

Innervation of the canine thoracolumbar vertebral column

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by

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INTRODUCTION

It has been estimated that 80% of all human beings will suffer from low back pain at sometime during their lives (Cailliet, 1981). One might expect that man would be particularly desirous of sparing his pets from the same fate, yet the proclivity for new and bizarre animals has resulted in the creation of canine breeds which suffer from a high incidence of intervertebral disc disease.

In man, the spinal cord does not extend beyond the level of first lumbar vertebra; thus the protrusion of a lumbar intervertebral disc cannot cause paralysis by exerting pressure upon the cord, although it may cause crippling pain. The spinal cord of the dog extends further caudally, usually to the level of caudal end of the sixth lumbar vertebra; therefore, protrusion of a disc may cause damage to the spinal cord. The resulting paralysis or paresis has naturally been a matter of great concern to veterinarians and considerable effort has been directed in understanding the pathogenesis, diagnosis, and treatment of this problem. However, the cause of the pain associated with intervertebral disc disease has been little studied.

The nature of discal pain cannot be discerned without a thorough knowledge of the innervation of relevant structures of the vertebral column. The purpose of this investigation was to describe the innervation of the caudal thoracic and cranial lumbar segments of the canine vertebral column, being the most common sites of intervertebral disc disease, as a basis for understanding the probable cause of discal pain. This finding may be compared with that of man for developing an

animal model for human research.

Further, it is hoped that the results accrued from this study will be helpful for clinical work both for diagnostic and prognostic purposes in dog and man.

LITERATURE REVIEW

Innervation of the Intervertebral Disc

Because degenerative changes of the intervertebral disc often result in pain in both man and dog, it would seem logical that the disc itself could be a major source of that pain. Prata (1981) noted that discogenic pain could be found in dogs with degenerated intervertebral discs in which discal material had not herniated into the vertebral canal. This statement would seem to imply that the disc is innervated, but apparently this has not been investigated in the dog.

Kumar and Davis (1973) examined the lumbar discs of cats, rats, and man using a variety of histologic stains (Table 1). They concluded that the discs were not innervated in those species. Stilwell (1956) was unable to find nerves in the discs of Rhesus and Cynomolgus monkeys, except for a few fibers from the posterior longitudinal ligament penetrating the outermost layer of the anulus fibrosus. He noted, however, the presence of nerves within the thin connective tissue layer surrounding the anulus.

Luschka in 1850 (cited in Edgar and Ghadially, 1976) attempted unsuccessfully to trace nerves into the intervertebral disc of man using gross dissection. Since then, there has been much disagreement concerning the innervation of the human anulus fibrosus.

Many early investigations failed to demonstrate the presence of nerves within the anulus fibrosus. The histologic study of Jung and Brunschwig (1932) was the earliest such search. Examinations of the anulus fibrosus were also performed by Wiberg (1949), Hirsch and

Table 1. Structures innervated by the sinu-vertebral nerve

Author(s)	Species	Blood vessels	Posterior longitudinal ligament	Intervertebral disc	Dura mater	Periosteum of vertebra
Luschka, 1850 (cited in Edgar and Ghadially, 1976)	Man	+	+	-	on outer surface	+
Hovelacque, 1925	Man	+	+	N.D. ^a	+	+
Stilwell, 1956	Monkey	+	+	(outer surface)	+	+
Lazorthes, Poulhes, and Espagno, 1948	Man	+	+	+	+	+
Wiberg, 1949						
Pedersen, Blunck, and Gardner, 1956	Man	+	+	?	+	+
Bridge, 1959	Man	N.D.	+	+	-	+
Cloward, 1960	Man	+	N.D. ^b	N.D.	+	N.D.
Edgar and Nundy, 1966	Man	N.D.	N.D.	N.D.	+	N.D.
Kumar and Davis, 1973	Man, cat, rat	N.D.	N.D.	-	N.D.	N.D.
Bogduk, Tynan, and Wilson, 1981	Man	N.D.	+	+	+	N.D.

^aNot determined.

^bThe posterior longitudinal ligament was not yet developed in the fetuses examined.

Schajowicz (1952), Ikari (1954), and Pedersen, Blunck, and Gardner (1956), who concluded that this structure was devoid of nerves.

Other investigators claimed to have observed nerve fibers within the anulus fibrosus of man (Tsukada, 1939; Roofe, 1940; Lazorthes, Poulhes, and Espagno, 1948; Malinsky, 1959; Ferlic, 1963; Hirsch, Ingelmark, and Miller, 1963; Jackson, Winkelmann, and Bickel, 1966; Shinohara, 1970). Two recent investigations of Bogduk, Tynan, and Wilson (1981) and Yoshizawa et al. (1980) have reaffirmed the existence of nerves within the anulus.

The innervation of the nucleus pulposus has also aroused controversy. The nucleus pulposus of the dog has not been examined for the presence of nerves, but the study of Kumar and Davis (1973), using lumbar discs of cats, rats, and humans, failed to demonstrate such nerves. However, it is unclear whether these authors actually examined the nucleus pulposus or limited their observations to the anulus fibrosus. Stilwell's investigation using monkeys did not find nerve fibers extending beyond the outermost parts of the anulus fibrosus but the nucleus pulposus itself was not specifically examined.

Tsukada (1939) was the only investigator who claimed to have found nerves within the nucleus pulposus of healthy human discs. Numerous studies have failed to confirm his findings (Table 1).

It has been speculated that nerve fibers may accompany the granulation tissue which grows into ruptured intervertebral discs (Hirsch and Schajowicz, 1952). Apparently no one has investigated this possibility in animals, but one study in man revealed the presence of

nerve fibers extending all the way into the degenerated nucleus pulposus (Shinohara, 1970). However, studies by Jackson, Winkelmann, and Bickel (1966) and Yoshizawa et al. (1980) were unable to demonstrate the existence of such nerves in degenerated human discs.

Dorsal Longitudinal Ligament

Most disc herniations in the dog occur dorsally (Hoerlein, 1978). One would expect that the dorsal longitudinal ligament would therefore commonly be compressed in intervertebral disc disease. Thus, if the dorsal longitudinal ligament is supplied with pain fibers, it could be a major source of pain associated with disc disease.

The presence of nerves in this structure has not been histologically confirmed in the dog, but there is some evidence of their existence. Hukuda and Wilson (1972) performed experiments in which a screw-device was implanted through the body of a cervical vertebra and brought out through the skin ventrally. The top of the screw was implanted at a level even with that of the ventral surface of the vertebral canal. After recovery from the surgery, the screw was advanced to compress the spinal cord so as to create an experimental myelopathy. In some dogs the dorsal longitudinal ligament was not completely removed during screw implantation (apparently inadvertently), so that when the screw was advanced it was forced dorsally into the ligament. These dogs showed unmistakable evidence of pain while the ligament was being compressed, which was interpreted to mean that the ligament was supplied with nerve fibers.

Although there has been no morphologic evidence for the existence of nerves within the dorsal longitudinal ligament of the dog, Stilwell (1956) found this ligament to be profusely innervated in the monkey. Even investigators who were unable to demonstrate the presence of nerve fibers in the human anulus fibrosus succeeded in finding them in the posterior longitudinal ligament (Table 1).

The Sinu-vertebral Nerve

As there can be no doubt that at least some of the structures within the vertebral canal are innervated, the question arises whence the nerve fibers originate. It is commonly believed that these fibers are derived from the meningeal rami of the spinal nerves (International Committee on Veterinary Anatomical Nomenclature, 1973; International Anatomical Nomenclature Committee, 1977). The meningeal ramus is considered to be one of the four principal branches of spinal nerve, the others being the dorsal and ventral rami and the ramus communicans. The meningeal ramus is synonymous with the sinu-vertebral nerve in the human literature.

Complete descriptions of the sinu-vertebral nerve in domestic animals appear to be lacking (Prata, 1981). Zietzschmann, Ackerknecht, and Grau (1943) mentioned that this nerve was difficult to demonstrate; they stated that it arose from either the spinal nerve or the ramus communicans. Nickel, Schummer, and Seiferle (1975) gave a similar description. In neither of the above reports did the authors state whether these descriptions were adapted from the human literature or were based on personal observations, or for what species the descriptions were applicable. Jenkins (1978) and Kitchell et al. (1980) described a spinal

nerve origin for the sinu-vertebral nerve of the dog seemingly without having observed the nerve by themselves. Evans and Christensen (1979) merely gave reference to Pedersen, Blunck, and Gardner (1956) who described the sinu-vertebral nerve of man; they made no claims, however, on the existence of this nerve in the dog. Bogduk (1976) could not find the sinu-vertebral nerve in the lumbar region of three cats which he dissected.

Although little information exists concerning the sinu-vertebral nerve of the dog, this nerve has been carefully examined in the monkey (Stilwell, 1956). The sinu-vertebral nerve was found to be formed by two branches, one arising from a paravertebral autonomic plexus, the other from a spinal ganglion, spinal nerve, or dorsal or ventral spinal root before reentering the intervertebral foramen.

The sinu-vertebral nerve of man was first described by Luschka in 1850 (cited in Edgar and Ghadially, 1976). He found that this nerve derived mainly from the spinal nerve but also received contributions from the ramus communicans. Hovelacque (1925), who examined the human thoracic vertebral column, agreed with the findings of Luschka that the sinu-vertebral nerve was formed by a branch from a spinal nerve and from sympathetic branches which united as they passed into the intervertebral foramen.

Spurling and Bradford (1939) maintained that the sinu-vertebral nerve of man arose distal to the posterior root ganglion. Wiberg (1949) and Pedersen, Blunck, and Gardner (1956) confirmed that the nerve arose in part from the spinal nerve distal to the posterior root ganglion.

Further, Wiberg believed that additional fibers were usually derived from the sympathetic chain, while Pedersen and co-workers reported that these fibers arose from the gray ramus communicans. Roofe (1940) was unable to determine the origin of the sinu-vertebral nerve by gross dissection. Kimmel (1961) and Edgar and Nundy (1966) both emphasized that the sinu-vertebral nerve in humans arose from a confluence of fibers from the spinal nerve, gray ramus communicans, and sympathetic trunk which then passed through the intervertebral foramen. Taylor and Twomey (1979) found one or more branches arising from the ventral rami in the lumbar region of man. Bogduk, Tynan, and Wilson (1981) found the human sinu-vertebral nerve to have arisen from two roots, one from the ventral primary ramus, the other from the ramus communicans.

The course of the sinu-vertebral nerve upon entering the vertebral canal through the intervertebral foramen is largely unresolved. Zietzschmann, Ackerknecht, and Grau (1943) believed that the nerve communicated with the sinu-vertebral nerve from the opposite side of the body within the vertebral canal; species variations were not described, nor was it stated if these communications existed at all levels of the vertebral canal. Stilwell (1956) found that each sinu-vertebral nerve in the monkey would branch upon entering the intervertebral foramen; fibers were found to ascend and descend in the vertebral canal, overlapping the fibers of the sinu-vertebral nerves arising one segment cranial and caudal. Fibers also crossed the midline to the opposite side of the canal.

The course of the sinu-vertebral nerve in man has been highly controversial. Luschka (cited in Edgar and Ghadially, 1976) believed that each sinu-vertebral nerve communicated with sinu-vertebral nerves of contiguous segments. Hovelacque (1925) was unable to find such communications.

Spurling and Bradford (1939) illustrated a sinu-vertebral nerve descending from its origin at the nerve roots of the second lumbar nerve. This nerve was shown passing along the floor of the vertebral canal caudally for a distance of two lumbar vertebrae, presumably overlapping with the sinu-vertebral nerves of the third and fourth lumbar nerves as it descended.

Roofe (1940) was unable to follow the sinu-vertebral nerve of man by gross dissection. Lazorthes, Poulhes, and Espagno (1948) claimed to have had more success with their dissections; they found that the nerve ascended for one segment. If true, this ascending nerve should overlap the sinu-vertebral nerve entering the vertebral canal one segment cranially. No such overlap was reported, however.

The dissections of Wiberg (1949) did not reveal how far each nerve was distributed in the human vertebral column, but some clues were obtained during laminectomies performed on conscious patients, under local anesthesia. If an intervertebral disc was prodded, the patient felt pain, even if the dorsal root at that level had been anesthetized (e.g., if the third lumbar dorsal root was blocked on the left side, the patient still felt pain when the left dorsal surface of the L3-L4 disc was stimulated). It was concluded that the disc must receive innervation

from sinu-vertebral nerves other than those arising at the intervertebral foramen over the disc. Wiberg pointed out that these nerves could be arising from the sinu-vertebral nerves above or below that level or from the sinu-vertebral nerve at the opposite side which crossed the midline.

Pedersen, Blunck, and Gardner (1956) reported that the sinu-vertebral nerve in the lumbar region of man branched into ascending and descending fibers which communicated with the nerves above and below. In one case, the nerve communicated with its counterpart from the opposite side.

Bridge (1959) stained human vertebral columns using the method of Penfield and the Schiff reaction prior to gross dissection. He concluded that the major branches of the sinu-vertebral nerve descended for one or two segments.

Cloward (1960) operated under local anesthesia on patients with ruptured cervical discs. He approached the vertebral column from an antero-lateral approach and drilled holes through the discs so as to expose the posterior longitudinal ligament. He found that when he stimulated the ligament the pain was felt over a broad area; this area would overlap the areas of distribution of pain felt when he stimulated other segments of the posterior longitudinal ligament. Cloward believed that this overlap meant that each disc was innervated by more than one sinu-vertebral nerve, and suggested that each nerve might innervate the disc at its own level plus the one below.

Kimmel's histologic study of human fetuses (1961) showed each sinu-vertebral nerve to cross the midline to the opposite side of the

vertebral canal. In the lumbar region, the nerves overlapped each other cranio-caudally as well. In three-month-old fetuses, the sinu-vertebral nerve extended one-half vertebral segment cranially and one to two segments caudally.

Edgar and Nundy (1966) believed that in man the nerve ascended for one segment and descended for two. Subsequently, this pattern of distribution was reiterated by Edgar and Ghadially (1976), while Bogduk, Tynan, and Wilson (1981) have indicated that most of the nerve was distributed cranially for at least one segment; smaller branches passed caudally and medially.

A summary of the investigations of the course of the sinu-vertebral nerve has been presented in Table 2.

If a sinu-vertebral nerve does exist in the dog, its distribution would be of great interest. Although Bridge (1959) demonstrated the presence of nerve fibers on the surface of the ventral dura mater of the dog, cat, and man, he did not determine that they arose from the sinu-vertebral nerve. As discussed previously, the presence of nerves in the intervertebral disc and dorsal longitudinal ligament of the dog has not been explicitly shown; it is likewise uncertain if the blood vessels in the vertebral canal or the vertebral periosteum are innervated by this nerve.

Stilwell (1956) and Lazorthes, Poulhes, and Espagno (1948), using simian and human specimens, respectively, demonstrated that the sinu-vertebral nerve was distributed to the blood vessels within the vertebral canal (e.g., the ventral internal vertebral venous plexus), the posterior

Table 2. Disposition of the sinu-vertebral nerve

Authors	Species	Disposition of sinu-vertebral nerve	
		Presence of ascending fibers	Presence of descending fibers
Luschka, 1850 (cited in Edgar and Ghadially, 1976)	Man	+	+
Hovelacque, 1925	Man	+	+
Spurling and Bradford, 1939	Man	+(2 segments)	+
Roofe, 1940	Man	Unable to determine disposition	
Lazorthes, Poulhes, and Espagno, 1948	Man	+	+
Wiberg, 1949	Man	+	+
Pedersen et al., 1956	Man	+(Most fibers were ascending.)	+
Stilwell, 1956	Monkey	+	+
Bridge, 1959	Man	+	+(1-2 segments)
Cloward, 1960 ^C	Man	+?	+?
Kimmel, 1961	Man	+	+
Edgar and Nundy, 1966	Man	+(1 segment)	+(2 segments)
Bogduk, Tynan, and Wilson, 1981	Man	+(at least 1 segment)	+(small number)

^aNot described.

^bNot seen grossly; postulated based on stimulation during surgery.

^cCervical region.

^dNot described but should logically occur based upon the distribution given by the author.

Disposition of sinu-vertebral nerve		
Overlapping of fibers of contiguous segments	Presence of fibers crossing midline	Method used
+	-	Gross dissection.
-	-	Gross dissection
+	-	N.D. ^a
Unable to determine disposition		Gross dissection
+	N.D.	Gross dissection
-? ^b	-	Gross dissection, stimulation during surgery
+	+	Gross dissection
+	+	Intravital methylene blue staining
+	N.D.	Gross dissection after staining with method of Penfield and Schiff reaction
+	N.D.	Stimulation of posterior longitudinal ligament during surgery
+	+	Histologic examination of fetuses
+	N.D.	Gross dissection
+ ^d	N.D.	Gross dissection

longitudinal ligament, the intervertebral disc, the dura, and the periosteum of the vertebral canal. Hovelacque (1925) found a similar distribution in man but did not discuss the innervation of the disc.

Luschka (1850) and Pedersen, Blunck, and Gardner (1956) reported a distribution of the sinu-vertebral nerve similar to that described above but were unable to trace fibers to the disc of man with any certainty using gross dissection.

Results of investigations into the distribution of the sinu-vertebral nerve are summarized in Table 2.

Innervation of the Articular Facets

With intervertebral disc degeneration, it would seem logical that a certain amount of instability might develop within the vertebral column. Consequently, this could easily put stress upon the articular facets (zygapophyseal joints) resulting in degenerative changes, which, in turn, could be a source of pain. Ghormley (1933) first advocated this idea. Later, as evidence accumulated in support of the concept that the articular facets play a role in back pain, many investigators studied the innervation of these joints.

The innervation of the articular facets in the dog has not been elucidated. Evans and Christensen (1979) stated that the medial branches of the dorsal rami of the thoracic spinal nerves probably supplied the thoracic vertebrae, their associated ligaments, dura mater, and epaxial muscles. Their description of the lumbar medial branches was even less specific; they pointed to Pedersen, Blunck, and Gardner (1956), noting that, in humans, branches entered the vertebral canal to supply the

posterior longitudinal ligament, dura, periosteum, and blood vessels. No claim was made that the distribution was similar in the dog, and their recapitulation of this study was misleading. Pedersen, Blunck, and Gardner described the innervation of the above structures as having originated from the sinu-vertebral nerves via branches of the spinal nerves near the rami communicantes, or from the rami communicantes themselves, and not from the medial branches of the posterior rami as implied by Evans and Christensen.

Bogduk (1976) was unable to trace nerves from the medial branches of the dorsal rami to the articular capsules in the lumbar region of the cat, presumably due to their small size. A nerve to the intertransversarii muscles was the only branch which could consistently be found leaving the medial branches of the dorsal rami.

Bogduk also noted that the traditional description of the dorsal ramus branching into a medial and lateral division did not seem applicable to the lumbar region of the cat. He found that more commonly a dorsal ramus would trifurcate into a medial division to the multifidi and intertransversarii, an intermediate division to the longissimus, and a lateral division to the iliocostalis and skin. Bogduk's observations on the cat were similar to those he had previously made based on dissections of two dogs and two monkeys (Bogduk, 1974); no mention was made of articular branches in this abstract.

The innervation of the articular facets in the monkey was studied by Stilwell (1956). He showed that each thoracic or lumbar dorsal ramus gave off a branch to the articulation at the level at which it originated and then continued to innervate the joint one segment caudal.

Badgley (1941) reported that the human lumbar articular facets received innervation from the medial branches of the posterior division of the lumbar nerves, and possibly also from the sinu-vertebral nerves. This idea was subsequently discredited by Pedersen, Blunck, and Gardner (1956).

Pedersen, Blunck, and Gardner also examined the medial branch of the posterior ramus in man and depicted it coursing not only to the facet joint at the level of origin of that branch (e.g., the medial branch from L3 would innervate the L3-L4 articulation) but possibly also to the facet articulation one segment caudal. Thus, each medial branch was believed to innervate two facet joints. This was later confirmed by Edgar and Ghadially (1976), who found that as the medial branch descended posteriorly it would give off twigs to the inferior part of the joint capsule at its level of origin before sending similar branches to the superior part of the joint capsule one segment below.

Jackson, Winkelmann, and Bickel (1966) confirmed the presence of nerve fibers in the articular facets of man using silver stains but did not examine the gross distribution of the nerves.

Knowledge of the innervation of these articulations has been applied by orthopedic surgeons to treat patients suffering from low back pain resulting from facet disease. Using insulated needles inserted percutaneously, it has been possible to destroy the major nerves to the lumbar facets, providing relief from pain (Shealy, 1976). A hitherto undescribed nerve has recently been reported (Anon., 1980) which arose from the medial branch of the posterior ramus and passed superiorly to innervate the facet one segment cranially. If this preliminary

observation proves to be correct, each medial branch would be supplying a total of three facet articulations: the one at its level of origin, plus the facets one segment inferior and superior. Each of these joints would in turn be supplied from the medial ramus of three different lumbar nerves. Failure to take into account the superior branch may account for some of the failures of facet deneurectomy to totally relieve pain (Anon., 1980). One report skirted the issue of the existence of a superior branch of the medial ramus by merely noting that each facet articulation is innervated by more than one segment (Nade, 1980). Bogduk, Wilson, and Tynan (1982) did not find a superior branch in their dissections of six human cadavers.

MATERIAL AND METHODS

Gross Dissections

Twelve healthy, adult dogs (six males, and six females) were obtained from a local pound for gross dissection--two Doberman Pinschers, one German Shepard dog, and one Laborador Retriever, the remainder being mongrels. All of them weighed at least 25 kg.

Ten dogs were anesthetized with an intravenous injection of sodium pentobarbital. The right common carotid artery and external jugular vein were then surgically isolated and cannulated. Using a roller pump, physiologic saline solution was then infused into the external jugular vein while the dog was bled out through the common carotid artery. When the heart became sufficiently weak that death appeared to be imminent (as evident from the rate of flow of blood from the cannula in the common carotid artery), 10% buffered neutral formalin (BNF) was infused into the external jugular vein in place of the saline. After the heart had altogether stopped, the dog was then perfused with 10% BNF through the common carotid artery while the external jugular vein remained open to permit egress of the remaining blood and saline. When the fluid flowing from the external jugular vein became clear and appeared to consist mainly of BNF, the external jugular vein was clamped off and perfusion through the common carotid artery was continued until the limbs, tail, and ears were sufficiently stiff to indicate that adequate fixation had occurred. The dogs were then stored in a cooler at 8⁰ C until dissected.

Two dogs were killed by an intravenous overdose of sodium pentobarbital and the specimens were immediately dissected without

fixation.

Dissections were begun by locating the lateral branches of the dorsal rami of the tenth thoracic to the fifth lumbar spinal nerves. The lateral branches were traced proximally to their points of detachment from the corresponding medial branches of the dorsal rami; a search was made for any nerves leaving the lateral branches proximally and coursing towards the vertebral column. The medial branches were then traced, with particular attention to nerves passing from the medial branches to the articular facets (*Processus articularis cranialis et caudalis*).

After examination of the spinal nerves, the spinous processes and laminae of eight embalmed and two fresh dogs were removed from the ninth thoracic to the sixth lumbar vertebrae to expose the vertebral canal. A search was then made for the presence of any nerves within the canal. The dorsal and ventral nerve roots were then severed at their sites of attachment to the spinal cord and the cord and its meninges were removed, leaving the undisturbed nerve roots in situ. A 40X binocular dissecting scope was then used again in searching the spinal canal for nerves. The nerve roots were examined closely for any nerves which separated and passed through the intervertebral foramina into the vertebral canal. Dissections continued through the intervertebral foramina to permit a similar examination of the proximal portion of the rami communicantes.

After examination of their spinal nerves, midsagittal sections of the vertebral canals of two embalmed dogs were made using a band saw. The spinal nerve roots were severed adjacent to the spinal cords prior to removal of the cords. The vertebral canals were again searched for

nerves using a 40X binocular dissecting scope.

Histologic Examinations

Four large healthy, adult mongrel dogs (two males, two females) and a nine-week-old mongrel male puppy were obtained from a local dog pound. These dogs were perfused with 10% BNF solution as described previously. Samples of the intervertebral discs from T12-T13 to L3-L4, including the dorsal longitudinal ligaments, were then removed and fixed in 10% BNF solution for two days to two months prior to sectioning.

In order to determine which technique would be most useful in demonstrating the presence of nerve fiber, preliminary studies were made on samples from a small number of intervertebral discs stained with Schofield's silver impregnation method and Bielschowsky's method (after Drury and Wallington, 1967) and Bodian's method (after Humason, 1979). Based on the results of these staining trials, Schofield's silver impregnation method was found to be suitable for staining of the intervertebral discs and dorsal longitudinal ligaments. Sagittal sections were cut from the discs and attached ligaments from two dogs, while the discs and ligaments of the remaining three were cut in cross (transverse) sections prior to staining with Schofield's technique, as described by Drury and Wallington (1967):

1. The frozen sections were cut at 40-80 μ m and placed in distilled water.
2. The sections were transferred to a dish of distilled water containing marble chips for one hour at 37^o C.
3. The sections were rinsed briefly in distilled water.

4. The sections were placed in 20% silver nitrate solution for 20-30 minutes at room temperature in the dark.
5. After blotting the sections, they were then passed through three baths of 10% formalin and one bath of 2% formalin for 30 seconds in each bath.
6. The sections were rinsed rapidly in distilled water and blotted.
7. The sections were placed in ammoniacal silver solution (made by adding strong ammonia to a 20% silver nitrate solution until the precipitate was just dissolved) for 30-40 seconds, constantly agitating, then blotted.
8. The sections were then placed in 1% formalin and agitated until they were a light brown to yellow color.
9. The sections were washed in tap water.
10. The sections were fixed in 5% sodium thiosulphate solution for five minutes.
11. The sections were washed in water, dehydrated in alcohol, cleared in xylene, mounted on slides with a synthetic resin.

Another four healthy, large, adult dogs (three males, one female) were obtained from a dog pound. Two of them were anesthetized with an intravenous injection of sodium pentobarbital and then infused with methylene blue using the technique of Stilwell (1956) for vital staining of the nerves within the dorsal longitudinal ligament. Both dogs died approximately one hour after the beginning of the infusion. As Stilwell had reported that a minimum infusion time of three hours was necessary

for success, a different methylene blue staining technique was performed for demonstrating nerves within the dorsal longitudinal ligaments of the remaining two dogs. These dogs were killed by an intravenous overdose of sodium pentobarbital; the dorsal longitudinal ligaments from T10 to L5 were then removed and immersed in a 0.0005% solution of methylene blue, and processed following the technique of Hirsch, Ingelmark, and Miller (1963).

Investigations Using Horseradish Peroxidase (HRP)

Five adult dogs weighing between 10 and 20 kg were obtained from a dog pound; included in this group were three female beagles and two male mongrels.

Each dog was anesthetized with sodium pentobarbital and prepared for sterile surgery. In dogs one and two, a surgical approach was made to the left facet (zygapophyseal) joint at T13-L1 by separating the multifidi muscles dorsally. Fifty μ l of a 30% solution of horseradish peroxidase (HRP Sigma Type VI; Sigma Chemical Company, St. Louis) dissolved in sterile physiologic saline and 2% dimethyl sulfoxide solutions were then injected into the joint cavity using a microliter syringe directed through the most dorsal part of the joint capsule. The surgical incision was then closed with sutures.

In dogs three, four, and five, the epaxial muscles were reflected by blunt dissection from the left side of the vertebral column to permit a hemilaminectomy at T13-L1. This facilitated the exposure of the intervertebral disc at that site. Using a microliter syringe, 30 μ l of a 30% solution of HRP were injected into the intervertebral disc. The

injections were made at the junction of the lateral edge of the dorsal longitudinal ligament medial to the ventral internal vertebral venous plexus. An attempt was made to restrict the injection to the outermost layer of the disc (i.e., anulus fibrosus) and its overlying connective tissue. Great care was taken to ensure that hemostasis was complete prior to a routine surgical closure.

All five dogs were kept alive for three days for the retrograde transport of the HRP. Subsequently, each dog was anesthetized with sodium pentobarbital. A clamp was placed on the aortic arch and the descending aorta was then rapidly cannulated for perfusion of the thoracolumbar vertebral column with warm physiologic saline using a roller pump. The abdominal aorta was clamped off just cranial to the renal arteries to prevent fluid from passing to the caudal regions of the body. The azygos vein was divided to permit egress of the perfusate. After perfusion with 2000 ml of saline, each dog was perfused with 2000 ml of a cold 3% glutaraldehyde solution in 0.1 M phosphate buffer.

The left dorsal root ganglia of the eleventh, twelfth, and thirteenth thoracic and first and second lumbar nerves were removed from dogs one and two. Both right and left dorsal root ganglia from the tenth thoracic through the third lumbar nerves were removed from dogs three, four, and five. The ganglia were fixed in cold 3% glutaraldehyde in 0.1 M phosphate buffer for several hours. They were then stored overnight in a 30% sucrose solution.

The next morning, 40 μ m sections were cut using a freezing microtome. The sections were prepared using the technique of Mesulam

(1978). Then the sections were mounted on slides with a mounting medium (Permount, Fisher Scientific Company, Pittsburgh), and examined microscopically to detect the presence of HRP reaction product within the nerve cell bodies of the ganglia.

RESULTS

Gross Observations

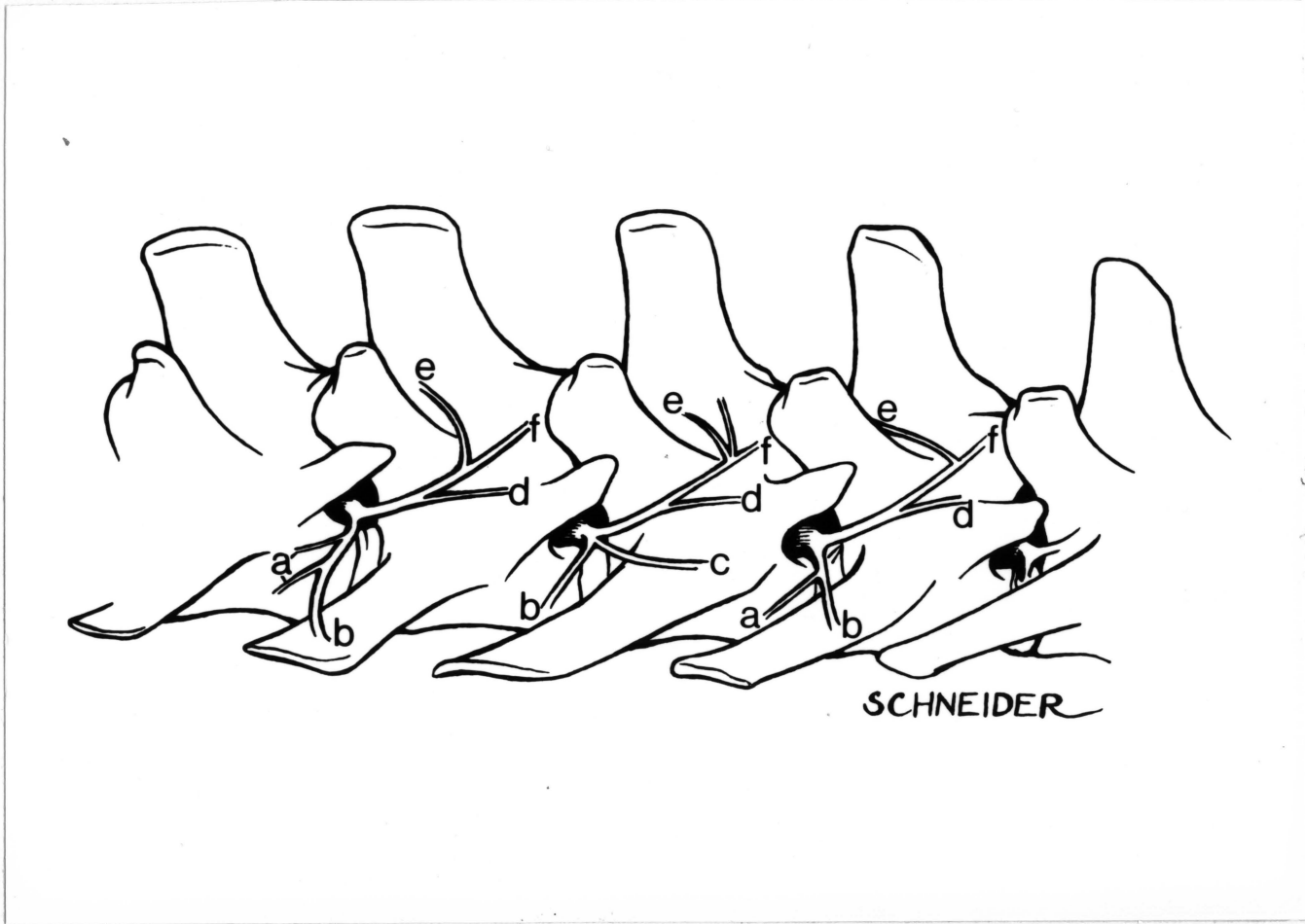
In the dog, the dorsal rami of the spinal nerves in the caudal thoracic and cranial lumbar regions usually divided into a medial and a lateral branch. Nerves to the longissimus muscles usually branched off the proximal portion of each lateral branch which then continued laterally to innervate the iliocostalis muscles, finally ending as a cutaneous nerve (Figure 1).

The medial branches passed caudodorsally, largely covered by the intertransversarii and multifidi muscles. No nerves were seen passing from the medial branches to the longissimus muscles. In most specimens, a distinct branch to the intertransversarii muscles were separated from the proximal portion of each medial branch; this nerve appeared to ramify solely within these muscles. The medial branches continued caudodorsally on the surface of the vertebral laminae satellite to an artery of the dorsal branch of the dorsal intercostal or lumbar (segmental) arteries. The medial branches gave off branches to the multifidi and, more dorsally, to the interspinales muscles. No cutaneous branches were seen.

During its course, a variable number of branches were given off by the medial branches which passed craniodorsally towards the caudal portion of the facet (zygapophyseal) joint of the same vertebral segment (e.g., the branch from the medial branch of L1 would pass towards the caudal portion of the L1-L2 facet joint). These branches (or in a few instances, one large branch) mainly ramified in the multifidi, but in most cases they could be traced to the level of the caudal surface of the

Figure 1. Dorsal branches of spinal nerves in the lumbar region of the dog (ventral branches are not shown)

- a. Branches to the longissimus muscles
- b. Lateral branches
- c. Intermediate branch
- d. Branches to intertransverarii muscles
- e. Branches to multifidi muscles and to the caudal parts of the articular facets
- f. Continuation of the medial branch into the multifidi and interspinales muscles and to the cranial parts of the articular facets



facet joint. Because of the small size and extreme delicacy of the nerves they could not, however, be followed grossly into the joint capsules with certainty. The medial branches continued caudodorsally, detaching branches to the multifidi and to the vicinity of the cranial part of the facet joint one segment caudal to that from which the nerve had originated (Figure 1). It was again impossible to grossly demonstrate that these nerves entered into the joint capsules. The medial branches ramified within the multifidi and interspinales muscles.

No branches of the medial branches could be traced to the cranial part of the facet joint at its level of origin (e.g., the L1 spinal nerve appeared to supply the caudal but not the cranial part of the L1-L2 facet joint). Neither nerves could be traced from the medial branches to the facet joints one segment cranial to their origin.

A variation commonly seen was the division of the dorsal ramus into medial, intermediate, and lateral branches instead of its bifurcation into medial and lateral branches (Figure 1). The intermediate branch, when present, supplied the longissimus muscles, replacing the muscular branches to the longissimus from the proximal part of the lateral branch. Occasionally the dorsal ramus divided into more than three branches.

No sinu-vertebral nerves (meningeal rami) as described in the literature were identified by gross dissection in these specimens. Examination of the ventral roots and proximal portion of the spinal nerves often revealed minute fibers extending from these nerves into the vertebral canal, which, on further examination, were found to be tiny veins off the ventral internal vertebral venous plexus. Until fully

dissected, in many instances connective tissue strands within the vertebral canal were frequently mistaken for small nerves. Occasionally, small linear strands of tissue could be seen, within or just outside of the intervertebral foramina, which appeared to leave the spinal nerves or their branches, but they were too delicate to be successfully traced. Such strands were likewise too delicate to be collected for histologic examination. Furthermore, no nerves could be found within the vertebral canal itself.

Histologic Observations

The dorsal longitudinal ligament was found to be profusely innervated (Figure 2). The nerves were more obvious on sagittal than on cross sections, due to their longitudinal orientation within the ligament. The largest nerves were usually found in its dorsal half, but along their course many branches were given off which passed ventrally towards the intervertebral discs. Artifactual separation of the ligaments and discs was common, making it difficult to trace such branches to their endings.

The intervertebral discs at all levels examined were found to be only sparsely innervated. Nerves were visible within the loose connective tissue and fat present on the dorsal surface of each disc, and along the course of blood vessels. Small blood vessels were found regularly in the more superficial layers of the disc (Figure 3); it was usually not possible to demonstrate the presence of nerves in company with these intradiscal vessels. Rarely, a nerve was found to penetrate the outermost layers of the anulus fibrosus (Figure 4). Nerves were

Figure 2. Nerve within dorsal longitudinal ligament

Figure 3. Blood vessel within anulus fibrosus

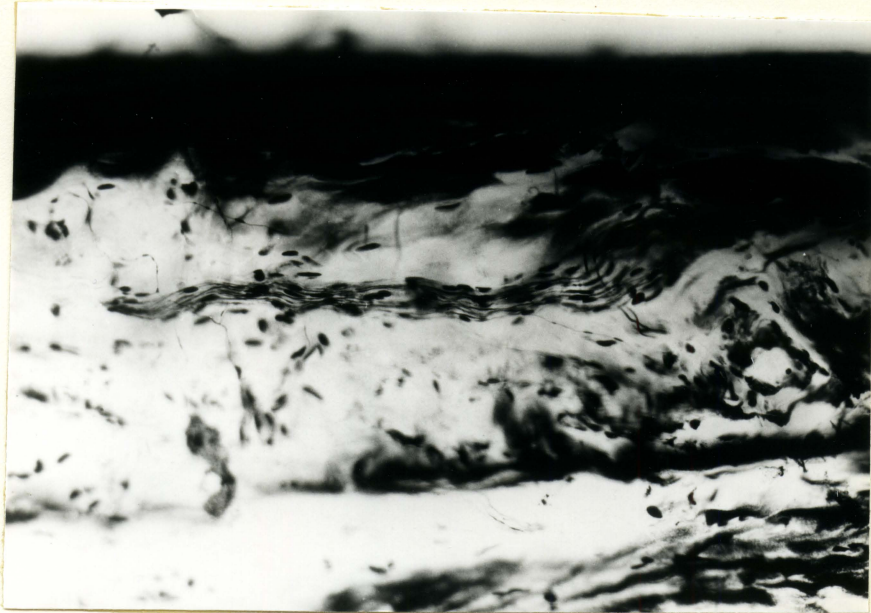
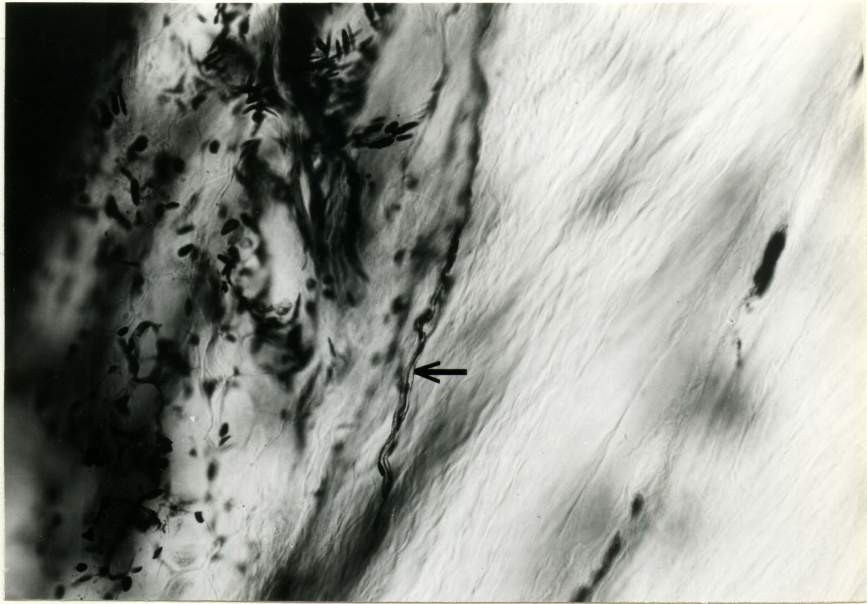


Figure 4. Nerve (arrow) penetrating anulus fibrosus from the surrounding connective tissue



neither detected in the deeper layers of the anulus fibrosus nor within the nucleus pulposus. Such nerves, as were seen within the disc, gave rise only to free, naked nerve endings.

Nerve fibers were not selectively stained by either of the methylene blue staining techniques attempted so that nerves could not be traced within the dorsal longitudinal ligaments of intervertebral discs of such specimens.

Observations with Horseradish Peroxidase (HRP)

The labelling of the dorsal root ganglia in the five dogs injected with HRP is shown in Table 3.

Table 3. Labelling of dorsal root ganglia with HRP

Dog number ^a	Presence of label in ganglionic cells													
	T10		T11		T12		T13		L1		L2		L3	
	L ^b	R ^c	L	R	L	R	L	R	L	R	L	R	L	R
1	N.E. ^d	N.E.	-	N.E.	+	N.E.	+	N.E.	-	N.E.	N.E.	N.E.	N.E.	N.E.
2	N.E.	N.E.	-	N.E.	+	N.E.	+	N.E.	-	N.E.	N.E.	N.E.	N.E.	N.E.
3	-	-	-	-	+	-	+	+	+	+	+	-	-	-
4	-	-	+	-	+	+	+	+	+	+	+	-	-	-
5	-	-	-	-	+	+	+	+	+	+	+	-	-	-

^aDogs 1 and 2 were given an HRP injection in the T13-L1 facet joint. Dogs 3, 4, and 5 were given an HRP injection in the left dorsal portion of the anulus fibrosus at T13-L1.

^bLeft side.

^cRight side.

^dNot examined.

DISCUSSION

Little information is available in the literature about the innervation of the thoracolumbar segments of the vertebral column in the dog. In the course of dissections, it became apparent that the available descriptions of the branching of the spinal nerves of the thoracolumbar regions of the dog were also largely inadequate. The usual description of each dorsal branch of thoracolumbar spinal nerves dividing into a medial and lateral branch was not consistently followed. Evans and Christensen (1979) reported that multiple branches were often seen arising from the dorsal branches of the nerves, but the authors did not describe the presence of a distinct intermediate branch, which was first described by Bogduk (1974, 1976) in the dog and cat, respectively. Based on his dissection of two dogs, Bogduk stated that an intermediate branch was typically present. In this study, although the presence of an intermediate branch was a frequent variation, the dorsal branch of thoracolumbar spinal nerves more commonly split only into medial and lateral branches. When present, the intermediate branch innervated the longissimus muscles. Of dorsal branches having only a medial and lateral branch, the lateral branch was found not only to provide innervation to iliocostalis muscles and the overlying skin, but also to the longissimus muscles, which are usually said to be innervated solely by the medial branch (Evans and Christensen, 1979). This finding is concordant with the previous observations of Bogduk (1974, 1976) on the dog and cat, respectively.

The articular facets were innervated by the medial branches. In the absence of a distinct articular nerve, the capsules of the facet joints were innervated mainly by the muscular branches to the multifidi, which, after coursing through the muscle fascicles, ramified to the adjacent facet joint. The innervation of the facet joints has been of great interest in man owing to pain resulting from degenerative changes involving them. Many investigators have alluded to that each human lumbar facet joint receives innervation from the nerve at that segmental level plus the nerve arising one segment cranially (Bogduk, Wilson, and Tynan, 1982). Wyke (1980) claimed that each lumbar facet was innervated by three segmental nerves without any anatomical evidence. The limitations of gross dissection have not permitted confirmation of a bisegmental innervation of facet joints, although the finding of a bisegmental pattern in the monkey (Stilwell, 1956) lends support to a belief for a similar pattern in man.

The bisegmental pattern of innervation of the facet joint described for man was also found on gross dissection of the thoracolumbar regions of the dog. Thus, each medial branch gave off nerves to two facets (one at its level of origin plus another one segment caudal), and each facet was, therefore, innervated by two segmental nerves. Further confirmation of this pattern was obtained from two dogs by injecting HRP into the left facet joint at T13-L1. Reaction (labelled) product was subsequently demonstrated in the left dorsal root ganglia of T12 and T13, demonstrating that they provided innervation to those structures. No HRP reaction product was found in either T11 or L1 dorsal root ganglia;

therefore, they did not provide innervation to the T13-L1 facets.

Although the results of the investigations using HRP corroborated those of the gross dissections, the possibility of individual variations must not be overlooked. The agreement between the HRP labelling and the gross dissections indicated that dissection can be successfully used, so that the bisegmental innervation of the other thoracolumbar facets, as determined by gross dissection, is probable.

Sometimes surgeons have successfully alleviated back pain in human patients by destroying the nerves to the articular facets. Perhaps, it would be possible to do the same in a dog by destroying the medial branch (possibly by percutaneous injection of a chemical under radiographic guidance) prior to the origin of the first branch which passes to the caudal portion of the facet of the corresponding thoracolumbar segments (Figure 1). Although execution of this procedure would probably not be difficult, it is uncertain if it could be clinically useful. First, the nature of involvement of the facets in back pain of the dog is unknown; it is possible that they may be a much less important source of back pain than in man. Second, even if it could be demonstrated that the facets were a source of pain and that destruction of the nerves to the facets could be easily done, the consequences might be dangerous. Once the pain was relieved by denervation, the dog might increase its activity, thereby increasing the chance of rupture of the degenerated discs which are commonly associated with diseased facets. It should be noted that, although a bisegmental facet innervation exists in the thoracolumbar regions of the dog, the same may not be true for other areas of the

vertebral column. Wyke (1979) claimed that the facets in the cervical region of the cat, monkey, and man were innervated by three rather than two nerves. This has not been conclusively proven. Although Wyke cited Stilwell (1956) as his source of information about the triple innervation for the facet joint in the monkey, in fact Stilwell only reported a dual innervation of the facets in the cervical region.

The articular facets may eventually be proven to be a potential source of pain in dogs with intervertebral disc disease, but it is quite likely that much of the pain originates from structures within the vertebral canal itself, including the meninges, periosteum, dorsal longitudinal ligament, intervertebral discs, blood vessels, and nerve roots. In man, pain from many of these structures is known to be carried to the spinal cord via the sinu-vertebral nerve. Further, it has been assumed that this nerve, often called the meningeal ramus (International Committee on Veterinary Anatomical Nomenclature, 1973), exists in animals, but in this study it was not found in the thoracolumbar regions of the dogs examined.

The sinu-vertebral nerve is not only absent in the thoracolumbar segments of the dog, it seems not to be present in the cat either (Bogduk, 1976). Indeed, the literature seemingly contains no specific account of any investigator actually having seen this nerve in domestic animals (personal communications from Prof. J. Frewein, former Secretary General of the World Association of Veterinary Anatomists, and Prof. O. Schaller, former President of the World Association of Veterinary Anatomists, to Prof. N. G. Ghoshal). Yet it is commonly shown in

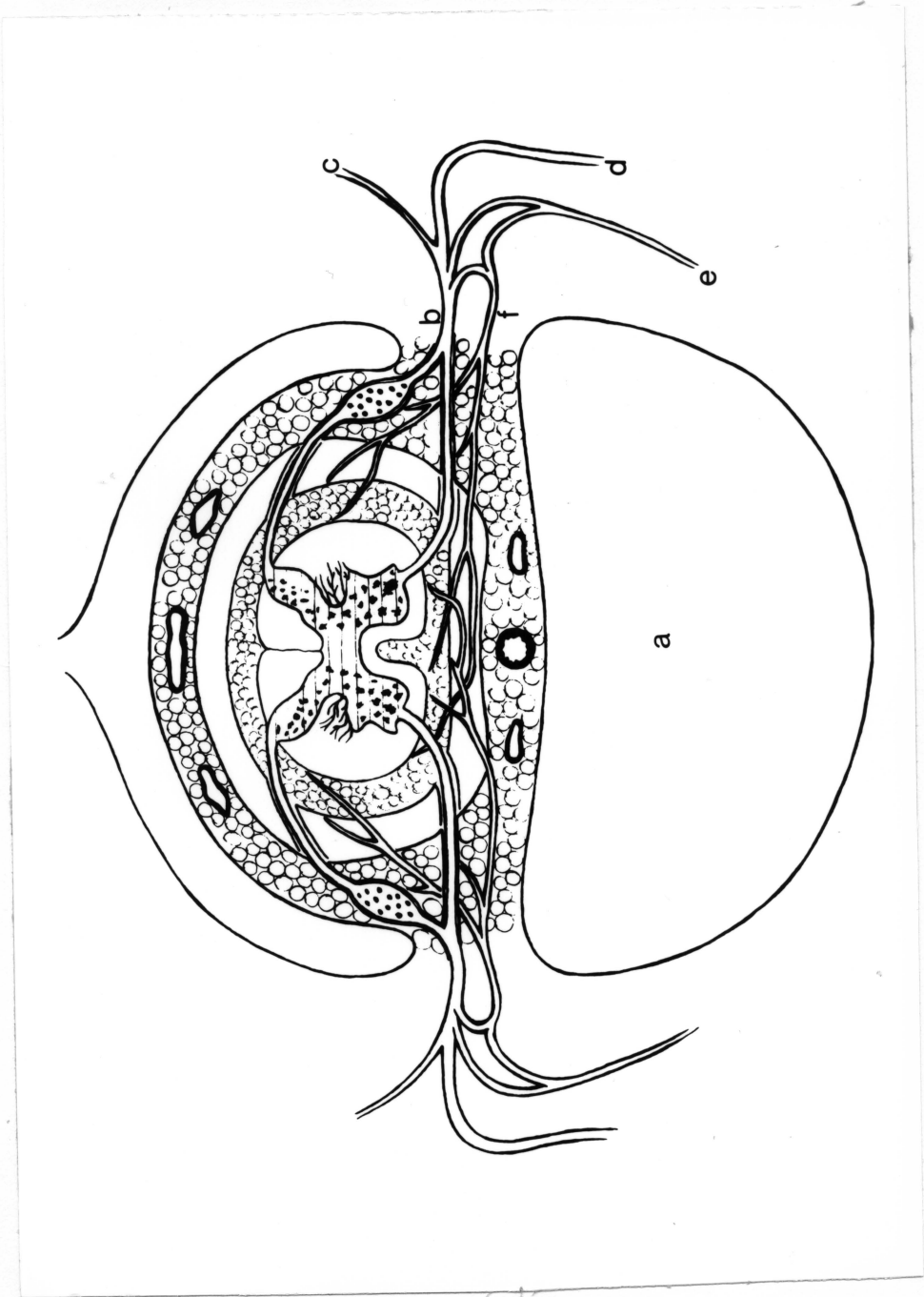
drawings in veterinary textbooks (Figure 5). Usually these drawings resemble those available in the human literature, from which they were probably inspired. Since the sinu-vertebral nerve of the dog (and probably the cat) does not exist in the thoracolumbar regions morphologically similar to that of man, the drawings are somewhat misleading. One might also question the appropriateness of the term meningeal ramus, since there is no distinct ramus present of a typical spinal nerve in the dog.

As there can be no doubt that there are nerves present within the vertebral canal, the question arises as to their origin since there is no sinu-vertebral nerve per se in the dog. It seems likely that the dog resembles the monkey (Stilwell, 1956) in which small numbers of nerve fibers enter each intervertebral foramen (after branching from a paravertebral plexus of nerves) but do not aggregate to form a grossly discernible sinu-vertebral nerve. It is, of course, impossible to say how these nerves branch in the dog, but if the disposition is similar to that of the monkey some might be derived from the spinal nerves and others from the rami communicantes.

Schofield's silver impregnation method did not consistently yield good staining of both discs and dorsal longitudinal ligament sections. Poor differentiation between connective tissue and nerves was a common problem; in many instances the tissues were stained too darkly to be useful. Nevertheless by staining numerous sections from each disc and associated ligaments, adequate numbers of well-stained specimens were obtained for microscopic examination. Similarly, the methylene blue

Figure 5. A typical illustration of the sinu-vertebral nerve (redrawn from Ellenberger and Baum, 1943)

- a. Centrum
- b. Spinal nerve
- c. Dorsal branch
- d. Ventral branch
- e. Ramus communicans
- f. Sinu-vertebral nerve (meningeal ramus)



staining of tissues provided no useful information due to its inability to differentiate nerve fibers from the surrounding structures. Kumar and Davis (1973) were similarly unsuccessful in applying this technique to staining of human discal material. The technique was, therefore, abandoned after four attempts, although with repeated experimentation it might have been modified to permit identification of nerve fibers.

The large number of nerves found within the dorsal longitudinal ligament of dog and man make this structure a potential source of pain in both species. Pain could result from direct pressure upon the ligament by a dorsally protruding disc, or by an inflammatory process occurring on the floor of the vertebral canal due to extruded disc material. It is also possible that increased mobility of the vertebrae may occur at the site of degenerating discs resulting in stretching of the ligament, which could cause pain.

Because the nucleus pulposus and most of the anulus fibrosus were devoid of nerves, it seems that degenerative changes limited to the disc itself are not likely to be a direct source of pain. However, when a disc ruptures dorsally, the nerves in the outermost layers of the anulus fibrosus and the surrounding loose connective tissue would certainly be involved. A morphologic study cannot prove that these nerves are pain fibers, but the nerves were seen terminating as naked nerve endings; such endings have been shown to be associated with pain reception in other areas of the body. These tissues may, therefore, account for some discal pain. It is also possible that these nerves may accompany granulation tissue growing deeper into a ruptured disc, but this remains yet to be

investigated in the dog.

The disc itself may indirectly cause pain due to vertebral instability accompanying discal degeneration. As mentioned previously, this may stretch the dorsal longitudinal ligament and the capsules of the facet joints. In addition, it could result in increased movement of the connective tissue surrounding each disc, and the stretching of the periosteum of the vertebral bodies along the floor of the vertebral canal which is contiguous with this connective tissue. The nerves within the connective tissue obviously could be affected as could any nerves present within the periosteum. Although the periosteum of the canine vertebral bodies has not been examined for the presence of such nerves, they are known to exist in the monkey (Stilwell, 1956).

Gross dissections failed to conclusively reveal how the nerves to the intervertebral discs and to associated dorsal longitudinal ligaments were distributed, but HRP experiments provided useful information about this subject. Injections into the most superficial layer of the left side of the anulus fibrosus at T13-L1 resulted in the presence of labelled cells in the left dorsal root ganglia at T12, T13, L1, and L2 in all three dogs (Table 3). Thus, all four of these spinal nerves contributed to the innervation of the T13-L1 disc. In dog #4, T11 also sent fibers to the disc. Therefore, each disc was innervated by nerves from four or five segmental levels. As shown in Table 2, the segmental distribution of nerves in man has been subjected to much disagreement. The pattern observed in the dog most closely resembles the description of Edgar and Nundy (1966) in man, who believed that each nerve ascended for

one segment and descended for two (as seen in dogs #3 and #5).

The great degree of overlapping of nerves found in this study may explain in part why discal pain is often poorly localized. A protruded disc would not only affect the nerves arising at that segmental level (e.g., a diseased disc at T13-L1 would affect the T13 nerve) but would also affect the nerves which ascend to and descend from that disc. Since nerves from four or five different levels would be affected, the pain might be felt over several metameric segments. It should also be noted that left sided injections resulted in labelling of some of the right dorsal root ganglionic cells. Thus, fibers from the right side crossed the midline to the left side of the disc. Pain arising from a protruded disc on one side could, therefore, also be felt on the opposite side of the body.

HRP is useful in tracing the distribution of nerves only if it does not leak from the site of injection. If the HRP leaks to other areas it may then be picked up by neurons in those areas and label them, leading to the erroneous conclusion that they were present at the injection site. Due to the small quantities of HRP injected and the small diameter needle used, leakage from the annulus fibrosus seems unlikely. If leakage occurred, it would likely have been trapped in the connective tissue on the surface of the disc. Post mortem examination of the vertebral canals did not reveal any surgically induced hemorrhage which could have disseminated leaked HRP to other levels within the vertebral canal. Therefore, it is felt that the results reflect the true distribution of the nerve fibers in those vertebral segments investigated.

CONCLUSIONS

The objectives of this study, as stated previously, were to investigate the innervation of the canine thoracolumbar spinal column as a basis of understanding the origin of pain associated with intervertebral disc disease in the dog and man. Within the limitations of this study, the following conclusions can be drawn.

1. The spinal nerves within the caudal thoracic and cranial lumbar regions of the dog commonly divide into medial and lateral branches, the medial branches supplying innervation to the multifidi, intertransversarii, and interspinales muscles and articular facets while the lateral branches supply the longissimus and iliocostalis muscles and the overlying skin. A common variation is the presence of an intermediate branch, which supplies the longissimus muscles.

2. Each articular facet in the caudal thoracic and cranial lumbar regions is bisegmentally innervated, receiving fibers from the spinal nerve at its own segmental level plus from another spinal nerve one segment cranially, as revealed by gross dissection and HRP tracing. This is similar to the pattern believed to exist in man.

3. The dorsal longitudinal ligament is profusely innervated.

4. Only the outermost layers of the anulus fibrosus are innervated. Nerves are also found in the connective tissue on the surface of the intervertebral discs.

5. No grossly discernible sinu-vertebral nerve (meningeal ramus) exists in the caudal thoracic and cranial lumbar regions of the dog.

6. The T13-L1 intervertebral disc, and presumably the intervertebral discs at other levels, is innervated by nerves arising from four or five segmental levels, as revealed by HRP tracing. Nerves cross the midline to contribute to the innervation of the opposite side of the disc.

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