid occlusion. They found an inhibition of the carotid occlusion response, a decrease in arterial pressure, and bradycardia. These effects were attributed to reduction of tonic and chronotropic sympathetic activity by central nervous system inhibition of the nerves innervating heart and peripheral blood vessels.

Haddy et al. (1959) were in accord with, and provided an explanation for, the earlier findings of Page and McCubbin (1956). By nervous and humoral means, they varied the vascular tone within the foreleg of the dog. Tone was lowered by denervation of the foreleg, by intra-arterial infusion of phentolamine (α- adrenergic blocker) plus denervation, and by intra-arterial infusion of the vasodilator methacholine. Elevation of vascular tone was accomplished by bilateral vagotomy and by I.A. infusion of norepinephrine.

The results showed that serotonin antagonizes extremes of vascular tone when the changes are produced neurogenically. Total resistance is lowered when vascular tone is high, and elevated when it is low. They remarked that the bidirectional response derives ultimately from the fact that changes in nervous activity change the calibers of small vessels (vessels less than 0.5 mm in diameter) without greatly altering the calibers of large vessels (0.5 mm to 5.0 mm in diameter). They elevated the blood pressure in the neurogenic hypotensive dogs by constricting the large vessels and leaving the already neurogenically dilated small vessels essentially unaffected. A net constriction was thus produced. When the small vessels were highly constricted in the neurogenic hypertensive animals, serotonin dilated them more than it constricted the large vessels.

In contrast, 5-HT could not produce its bidirectional effect when the pressor and depressor changes were induced by the humoral agents, methacholine and norepinephrine. Methacholine worked to reduce tone by dilating large arteries; the presence of the dilator agent reduced serotonin's constriction effect upon these vessels. As a result, the net increase in resistance in the humoral-induced hypotensive dogs, after infusion of 5-HT, was relatively insignificant.

In analyzing norepinephrine's role in determining the results they obtained, Haddy et al. cited a previous study which Haddy, Fleishman and Emanuel completed in 1957. The finding in the earlier study revealed that norepinephrine increases vascular tone in part by constricting arteries and veins. In the 1959 study, the presence of norepinephrine was concomitant with a greater constriction effect of 5-HT on the larger vessels, even though 5-HT continues to dilate smaller vessels, resulting in an increase in resistance. A drop in resistance was necessary, in this case, for a bidirectional response to occur.

The effect of serotonin differs markedly from vascular bed to vascular bed. Vasodilation occurs in the vessels of skeletal muscle and superficial vessels of the skin (Goodman and Gilman, 1971). When 5-HT is applied to renal vessels (Page and McCubbin, 1953b and Emanuel et al., 1958), umbilical, meningeal and pulmonary vessels, and arterioles of the skin or vessels in which nervous control has been destroyed, constriction occurs (Goodman and Gilman, 1971).

In summary, the following mechanisms are known to contribute to the amphibaric actions of single injections of serotonin: (1) direct vasoconstrictor action, (2) chemoreceptor stimulation (Bezold-Jarisch reflex), (3) dilation accomplished indirectly by the release of histamine, and (4) transient autonomic ganglion blockade. Other mechanisms which may play a part are cardiac stimulation and central nervous system inhibition of neurosensory transmission of nerve fibers controlling blood pressure by some unknown phenomena (Page and McCubbin, 1956).

2. Other Studies

Swank (1961) developed a method for calculating relative amounts of aggregated blood elements. Swank's method determined the screen filtration pressure (SFP) of blood components when blood is passed through a standardized screen with multiple openings. The SFP increases when blood elements are aggregated.

Using the preceding method, Swank et al. (1963) were able to clarify some aspects of the role 5-HT plays in the aggregation of blood elements. They added serotonin, at concentrations ranging from 0.1 μg to 20 μg/ml, to the blood of dogs *in vitro* and found that the response was dose dependent and biphasic. At concentrations from 0.1 μg to 0.2 μg/ml of blood, SFP was observed to decrease. Higher concentrations of 5-HT caused an increase in SFP; a maximum SFP was reached at concentrations of 5 μg to 10 μg of 5-HT/ml of blood.

Swank et al. supplemented their in vitro experiments with observations of the vascular beds in the conjunctiva of cats, dogs and rabbits after injections of 5-HT (0.05 mg to 1.0 mg) in the common carotid artery. They found slowing of flow and slight aggregation at concentrations of 0.05 mg to 0.1 mg (ten seconds following injection), and marked rbc aggregation at a concentration of 0.2 mg. A more generalized aggregation, which was observable by the naked eye, occurred at a concentration of 1.0 mg.

These investigators also observed small light gray masses in small venules and thought them to be aggregated platelets and leucocytes. LSD-25 (lysergic acid diethylamide), UML-491 (methysergide maleate), and BOL-148 (bromolysergic acid diethylamide) prevented the rise in screen filtration pressure. UML-491 (1 μg/ml) was found to be the most potent inhibitor.

Having removed the platelets by passing the blood through glass wool, they observed that 5-HT could not produce any change in SFP. Because of this finding, Swank et al. suggested that platelets could possibly serve as a nidus for red blood cell adherence. However, they thought a more likely possibility for this phenomenon was that 5-HT causes an increase in the adhesiveness of the blood elements.

After microinjections of 5-HT (1 μg) and histamine (1 μg) into the endoneurium of rat sciatic nerves, Olsson (1966) observed an increase in permeability of the endoneural vasculature. An increase in epineural and perineural vascular permeability, but no noticeable change in the endoneural vascular permeability, occurred after local application of 5-HT (0.2 μg) and histamine (40 μg). In both the microinjection and local application experiments, animals which were treated with methysergide (0.1 mg/100 g bodyweight, subcutaneously) had no change in permeability following 5-HT administrations. Similarly, pretreatment of the rats with mepyramine maleate prevented alterations in vascular permeability due to histamine.

CHAPTER II

METHOD

In developing the experimental technique, it was advantageous to explore different vascular beds in three kinds of animals: the cat, rabbit and rat. Approximately forty animals were used in developing the experimental procedures. Of these forty animals, twenty-four were used in experiments with serotonin. A total of seventy vascular beds were observed, and twenty-five were used in recording data (Table 1).

Table 1. Vascular beds studied.

Animal	Vascular Bed	No. of Vascular Beds
Cat	Vagus Nerve	8
	Common Peroneal Nerve	3
	Tibial Nerve	6
Rabbit	Tibial Nerve	1
Rat	Sciatic Nerve	7

The duration of the experiments ranged from six to fourteen hours. Intra-arterial (I.A.), intravenous (I.V.), intramuscular (I.M.), intraperitoneal (I.P.), and topical applications were all studied, with emphasis on intra-arterial and topical administrations. In addition, photomicrographs and motion pictures were taken to supplement the recorded data.

A. Anesthetic Technique

1. Cats

Cats weighing between 4.4 and 6.2 pounds were initially anesthetized with intraperitoneal injections of sodium pentobarbital anesthetic solution (halatal, Jen-Sal), administered at dosages of 1 cc/5 lb. The surgical plane of anesthesia was maintained by administering the halatal in 0.1 cc to 0.2 cc amounts I.V.

2. Rabbits

In most of the experiments involving rabbits, the halatal anesthetic and the procedures described above were used. However, the following difficulties were encountered:

1. the animal would sometimes die before reaching a consistent plane of surgical anesthesia;
2. once a surgical plane of anesthesia was reached, the animal would sometimes die with additional maintenance dosages of halatal;
3. diffuse tremors frequently occurred in animals during observations of the vascular bed. This problem presented difficulties with the microscopic observations, with photography, and occasionally resulted in minor trauma to the nerve.

Because of the above difficulties, another anesthetic agent, dial, was used. Dial is composed of the following constituents: dial crystals, 10 gms (all amounts per 100 cc); monoethyl urea, 40 gms; urethane, 40 gms; disodium

calcium ethylene diamine tetra-acetate, 50 gms; distilled water, 100 gms. The anesthetic was injected intraperitoneally in 1 ml/kg amounts. Achieving a surgical plane of anesthesia was a major difficulty in using dial. In the three experiments in which dial was used, it took from three to four hours to achieve a level of anesthesia necessary for surgery. However, once the surgical plane was reached, the animals were maintained very well by the initial dose of dial. Additional experiments with dial were not tried in the rabbit, because visualization of the vascular bed in the rabbit's tibial nerve was difficult. Accordingly, a decision was made to observe the sciatic nerve of the rat.

3. Rats (Sprague-Dawley)

The anesthetic technique found most effective in experiments with the rat involved a combination of ether and halatal. These animals were initially placed in a five liter jar which contained a gauze pad saturated with ether. When the animals began to show signs of anesthesia, they were taken out, and injected intraperitoneally with halatal (0.1 cc/250 gms). A comparison with animals which received only halatal showed that prior administration of ether seemed to hasten the effect of halatal. Because the animals' resistance was reduced, ether also made the I.P. injections of sodium pentobarbital easier.

Rats also demonstrated a tendency to die after the accumulation of variable amounts of sodium pentobarbital.

The average amount of halatal accumulation over the course of the experiment which caused death was approximately 1.5 cc of halatal solution (0.97 mg sodium pentobarbital).

B. Surgical Technique

1. Cannulation of the External Iliac Artery in the Cat

In these experiments, one of the external iliac arteries was cannulated. The right external iliac was chosen most commonly. Thus, the injected material passed via the right (or left) external iliac to the blood vessels of the right (or left) leg supplying the vasa nervorum of the respective tibial and common peroneal nerves (Figure 4).

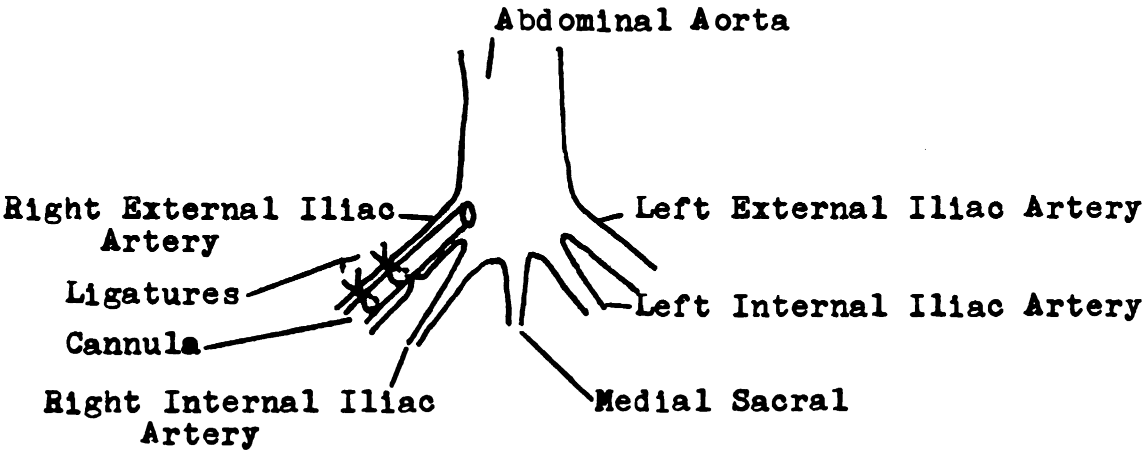
Abdominal Aorta
Right External Iliac Artery
Ligatures
Cannula
Right Internal Iliac Artery
Left External Iliac Artery
Left Internal Iliac Artery
Medial Sacral

Figure 4. Cannulation of the external iliac artery.

The external iliacs were approached ventrally, and an incision along the linea alba, extending from 3 cm below the umbilicus to 4 cm above it, was made. Two incisions at each end of the main incisions and at approximate right angles to it were also made. Next, the muscular abdominal flaps were reflected laterally with hemostats. The loops of small intestine, which covered the branching point of the external iliacs, were pulled back with hemostats that were clamped to the serosa and surrounding connective tissue associated with the outside wall of the small bowel. Special care was taken not to extend the bowel more than was necessary. Fat and connective tissue that surrounded the major blood vessels in the surgical field were removed, and the right or left external iliac was freed for cannulation. Cannulation was made with polyethylene tubing (PE 190) and tied twice with M-404 Ethicon black silk suture, in a manner depicted in Figure 4. Note that the cannulated external iliac has been completely blocked of blood flow.

Next, the two muscular flaps were sutured with 4-0 mersilene braided polyester suture so that the polyethylene tubing extended out the caudal end of the incision (Figure 5).

2. Isolation of the Tibial Nerve in the Cat and Rabbit

An incision parallel and 1 cm medial to the tibia was made on the medial side of the left leg, extending from the medial malleolus to the distal edge of the gracilis muscle. The reflection of skin flaps on both sides of the incision, and removal of the crural fascia, exposed the tibial nerve lying between the medial head of the gastrocnemius muscle and the flexor digitorum longus muscle. A pocket under the tibial nerve, which opened out onto the posterior aspect of the leg, was made with a hemostat and a glass probe,

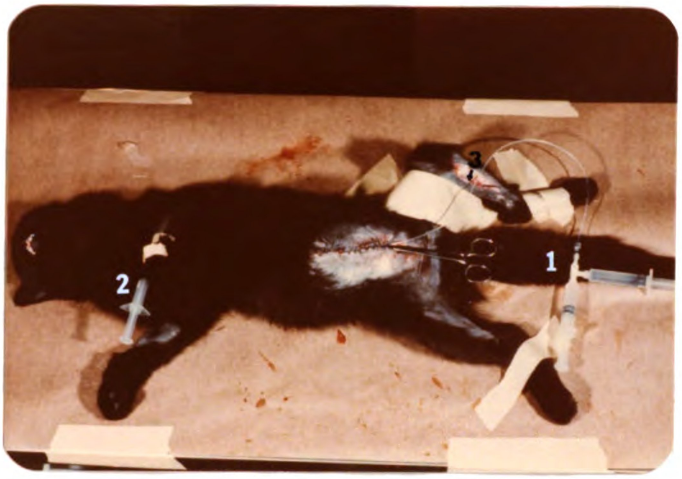

Figure 5. Exposed tibial nerve with catheter in place.

(1) Syringe and tubing for I.A. injections, (2) syringe with halatal inserted in the cephalic vein, (3) exposed tibial nerve.

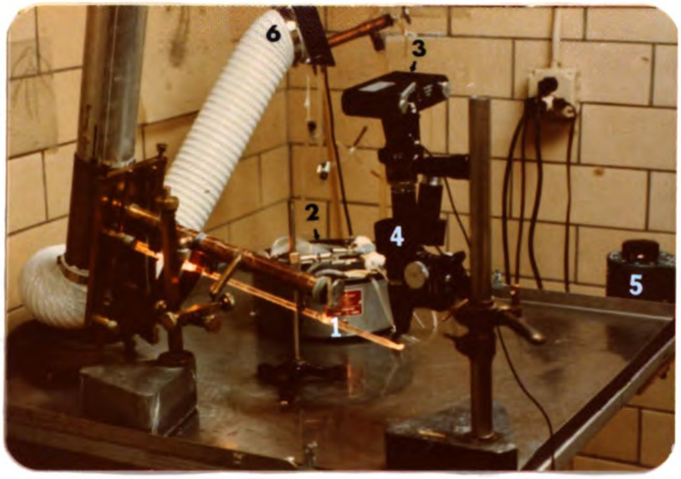

Figure 6. Quartz rod transilluminator.

(1) Quartz rod, (2) warm water bath containing tygon tubing filled with mammalian Ringer's solution, (3) Nikon camera, (4) Leitz dissection microscope, (5) rheostat, (6) fan and coil, which act to cool the light source.

maintaining the nerve in a layer of connective tissue. The pocket for placement of the quartz rod was easily prepared with no apparent trauma to the nerve (Figures 6-8).

3. Common Peroneal Nerve Isolation in the Cat and Rabbit

Initially, an incision was made through the skin, on the lateral surface of either the right or left leg, extending in an anterior-posterior direction, and level with the superior border of the knee. Next, the underlying biceps femori muscle was bisected and the two pieces of muscle were reflected back, revealing the common peroneal nerve. The right or left common peroneal nerve was found lying along the lateral surface of the lateral head of the gastrocnemius. From this point, the nerve coursed deeply and was hidden from sight by an overlying slip of the gastrocnemius which attaches to the fascia of the shank. Using a posterior approach, a pocket was made for insertion of the quartz rod in the same manner as that described for the tibial nerve.

4. Isolation of the Vagus Nerve in the Cat and Rabbit

A 3 cm long incision was made parallel to the trachea (the side that was involved in the nerve isolation varied from experiment to experiment). The incision was located 1 cm lateral to the trachea and extended from a line parallel to the inferior border of the larynx to about 1 cm from the clavicle, approximately 0.3 cm medial to the external jugular vein. The sternomastoid muscle, located just

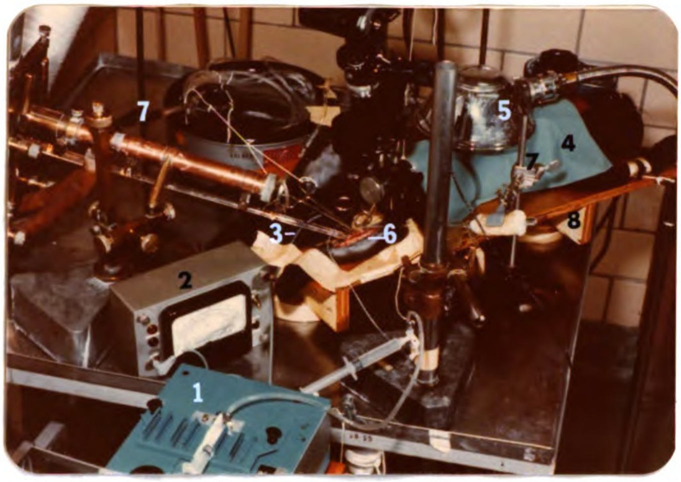

Figure 7. Instrumentation used during an intravascular infusion.

(1) Harvard infusion pump, (2) telethermometer temperature gauge, (3) temperature sensor, (4) cotton blanket, (5) light used in warming animal, (6) exposed tibial nerve, (7) adjustable ring stands, (8) wood platform.

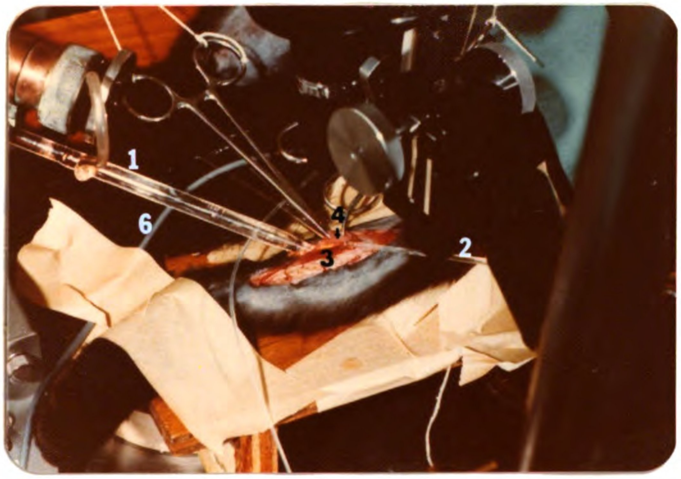

Figure 8. Exposed cat tibial nerve transilluminated by the quartz rod.

(1) Quartz rod, (2) dripper supplying Ringer's solution to superior surface of the nerve, (3) tibial nerve, (4) connective tissue flap, (5) thermometer.

beneath the skin, was transected, and the two segments of muscle were reflected back with hemostats. The vagus nerve, enveloped in the carotid sheath, was exposed just beneath this muscle. In order to maintain the vagus nerve along with the carotid artery in a connective tissue sheet, the cleidomastoideus muscle, or a portion of the sternomastoideus muscle, was used to secure the external edge of the medially attached connective tissue sheet.

Often, connective or fatty tissue had to be freed from the anterior and posterior surfaces of the connective tissue flap to allow for complete and clear visualization of the nerve. Upon distension of the connective tissue flap with hemostats which were attached to the external muscular edge and tied to adjustable ring stands, the quartz rod tip could easily be placed below the nerve.

5. Isolation of the Sciatic Nerve in the Rat

The incision made for this isolation was 1 cm above the caudal edge of either thigh. The incision was 3 cm long and parallel to the caudal edge. The adductor magnus and semimembranosus muscles were found lying next to each other beneath the skin. Their juxtaposing borders were freed. This was done very simply with a hemostat. The sciatic nerve was found immediately beneath this separation, and usually no further dissection was needed in order to free the nerve. Also, a natural pocket was present for placement of the quartz rod tip.

C. Subsequent Experimental Technique

After the surgical preparation was completed, the cat was placed on a platform (refer to Figure 7) to help position the animal, and secured with ties. The nerve was bathed with mammalian Ringer's solution, and was maintained at 37°C. The Ringer's solution coating the undersurface was supplied through the tip of the quartz rod. Ringer's solution coating the superior surface was supplied by a glass dripper which extended from a ring stand (refer to Figure 8). Figure 9 demonstrates the apparatus which maintained the Ringer's solution at a constant temperature.

1. Experiments Involving Intra-arterial Injections

Before any injections were made, the following procedures were carried out:

1. Each vascular bed was viewed for at least twenty minutes through a Nikon or a Leitz dissection microscope.*

2. A description of the rate of flow was made, using the following criteria. If individual red blood cells could not be seen, flow was designated as fast. Slow flow was used if red blood cells could be seen, but the flow moved at a regular pace. Very slow flow designated that the red blood cells not only could be seen, but that

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*These vascular bed observations, plus observations of animals used in which no injections were made, served as controls. The vascular beds of the latter group of animals were watched for intervals of time varying from six to eight hours (the approximate average time of experiments involved in drug administrations).

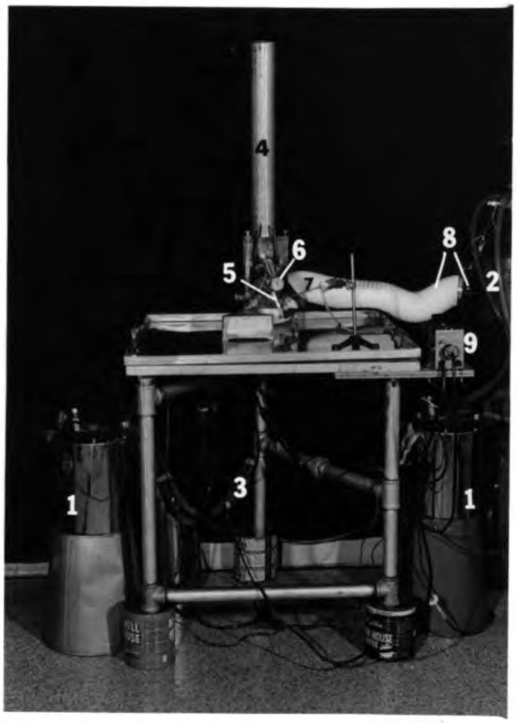

Figure 9. Complete quartz rod apparatus.

(1) Metal basins containing water, heating coils and water pumps, (2) polyethylene tubing housing tubing supplying saline to one dripper, surrounded by temperature regulated tap water, (3) rubber tubing containing the same constituents as no. 2, (4) aluminum tube conducting heat away from light source, (5) quartz rod, (6) temperature gauge, (7) saline dripper, (8) fan and coil, (9) rheostat.

flow was slowed down to the point where it appeared to be almost stopped.

3. Vessels in the bed were sketched, and their respective diameters were recorded with the use of an ocular micrometer.

4. White emboli counts in each of the observed vessels were recorded.

The times of the initial injection, the subsequent vascular events, and the rate of injection were recorded with the use of a stop watch and tape recorder.* After the injection, the vascular beds were usually viewed from twenty to thirty minutes. However, in order to detect any long term or delayed effects of the injectate, some vascular beds were observed for periods ranging from one to four hours. Intervals of time less than twenty minutes were selected when very small quantities of the drug were used, and when observation of the effects of accumulated amounts of the injected drug was desirable over shorter periods of time. White emboli counts, vessel diameters, and the status of blood flow in the vessels observed were recorded periodically. Injections of methysergide maleate (UML-491, Sandoz) were made at varying intervals following 5-HT I.A. administrations.

The solutions of 5-HT were prepared by dissolving serotonin (complexed with creatinine sulfate) in Ringer's solution. Just before injection, the solutions were heated to 37°C. The temperature of the animal was measured rectally with a temperature sensor (Tele-Thermometer, model 437 A). The animal was kept warm by a cotton blanket, and if the animal's temperature fell below 36°C, external heat was provided with a 75-watt light bulb inserted in an adjustable light stand.

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*A Harvard Infusion Pump was used to measure injection rate in some of the experiments.

2. Experiments Involving Topical, Intramuscular, Intraperitoneal and Intravenous Administrations of Serotonin

The procedure involved in these experiments is the same as that described for the I.A. injections, with the exception of the method of 5-HT administration. In the topical application experiments, solutions of 5-HT heated to 37°C were applied directly to the nerve with 1 ml pipettes. Only two I.V. injections were made during one experiment, and just one experiment involving a single injection of 5-HT I.M. and I.P. was made. In the experiment involving I.V. administration of 5-HT, the great saphenous vein was used. The biceps femoris muscle was the site chosen for the I.M. injection. The lower abdomen received the I.P. injection.

The total number of 5-HT administrations was 73. The distribution of the various kinds of administrations is as follows:

I.A.	23
Topical	46
I.V.	2
I.M.	1

I.P.	1
Total	73

The average number of administrations used for each animal was 3.4. The distribution of the amount of 5-HT used, according to range of concentration, for each vascular bed is listed in Table 4.

CHAPTER III

RESULTS

Nerve dissections varied in both the degree of difficulty and in the apparent trauma to the animal. Gauging the degree of trauma according to the amount of vascular, nervous, muscle and connective tissue which needed to be displaced in order to isolate a nerve, it appeared that the tibial nerve dissection in the cat and rabbit produced the least amount of trauma. This dissection involved isolating a nerve which was located just beneath the skin and had relatively little vascular and connective tissue surrounding it. In order of the least traumatic to the most, the other dissections are listed as follows: sciatic nerve (rat), common peroneal nerve (cat), and vagus nerve (cat and rabbit). The sciatic nerve dissection also seemed to induce relatively little trauma to the animal. Best resolution of the vascular beds was obtained through the use of the Leitz dissection microscope.

The complexity and general structure of vascular beds varied from animal to animal. However, in every group of vascular beds studied, each of the characteristics listed below were observed:

1. epineural and occasionally perineural vasculature;

2. vessels oriented along the longitudinal axis of the nerve;
3. intercommunicating transverse anastomoses;
4. capillary plexuses, many of which took the form of fine, glomerular-like capillary formations similar to those described by Lindström (1963).

The sciatic nerve of the rat was found to have the most visible, as well as the most intricate, vasa nervorum. Clear visibility of a complex vasculature was also true of the tibial nerve in the cat, although not to quite the same extent as the rat's sciatic nerve. Except for the vasculature of the rabbit's tibial nerve, the rest of the vascular beds were slightly less clearly observed. Visualization of the tibial nerve vasa nervorum in the four relatively young (less than one year old) rabbits studied was very difficult, and usually only a few longitudinal vessels were observed clearly.

A total of 219 vessels were utilized for data collection. Vessels diameters ranged from 7 to 126 microns, with 67% lying between 25 and 50 microns (see the Appendix for the mathematics applied to determine vessel size). The majority of vessels observed fell into venule or small vein status, and approximately 25% of all vessels were metarterioles, arterioles, or small arteries. This estimate, with slight variation, was found to be consistent in all vascular beds studied. However, the exact number of arterioles and venules is not known, because differentiating the two *in vivo* was not always possible. Distinction of vessels was made primarily on:

1. whether or not the flow in smaller vessels joining a particular larger vessel was leading into or away from the larger vessel (the major vessel under observation);
2. when no branches could be seen, nor the direction of flow could be determined, then an estimate was made on the bases of: (1) the relative diameter of the vessel (vessels in microvascular beds with diameters greater than 25 microns are described as larger venules or small veins, Zweifach, 1961), and (2) the relative size of the wall, e.g. relatively thick walls of arterioles.

Because of inherent experimental difficulties which were encountered, investigations involving the rabbit and rat were limited. Consequently, the results obtained from experiments involving the cat will be emphasized.

Blood flow in the vasa nervorum could usually be seen, particularly in vessels with diameters of less than 60 microns. In vessels with diameters larger than 60 microns, blood flow became more difficult to observe. In the normal vasculature, flow occasionally slowed, stopped, and changed direction. Also, during two observations of the normal, constrictions occurred prior to the onset of the observation period. During one observation of the normal vascular bed, blood flow slowed immediately adjacent to one of the above mentioned constrictions. Within one minute, blood flow in this vessel returned to its initial rate. See the Appendix for results and photographic records of an experiment typical of those which provided the results presented in this section.

The following findings are summarized in Tables 5 and 6, in the Appendix.

A. Intra-arterial Injections of 5-HT

Slowing of flow occurred in 21 of 69 vessels monitored for this phenomenon. Slowing of flow occurred at 5-HT injections ranging from 0.4μg to 900μg. Time of onset ranged from 15 sec. to 6 min. 30 sec. (av. = 2 min. 42 sec.). Stasis followed slowing of flow in 14 of the 69 vessels, with a range of onset of 16 sec. to 5 min. (av. = 2 min. 38 sec.). The range of amounts of 5-HT was 0.4μg to 54.6μg. The time of onset, the 5-HT amounts, and the number of vessels involved in rbc aggregation are the same as those for stasis of blood flow.

Constriction occurred in 6 of 40 vessels observed, and ranged in onset from 2 min. 30 sec. to 5 min. 45 sec. (av. = 4 min. 15 sec.). Constriction occurred at 0.6μg, 0.9 μg, 54.6μg and 900μg. In two of the constrictions, at 5-HT amounts of 54.6 μg and 900μg, complete closure of the vessels along most of their observable lengths occurred. Constriction was visualized in vessels ranging from 25 to 50 microns in diameter, and the extent of constriction ranged from 6.2 to 45.0 microns (av. = 26.8μ). Both slowing of flow and stasis were observed to follow the 54.6μg constriction.

Dilation occurred in 4 of the 40 vessels observed; the range of onset was 3 min. 30 sec. to 19 min. 30 sec. (av. = 9 min. 20 sec.). In all 4 vessels, slowing of flow occurred prior to, or at approximately the same time as, the dilation. In three of the preceding four vessels, stasis occurred within seconds after slowing of flow. The 5-HT amounts injected prior to the observations of dilations were 0.4 μg, 0.8 μg, 9.9 μg and 54.6 μg.

An increase in white emboli counts occurred in 10 of 35 vessels monitored for this entity. Time of onset was 45 sec. to 6 min. (av. = 2 min. 38 sec.). The amounts of 5-HT involved ranged from 0.6 μg to 54.6 μg.

B. Topical Applications of 5-HT

Slowing of flow occurred in 36 of 83 vessels observed. Time of onset varied from 3 sec. to 7 min. (av. = 3 min.). Stasis occurred in 24 of these vessels, and varied in time of onset from 45 sec. to 5 min. (av. = 2 min. 53 sec.). Red blood cell aggregation occurred in 20 of the 70 vessels monitored for this phenomenon, and range of onset was 3 sec. to 7 min. (av. = 3 min.). As summarized in Table 6, each of the three preceding vascular events was found to occur in all of the groups of topically administered 5-HT amounts studied (range was 1 to 86 μg).

Increases in white emboli counts occurred in 43 of 74 vessels monitored for this entity. Time of onset was 4 sec. to 12 min.; av. = 2 min. 41 sec. Like slowing of flow, stasis and aggregation, increases in the number of white emboli were observed in every group of 5-HT dosages studied (range was 1 to 86 μg).

Constriction occurred in 16 of 83 vessels observed, and time of onset ranged from 1 min. 30 sec. to 5 min. 15 sec. (av. = 2 min. 51 sec.). As demonstrated in Table 6, constriction occurred over a broad distribution of 5-HT amounts (range of 1 to 86 μg). The vessels that were involved ranged from 27.3 to 126 μ in diameter, with 82% lying between 27.3 and 50 μ. There were five constrictions which were followed by, or occurred at, approximately the same time as slowing of flow and rbc aggregation. In three of these vessels, stasis occurred. The range of the extent of constriction was 5.7 to 57.0 μ (av. = 27.2 μ).

Dilation occurred in 4 of 83 vessels; the average time of onset was 5 min., and the average extent of dilation was 12.5 μ. Dilation was observed at 5-HT amounts of 1 μg, 3 μg, and 86 μg. Dilation was preceded by slowing of flow in two of the four vessels, and slowing of flow occurred at approximately the same time in the other two vessels.

C. Experiments Involving UML 491 Administrations Following 5-HT Administrations

UML 491 was injected intra-arterially or applied topically, and varied in amounts from 3.6 μg to 30 μg. In each experiment, the mode of UML 491 administration was the same as that of each preceding 5-HT administration. The time between the last 5-HT administration and first administration of UML 491 varied between 6 min. and 58 min. 30 sec. The vascular events observed after 5-HT administration, and their modifications with subsequent UML 491 applications, are described in Tables 2 and 3.

In one experiment (6/6. Table 7) in which UML 491 I.A. injections (totaling 35 μg) followed 5-HT I.A. injections (totaling 25 μg), and alterations in the vasculature were corrected, subsequent 5-HT I.A. injections of 12.6 μg, 9.9 μg, and 9.9 μg, consecutively, produced only a slight slowing of flow in one vessel (see Figures 19-21). In another very similar experiment (5/4, Table 7), 5-HT I.A. injections (totaling 12.6 μg) were followed by no changes in the vasculature. A more detailed account of the dosages of both 5-HT and UML 491 can be found in Tables 7 and 8, in the Appendix.

Table 2. Intra-arterial injections.

Vascular Events Following 5-HT Injections	No. of Vessels Involved	Vascular Events Following UML 491 Injection	No. of Vessels Involved
Slow flow	6	Increase in the rate of flow such that flow returned to or was at approximately the same rate as the control value	6
Stasis	8	Increase in the rate of flow such that flow returned to or approached the control rate	6
RBC aggregation	8	Breaking up of the rbc aggregates	6
Increase in white emboli count	2	Return of white emboli count to approximately the control value	1
Constriction	12	Return of diameter of vessel to approximately the control vessel's diameter	2
Dilation	0	-	0

Table 3. Topical applications of UML 491 and 5-HT.

Vascular Events Following 5-HT Applications	No. of Vessels Involved	Vascular Events Following UML 491 Applications	No. of Vessels Involved
Slow flow	2	Increase in the rate of flow such that flow returned to or was at approximately the same rate as the control value	2
Stasis	1	Increase in the rate of flow such that flow returned to or approached the control rate	1
RBC aggregation	1	Breaking up of the rbc aggregates	1
Increase in white emboli count	0	Return of white emboli count to approximately the control value	0
Constriction	1	Return of diameter of vessel to approximately the control vessel's diameter	0
Dilation	0	-	0

CHAPTER IV

DISCUSSION

Fahreus (1929) described intravascular agglutination, or red blood cell clumping, in some detail, and he initiated the idea that this circulatory disturbance may lead to organ pathology. Later, Knisely and co-workers (1950) originated the terminology "sludged blood" to describe the agglutination of erythrocytes which blocked the flow of blood through terminal arterioles and capillaries. They attributed this finding to changes in the blood or in the vessel wall, and they provided additional evidence that sludging of blood is pathological.

The findings presented in this thesis reveal that in the vasculature of the peripheral nerves studied, 5-HT does in fact cause sludging or, as designated in the results section, rbc aggregation.* There are at least four different possible causes of sludging subsequent to 5-HT administration, of which all may play a part:

1. Increased permeability of the endothelial lining of vessels followed by a decreased volume of the fluid

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*See Appendix for statistical proof of this event as well as constriction, slowing of flow, stasis, dilation and increase in white emboli observed after I.A. and topical 5-HT administrations.

component of the blood and a slowing of flow. This event is supported by Olsson's (1966) studies outlined in the literature review.

2. An increased adherence of the rbc's due to the direct effect of 5-HT on individual red blood cells (Swank, 1963).

3. The inducement of platelets by 5-HT to act as a nidus for rbc adhesion (Swank, 1963).

4. A slowing in the velocity of blood flow due to constriction was proposed by Swank (1963) as a possibility. However, he did not observe constriction following 5-HT I.A. injections in the conjunctivae of cats and other animals in which sludging occurred.

The following findings by a number of investigators contribute to an understanding of sludging. An implication of the sludging phenomena observed is the development of various degrees of anoxia suffered by the vascular endothelium and the accumulation of endoneural edema fluid. This event was described by Knisely in 1950. Two examples of entities which are known to cause release of 5-HT from platelets in enough magnitude to induce sludging of blood are anaphylaxis (Waalkes and Coburn, 1959) and bacterial endotoxin (Des Prez et al., 1961). The direct effect of 5-HT on the capillary endothelium may contribute to the accumulation of the proteinaceous edema fluid (Olsson, 1966). A logical sequela of 5-HT's effect on the nerve is the occurrence of hypoxia involving Schwann cells with subsequent demyelination, and finally alteration in nerve function.

This conclusion is supported by Seneviratne (1972b), who stated that the effect of increased endoneural capillary permeability and edema fluid accumulation in his aloxan-diabetic rats is tissue hypoxia and segmental demyelination.

The present study has determined that constriction occurs in some vessels of the nerve following both 5-HT I.A. injections and topical applications. In five of twenty vessels constricted, rbc aggregation occurred behind the constriction and slowing of flow occurred at approximately the same time, or subsequent to, the constriction in most of the vessels in which constriction was visualized. These findings suggest that constriction probably contributes, at least in part, to some of the sludging phenomenon.

Since the onset of constriction was observed 1 min. 30 sec. to 5 min. 15 sec. after topical 5-HT applications, and 2 min. 30 sec. to 5 min. 45 sec. after I.A. 5-HT injections, the possibility arises that the constriction occurs via a sympathetically mediated reflex response of the animal to 5-HT. However, because constriction was observed at low μg levels of 5-HT, inclusing amounts less than 1 μg, and because 5-HT is removed very quickly from the blood by the liver, lung and platelets (Page, 1968 and Thomas and Vane, 1967), it is the belief of this investigator that a sufficient amount of the drug would not be present in the systemic circulation long enough to elicit a reflex response. With the above facts and theory in mind, it seems plausible to conclude that 5-HT causes constriction in the vasa nervorum by a direct action on the vascular smooth muscle. A possible explanation for the later onset of constriction when compared to that of sludging (an event which has been observed to occur within seconds after 5-HT administration) is that constriction may require more accumulated amounts of 5-HT.

As noted in the results section, dilation was invariably accompanied by slowing of flow; sludging and stasis occurred with dilation most of the time. Combining the evidence presented in the study by Knisely et al. (1950) regarding the blockage of blood flow by blood element aggregates with the above results on constriction and the knowledge of the events which accompanied dilation, the dilation may be explained by an increase in intraluminal pressure due to blood element aggregates blocking vessels, supplemented by an occasional constriction. The blood element aggregates may be composed of red blood cells, platelets and white blood cells. The white emboli observed in these experiments are similar to the "gray masses" which Swank (1963) described and believed were comprised primarily of platelets and white blood cells.

When UML 491 was administered I.A. or topically, following 5-HT administration, a reversal of slow flow, sludging, and stasis to approximately the control state occurred at all g levels of UML 491 used. However, the correction of constrictions by UML 491 was not as marked an effect: only two of thirteen vessels constricted returned to their control diameters. This investigator believes that there were not enough increases in white emboli count and vascular dilations in the experiments involving UML 491 to draw any conclusions about UML 491's effect in causing the reversal of these two events.

SUMMARY AND CONCLUSIONS

1. Dissection techniques for isolating the following nerves were developed to the point where they afforded relatively little trauma to the animals involved: (1) common peroneal, vagus, and tibial nerves in the cat and rabbit; (2) sciatic nerve in the Sprague-Dawley rat. However, because of inherent experimental difficulties which were encountered in working with the rat and rabbit, only the cat was chosen for data collection.

2. The majority of vasa nervorum visualized (approximately 75%) in the epineurium appeared to be venules and small veins.

3. Both I.A. and topical administrations of 5-HT were found to induce sludging of blood, constriction, slowing of blood flow, stasis, dilation and increased white emboli.

4. Serotonin probably acts to cause constriction in the vasa nervorum through the direct action of serotonin on the vascular smooth muscle. The possible mechanisms by which 5-HT causes the other events listed above are discussed extensively in the previous section.

5. Some possible implications of increased blood levels of 5-HT are vascular endothelial damage, accumulation of endoneural edema fluid, and nerve damage.

6. UML 491 administration following 5-HT administration enabled vasculature which demonstrated blood sludging, stasis and slow flow to return to the control state. In contrast, it had relatively little effect in reverting constriction to the control state.

APPENDIX

Table 4. Vascular beds and 5-HT administrations utilized.

Animal	Nerve		Total number of applications	AMOUNT (μg)								
				TOPICAL APPLICATIONS								
				0-1	2-10	11-30	31-50	51-70	71-90	91-100	101-210	Other
Cat	Vagus		9						9			
Cat	Common Peroneal		13	1	7		2	2	1			
Cat	Tibial	Number	5	2	3							
Rabbit	Tibial		4	2	2							
Rat	Sciatic	Of	15	2	7		2		2		2	
				INTRA-ARTERIAL ADMINISTRATIONS								
Cat	Tibial	Adminis-trations	23	10	6	2		2				3 (450, 750 & 900)
				OTHER ADMINISTRATIONS								
Cat	Vagus		I.M. 1								1 (150)	
Cat	Vagus		I.P. 1								1 (110)	
Cat	Vagus		I.V. 2									2 (344,420)

Table 5. The *in vivo* effects of 5-HT on feline vasa nervorum.*

	TOPICAL (No. of vessels)	INTRA-ARTERIAL (No. of vessels)
A. VESSEL DIAMETER:		
1. No Change	63	30
2. Constriction**	16	6
3. Dilation***	4	4
Total	83	40
B. FLOW:		
1. No Change	47	18
2. Slowing	36	21
3. Stasis	24	14
4. Increased Rate	0	2
C. RED BLOOD CELLS:		
1. No Change	50	21
2. Aggregation	20	19
D. WHITE EMBOLI:		
1. No Change	31	25
2. Increased Number	43	10

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*Constriction, slowing of flow, rbc aggregation, stasis and increased white emboli count were statistically valid at a .03 level of significance, and dilation at a .06 level of significance in both I.A. and topical administrations.

**Range of extent of constriction is 5.7 to 57.0μ; av. constriction = 27.2μ. Constriction also involved a complete shut down in two vessels.

***Dilation: range = 6.3 to 12.5μ; av. = 8.4μ.

CALCULATIONS USED IN DETERMINING VESSEL SIZE

The following mathematics were applied to determine the vessel size:

e.g. oculars = 20X   objective = 1.5X

$$C = \frac{X}{Y}$$

C = diameter of the vessel

X = distance between marks on the micrometer = 0.1mm

Y = number of divisions on eye piece micrometer

e.g. Y = 2.75 marks

$$C = \underline{X} = \frac{0.1\text{ mm}}{2.75\text{ marks}} = 0.0364\text{ mm}$$

To convert mm to microns:

$$0.0364\ \cancel{\text{mm}} \times \frac{1000\text{ microns}}{1\ \cancel{\text{mm}}} = 36.4\text{ microns}$$

STATISTICAL ANALYSIS

In the statistical analysis of the experimental data, the following hypotheses were made:

H_0 (null hypothesis): Proportion of vessels that changed (slowing of flow, constriction, etc.) under the influence of 5-HT (P_d) $\leq$ the proportion of vessels that a particular change was observed in control conditions (P_c); $P_d \leq P_c$ or $P_d - P_c \leq 0$

H_1 (alternative hypothesis): $P_d > P_c$ or $P_d - P_c > 0$

$\bar{p}$ (pooled proportion or mean of $P_d + P_c$) is set at $.05 < \bar{p} < .95$. When $\bar{p}$ fell within these limits, the Z test for the difference of two proportions was used. The Z test is defined by the following formula:

$$Z = \frac{P_d - P_c}{\sqrt{\bar{p}(1-\bar{p})\left(\frac{1}{M_d} + \frac{1}{M_c}\right)}}$$

M_d = # of experimental vessels monitored for a particular vascular event

$$\bar{p} = \frac{P_d M_d + P_c M_c}{M_d + M_c}$$

M_c = # of control vessels

Subsequent determinations of the Z value resulted in rejection of H_0 at a .03 level of significance in constriction, slowing of flow, rbc aggregation, stasis and increased white emboli count, in I.A. and topical administrations. It is therefore statistically valid to conclude that 5-HT had an effect in causing these events.

In I.A. and topical experiments in which dilation was observed, p did not fall within the interval $.05 < \bar{p} < .95$, the Fischer exact test was used. The Fischer exact test is defined by the following formula:

$$P = \sum_{i=1}^{a+1} \frac{(a+b)!(c+d)!(a+c)!(b+d)!}{(a+1-i)!(b-1+i)!(c-1+i)!(d+1-i)n!}$$

$$P_d = \frac{a}{a + b}$$

$$P_c = \frac{c}{c + d}$$

a = # of vessels in which changes were observed after the administration of 5-HT
b = # of vessels in which no change was observed after the administration of 5-HT
c = # of vessels in which changes were observed in the control
d = # of vessels in which no changes were observed in the control

A digital computer was used to calculate the following significance levels: .052 (dilation, I.A.) and .057 (dilation, topical). Therefore, H_0 is rejected at a .06 level of significance and the conclusion that 5-HT caused dilation can be made with reasonable certainty.

Table 6. Vascular events associated with varying 5-HT µg amounts.

Ranges of 5-HT Amounts (µg)	0-1	2-10	11-30	31-50	51-70	71-90	91-100	450	750	900
INTRA-ARTERIAL INJECTIONS										
No. of Trials	9	3	2	0	2	0	0	1	1	1
No. of Constrictions	3	0	0	-	3	-	-	0	0	2
No. of Dilations	2	1	0	-	2	-	-	0	0	0
No. of vessels involved in slowing of flow	14	4	1	-	6	-	-	1	0	1
No. of vessels in which stasis occurred	9	1	0	-	4	-	-	0	0	0
No. of vessels in which an increase in white emboli occurred	7	1	1	-	2	-	-	1	1	0
TOPICAL APPLICATIONS										
No. of Trials	4	10	0	2	2	10	0	0	0	0
No. of Constrictions	2	6	-	0	0	8	-	-	-	-
No. of Dilations	1	1	-	0	0	2	-	-	-	-
No. of vessels involved in slowing of flow	3	15	-	7	1	10	-	-	-	-
No. of vessels in which stasis occurred	3	10	-	6	0	4	-	-	-	-
No. of vessels in which an increase of white emboli occurred	6	17	-	12	12	7	-	-	-	-

Table 7. Intra-arterial injections of serotonin followed by intra-arterial injections of UML 491.

Total amount of 5-HT injected prior to UML 491 injection (μg)	Amount of last 5-HT injection (micrograms)	UML 491 injection no.	Total amount of UML 491 injected so far (μg)	Amount of UML injected and Time injected (Timed from previous injection)	State of vascular bed before UML 491 was injected	State of vascular bed after UML 491 was injected
4/20 109.2	54.6	1	0	26 μg at 15 min.	1. Complete stasis in vessels 1 and 2 (6 vessels observed). 2. Constricted areas found in vessels 1 and 2. 3. Increased number of white emboli in vessel z.	1. Increased rate of flow with breaking up of the rbc aggregates in vessel 2 (3 min. 50 sec.). 2. Decrease in the number of white emboli seen in vessel z.
109.2	54.6	2	26	26 μg at 26 min. 35 sec.	1. Vascular stasis and constriction reappeared in vessels 1 and 2 (4 min. 30 sec.).	1. Breaking up of rbc aggregates, accompanied by a small increase in flow in vessel 2 (30 sec.). 2. Stasis returned to vessel 2.
5/1 67.0	9.9	1	0	30 μg	1. Stasis in vessel 1. 2. Slow flow in vessel 5.	1. Increase in rate of flow in all vessels with slow flow prior to injection.

Table 7 (cont'd.).

Total amount of 5-HT injected prior to UML 491 injection (µg)	Amount of last 5-HT injection (micrograms)	UML 491 injection no.	Total amount of UML 491 injected so far (µg)	Amount of UML injected and Time injected (Timed from previous injection)	State of vascular bed before UML 491 was injected	State of vascular bed after UML 491 was injected
5/4 5.4	0.9	1	0	3.6µg at 27 min.	1. Constriction in vessels 2 and 4a. 2. Stasis of flow in vessel 2. 3. Increase in white emboli in vessel 5.	1. Increase in rate of flow in vessel 2 (30 sec.).
5.4	0.9	2	3.6	4µg at 5min.	1. Slow flow in vessel 3.	1. Increase in rate of flow in vessel 3.
Subsequent injections of 5-HT, totalling 12.6µg, produced no additional changes in the vasculature						
6/6 19.8	0.9	1	0	25µg at 6 min.	1. Stasis and constriction in vessels 1 and 2. 2. Decreased flow in vessel z. (See Figure 15.)	1. Increase in rate of flow in vessel z (5 sec.). 2. Some increase in flow in vessels 1 and 2. (See Figure 16.)

Table 7 (cont'd.).

Total amount of 5-HT injected prior to UML 491 injection (μg)	Amount of last 5-HT injection (micrograms)	UML 491 injection no.	Total amount of UML 491 injected so far (μg)	Amount of UML injected and Time injected (Timed from previous injection)	State of vascular bed before UML 491 was injected	State of vascular bed after UML 491 was injected
6/6 (cont'd) 19.8	0.9	2	25	10μg at 6 min.	1. Flow in vessels 1 and 2, although slightly increased, was still slow and constriction was still present in both of these vessels. (See Figure 17.)	1. Increase in rate of flow in vessels 1 and 2 (1 min.). 2. Reduction of the constricted area in vessels 1 and 2 (4 min. 30 sec.). 3. A further increase in the rate of flow, and reduction of the constrictions in vessels 1 and 2 (8 min.). (See Figure 19.)
Subsequent injections of 5-HT, totalling 32.4μg, produced slowing in the larger vessel, vessel z, but was not followed by changes in vessels 1 and 2.						

Table 8. Topical applications of UML 491 following topical applications of serotonin.

Total amount of 5-HT applied prior to UML 491 application (μg)	Amount of last 5-HT application (μg)	UML 491 application no.	Total amount of UML 491 applied (μg)	Amount of UML applied and Time applied (Timed from previous application)	State of vascular bed before UML 491 application	State of vascular bed after UML 491 application
4/25 4	3	1	0	10 μg at 58 min. 30 sec.	Stasis of flow and constriction in vessel 1 (present since 5 min. past last 5-HT application).	Increase in rate of flow in vessel 1 (3 min. 15 sec.).
4	3	2	10	10 μg at 5 min. 15 sec.	Flow in vessel 1 has slowed and approached stasis again.	Flow in vessel 1 increased in rate (15 sec.).
4	3	3	20	10 μg at 10 min.	Flow in vessel 1 has slowed and approached stasis again.	Flow in vessel 1 increased in rate (20 min.).

SUMMARY OF A TYPICAL EXPERIMENT INVOLVING I.A. INJECTIONS OF 5-HT, 5-HT FOLLOWED BY UML 491, AND 5-HT FOLLOWED BY UML 491 WITH SUBSEQUENT 5-HT INJECTIONS

This experiment involved injection of 5-HT into the left external iliac artery and observation of the vascular bed in the left tibial nerve of the cat. The vascular bed, as pictured in Figure 10, was observed for approximately forty minutes, and the following observations were noted:

1. Blood flow was found to be traveling fast* in vessels labeled 1, 2, and z. Also, blood flow was observed to be traveling slightly slower in the smaller vessels 3 and 4.
2. No white emboli were observed.
3. Vessel 1 measured 33.2 microns in diameter.
   Vessel 2 measured 24.9 microns in diameter.
   Vessel z measured 102.6 microns in diameter.
   Vessels 3 and 4 measured 8 microns in diameter.
4. Flow was observed to travel in the directions indicated with arrows.

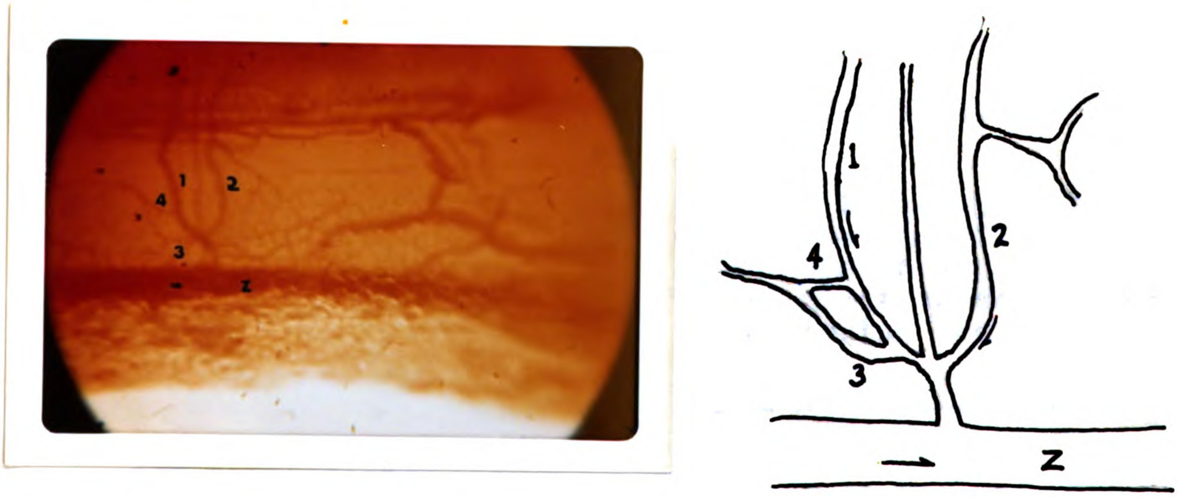

Figure 10. Normal vascular bed.

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*Fast is used here to designate that the blood stream was traveling at a speed to prevent viewing of individual rbc's.

INJECTIONS:	OBSERVATIONS:
A. Serotonin injections	
1. 9.9 micrograms (0.9 micrograms % injected at a rate of 1.1 ml/min. for 1 min.)	1. Increase in white emboli. (40 sec.) 2. Constriction in vessels 1 and 2 (2 min. 45 sec.)*, followed immediately after by dilation in vessel 1 (dil. dia. = 40 microns; previous dia. = 33.2 microns) at 2 min. 46 sec. 3. Decrease in flow in all labeled vessels except vessel z, occurring at approximately the same time as the constriction and dilation.

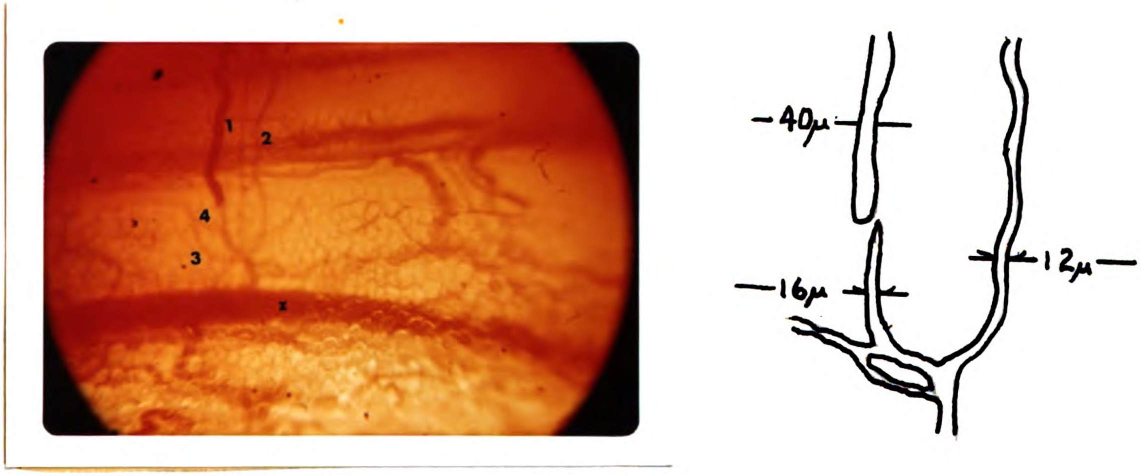

Figure 11. Vascular bed at 3 min. 10 sec. following 5-HT injection.

The vascular bed, following Injection 1, began to return to normal at 4 min., at which time the flow picked up and the vessels began to approach their original diameters. Figure 12 depicts the vascular bed just after these changes were noticed, at 4 min. 45 sec. The following observations were made at that time:

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*The constriction measured near zero at the point where vessel 1 begins to dilate, and 16 microns at the midway point between the constriction and the point where it joins vessel 2 (as indicated on the diagram). Vessel 2 constricted down to 12 microns, approximately one half its original size of 24.9 microns.

1. The constricted portions of vessels 1 and 2 returned to their original diameters.
2. Flow in all labeled vessels returned to its initial rate.
3. Flow in the surrounding capillaries also returned to the original state.
4. The portion of vessel 1 which remained dilated after the first injection still remained dilated at the time depicted in Figure 12.

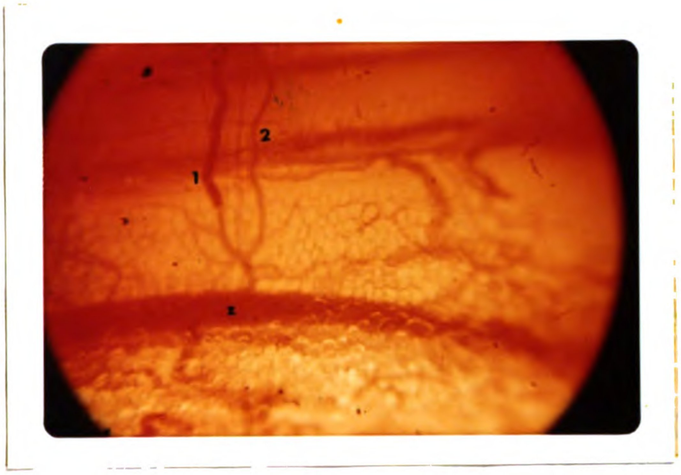

Figure 12. Vascular bed at 4 min. 45 sec. following 5-HT injection number 1.

At 20 min., the vascular bed appeared to be restored to normal, and the second injection was made soon after.

INJECTION:	OBSERVATIONS:
2. 9.9 micrograms (0.9 microgram % at 1.1 ml/min. for 1 min.	1. Increase in white emboli in vessel z. (15 sec.) 2. Increase in white emboli in vessels 1 and 2. (20 sec.) 3. Constriction followed by dilation and vascular stasis in vessels 1 and 2. (See diagram, Figure 13.) Flow appeared unaffected in vessel z. (3 min.) 4. Flow in surrounding capillary bed slowed and became static. (3 min.)

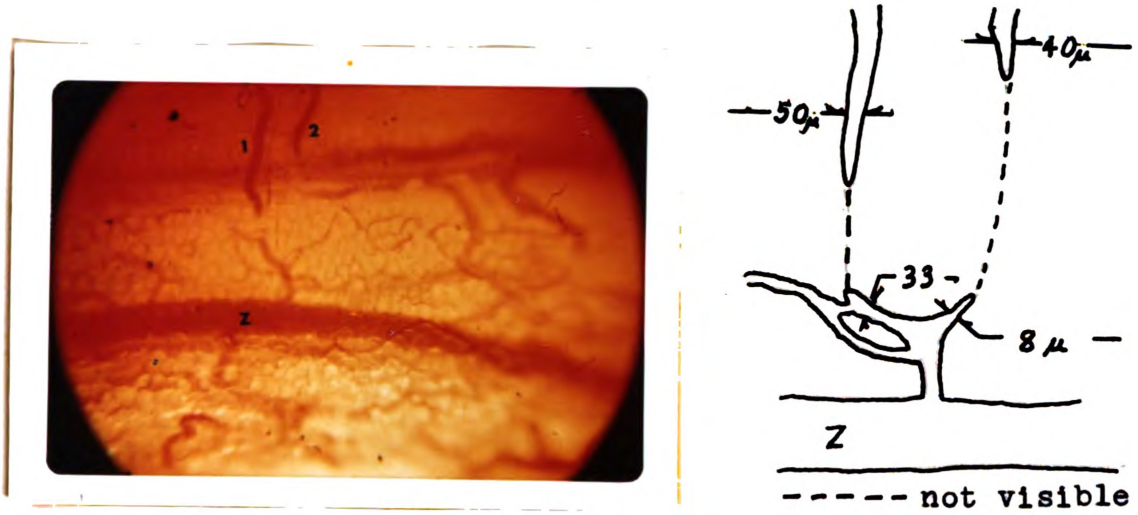

Figure 13. Vascular bed at 3 min. following 5-HT injection number 2.

5. The vascular bed began to return to its previous state (4 min.) Figure 14 depicts this time. The following vascular changes were observed: (a) flow and diameter of vessel 2 returned to approximately normal; (b) flow in vessel 1 remained static, and the portions of the vessel which dilated and constricted after the second injection remained at this time.

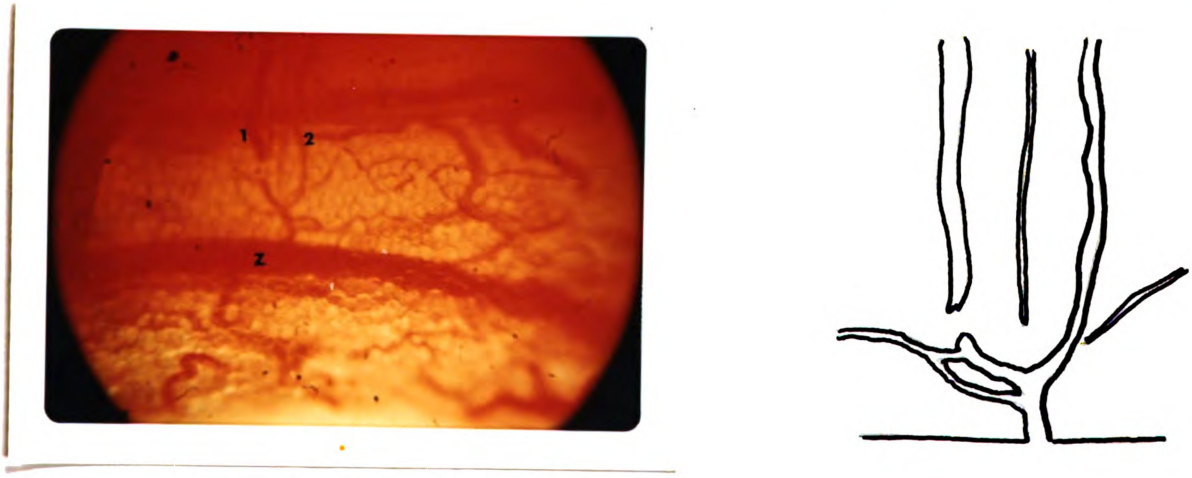

Figure 14. Vascular bed at 4 min. following 5-HT injection number 2.

INJECTIONS:	OBSERVATIONS:
	6. Without any additional applications of 5-HT, the flow in vessel 2 became static, and flow remained static in vessel 1 (17 min.). Constriction reoccurred in vessel 2 at this time. There was decreased flow in vessel z.
3. 3.6 micrograms of 5-HT (0.4 ml of 0.9 mg % 5-HT, injected as a bolus at 18 min. following previous 5-HT injection)	1. The vasculature did not appreciably change from the vascular bed observed at the end of the second injection, and described under observation (6) of that injection. (See Figure 15.) 2. Figure 16 depicts the vascular bed just before the subsequent UML 491 injection, and it also shows no visible change.

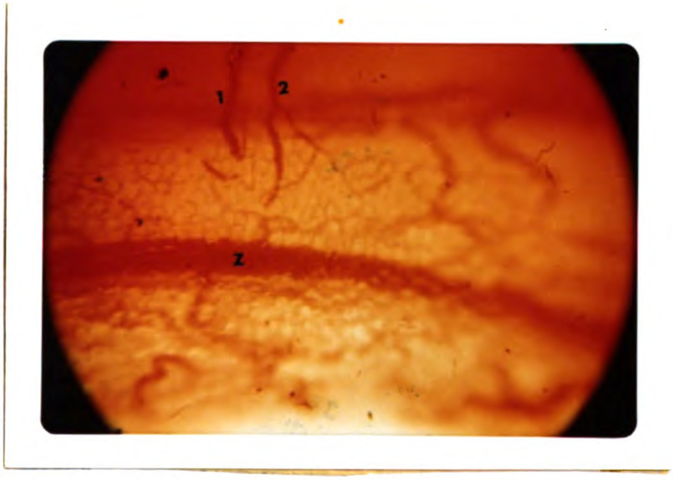

Figure 15. Vascular bed at 2 min. following 5-HT injection number 3.

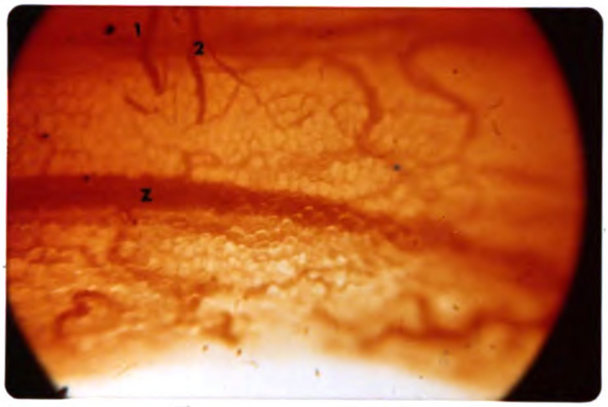

Figure 16. Vascular bed at 5 min. following 5-HT injection number 3.

INJECTIONS:

B. UML 491 injections

1. 25 micrograms (2.5 ml of a 1 mg % solution, injected as a bolus at 6 min. after the third 5-HT injection)

OBSERVATIONS:

1. Slight increase in flow in vessel z. (5 sec.)

2. Slight increase in flow in vessels 1 and 2 (1 min.). Figure 17 depicts the vascular bed at 1 min. 25 sec. (demonstrates only slight changes). Figure 18 shows essentially the same findings as Figure 17, but was taken at 3 min.

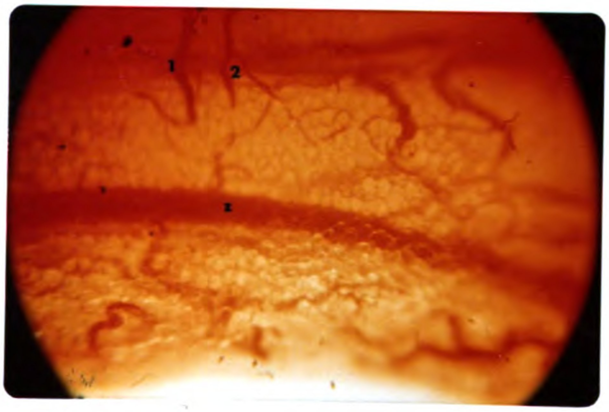

Figure 17. Vascular bed at 1 min. 25 sec. following UML 491 injection number 1.

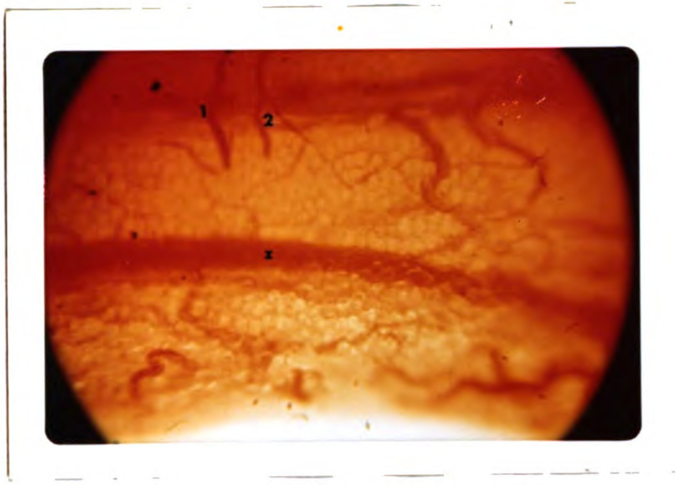

Figure 18. Vascular bed at 3 min. following UML 491 injection number 1.

INJECTIONS:	OBSERVATIONS:
2. 10 micrograms of UML 491 (1 ml of a 1 mg % solution, injected at 6 min. past the time of the initial UML 491 injection)	1. Increase in flow in vessel 1, but the area of constriction was still present in this vessel. Constriction remained constant in vessel 2. (1 min.)
	2. Increase in flow with reduction of constricted portions of both vessels 1 and 2 (4 min.). A further increase in flow, as well as reduction of constriction in vessels 1 and 2, occurred at 8 min., one minute prior to the time of Figure 19.

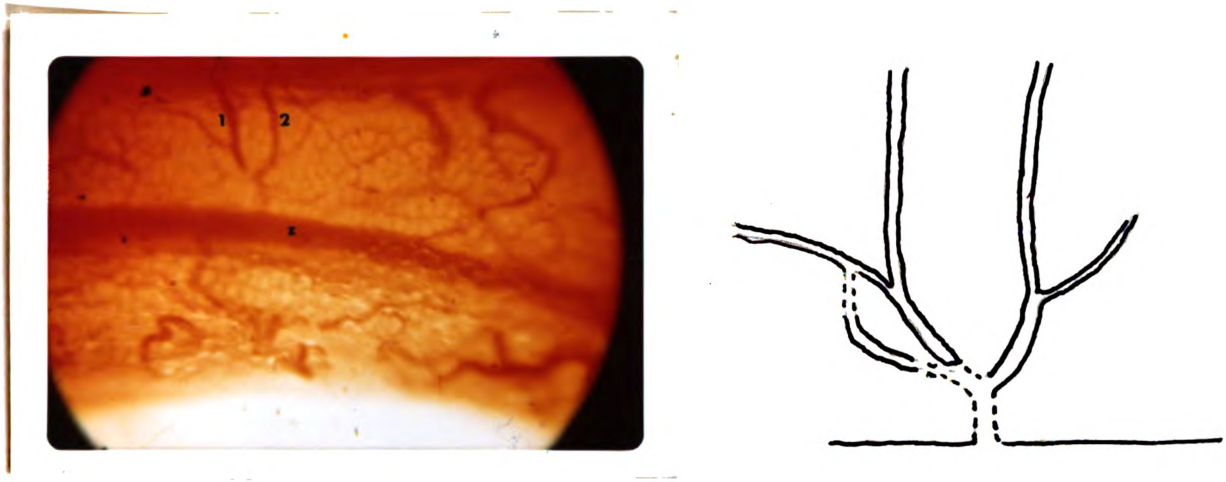

Figure 19. Vascular bed at 9 min. following UML 491 injection number 2.

INJECTIONS:	OBSERVATIONS:
"Special 1". 12.6 micrograms of 5-HT (1.4 ml of 0.9 mg % solution, injected at 11 min. following the preceding UML 491 injection)	1. No significant change. (See Figure 20.)

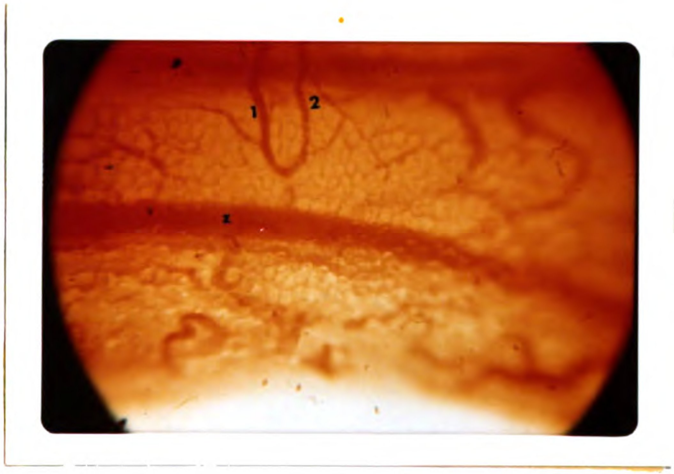

Figure 20. Vascular bed at 3 min. 15 sec. following 5-HT special injection number 1.

"Special 2". 9.9 micrograms of 5-HT (1.1 ml of a 0.9 mg % solution, injected at 6 min. past the preceding 5-HT injection)	1. No appreciable change. (See Figure 21.)

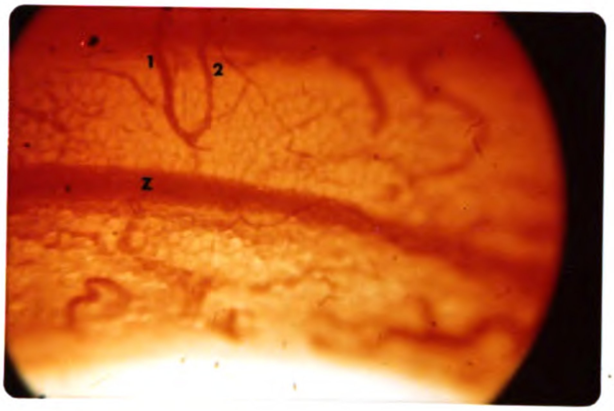

Figure 21. Vascular bed at 5 min. following 5-HT special injection number 2.

INJECTION:	OBSERVATIONS:
"Special 3". 9.9 micrograms of 5-HT (1.1 ml of a 0.9 mg % solution, injected at 5 min. 30 sec. following the preceding 5-HT injection)	1. Slight slowing of flow in vessel z (20 sec.), lasting only 10 to 20 sec. No subsequent change. (See Figure 22.)

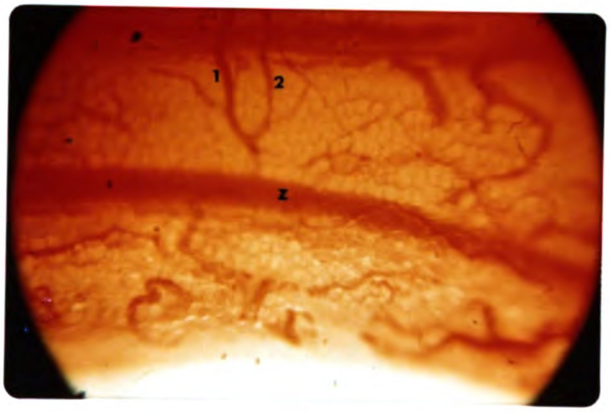

Figure 22. Vascular bed at 5 min. following 5-HT special injection number 3.

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