AN ANATOMIC STUDY OF THE MYOTOMAL ORGANIZATION OF THE ANTEBRACHIUM OF THE DOG (CANIS FAMILIARIS) A RETROGRADE HORSERADISH PEROXIDASE STUDY

By

RICHARD HAROLD JESSEN

Bachelor of Science

University of Wisconsin

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Thesis Approved:

J E Breazile
Thesis Advisor

Subhash Sangvi

Robert P Wettemann

Theodore E Stice

Norman N Durham
Dean of the Graduate College

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CHAPTER I

INTRODUCTION

A truly comprehensive understanding of the structure and function of the vertebrate spinal cord did not begin to focus until the publication of Sherrington's paper in 1892 (92) In this paper, Sherrington combined the concepts of reflex arcs, the neuron theory, and the parcellation of spinal cord function to give a more unified understanding of the spinal cord. This paper also delineated the myotomal organization of the peripheral nervous system and demonstrated the organization of neurons into cell columns within the cervical and lumbosacral spinal cord enlargements of the monkey Sherrington's paper stimulated interest in the organization of the spinal cord that remains an impetus for further study even today.

In 1928, Goering (38) continued along the same lines as Sherrington and demonstrated that the cervical enlargement of the white rat is also organized into distinct cell columns After Goering's paper, work on the organization of the spinal cord foundered until Elliot's papers in 1942 and 1943 (28,29), which rekindled interest by demonstrating a nuclear organization in the human spinal cord Elliot also contributed a method of mapping the location of spinal cord nuclei which helped to standardize a much disoriented field. Elliot's method consisted of projecting slides of the spinal cord with its nuclei and then tracing

consecutive slides onto a single sheet of paper, thus visualizing the columnar organization of the spinal cord nuclei

Romanes (88) enhanced Elliot's work by demonstrating that the individual identified columns supplied different muscles depending on which level of the spinal cord examined.

These early studies laid a foundation for our understanding of spinal cord structure and function but the early techniques lacked specificity and thus limited our understanding the organization of spinal cord motor columns Sherrington used crude electrophysiological techniques combined with anatomical dissecting skills in his studies Romanes and Elliot used chromatolytic studies. This technique damages a nerve fiber resulting in anatomically identifiable changes in the nerve cell bodies of the transected axons. The major problem with these methods is their lack of specificity and accuracy. Even Romanes, who used only chromatolytic techniques questioned its accuracy (88)

With the need for more accurate results Erlanger and Gasser (36) developed a method for recording nerve action currents using an oscilloscope With this instrument, all action currents could be made visible even if the effector muscle didn't twitch when its nerve was stimulated. Microelectrodes were developed and used by Jankowski and Roberts (45) to demonstrate the axonal projections of a single spinal interneuron Microelectrode techniques can be used to demonstrate peripheral nerve pathways but the complications and expense involved make this method almost prohibitive

The development of nerve tracer compounds revived interest in the study of spinal cord organization. Horseradish peroxidase (HRP) was found to be an almost ideal substance to use as a nerve tracer, it

produces an extremely sensitive technique, is inexpensive and relatively easy to use When Mesulam (73) pioneered the use of tetramethylbenzidine as the identifying chromagen in the HRP reaction, he made an extremely sensitive and useful process available to researchers interested in tracing nerve axons.

The HRP technique has been used to dtudy limb innervation of many different species (61,63,73) With the HRP method, the location of neurons supplying the muscles of dogs antebrachium can be mapped with great accuracy This map will provide information about the organization and structure of the cervical enlargement of the dogs spinal cord and will allow for comparisons with others species The information provided by the map also has some clinical implications, when a cervical or cranial thoracic spinal injury occurs a diagnosis concerning which limb muscles will be affected is more easily made when the exact location of the neurons supplying these muscles is known

This paper describes the use of the HRP method for mapping the motor neuron pools of the canine antebrachium

CHAPTER II

REVIEW OF LITERATURE

Nerves

History records a long term human interest in the nervous systems of animals as well as much confusion in understanding these systems. Aristotle confused nerves with tendons and ligaments and thought the brain bloodless and the heart supreme Galen's doctrine of communicating humors, formulated in the second century A D , held advances in medicine to a standstill for a thousand years Galen thought (about the nervous system) that the blood, manufactured in the liver, carried (in it) natural spirits The blood flowed to the heart where the natural spirits became vital spirits that moved with the blood to the rete mirabile (the terminal branches of the carotid arteries at the base of the brain) Here, they changed into soul substance, which then flowed out to the body through hollow nerves (34).

In the 17th Century, Descartes bequeathed to generations of neurophysiologists the notion that impressions from the external world moved via material, animal spirits, to the ventricles of the brain, then the pineal gland directed these spirits into outgoing tubular nerves to the desired site of action Descartes thought that the direction provided by the pineal gland was purely mechanical in animals, but in

man the soul resides in this gland and controls the direction taken by the vital spirits. Descartes recognized, however, that some of these actions lay outside the control of the will, for example, involuntary blinking and withdrawing the hand from heat Descartes also argued the idea of reciprocal innervation, while animal spirits flowed into one set of muscles the opposing set relax. He thought this difference in muscle function was accomplished by valves that block the flow of spirits into the antagonist muscles (24)

By the middle of the 18th Century, Albrecht von Haller recognized that nerves were the source of all sensibility, and he differentiated between motor and sensory nerves (101) This advance did not displace Galen's 1600 year old concept of humors, as von Haller still thought that nerves functioned via a nervous fluid flowing within a hollow nerve The experiments of Volta (99) and Galvani (35) at the end of the 18th Century led to the discovery and acceptance of animal electricity and its role in neuronal transmission The middle of the 19th Century brought instruments sensitive enough to measure the small currents flowing in nerves With these instruments, du Bois-Reymond demonstrated that the nervous principle is electricity and that this electricity causes the action current in muscle Bernstein expanded on du Bois-Reymond's work, demonstrating a resting membrane potential in nerves and that the action current or potential is a self-propagating depolarization of the nerve membrane Bernstein thought that the action potential resulted from the nerve membrane's selective permeability to potassium ions At the end of the 19th Century, Willy Kuhne suggested that the action currents of the nerve invaded the muscle and thereby caused the muscle to contract. In 1921, Lowei working with a frog's

heart first demonstrated a neurotransmitter agent, which he named Vagustoffe (64)

Spinal Cord

As long as the belief persisted that every nerve in the body required its own canal leading directly to the brain, the spinal cord appeared only a prolongation of the peripheral nervous system, a channel into the brain By the middle of the 19th Century, von Helmholtz established the connection between peripheral nerves and spinal ganglia (100) During this same period, Waller demonstrated that axons degenerate when cut from their cell bodies This discovery of The Wallerian degeneration led to the development of a specific stain for the degenerating myelin sheaths of these cut axons, giving anatomists a technique for tracing nerve tracts both in the peripheral and central nervous system.

Waldeyer-Hartz presented a clear formulation of the Neuron Theory in 1891, leading to a functional understanding of the spinal cord (102) This doctrine states that the individual nerve cell constitutes the genetic, anatomic, trophic, and functional unit of the nervous system All neural pathways, circuits and reflex arcs are composed of individual nerurons arranged in simple or complex patterns. Full comprehension of the spinal cord's role, however, required a differentiation of motor and sensory function. Galen's doctrine prevented recognizing the separation between motor and sensory nerves for centuries because he associated the presence of spinal ganglia with motor function In 1822 Magendie reported experiments that established the dorsal roots of spinal nerves as sensory and the ventral roots as

motor(67). Bell claimed full priority and the Bell-Magendie law was born(8)

Combining the knowledge that reflex activity takes place in the cord with the knowledge that muscle inhibition occurs (even though there are no inhibitory nerves to vertebrate skeletal muscle) focused attention on the connections between incoming sensory nerves and the outgoing motor nerves Sherrington called this connection the synapse At the start of the 20th Century, Sherrington combined the ideas of the Neuron Theory, the parcellation of spinal cord function (the concept dividing the spinal cord anatomically into identifiable segments; each segment contains only the neurons whose axons form the dorsal and ventral rootlets that eventually combine to form a single spinal nerve), and the concept of reflex arcs to give us the modern concept of spinal cord structure and function Sherrington (92) in 1892 published a paper that consolidated and clarified the few preceding reports on the spinal cord and the peripheral nervous system. This paper added some significant new data that were and remain a current impetus for continuing study of the spinal cord Sherrington was the first to demonstrate myotomal organization of the peripheral nervous system (a myotome is all the muscles innervated by a single ventral root), using electrophysiological techniques. Sherrington's work demonstrated nerve cell columns within the cervical and lumbosacral spinal cord enlargements, these columns provided the main interest of the paper.

Sherrington identified two distinct columns to which he gave different and distinct functions. He said that the poster-lateral column provides nerve cells that supply the more distal elements of the limbs and the antero-lateral columns innervate the more proximal elements of

the limbs and the muscles of the trunk Sherrington's work added greatly to our understanding of muscle innervation and demonstrated that the same spinal cord ventral root often innervates opposing muscles This information allowed Sherrington to conclude that the coordinated natural movements are not the sole result of one ventral root's excitation Two additional and important pieces of information were brought to light. (1) the outflow for any individual main movement involves at least three segments of the cord, (2) excitation of the same spinal nerve root does not always produce the same movement, even in individuals of the same species, the nervous system is not a rigid, fixed map It may not be true that Sherrington's work stimulated a flurry of papers, but it was instrumental in initiating interest in the organization of the spinal cord.

Goering (38) in 1928, using the Nissl method, found that the gray matter of the cervical enlargement in the albino rat is organized into five distinct longitudinal cell columns He assigned a distinct function for each of these columns One column contained the cells innervating the muscles producing flexion of the forelimb, two columns extension, one column rotation and one possibly adduction, all of the same limb Other works on the anatomy of the spinal cord, with the purpose of more precisely identifying the functions of Goering's columns produced basic and irreconcilable differences between authors in describing motor cell distribution in the spinal cord. These differences resulted mainly from the use of nonstandardized techniques and nomenclature Little progress was made until Elliott, (28) in 1942 which clearly demonstrated a nuclear organization of the human spinal cord. Using chromatolytic techniques, Elliott demonstrated eight

distinct nuclei (with many subdivisions) in the lumbosacral enlargement and twenty-one in the cervical enlargement. Elliott also designed a precise method for mapping the location of these nuclei, a method still used today Elliott (29,30) later confirmed his previous works and identified a new nucleus in the cervical enlargement of human fetal spinal cords. Sprague (95) extended Elliott's technique and attempted to determine if Elliott's columns innervated completely different muscles or innervated synergistic muscles that were grouped or otherwise localized Sprague (95) demonstrated that a true lateral column, giving rise to a ventral rami (limb muscles) and a true medial column, giving rise to a dorsal rami, (vertebral muscles) exists but only in the limb areas and not for the total length of the spinal cord. Sprague also found that motor cells in the thoracic region extend at least one segment above and below their roots of exit and are chiefly those of dorsal rami while the ventral rami cells are limited to their root segments Romanes (88) in a study similar to that of Elliott and Sprague described seven cell columns, each ascending and descending varying distances or numbers of segments up and down the spinal cord from the spinal segment associated with the efferent nerve Using Sherrington's terms <u>prefixed</u> and <u>postfixed</u> (prefixed indicates that a spinal nerve originates cranial to its usual sites of origin in some of the animals examined and postfixed caudal to its usual sites of origin) and applying them to the cat's lumbosacral plexus, Sprague found that any two cats may vary as much as a complete spinal cord segment in the origin of nerve fibers that contribute to any specific nerve or portion of the plexus. Romanes (88) found that the different columns he identified supplied different muscles depending on which level (segment)

of the cord he examined and that the spinal nerve supplying a given muscle in one cat may supply a different muscle in another cat. Using chromatolytic techniques, he demonstrated that there is a standard topographical relationship between a given group of muscles and a given cell column and that the number of altered (chromatolytic) cells bears a relation to the size of the muscle denervated Romanes contributed to the work of Sherrington and Sprague by demonstrating that those cells in the most dorsal columns innervate the most distal part of a limb Straus (98) confirmed some of Romanes' findings, and demonstrated that there is nothing to indicate the existence of anything approaching a true nerve-muscle specificity between animals of different vertebrate classes Clark (19) introduced the idea of innervation ratio, which he defined as the ratio of the number of motor nerve fibers supplying a given muscle to the number of muscle fibers. This concept of the innervation ratio makes it possible to make direct comparisons between nerve components and the muscles they supply

In 1964 Romanes (88) reviewed the literature pertaining to nerve degeneration and chromatolytic techniques and summarized the findings pertaining to the motor cell groups in the cat's spinal cord. (1) Chromatolytic cells are always found in the same cell groups following the same peripheral nerve injury and are never found in these groups unless one or more of the muscles innervated from these groups is denervated (2) The cell groups innervating the morphological extensor muscles lie in the lateral part of the ventral horn, while the flexors are supplied by more medially placed cells. (3) In some cases (hamstring muscles) each muscle is innervated by a separate group of motor cells, but in the majority of cases the cells innervating a single

muscle are intermingled with cells supplying the other muscles innervated from the same column. A discrete nucleus for each muscle is not found. (4) In the cat, the flexor groups are so arranged that each main mass of cells tends to be concerned with the innervation of muscles that moves the same joint or joints and not necessarily with muscles that are topographically closely associated. (5) The nerve cells supplying flexor and extensor muscles activating the same joints tend to lie in the same horizontal plane in the ventral horn, the more distal the muscle the more distal the position of the cells supplying it. (6) The number of cells innervating a muscle or group of muscles is remarkably constant

Anatomy

Chromatolytic studies reveal the columnar and nuclear organization of the spinal cord. Efferent fibers to specific muscles originate from groups of neurons called nuclei and these nuclei occupy varying lengths of the spinal cord called columns. To determine the terminations of efferent fibers from these nuclei and columns and which nerves contain them, requires a variety of techniques. One such technique is to trace each ventral root mechanically to the plexus to which it contributes to and follow it through the plexus to the nerve or nerves it forms and then to the muscle or muscles it supplies Patterson (83) began this type of work, but another study by Miller and Detweiler (76) laid the ground work for all the succeeding studies They compared the embryological and anatomical differences of the origins of the brachial plexus between eleven different species, five of them vertebrates. This study included various classes of vertebrates but concentrated on the

primate order Using gross observation and microscopic sections, they determined the segmental levels of the spinal cord and the number of nerves from each segment entering into the brachial plexus. Following Miller's work, Allam et al. (4) described the brachial plexus of the "normal" dog After examining fifty-eight plexuses, they found that (1) thirty-four of the studied plexuses were formed by ventral roots from C6,7,8 and T1, (2) twelve are formed by roots from C5,6,7,8 and T1, (3) ten by roots from C6,7,8,T1 and T2, (4) two by roots from C5 through T2. Allam's work also provided the following information. (1) The subscapular nerve forms from the union of two radices derived from C6 and C7 (2) The musculocutaneous nerve is formed entirely by C7 (3) The axillary nerve also originates only from C7. (4) The dorsal thoracic nerve derives from C7 and C8 and occasionally from one or the other alone (5) The radial nerve is formed from C8 with a branch from C7 and T1 and when a root from T2 is present some fibers from it enter this nerve (6) The median and ulnar nerves are derived from C8, T1, and possibly T2

Electrophysiology

Sherrington (92), used an electric current to stimulate a nerve and hence its effector muscle, to determine the limit of myotomes. The method Sherrington used was very crude; he stimulated a nerve and watched for a twitching muscle. If a muscle twitched, that muscle was thought to be innervated by the stimulated nerve. This stimulate-observe technique led to many errors because it is easy to overlook a twitching muscle, or if using too large a stimulus, the

stimulating current can spread to another nerve giving false innervation identities

In 1922 Erlanger and Gasser (36) developed a method that used a low voltage cathode ray oscillograph to easily and accurately record nerve action currents. This technique provided a way to record action currents in nerves or in the muscles they supply and to make all action currents visible even if the effector muscle does not twitch. Using the oscillograph technique, Coombs et.al , (20) demonstrated that the spinal cord motoneuron functioned as an integrating unit Eccles et. al., (26), established that this integrating function is specifically aided by the low threshold of the initial segment of the neuron relative to the threshold of the soma-dendritic membrane. Eccles et al., (26) followed his first electrophysiological study with a second, which demonstrated the patterns of activation of Renshaw cells. The second study showed that Renshaw cells tend to be excited by collaterals from the axons of adjacent motoneuronal nuclei, demonstrating interrelationships between the different spinal cord nuclei.

Fletcher (33) used Sherrington's stimulate record technique but in a different way, he stimulated muscle nerves of the dog while recording form the ventral roots of spinal nerves L4 to S3 Using this method, Fletcher determined that the intrinsic musculature of the dog's pelvic limb consists of five myotomes L4,L5,L6,L7, and S1 Fletcher also found that three myotomes supply most pelvic limb muscles and no pelvic limb muscle is supplied by less than two myotomes.

Using microelectrodes, Jankowski and Roberts (45) demonstrated the axonal projections of a single spinal interneuron and showed that ventral horn interneurons excited from group Ia afferents mediate

reciprocal inhibition of motoneurons. These microelectrode techniques can be used to demonstrate nuclear organization and Akaike et. al , (3) used them to demonstrate the vestibulospinal reflex pathway in the rabbit

In 1974, an exciting series of unrelated experiments (18,32) began that changed and confused our understanding of spinal cord structure and function Coggeshall et al , (18) demonstrated anatomically that the ventral roots of the cat's lumbosacral enlargement contain a large population of unmyelinated fibers originating from the dorsal root ganglion cells Two papers, both measuring nerve conduction velocities, appeared next, one in 1974 (17) and one in 1976 (5), and both demonstrated that unmyelinated ventral root fibers did not have their cell bodies in the spinal cord These two papers also demonstrated that one-third of these unmyelinated ventral root fibers have receptive fields in somatic structures and two-thirds have receptive fields in the viscera. The inevitable conclusion that these papers advocate is that the cat's ventral root contains a major sensory component and that the Law of Bell and Magendie is therefore not an accurate description of the organization of the ventral roots in the cat. Emery, Ito, and Coggshall (32) substantiated these works and further demonstrated that more than half of the unmyelinated axons in the cat's T11 and T12 ventral roots arise from dorsal root ganglion cells.

Landmesser and Morris (56) made an interesting application of electrophysiologically recording from muscle to study the development of the functional motor innervation in chick embryos. They demonstrated that (1) a given muscle always received functional innervation from specific peripheral nerves, (2) most limb muscles or primitive muscle

masses become functionally innervated at the same time with no clearly defined proximal-distal sequence of limb innervation, (3) chick motoneurons are initially specified with respect to their peripheral destination. Electrophysiolical techniques are also used to delineate dermatomes as well as myotomes, and the dermatomes of both the horse and dog have been studied this way (46,47).

Motoneuron Pools

The population of motoneurons innervating an individual muscle is called a pool (21) Romanes (88) demonstrated that the pool of motoneurons supplying limb muscles of the cat were organized into longitudinal columns within the ventral gray of the spinal cord Recent work supports his observations. Burke et al (15), using HRP labeling of motoneurons showed that the neurons supplying the left medial gastrocnemius and the right soleus muscles of the cat formed a column of cells parallel with the long axis of the spinal cord in the lateral part of the ventral horn. The pools of neurons supplying these muscles overlap throughout most of their course, permitting close apposition of pools supplying related muscles. This arrangement may facilitate the organization of input common to these pools and of interconnection between them

Cullheim and Kellerth (23), using HRP injected directly into motoneurons traced the axons and recurrent collaterals of these labeled motoneurons to their terminations, which were frequently found within motor nuclei This work provided the best evidence to date that direct synaptic interconnection between motoneurons exist

There is a receptor in muscles which senses muscle length called the muscle spindle The primary afferent fiber from this receptor organ is called the Ia fiber Mendel and Henneman (41,42) doing electrophysiological studies, showed that single Ia fibers are distributed to virtually all the homonimus motoneurons (motoneurons supplying the same muscle) of a single pool Supplemental experiments by others (77,91,103) substantiated Mendel and Henneman's findings Anatomical studies by Scheibel and Scheibel (90) using Golgi techniques and sagittal sections in kitten spinal cords, reconstructed the entire intraspinal course of Ia fibers. Ia afferent fibers bifurcate upon entering the spinal cord from the dorsal root, divide and project in both cranial and caudal directions in the dorsao lateral fasiculus Several collaterals are given off from the dorsal horn and drop ventrally through the medial part of the dorsal horn into the ventral horn where collaterals form and provide terminals to motoneurons of the ventral horn All of these studies indicate considerable divergence of afferent Ia terminals to a motoneuron pool, resulting in a significant degree of correlated firing in the neurons of the pool

A number of descending systems project to motoneurons. Evidence exists for direct projection from vestibulospinal, reticulospinal, rubrospinal and corticospinal systems in various species (13). Even though, there is strong evidence that these systems have some fibers that terminate selectively on large alpha motoneurons there is, at present, little evidence that higher centers and their projections are used in isolation to achieve special patterns of neuronal excitation and recruitment within a pool (6,14).

Henneman and others (41,42), in studies on motoneurons demonstrated that as the Ia stimulus is increased to a specific pool progressively larger motoneurons are recruited into activity In general, the smaller of any two motoneurons compared was discharged at a lower intensity of stimulation regardless of whether the stimuli arises ipsilaterally or contralaterally, physiologically or electrically or whether the responses were elicited monosynaptically or polysynaptically In a further series of experiments (94), trains of electrical stimuli were applied to brain stem motor areas, cerebellum, basal ganglia, and motor cortex of the cat. Regardless of the site of stimulation the motoneurons supplied by these areas were recruited according to size. The smaller motoneurons were discharged at the lowest thresholds and other units were recruited in order of increasing size The results of these experiments suggested that the susceptibility of a motoneuron to discharge is strongly correlated with size regardless of the source of excitation and the neural circuits that transmit it to motoneurons Henneman (41) also showed that susceptibility to inhibition is also correlated with cell size In general, the larger the motoneuron, the more readily it was silenced by inhibition In response to these experiments, Henneman proposed a "size principle" (41) which makes the correlation between the size of the neuron and the neuronal energy required to discharge a motoneuron, the energy it transmits and releases in its muscle fibers, its excitability and inhibitability, its mean rate of firing and even its rate of protein synthesis (86).

Budingen and Freund (12) showed that the activation of the motoneurons in a pool is organized to achieve precise mechanical effects These effects are stable relations between the force of output

of the individual motor units at the time of their recruitment and the corresponding force output of the entire muscle.

Adrian and Bronk (2) noted that the force of a voluntary muscle contraction could be increased by two mechanisms The number of actively contracting motor units could be increased (recruitment) or the rates of the units already discharging could be accelerated Milner-Brown et al (72) demonstrated that in humans, increased rate produced about two-thirds and recruitment only about one-third the total force of muscle activity The wide spread distribution of Ia terminals to practically all homonimous motoneurons is an important factor in rate modulation Any increase in synaptic excitation to the pool would thus occur simultaneously in all motoneurons, including those already firing In a discharging motoneuron this would tend to cause an increase in firing rate Therefore, an increase in the number of recruited motoneurons would commonly be expected to be accompanied by some increase in firing rate among all discharging members of the pool with an increase in muscle force These experimental results reveal the basic principle involved in the grading of muscular tension. The motoneuron pool functions incrementally by adding progressively larger units with increased rate of smaller units already firing as the net excitatory input increases. As excitation decreases or inhibition increases, the pool operates decrementally by subtracting progressively smaller units and also decreasing its rate of firing These operations depend on the distribution of cell sizes in the pool and the correlates of cell size that determine susceptibility to discharge

The role of a motoneuron pool is to translate a large, heterogenous inflow of signals from peripheral receptors and from many parts of the

central nervous system (Barett (9) estimated the total number of synaptic endings at 20,000 to 50,000 per motoneuron depending on its size) into a much smaller and simpler output that will produce precisely controlled tensions in a particular muscle In spite of the great diversity of input, both excitatory and inhibitory, that they receive, motoneurons innervating the same muscle function as collective entities The activity of each cell in a pool is closely correlated with the activity of all the other cells in the pool. This is the result of the intrinsic properties of the motoneurons and by the distribution of their input. The motoneurons supplying a specific muscle could be scattered indiscriminately throughout the ventral gray of the spinal cord without organization into pools, but this would present insurmountable problems in supplying them with feedback input from peripheral receptors In this situation the central nervous system would have to selectively activate each motoneuron to accomplish voluntary movements, picking the proper combination of cells to yield the desired total output Circuits would be necessary to calculate what combinations of active units would produce the correct total tension. This circuitry would be formidable and produce considerable neural delay. If, for example, a muscle had 300 motoneurons the possible number of combinations the central nervous system would need to consider in recruiting these neurons would be greater than 10^{90} The solution that has evolved is the size rank-ordered pool which relieves the central nervous system of the necessity for selective activation of motoneurons and provides a simple rule for their combination

Nerve Tracers

Electrophysiological techniques made it possible to determine peripheral nerve pathways and spinal cord ascending and descending tracts The location of the nerve cell bodies of these tracts can be determined using microelectrode techniques, but this is time consuming, tedious and expensive. Chromatolytic technique can also pinpoint the location of these nerve cells, but this technique is very unreliable (39) Romanes (88) could not use the chromatolytic technique to determine if the small cells of the ventral horn send their axons into the ventral roots because he found the reaction of these small cells to injury to be difficult to ascertain The chromatolytic technique requires that the investigator examine sectioned tissue for injured cells but in the great majority of cases these cells are only slightly altered There is often considerable variation between animals similarly treated and even in individual animals (62,84,85,93). Because the degree of chromatolysis is not standard as a response to peripheral nerve injury, Romanes (88) felt that the validity of results from studies using this technique should be questioned A better technique than either electrophysiology or chromatolysis is required to determine the location of specific nerve cell bodies within the central nervous system

Nerve Tracer Compounds

In the early 1970's a variety of nerve tracer compounds were used to trace axons These tracers are placed near or in a axon The axon takes up the tracer and carries it in its cytoplasm to the nerve cell body where the tracer is localized and identified Tracer compounds can be divided into six groups (71). (1) amino acids labeled with radioactive atoms---a highly technical, time consuming and expensive method but very successful, (2) fluorescent compounds---methodology for the use of these compounds is not yet stabilized and these compounds have the potential for diffusing out of the axon or cell in which they initially were placed. (3) macromolecules with lectin properties---these compounds are identified with a radioactive label or can be detected immunohistochemically, (4) neurotransmitters ---these substances are radiolabeled and are used for anterograde as well as retrograde tracing however the level of specificity is low, (5) nucleosides---these molecules are radiolabeled and used for both anterograde and retrograde tracing, (6) horseradish peroxidase (HRP)---an enzyme that is both easy and inexpensive to use, but its most appealing trait is that it is the most sensitve of any of the other methods

Horseradish Peroxidase

HRP is a small molecule (40,000 D) that does not freely diffuse into intact cells but gains entry through the process of endocytosis which occurs at neuro-muscular junctions or at synapses The HRP molecule itself is not visible within a cell but an easily detectable reaction product (oxidized chromagen) can be formed by enzymatic action on a suitable substrate Four types of markers are used for detecting

the HRP in neural tissue, fluorescence, immunohistochemical, radiolabel, and HRP enzymatic reaction product formed by a suitable substrate The last of these is the most commonly used and the most technically developed method In experiments based on the direct histochemical detection of HRP, it is the distribution of enzyme reaction product at the time of microscopic examination that provides the information for determining the pattern of neural connectivity The accuracy in determining the HRP distribution is influenced by a number of factors related to the enzymatic activity of the HRP (1) Loss of enzyme activity during fixation, (2) Reactivity of chromagen, (3) Solubility of reaction product, (4) Visibility of reaction product, (5) Quantity of reaction product, and (6) Stability of reaction product. Because some low sensitivity procedures may fail to demonstrate reaction product (30) Mesulam (71) contrasted the six factors presented above and found the role of the chromagen to be the crucial factor in demonstrating the presence of HRP. Mesulam compared the sensitivity and ease of use of the five chromagens available in 1979, tetramethylbenzidine (TMB), diaminobenzidine (DAB), benzidine dihydrochloride (BDHC), o-dianisidine (OD), p-phenylenediamine (PPD) and found TMB to be the most sensitive and easy to use The technique he developed for retrograde transport using this chromagen is used today by the majority of researchers and provides the standard for comparison of results.

Tracing with HRP

Kristensson and others in 1971 (51,52) made the first definitive demonstration of neural connections within the central nervous system using HRP. Following their lead Holtzman et. al , (44) demonstrated

that the amount of HRP uptake at the neuromuscular junction of the lobster can be increased ten times by stimulating the nerve before placing the HRP in the muscle LaVail and LaVail (59) demonstrated a retrograde transport analogous to Kristensson's done in the chick nervous system. Krishnan and Sanger (50) showed that HRP is not readily picked up by intact nerves but enters the nerve only at the neuromusclar junction The injection of HRP directly into the tissue being studied (21,40,55,60,61,63) was the standard procedure for placing HRP, until the studies of DeVito et al (25), and Kristesson and Olsen (53) demonstrated that HRP is readily picked up and transported by sectioned (cut) axons. Several studies have (16,54,68) successfully used the technique of applying HRP to the proximal end of a cut nerve and localization of the cell bodies of origin HRP is used to study muscle innervation (7,11,15,43,48,57,58,79,80) and (81,82,87,89), central nervous system pathways (65), the organization and projections of cranial nerve nuclei (49) and autonomic nerve nuclei (10,31), the dorsal root ganglia of the peripheral nervous system (73), the comparison and distribution of alpha and gamma motoneurons (97) and the afferent nerve pathways to the spinal cord columns (69,78)

Conclusion

The number of HRP studies is expanding rapidly and contributing to our knowledge of the organization of the nervous system. The increasing number of studies leads to refinements in HRP techniques, which offer increased sensitivity and detail These rapidly improving techniques can provide for complete and accurate maps of the innervation of any body part of interest

CHAPTER III

MATERIALS AND METHODS

Animals

Thirty-one mongrel dogs of both sexes with ages ranging from two to ten weeks old and weights from one to ten pounds were used in these experiments. Before and after surgical procedures, the dogs remained in university kennels. If the dogs were not weaned, they stayed with their dam, if they were weaned, they received the same maintenance care as the other dogs in the kennel at that time. These experimental dogs received no special care except to attend to their surgical wounds.

Horseradish Peroxidase

The horseradish peroxidase (HRP) used in these experiments was Sigma Type VI obtained from Sigma Chemical Company St. Louis Mo (catalog # P 8375) Preparation of the HRP consisted of dissolving it in a 2% DMSO 01M phosphate buffered saline solution (pH 7 4) to a concentration of 25% Preliminary experiments demonstrated that maximum neuronal transport of the HRP occurred when using the 2% DMSO with a HRP concentration of 25%

Surgery

Before surgery, each dog received Pentobarbital (Nembutal) 50mg/ml anesthesia in a dose of 8 2 mg/Kg intraperitoneally (IP) The IP route proved to be the easiest, most efficient, and least stressful to these young dogs. In the original plan for this work, only one dog was to be used for each muscle studied with the same muscle injected on both limbs as a replication. Problems with lack of transport of the HRP mandated the use of several dogs for each muscle so that there was an average of three dogs used per muscle studied.

The muscles of the antebrachium were surgically exposed using the smallest possible incision that provided adequate access to the muscle to be infused. Once exposed, the muscle being infused was isolated from surrounding muscles by removing it from its fascia and elevating it slightly without disturbing its innervation or its blood supply. The muscle was infused with HRP using a ten microliter syringe with a tip diameter of 30u to 50u In all cases, the muscle required more than one injection to fill it with enough HRP to completely darken it (volume varied), but this was by design as this technique allowed for the HRP to be spaced equally throughout the muscle. The infusion was performed by first infusing the proximal end of the muscle, with each subsequent injection of HRP placed nearer the middle of the muscle. Then the distal end of the muscle was infused, again working toward the middle of the muscle with succeeding injections of HRP Any HRP that leaked or spilled was immediately removed from the area with a cotton swab. With perfusion completed, the muscle was returned to its original position and the incision closed with interrupted sutures On two different occasions, the technique used required muscles overlying the targeted

muscle to be cut and retracted to provide access to it. To expose the deep digital flexor (radial head), the flexor carpi radialis was bisected and retracted, and to expose the deep digital flexor (humeral head), the flexor carpi ulnaris and the superficial digital flexor muscles were bisected and retracted. After surgery, the dog was monitored until fully recovered from the anesthesia and if there were no complications, returned to the kennel.

Transport Time

Mesulam reported (74) a transport time of approximately 120mm per day for HRP in somatic motor neurons. He also stated that once the HRP localized in the nerve cell body, it would stabilize there for four to five days Studies done preliminary to the studies described here substantiated Mesulam's findings, leading to a selection of a transport time of three days (72hrs) The spinal cords of the experimental dogs were collected three days after the surgery to allow optimum transport and concentration of HRP in spinal neurons.

Perfusion and Fixation of the Spinal Cord

The dogs were anesthetized with pentobarbital 8 2mg/Kg IP. A catheter was placed into the jugular vein and 200 units per Kg of heparin sulfate were injected via this catheter five minutes before making a chest incision A longitudinal incision was made just to the left of the sternum, the ribs were cut and spread, the descending aorta at the level of the apex of the heart was clamped, the pericardium was incised, and a 12 gauge needle (attached with 1/4inch ID polyethylene tubing to the perfusing solution) was inserted into the left ventricle The tube attaching the needle to the perfusing solution was clamped to

prevent blood from the heart from entering the perfusion solution The right atrium was incised and the perfusing solution allowed to flow.

Initially, the perfusing solution was suspended one meter above the animal to provide an adequate perfusing pressure Just as the perfusing solution began to flow, a bolus of Xylocaine (40mg/Kg) was injected into the tube supplying the perfusing solution to the heart Xylocaine administered in this way gave excellent and dependable vasodilation In preliminary studies, Verapamil was tried as a vasodilator, but proved to be undependable. The first perfusing solution was one liter of normal saline buffered with 0 1M phosphate buffer to pH 7 4 This solution was allowed to flow at full pressure and rate of flow, providing excellent clearance of blood from the spinal cord The second perfusing solution consisted of one liter of a 2 5% solution of gluteraldehyde in 0 1M phosphate buffer This solution was also administered at full pressure (one meter above the dog) The second liter of perfusing solution was followed by three more liters of gluteraldehyde 2.5% but at decreased pressure so that the total perfusing time was one hour This technique allowed for complete in vivo fixing of the spinal cord, which was imperative for the success of the staining procedures. The gluteraldehyde solutions were followed with one liter of a 5% sucrose in 0 1M phosphate buffer administered at full pressure Following the 5% sucrose solution, one liter of a 0.1M phosphare buffered 30% sucrose solution was perfused at reduced pressure so that the total administration time was 30 minutes. The sugar solutions were used to cryoprotect the spinal cord tissue.

Collection of the Spinal Cord

Following the in vivo perfusion, fixing, and cryoprotection, a bilateral laminectomy from cervical vertebrae number 3 to thoracic vertebrae number 3 was done and the spinal cord from C4 to T2 was removed The spinal cord was embedded in an aqueous 10% gelatin solution The cord in the gelatin was cooled and when the gelatin hardened, it was trimmed to conform to the shape of the spinal cord. The spinal cord with its gelatin coat was placed in a 0.1M phosphate buffered 2 5% gluteraldehyde solution and allowed to soak until the gelatin fixed (about four hours). The cord was cut into segments using the origins of the dorsal and ventral rootlets as the markers The left side of each cord segment was marked by incising the gelatin coat the total length of each segment Each segment was placed into a labeled test tube containing 30% sucrose solution and allowed to soak at 4°C until the sections were saturated (about 12 hours). The sections were determined to be saturated when they no longer floated in the sucrose solution

Sectioning of the Spinal Cord

When fixation and cryoprotection of the spinal cord segments was complete, the individual segments were frozen and sectioned into 40 micron sections on an International-Harris-Cryostat Model CTD Every third section was saved and immediately mounted on a slide coated with 2% gelatin. The sections were mounted fifteen to a slide and each slide was labeled as to which spinal cord section it contained. This technique differs markedly from Mesulam's (74), but in preliminary experiments this method proved to be just as sensitive and much less

troublesome than his technique Mesulam's procedure resulted in many lost sections of spinal cord. The spinal cord sections (mounted on the slide) were reacted with tetramethylbenzide HCL (TMB,Sigma) and hydrogen peroxide This reaction produces an insoluble marker wherever the HRP had localized in the spinal cord.

Reacting the Spinal Cord Sections

All sections were treated at the same time to produce consistent staining First, the mounted sections were rinsed six times in distilled water, then immersed in the 2% tetramethylbenzidine solution for 20 minutes. The slides were removed and 3ml. of 0 3% hydrogen peroxide for each 100ml of incubation solution were added to the original solution. The slides were returned to the new solution and allowed to react for 20 minutes. They were then rinsed six times in the acetate buffer (ph 3 3) rinse solution for a total of 20 or 30 minutes and then dried in a vacuum desiccator for 12 hrs After drying, the slides were counterstained and dehydrated using the following procedure:

1 100% ethanol (10sec.)

2. dist water (10sec.)

3 1% buffered neutral red solution (8 minutes)

4 70% ethanol (10s)

5. 95% ethanol (10s)

6 100% ethanol twice (10s each)

7 two baths of xylene (2-5 minutes each)

8 cover slip.

Examination of Spinal Cord Sections

Each spinal cord section was examined with a microscope and maps were drawn of each section delineating the location of each neuron containing HRP reaction product. These maps demonstrate the columns of neurons that supply each muscle in which HRP was deposited.

CHAPTER IV

RESULTS

Extensor Carpi Radialis

Isolation and perfusion of the extensor carpi radialis muscle with horseradish peroxidase resulted in one-hundred-twenty-five labeled neurons in the dog being studied. Ninety (72%) of these neurons were found in spinal cord segment C7 and thirty-five (28%) in segment C8. These labeled neurons form bilateral columns within the gray matter of the spinal cord The columns are located in the most dorsal lateral part of the spinal cord ventral horn extending from the beginning of C7 to the end of C8 as depicted in figures 1, 1a, and 1b. As shown by the above figures, the columns formed by the neurons supplying the extensor carpi radialis are intermingled with the columns formed by the neurons supplying the other muscles of the antebrachium. The main difference between the columns formed by the neurons of the extensor carpi radialis muscle and the columns formed by the neurons of the other muscles of the antebrachium is that the columns of the extensor carpi radialis are confined solely to spinal cord sections C7 and C8

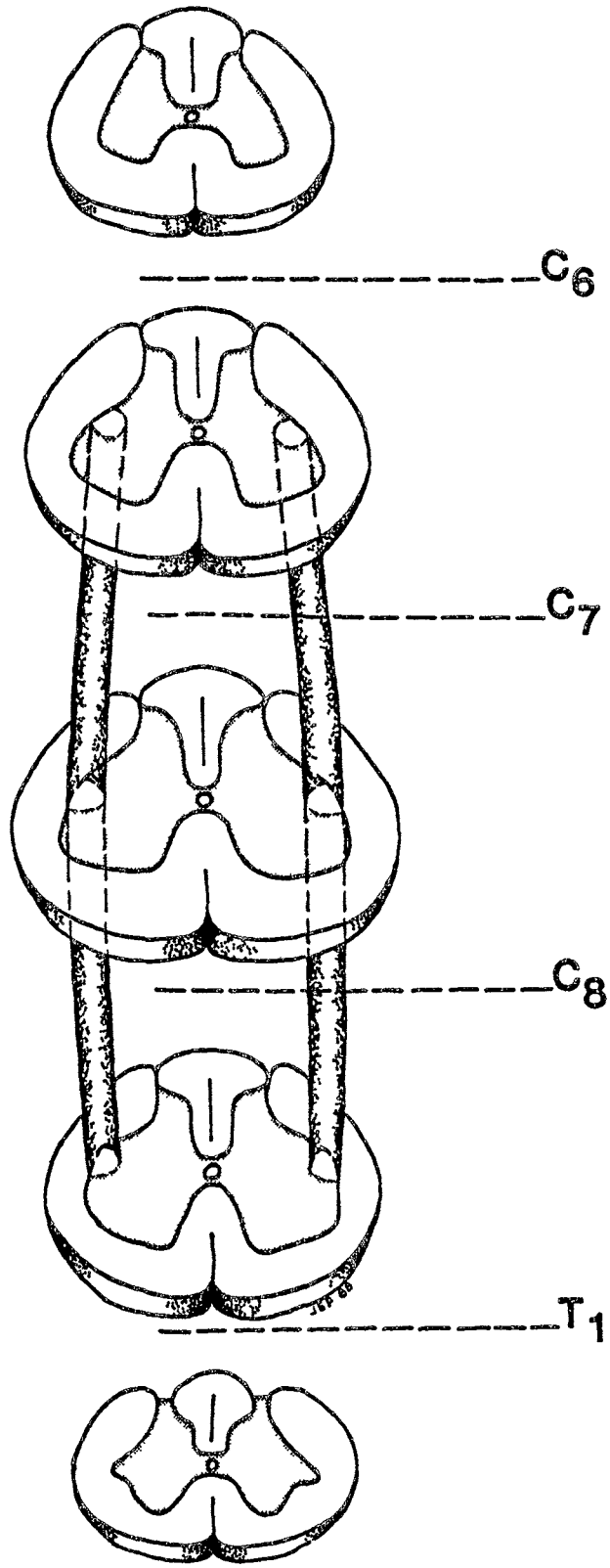

Figure 1. Extensor Carpi Radialis

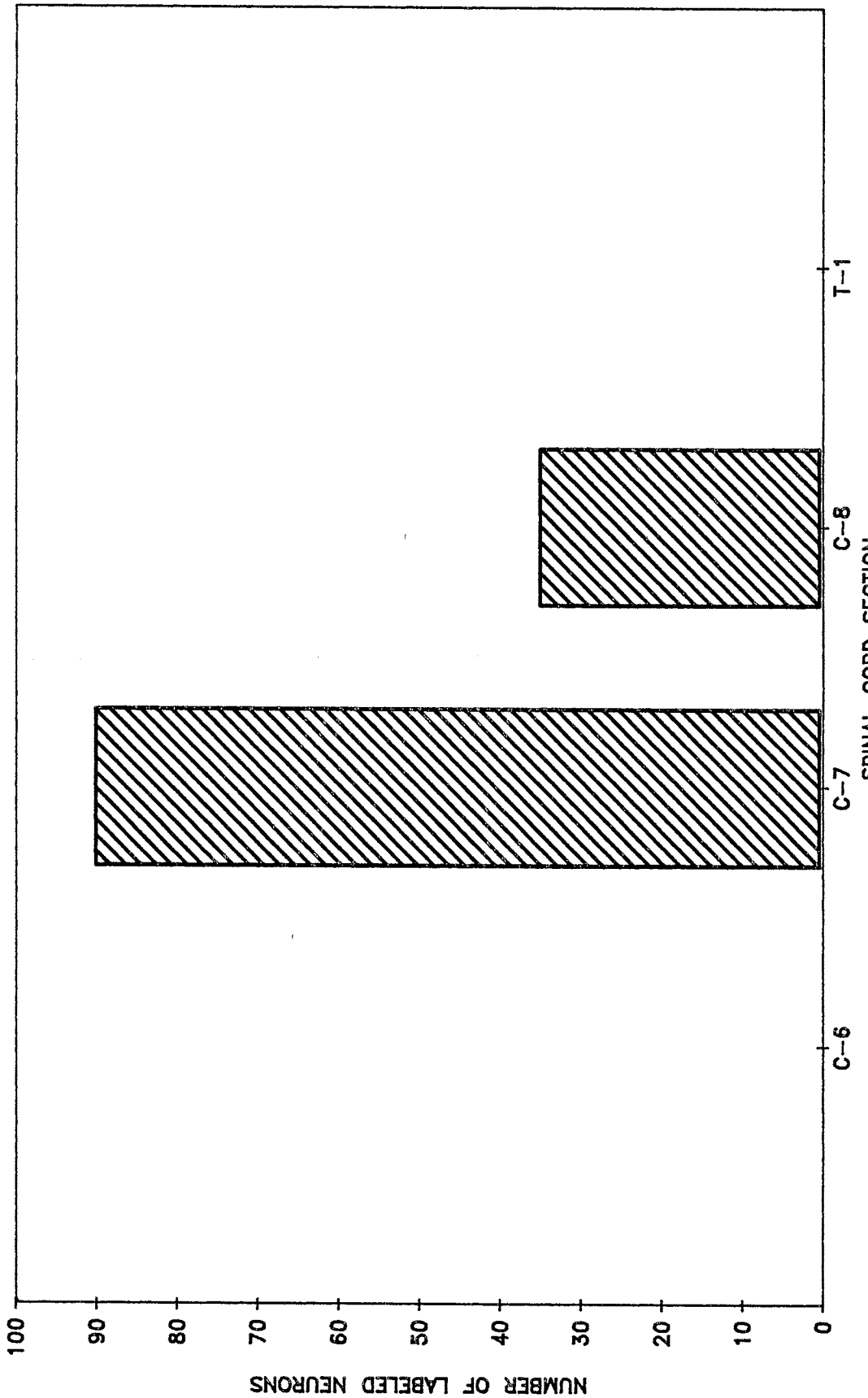

Figure 1a. Extensor Carpi Radialis

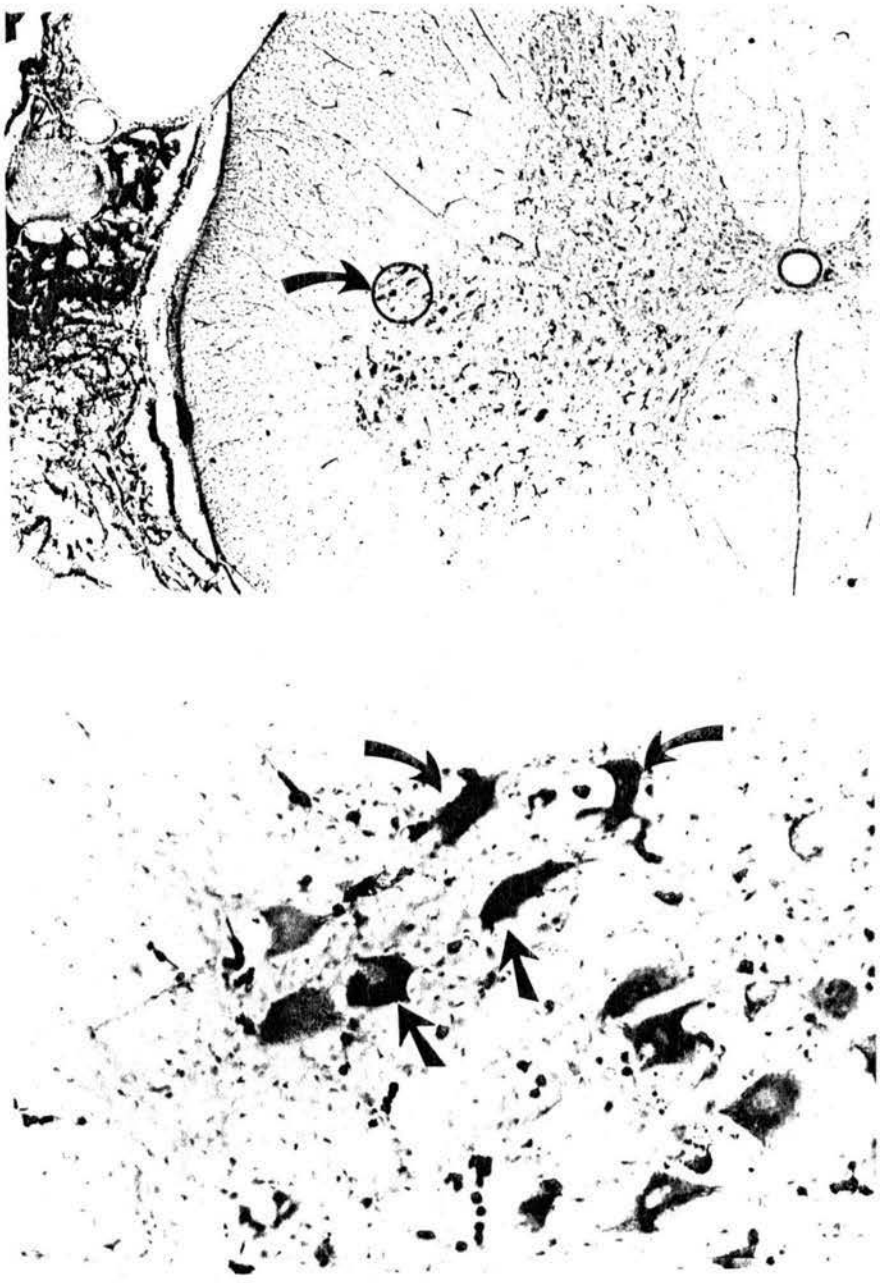

Figure 1b. Extensor Carpi Radialis
Upper photomicrograph shows a LPF demonstrating the location of labled neurons in the ventral horn. Lower photomicrograph shows a HPF of these same labled neurons.

Common Digital Extensor

The main component of the bilateral columns formed by the labeled neurons supplying the common digital extensor muscle are located more laterally and less dorsally than the columns of the extensor carpi radialis After isolation and perfusion of the common digital extensor muscle with horseradish peroxidase, five-hundred-thirty-three labeled neurons were found in the dog being studied Thirteen (2 4%) of these neurons were found in spinal cord segment C6, one-hundred-eight (20%) of these neurons in spinal cord segment C7, one-hundred-forty-four (25%) of these neurons in spinal cord segment C8, and two-hundred-sixty-eight (50%) in segment T1. The columns extend from the last one-eigth of C6 to the end of T1 as depicted by figures 2, 2a, and 2b As is readily seen by examining the above figures, the columns formed by the neurons supplying the common digital extensor are intermingled with the columns formed by the neurons supplying the other muscles of the antebrachium The columns formed by the neurons of the common digital extensor vary from the columns formed by the neurons of the other muscles of the antebrachium in that the columns of the common digital extensor encompass almost the entire length of the spinal cord from C6 through T1 and 50% of these neurons are located in segment T1

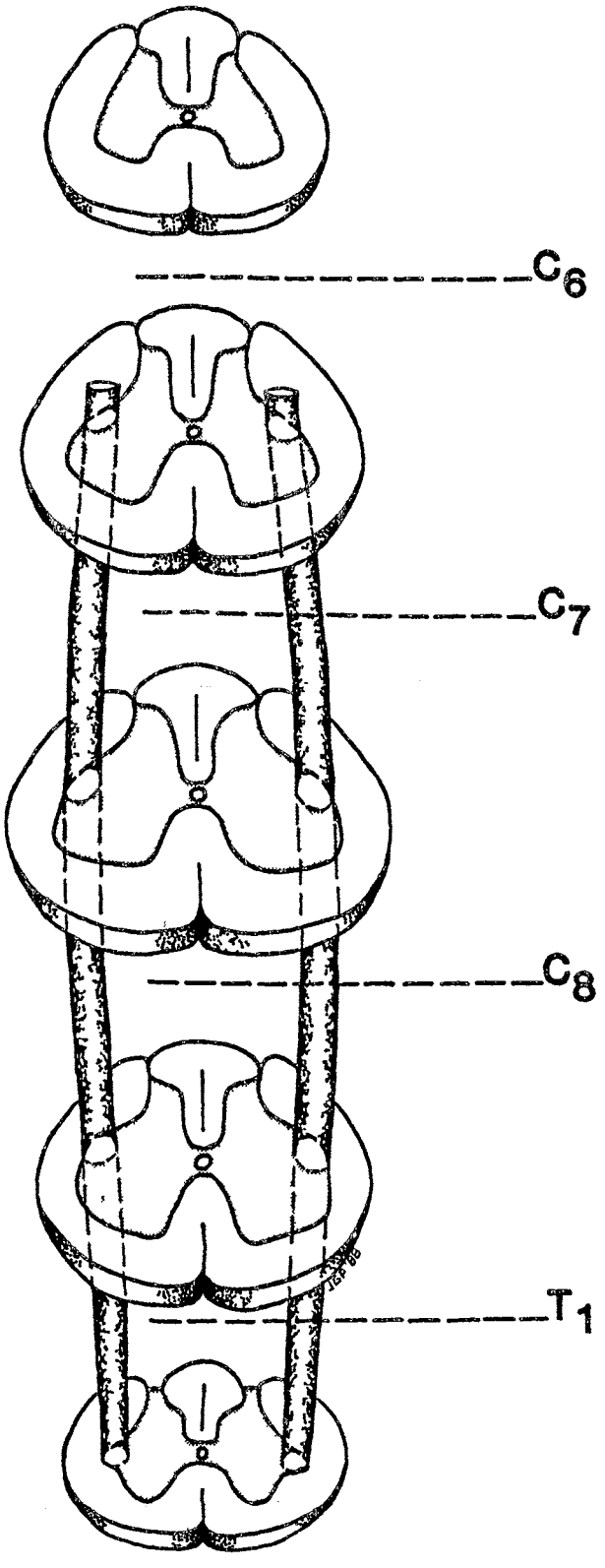

Figure 2. Common Digital Extensor

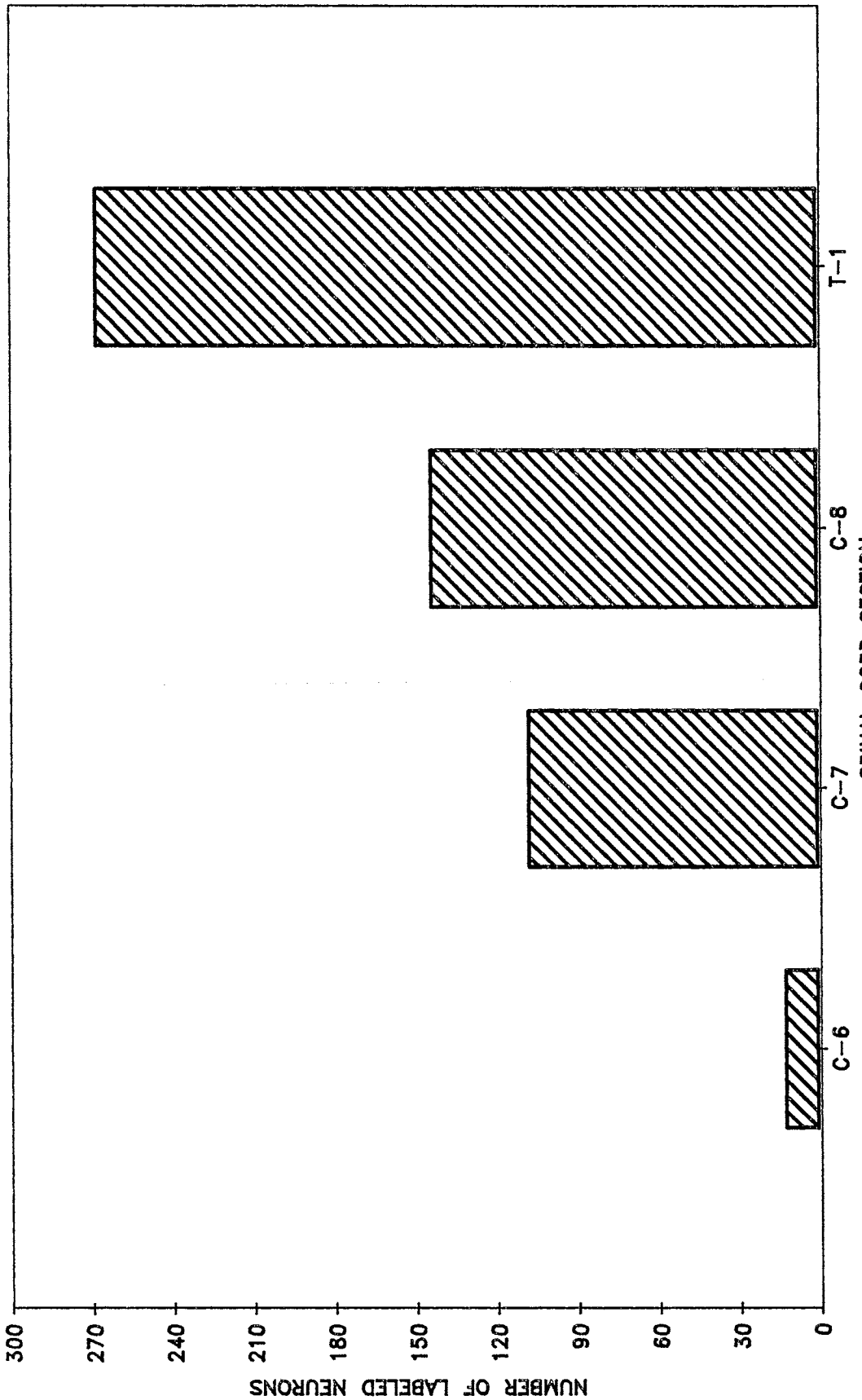

Figure 2a. Common Digital Extensor

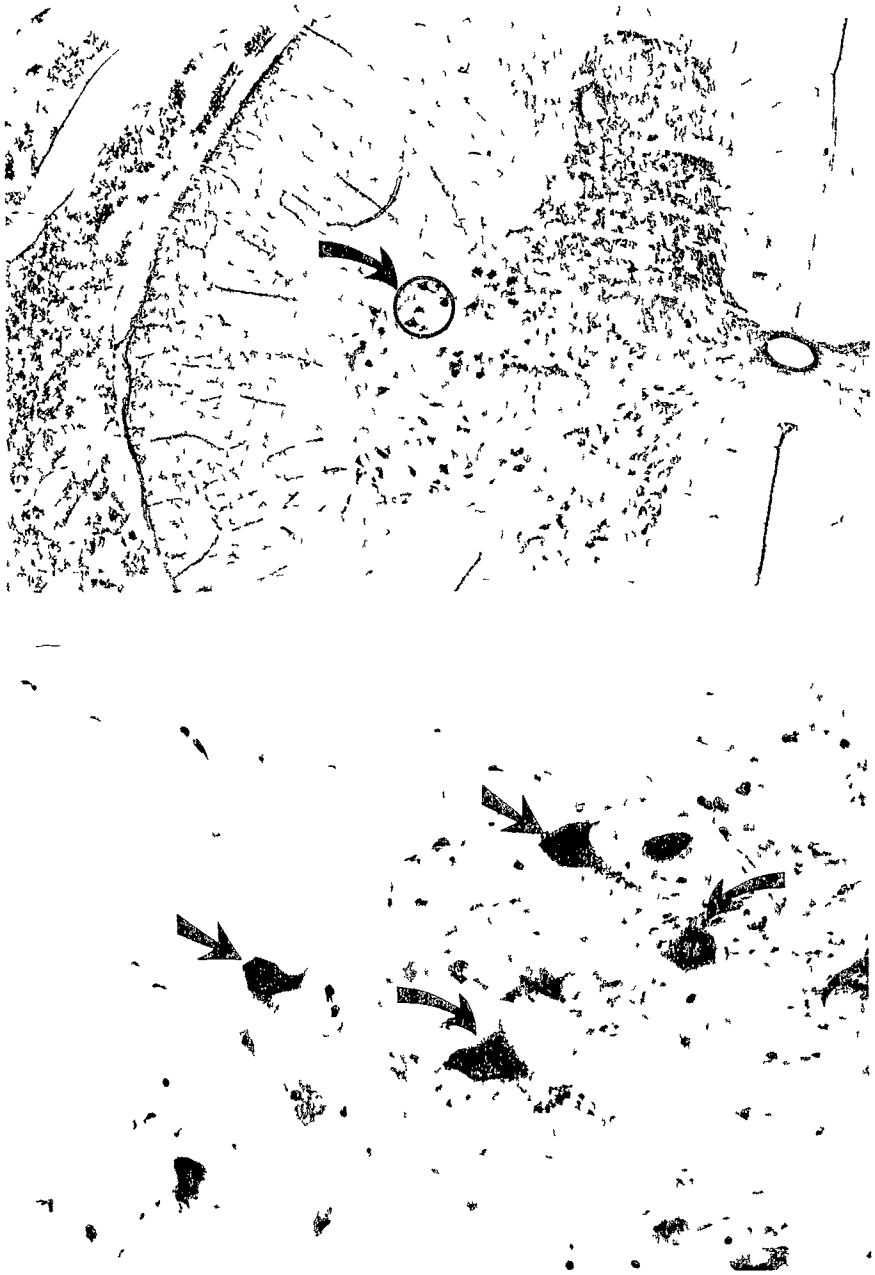

Figure 2b Common Digital Extensor
Upper photomicrograph shows a LPF demonstrating the location of labled neurons in the ventral horn
Lower photomicrograph shows a HPF of these same labled neurons

Lateral Digital Extensor

In the animal being studied, the lateral digital extensor muscle was isolated and perfused with horseradish peroxidase. This perfusion resulted in one-hundred-forty-one labeled neurons The main component of the bilateral columns formed by these labeled neurons is located dorsal laterally in the ventral horn. The columns are confined completely to spinal cord segment T1 and run from the beginning of T1 to its end as depicted by figures 3, 3a, and 3b As with the previous muscles, the columns formed by the neurons supplying the lateral digital extensor are intermingled with the columns formed by the neurons supplying the other muscles of the antebrachium. The main difference between the columns formed by the neurons of the lateral digital extensor and the columns formed by the neurons of the other muscles of the antebrachium is that the columns of the lateral digital extensor encompass the entire length of the spinal cord segment T1

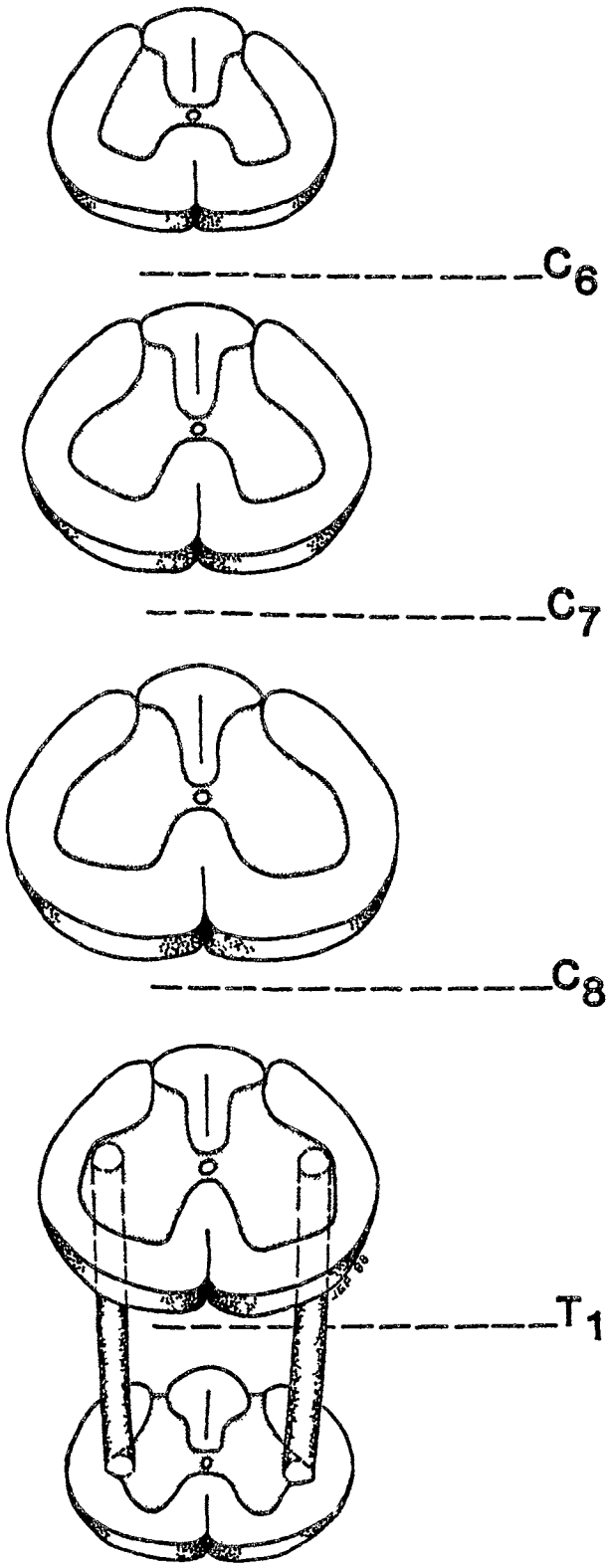

Figure 3. Lateral Digital Extensor

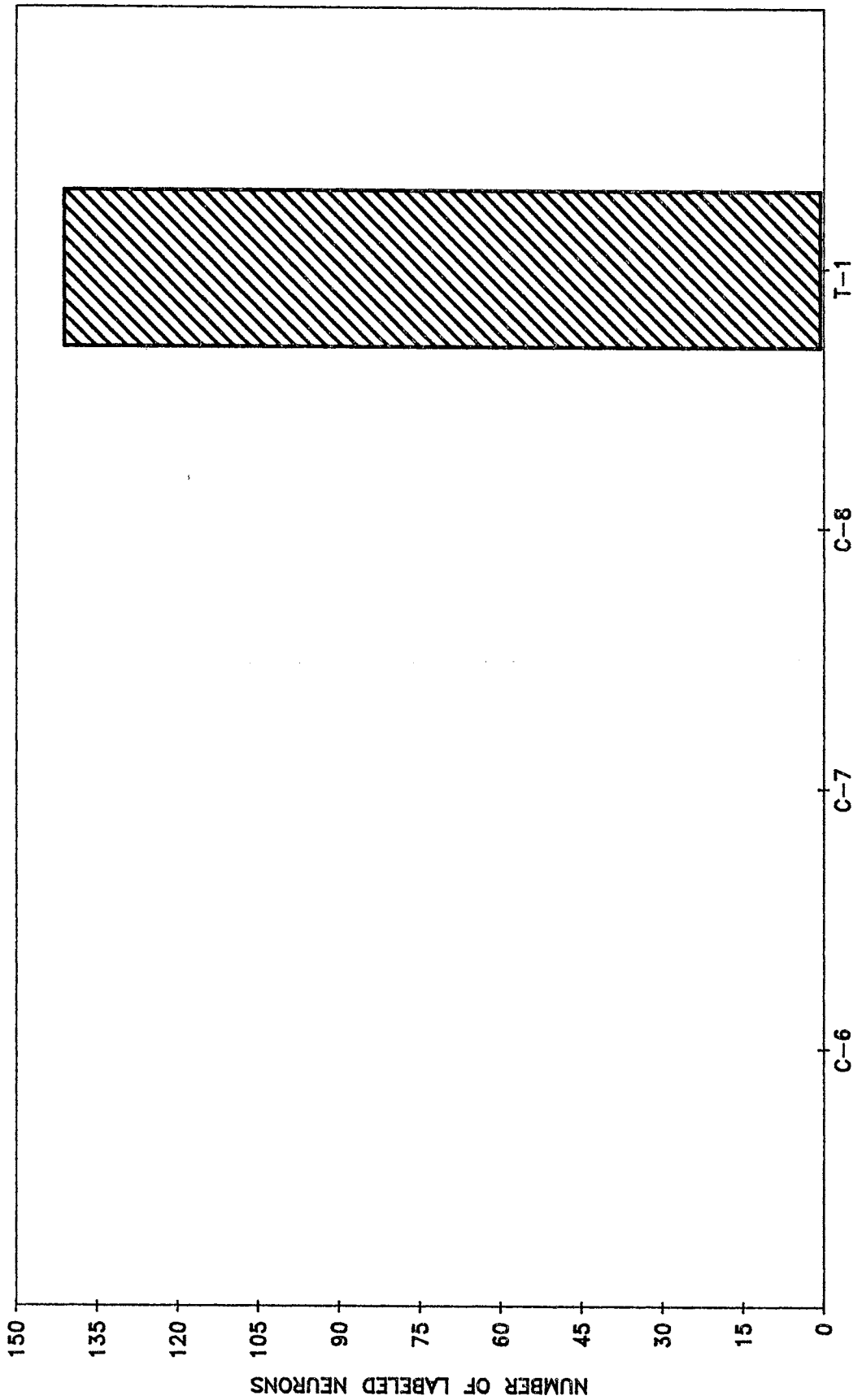

Figure 3a. Lateral Digital Extensor

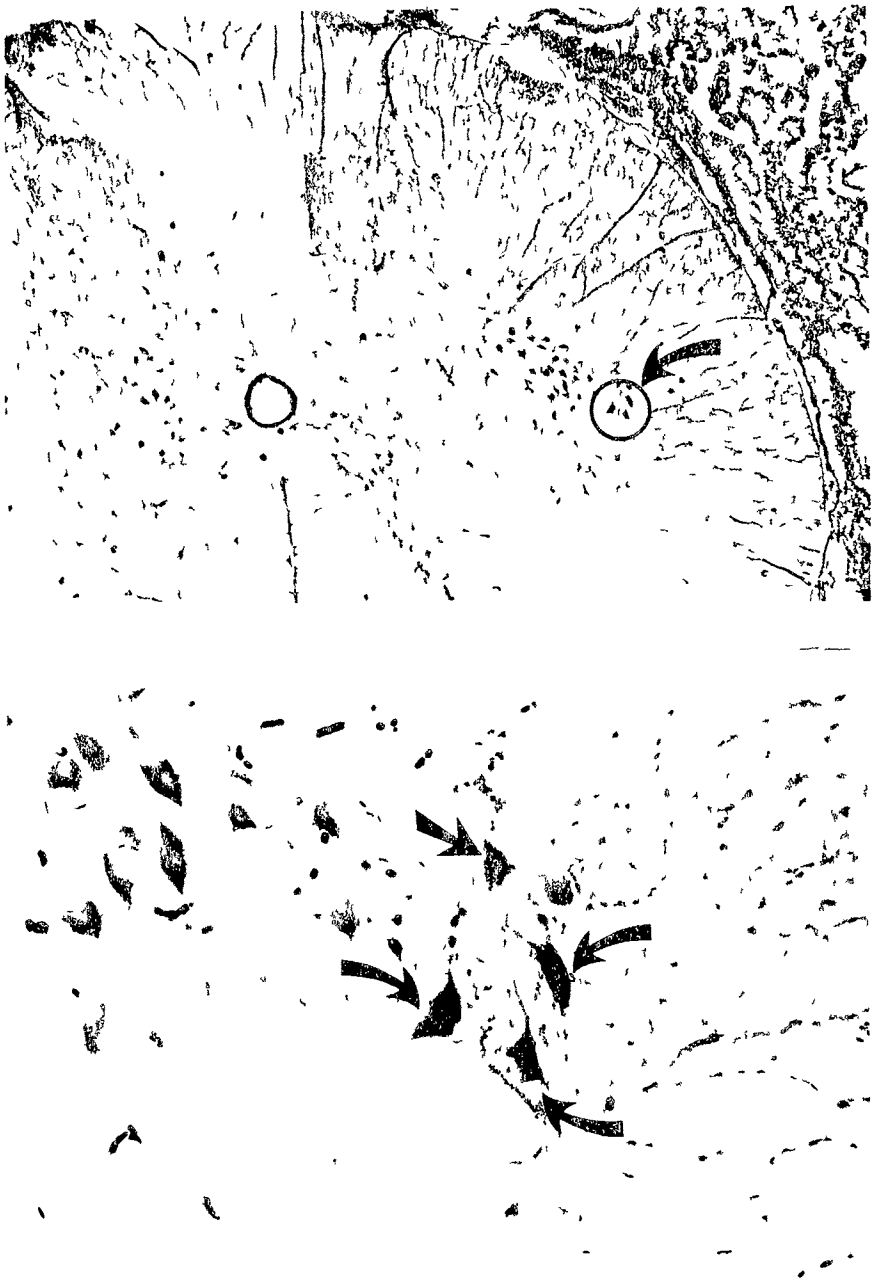

Figure 3b Lateral Digital Extensor
Upper photomicrograph shows a LPF demonstrating the location of labled neurons in the ventral horn
Lower photomicrograph shows a HPF of these same labled neurons

Extensor Carpı Ulnarıs

Two-hundred-fıfteen labeled neurons were found ın bılateral columns ın the gray matter of the spınal cord after perfusıon of the extensor carpı ulnarıs muscle wıth horseradısh peroxıdase The columns are located more medıal and mıdway between the top and bottom of the ventral horn extendıng from nearly the end of C7 to the end of C8 as depıcted ın fıgures 4, 4a, and 4b. Eleven (5%) of these neurons were found ın spınal cord segment C7 and two-hundred-four (95%) ın segment C8 The above fıgures show that the columns formed by the neurons supplyıng the extensor carpı ulnarıs are ıntermıngled wıth the columns formed by the neurons supplyıng the other muscles of the antebrachıum. The columns formed by the neurons of the extensor carpı ulnarıs muscle dıffer from the columns formed by the neurons of the other muscles of the antebrachıum ın that the columns of the extensor carpı ulnarıs are located more ventrally than the other columns and are confıned almost completely to segment C8

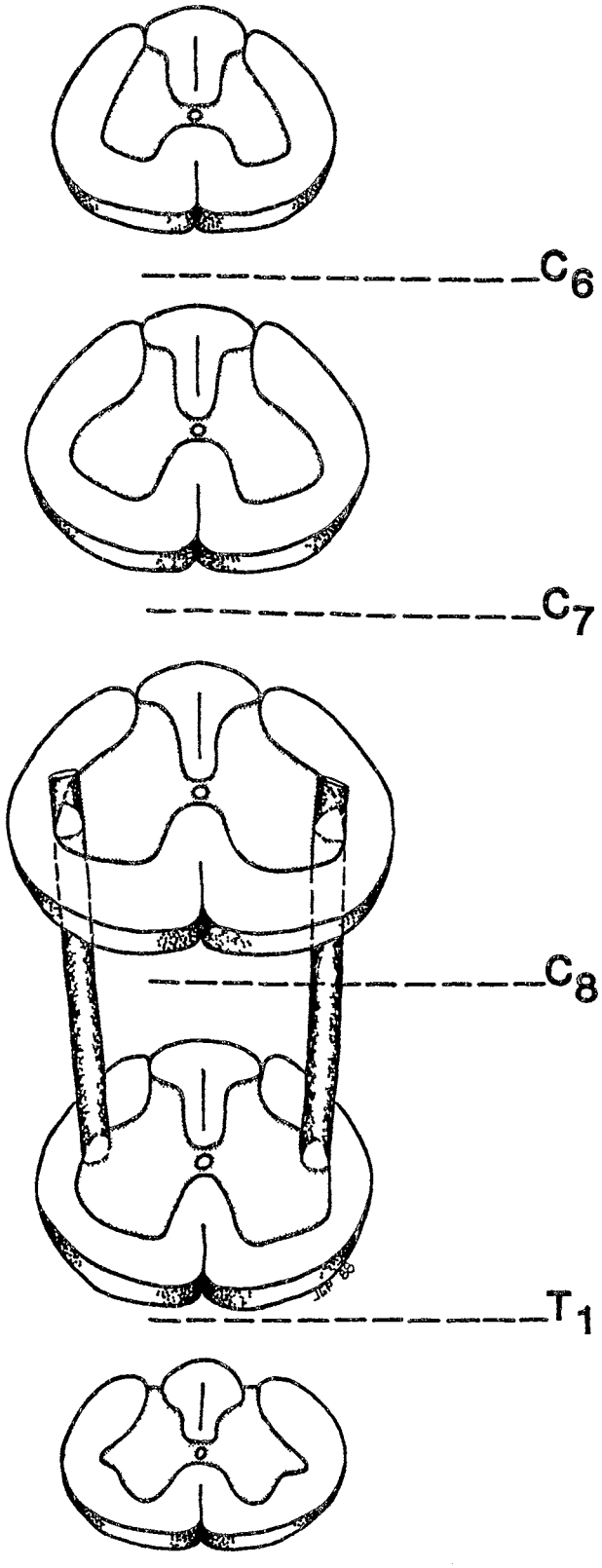

Figure 4. Ulnaris Lateralis

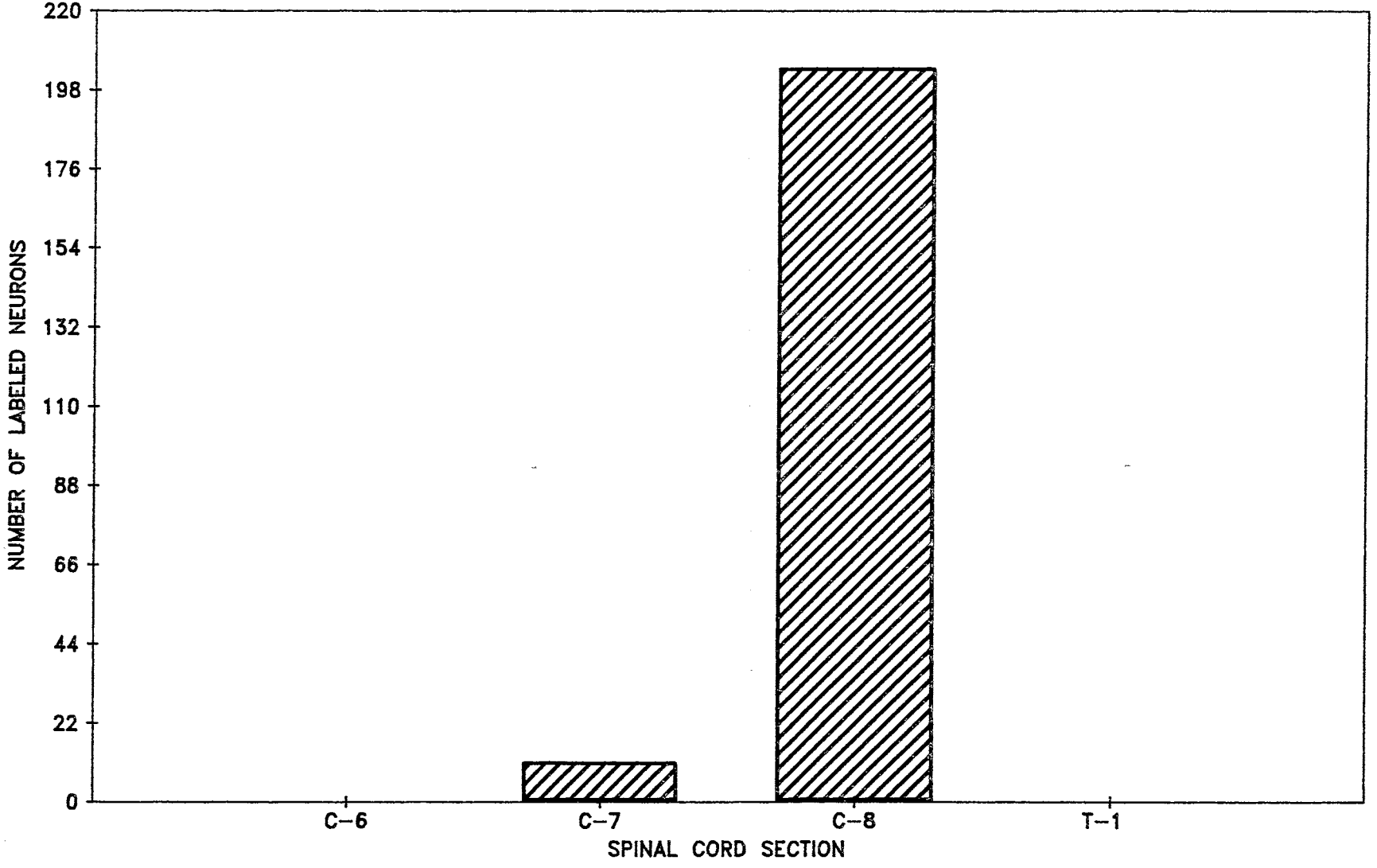

Figure 4a. Ulnaris Lateralis

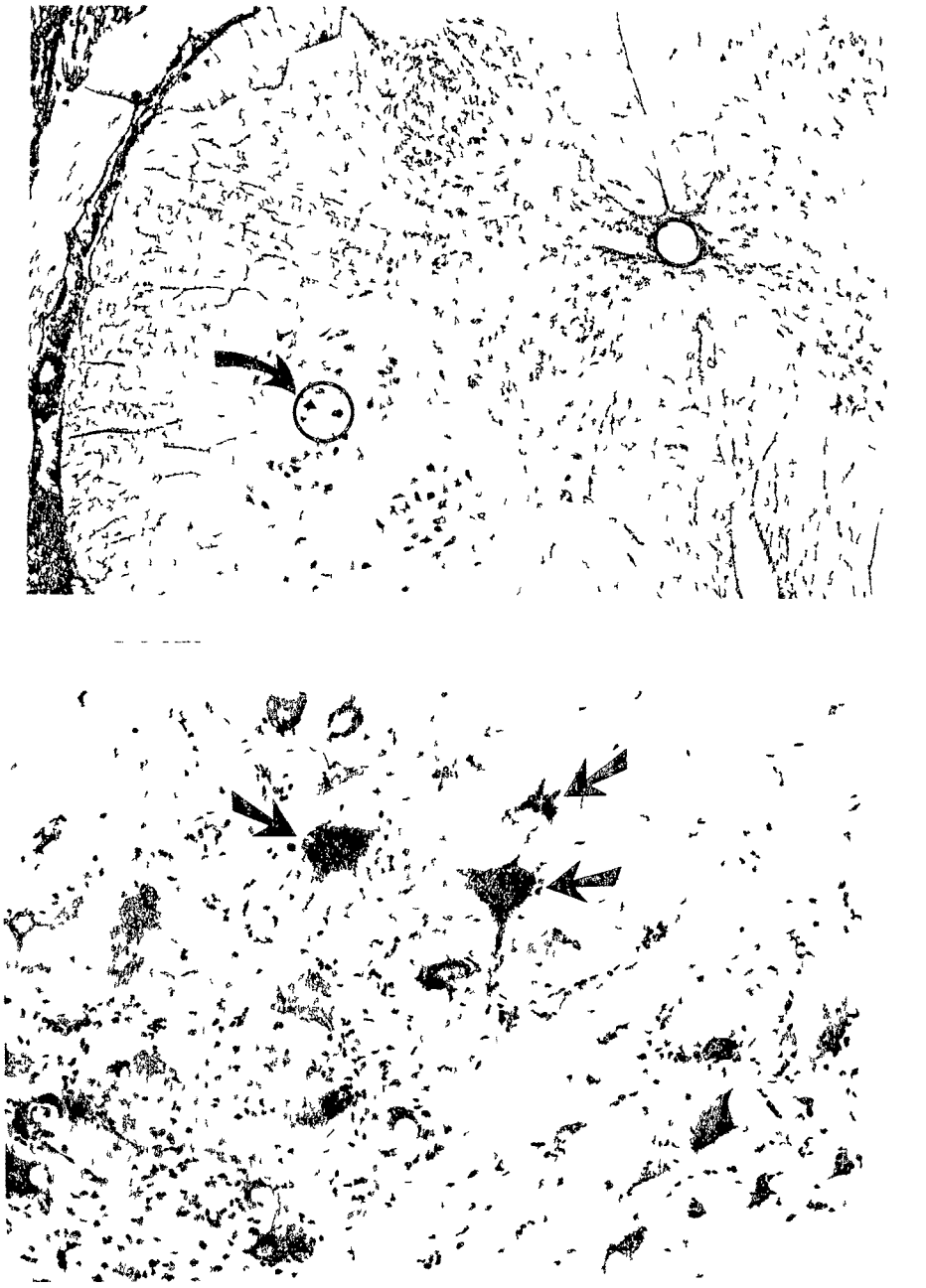

Figure 4b. Ulnaris Lateralis
Upper photomicrograph shows a LPF demonstrating the location of labled neurons in the ventral horn
Lower photomicrograph shows a HPF of these same labled neurons

Deep Digital Flexor (ulnar head)

In the dog used to study the deep digital flexor (ulnar head) muscle, bilateral columns of labeled neurons were found after isolation and perfusion of the muscle with horseradish peroxidase This procedure resulted in sixteen labeled neurons. Four (25%) of these neurons were found in spinal cord segment C8 and twelve (75%) in segment T1 The columns were located in the most dorsal lateral part of the spinal cord ventral horn extending from the last one-eigth of C8 to the last one-fourth of T1 as depicted in figures 5, 5a, and 5b. The neurons supplying the deep digital flexor (ulnar head) form columns which are intermingled with the columns formed by the neurons supplying the other muscles of the antebrachium. The most significant difference between the columns formed by the neurons of the deep digital flexor (ulnar head) muscle and the columns formed by the neurons of the other muscles of the antebrachium is that the columns of the deep digital flexor (ulnar head) are located in a small area of the cord between C8 and T1 and that the number of neurons labeled is small compared with the columns supplying the other muscles of the antebrachium

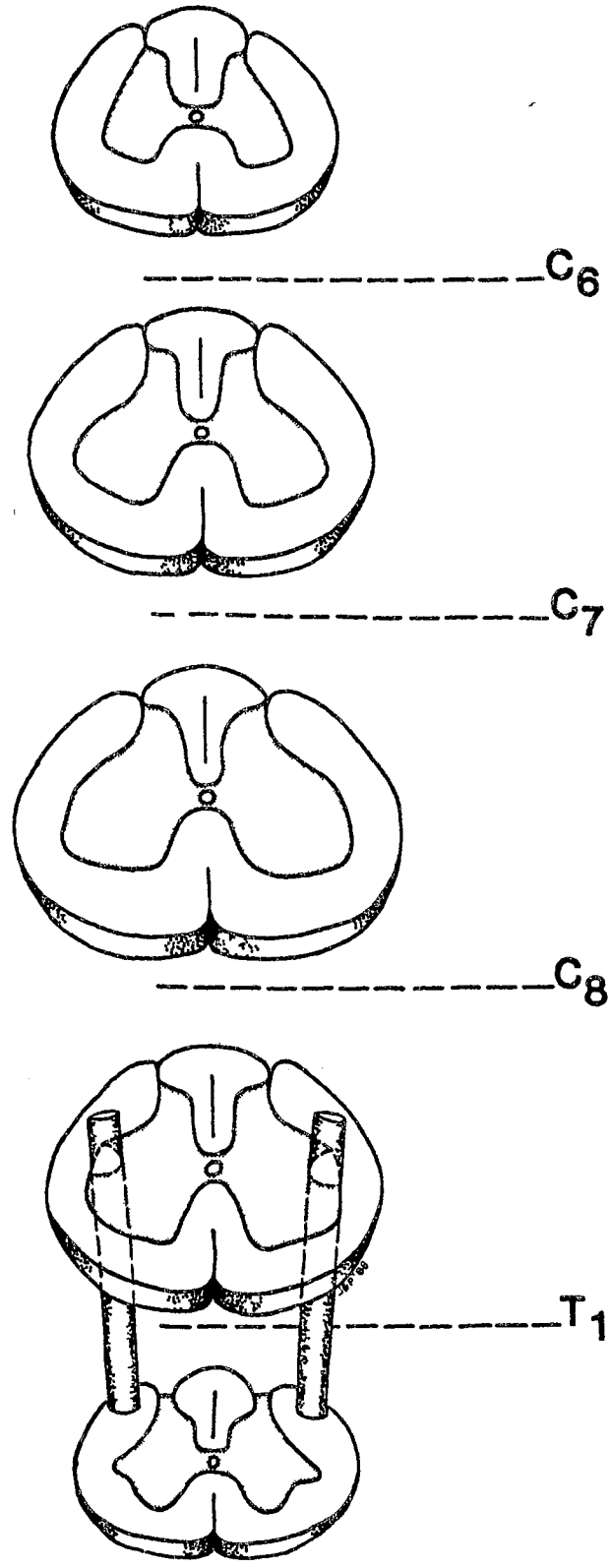

Figure 5. Deep Digital Flexor (Ulnar Head)

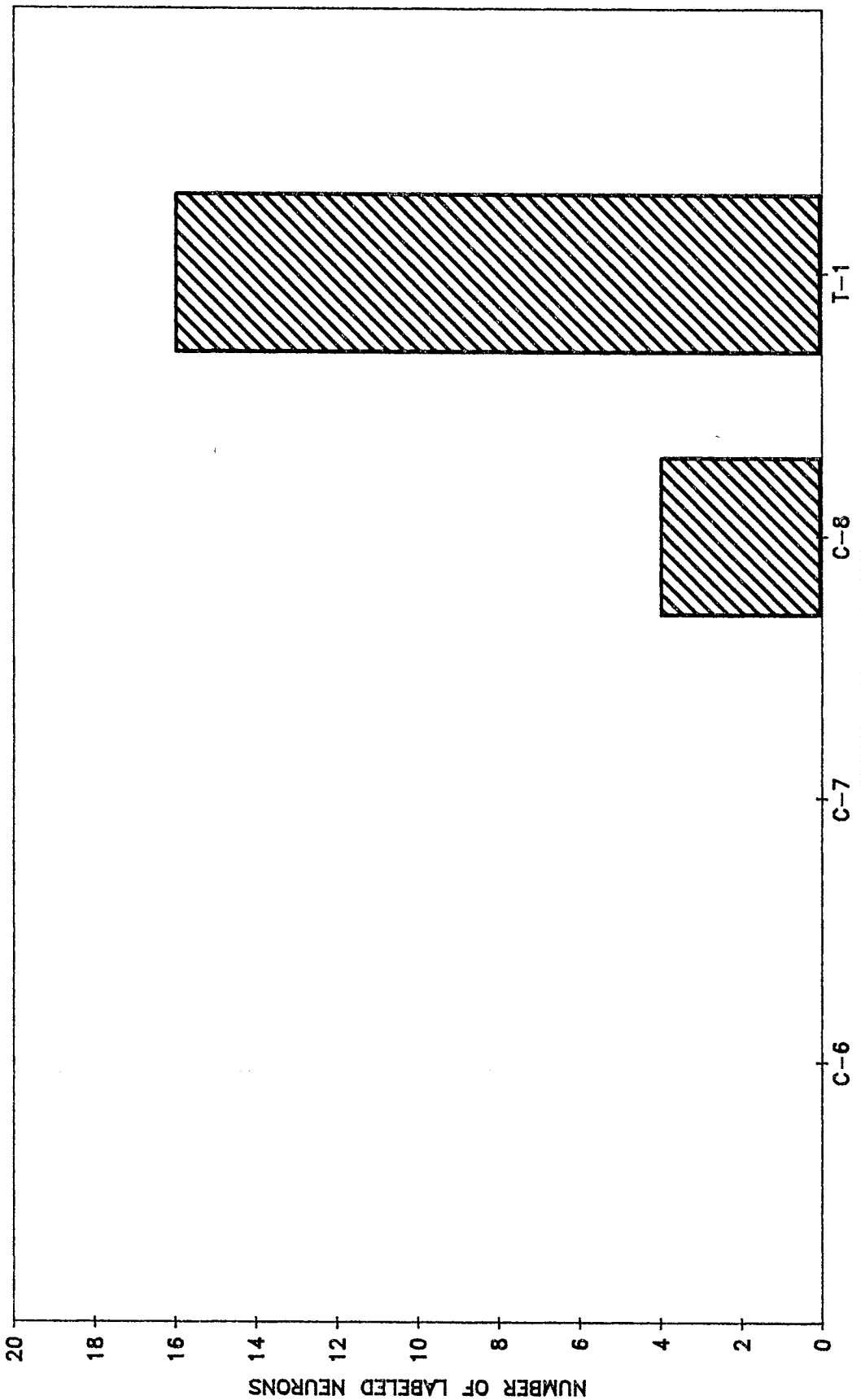

Figure 5a. Deep Digital Flexor, Ulnar Head

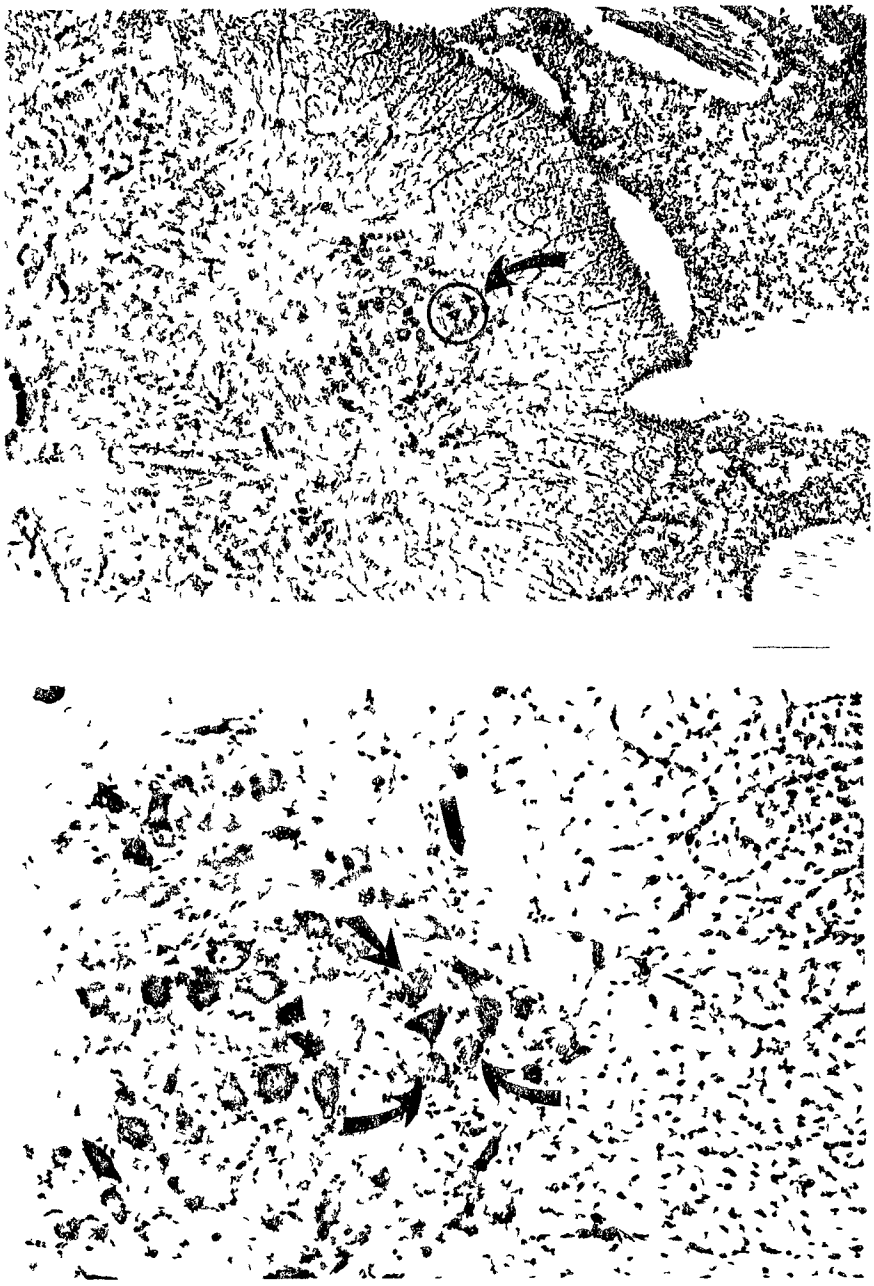

Figure 5b Deep Digital Flexor (ulnar Head)
Upper photomicrograph shows a LPF demonstrating the location of labled neurons in the ventral horn
Lower photomicrograph shows a HPF of these same labled neurons

Deep Digital Flexor (humeral head)

Seventy-seven labeled neurons were found in bilateral columns after isolation and perfusion of the deep digital flexor (humeral head) muscle with horseradish peroxidase Thirty-eight (49%) of these neurons were found in spinal cord segment C8, thirty-two (42%) in segment T1, and seven (9%) in segment T2 These labeled neurons form bilateral columns within the gray matter of the spinal cord which are intermingled with the columns formed by the neurons supplying the other muscles of the antebrachium These columns appear to be more separate than the columns formed by the neurons of the other muscles of the antebrachium The columns are located in the dorsal middle portion of the ventral horn and are not as dorsal nor as lateral as the columns discussed previously The columns begin in the first one-third of the ventral horn of C8 and continue through the first one-fourth of the ventral horn of T2 as depicted in figures 6, 6a, and 6b

The most striking difference between the columns formed by the neurons of the deep digital flexor (humeral head) and the other columns already discussed is the more medial position of the columns of the deep digital flexor (humeral head) and the extension of these columns into spinal cord segment T2.

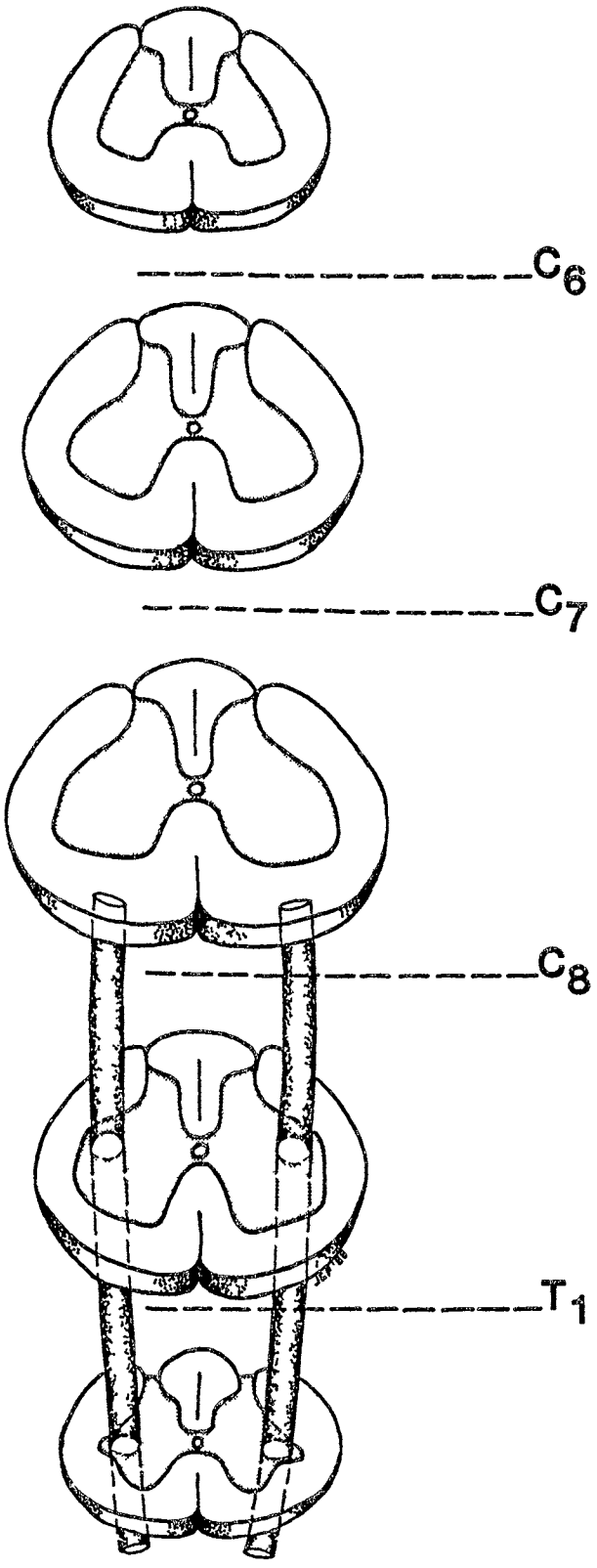

Figure 6. Deep Digital Flexor (Humeral Head)

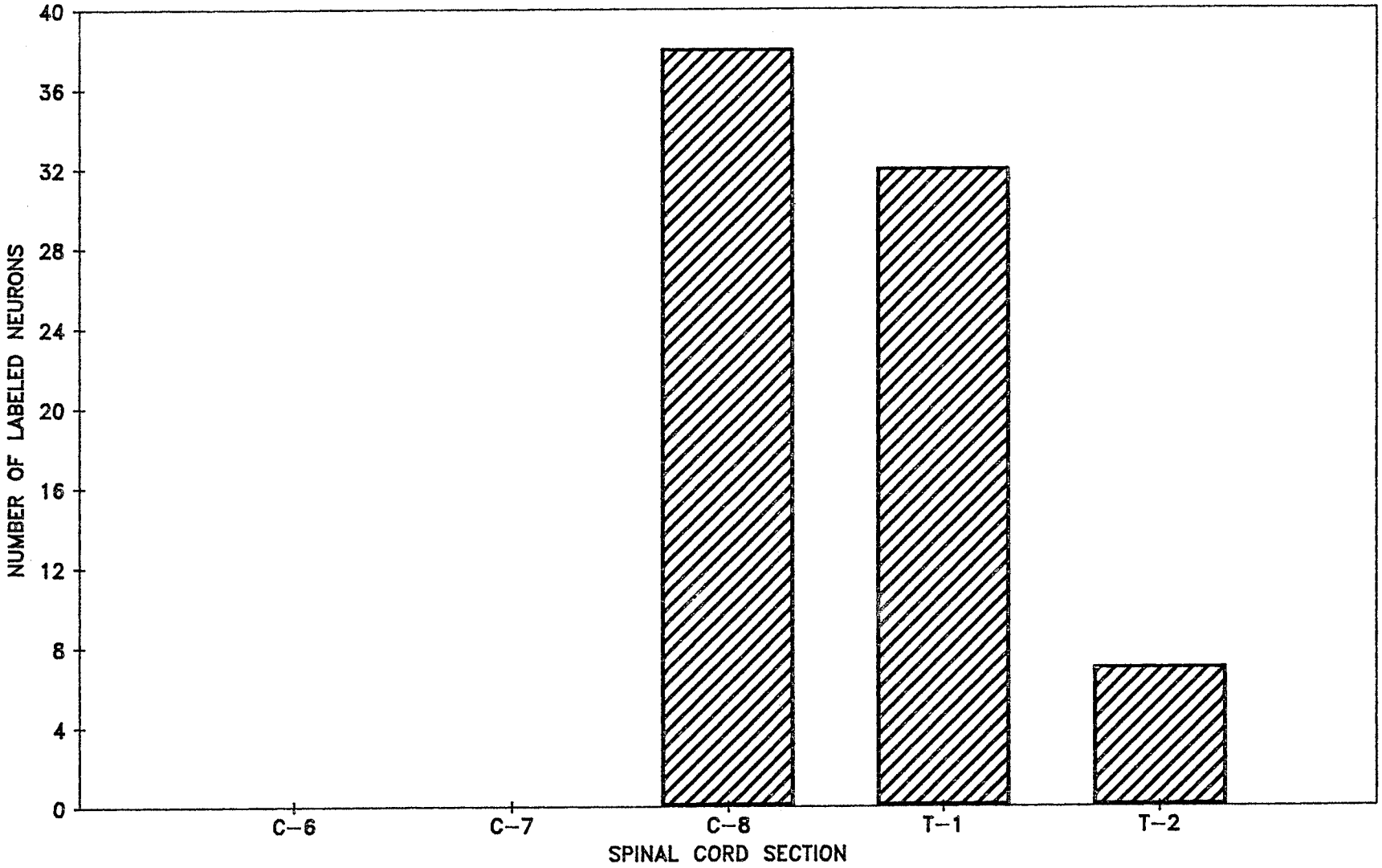

Figure 6a. Deep Digital Flexor, Humeral Head

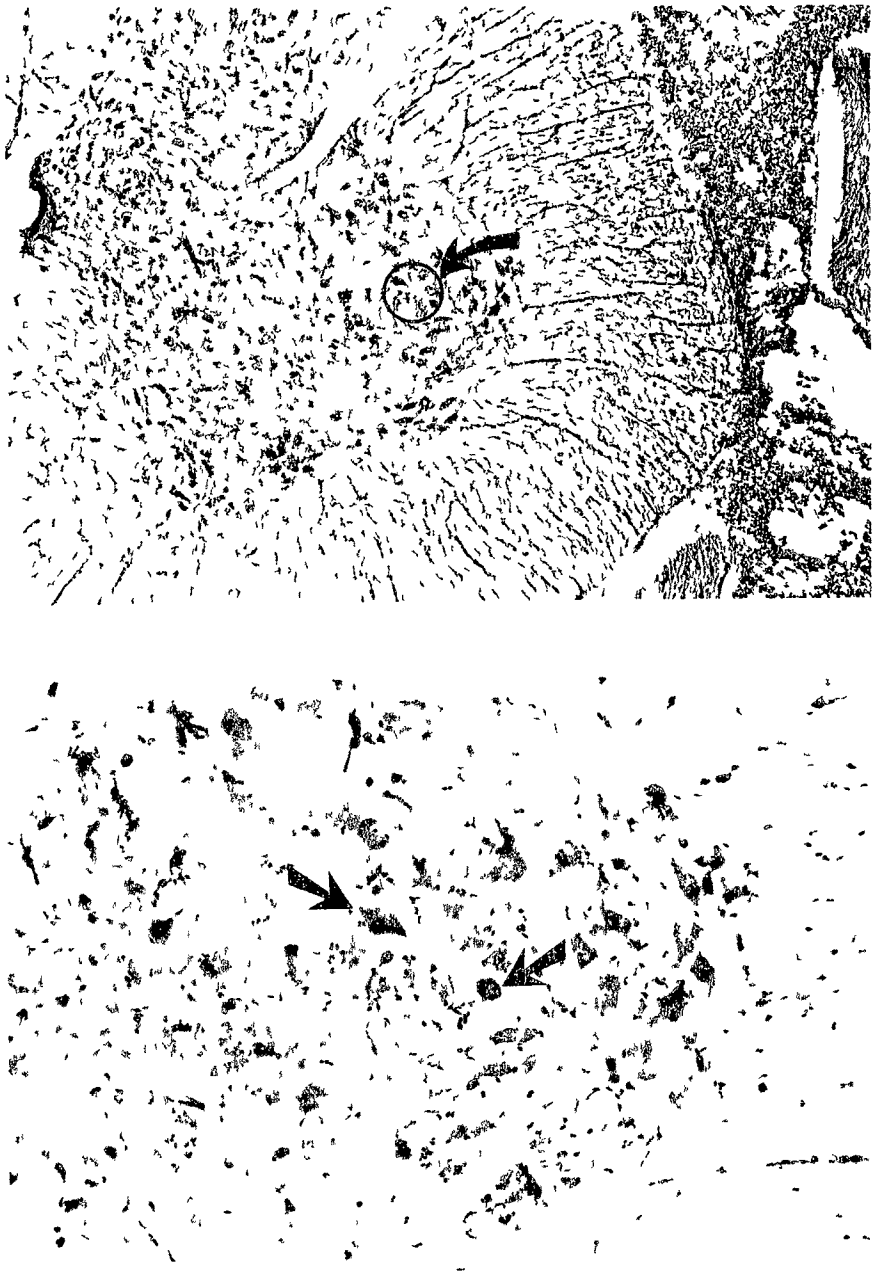

Figure 6b Deep Digital Flexor (humeral head)
Upper photomicrograph shows a LPF demonstrating the location of labled neurons in the ventral horn
Lower photomicrograph shows a HPF of these same labled neurons

Deep Digital Flexor (radial head)

The neuronal columns formed after isolation and perfusion of the deep digital flexor (radial head) muscle with horseradish peroxidase were located medially in a region midway between the most dorsal and most ventral extremes of the gray matter of the ventral horn of spinal cord segment T1, as depicted in figures 7, 7a, and 7b. The perfusion of this muscle resulted in twelve labeled neurons all confined to a small portion of spinal cord segment T1 These labeled neurons form bilateral columns within the gray matter of the spinal cord which are partly intermingled with the columns formed by the neurons supplying the other muscles of the antebrachium. As with the column formed by the neurons of the deep digital flexor (humeral head), these columns were more separate than columns formed by the neurons supplying the other muscles of the antebrachium The columns formed by the neurons of the deep digital flexor (radial head) muscle vary from the columns formed by the neurons of the other muscles of the antebrachium in that the columns of the deep digital flexor (radial head) are confined solely to a very small portion of spinal cord section T1

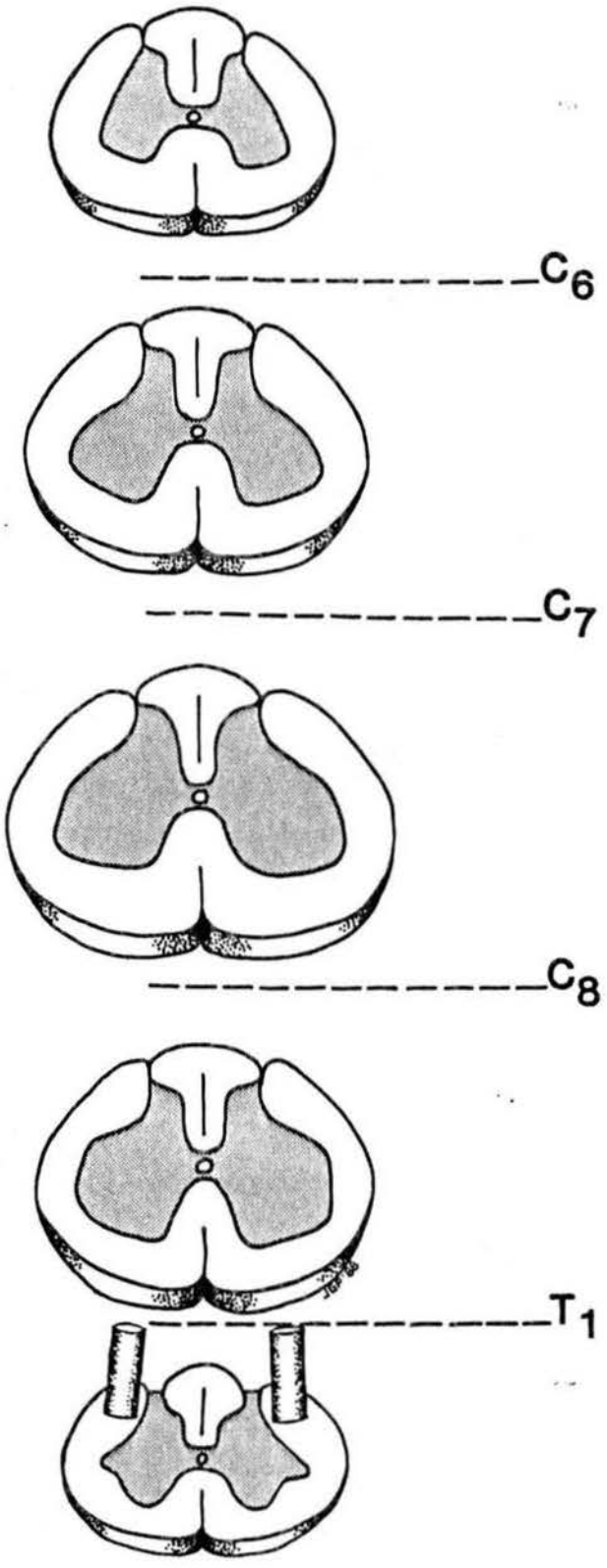

Figure 7. Deep Digital Flexor (Radial Head)

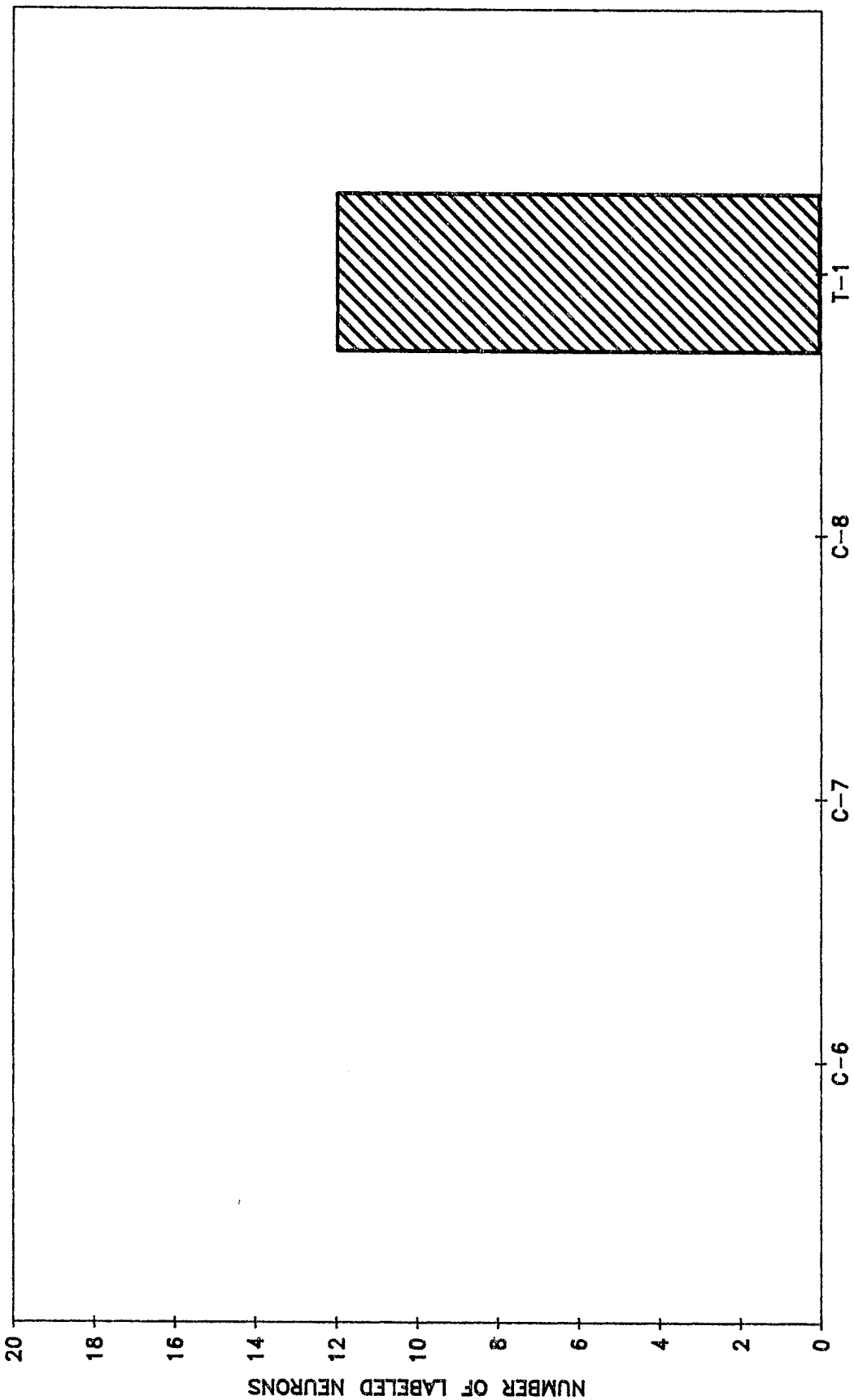

Figure 7a. Deep Digital Flexor, Radial Head

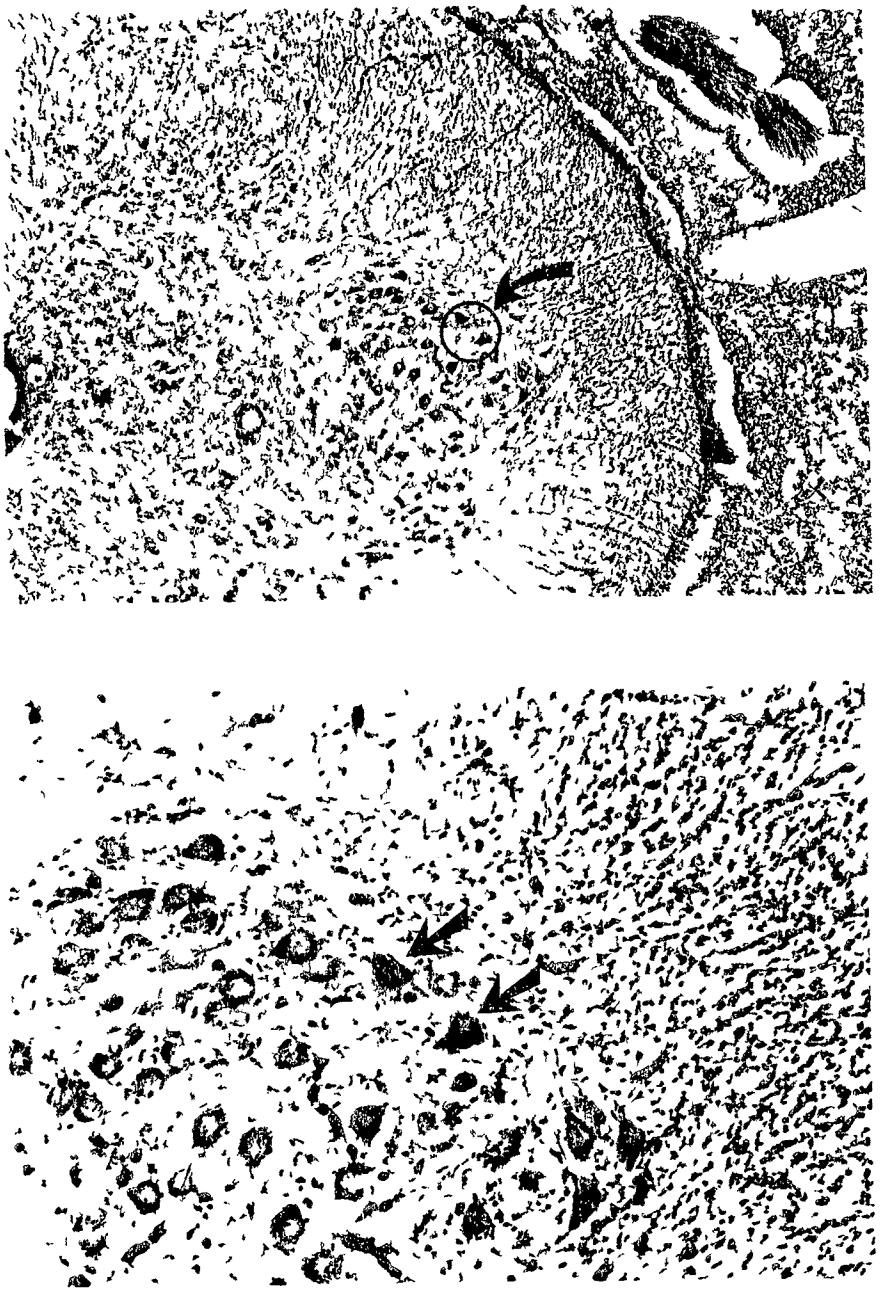

Figure 7b Deep Digital Flexor (radial head)
Upper photomicrograph shows a LPF demonstrating the location of labled neurons in the ventral horn
Lower photomicrograph shows a HPF of these same labled neurons

Flexor Carpi Ulnaris (humeral head)

The columns formed by the neurons supplying the extensor carpi ulnaris (humeral head) muscle were labeled after isolation and perfusion of the muscle with horseradish peroxidase This procedure resulted in one-hundred-six labeled neurons Sixty (57%) of these neurons were found in spinal cord segment C8 and forty-six (43%) in segment T1 The bilateral columns formed within the gray matter of the spinal cord by the labeled neurons begin in the dorsal middle area of the ventral horn gray matter in the first one-eighth of spinal cord segment C8 and end in the center of the ventral horn at the end of segment T1 This distribution is depicted in figures 8, 8a, and 8b. The columns formed by the neurons supplying the extensor carpi ulnaris (humeral head) are intermingled with the columns formed by the neurons supplying the other muscles of the antebrachium Even though these columns intermingle with the other columns, their location near the middle of the ventral horn sets them some what apart allowing them to be more easily visualized as distinct entities The columns formed by the neurons of the flexor carpi ulnaris (humeral head) differ from the columns formed by the neurons of the other muscles of the antebrachium by being more medial than any of the other columns formed by the neurons supplying the other muscles of the antebrachium.

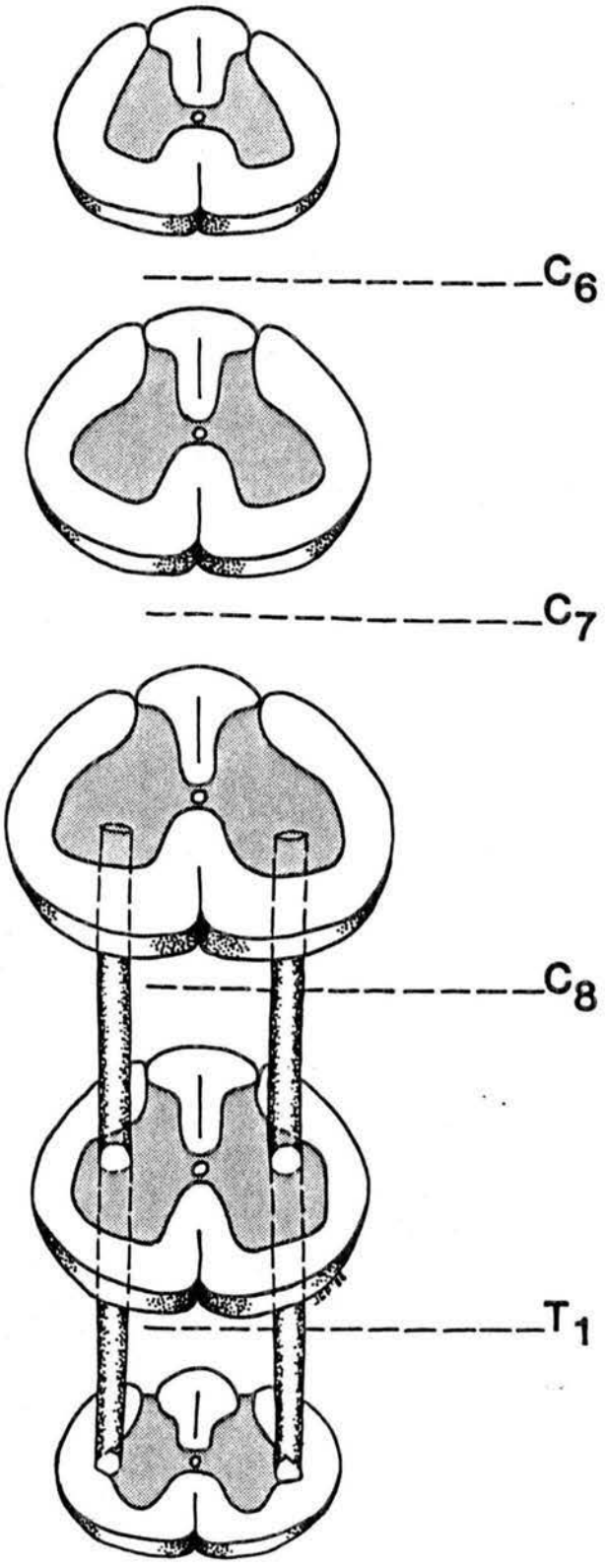

Figure 8. Flexor Carpi Ulnaris (Humeral Head)

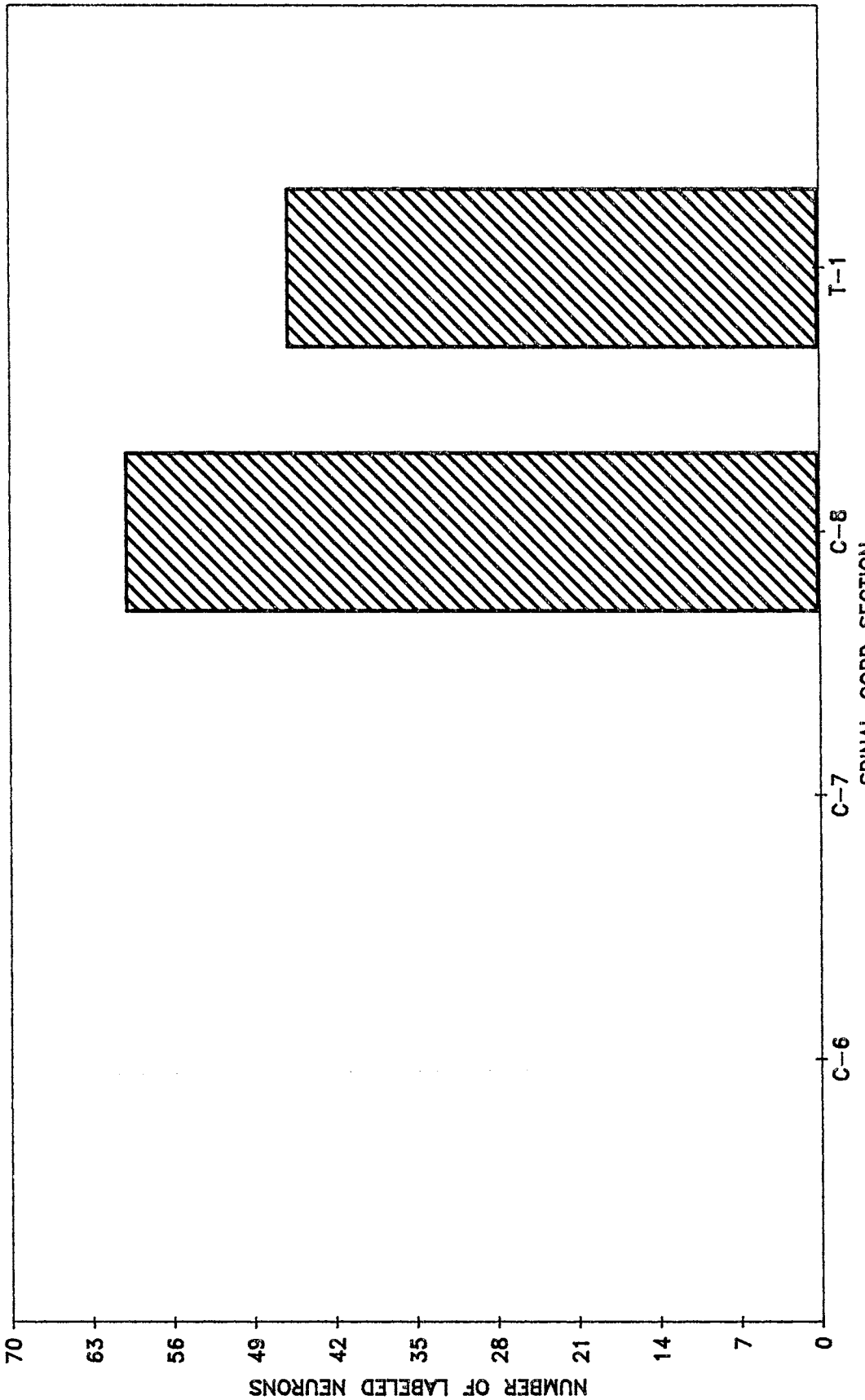

Figure 8a. Flexor Carpi Ulnaris, Humeral Head

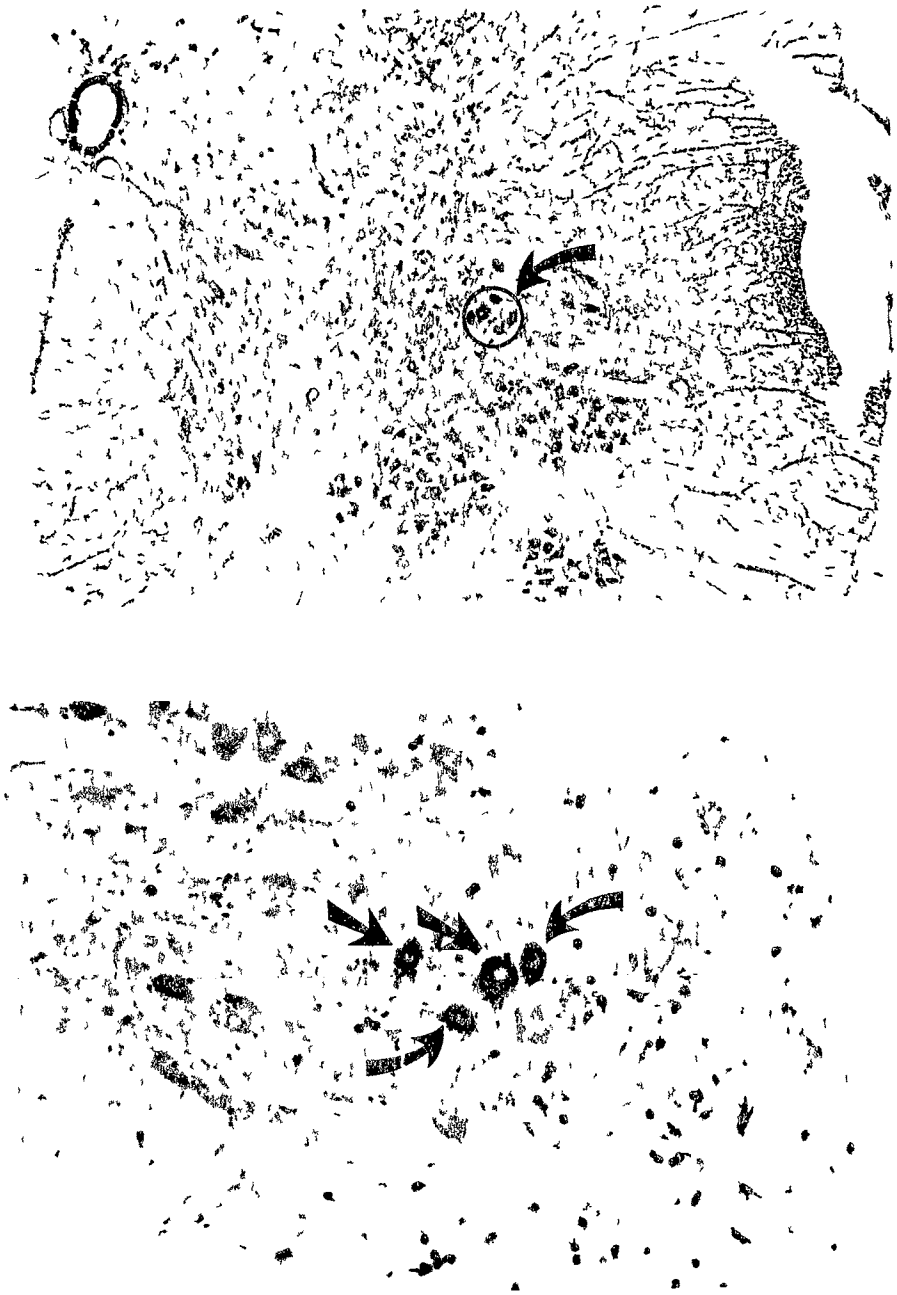

Figure 8b Flexor Carpi Ulnaris (humeral head)
Upper photomicrograph shows a LPF demonstrating the location of labled neurons in the ventral horn
Lower photomicrograph shows a HPF of these same labled neurons

Flexor Carpı Ulnarıs (ulnar head)

The columns formed by ısolatıon and perfusıon of the flexor carpı ulnarıs (ulnar head) muscle wıth horseradısh peroxıdase were located ın the most dorsal lateral part of the spınal cord ventral horn These labeled neurons form bılateral columns wıthın the gray matter of the spınal cord. The maın component of the columns movıng medıally ın segment C8 and extendıng from the mıddle of C7 to the end of C8 as depıcted ın fıgures 9, 9a, and 9b. The horseradısh peroxıdase perfusıon technıque resulted ın one-hundred-twenty-three labeled neurons Fıfty-two (42%) of these neurons were found ın spınal cord segment C7 and seventy-one (58%) ın segment C8 The columns formed by the neurons supplyıng the flexor carpı ulnarıs (ulnar head) are ıntermıngled wıth the columns formed by the neurons supplyıng the other muscles of the antebrachıum. These columns dıffer from the columns formed by neurons of the other muscles of the antebrachıum ın that the columns of the flexor carpı ulnarıs (ulnar head) move dıstınctly medıally as they progress from segment C7 through segment C8.

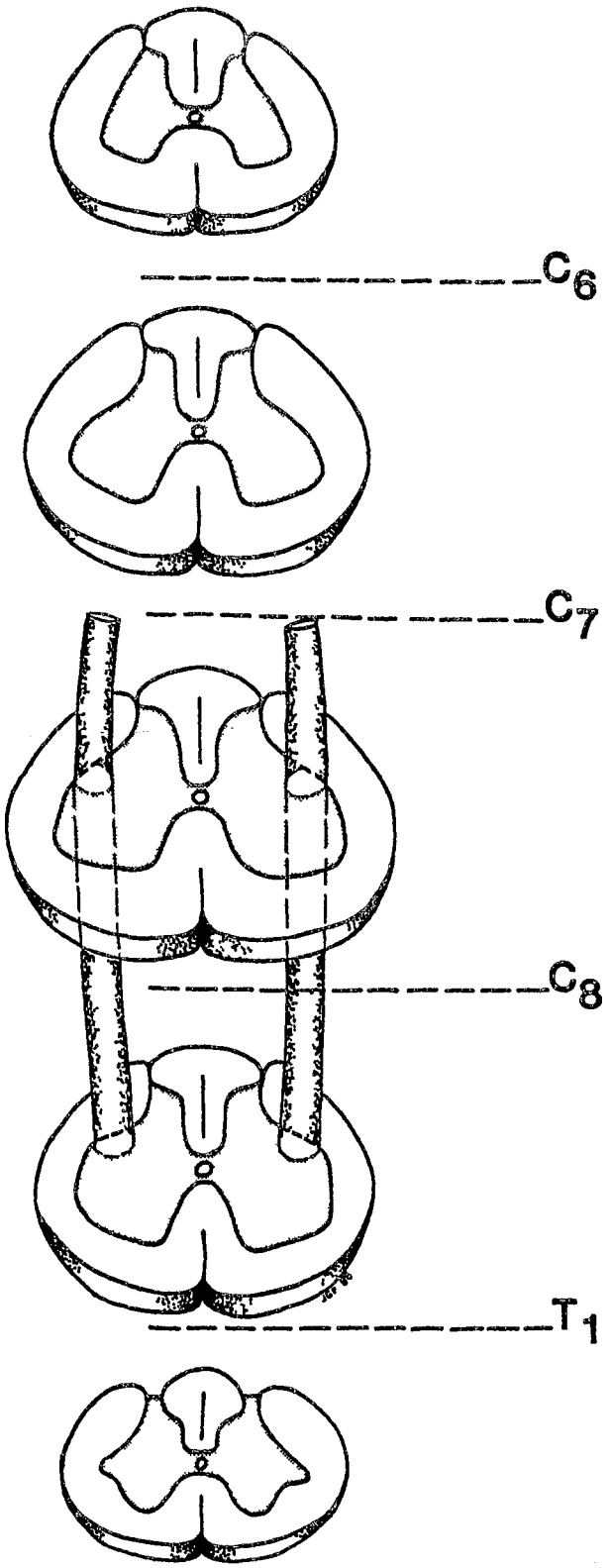

Figure 9. Flexor Carpi Ulnaris (Ulnar Head)

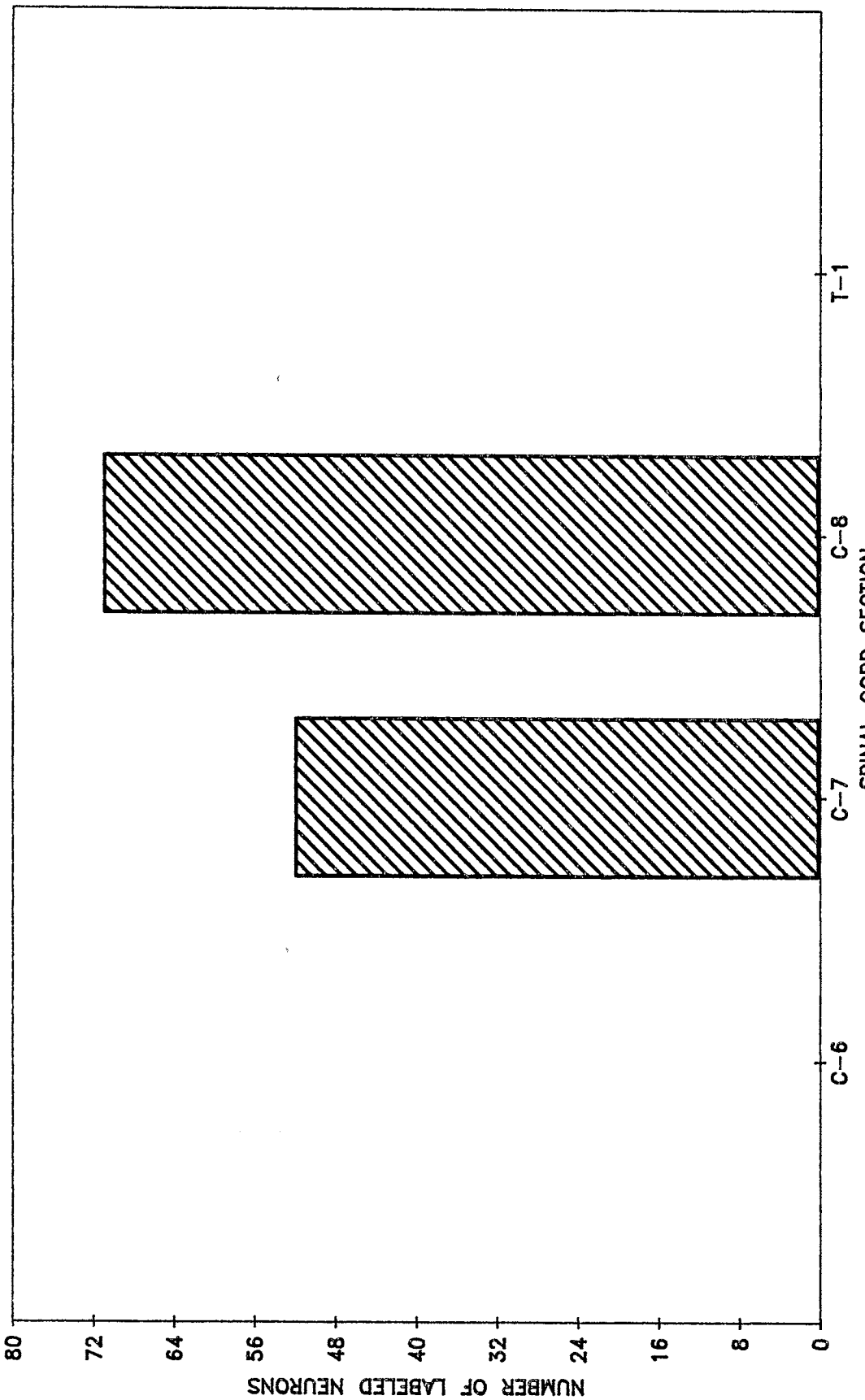

Figure 9a. Flexor Carpi Ulnaris, Ulnar Head

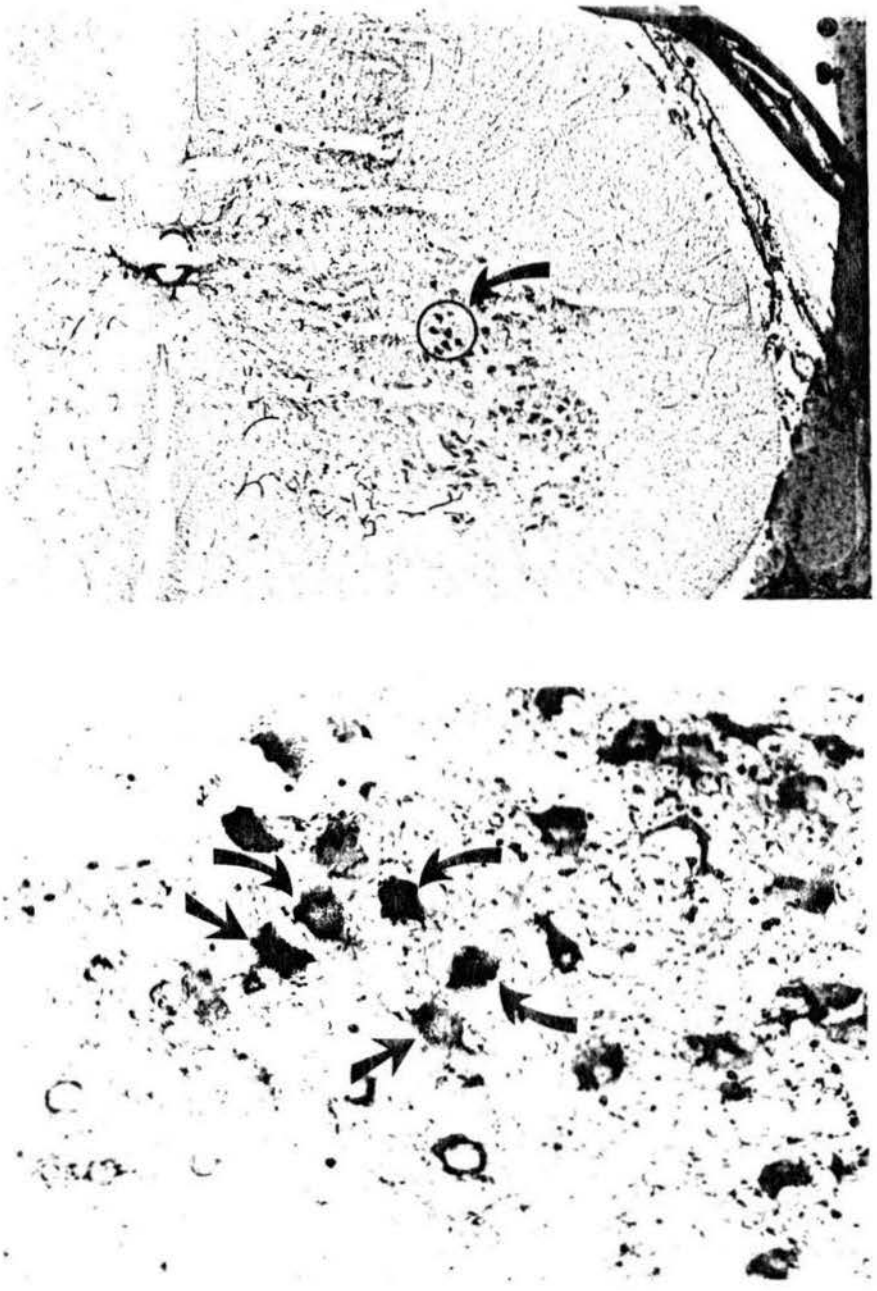

Figure 9b. Flexor Carpi Ulnaris (ulnar head)
Upper photomicrograph shows a LPF demonstrating the location of labled neurons in the ventral horn. Lower photomicrograph shows a HPF of these same labled neurons.

Superficial Digital Flexor

Isolation and perfusion of the superficial digital flexor muscle with horseradish peroxidase resulted in fifty labeled neurons Twenty-three (46%) of these neurons were found in spinal cord segment C8 and twenty-seven (54%) in segment T1. These labeled neurons form bilateral columns within the gray matter of the spinal cord which are intermingled with the columns formed by the neurons supplying the other muscles of the antebrachium. The columns are located dorsally and some what medially in the spinal cord ventral horn extending from the middle of segment C8 to the end of T1 as depicted in figures 10, 10a, and 10b These columns of neurons are different from the other columns formed by the neurons of the muscles of the antebrachium in that they begin dorsally in segment C8 and move ventrally in segment T1. Also, these columns were slightly medial in their location when compared to the other columns of antebrachial neurons.

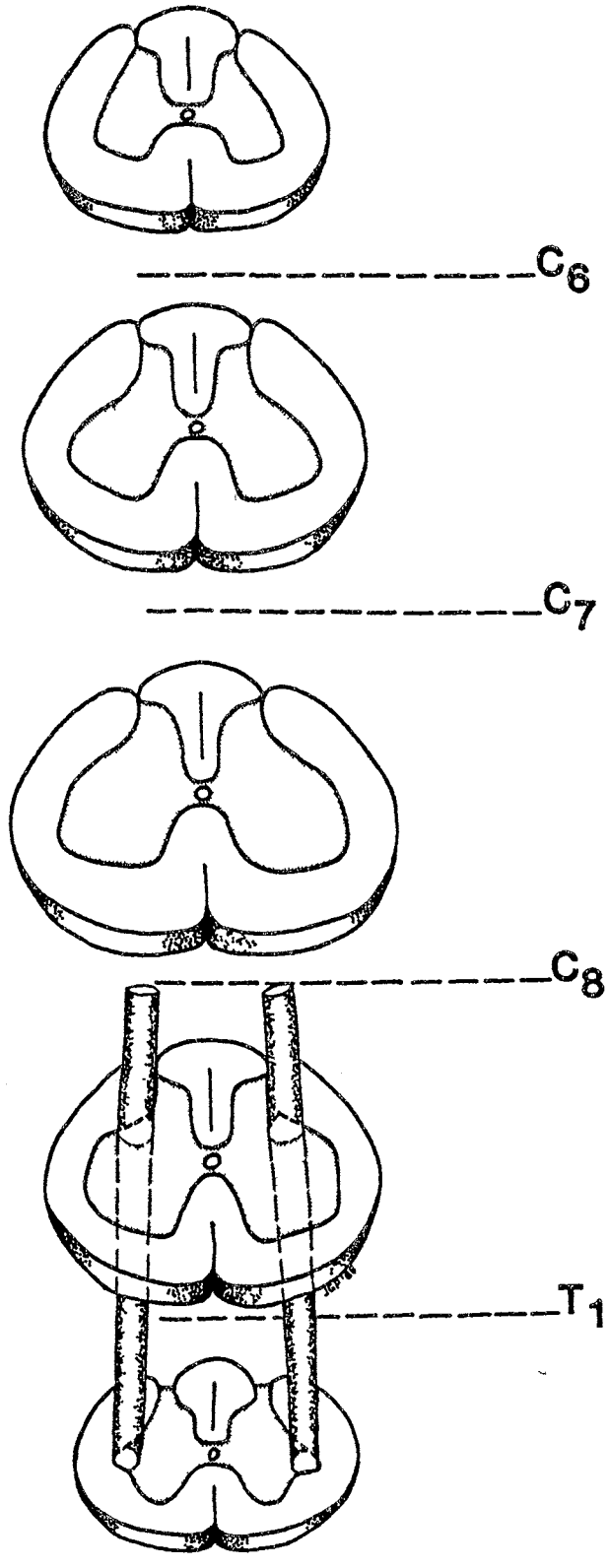

Figure 10. Superficial Digital Flexor

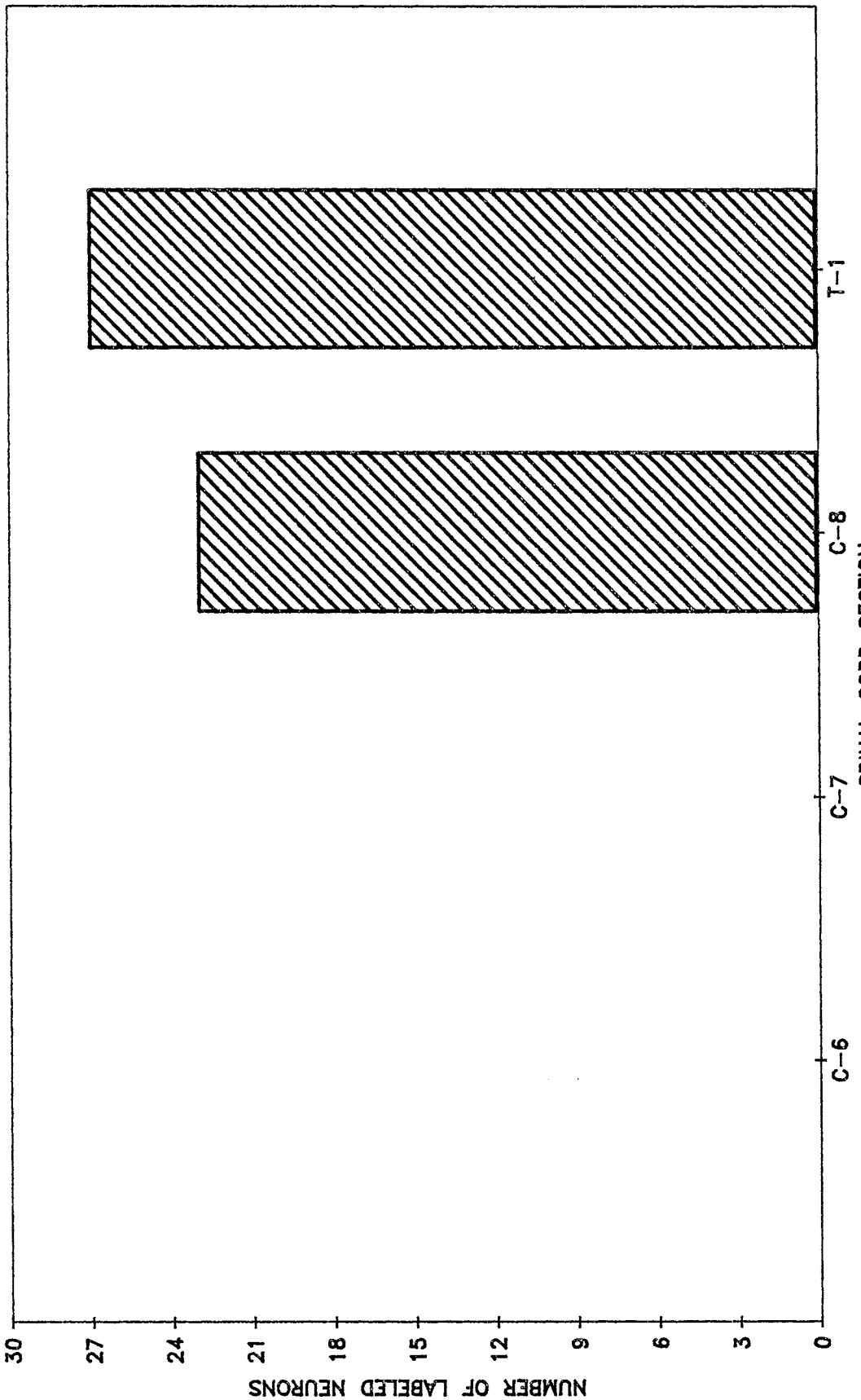

Figure 10a. Superficial Digital Flexor

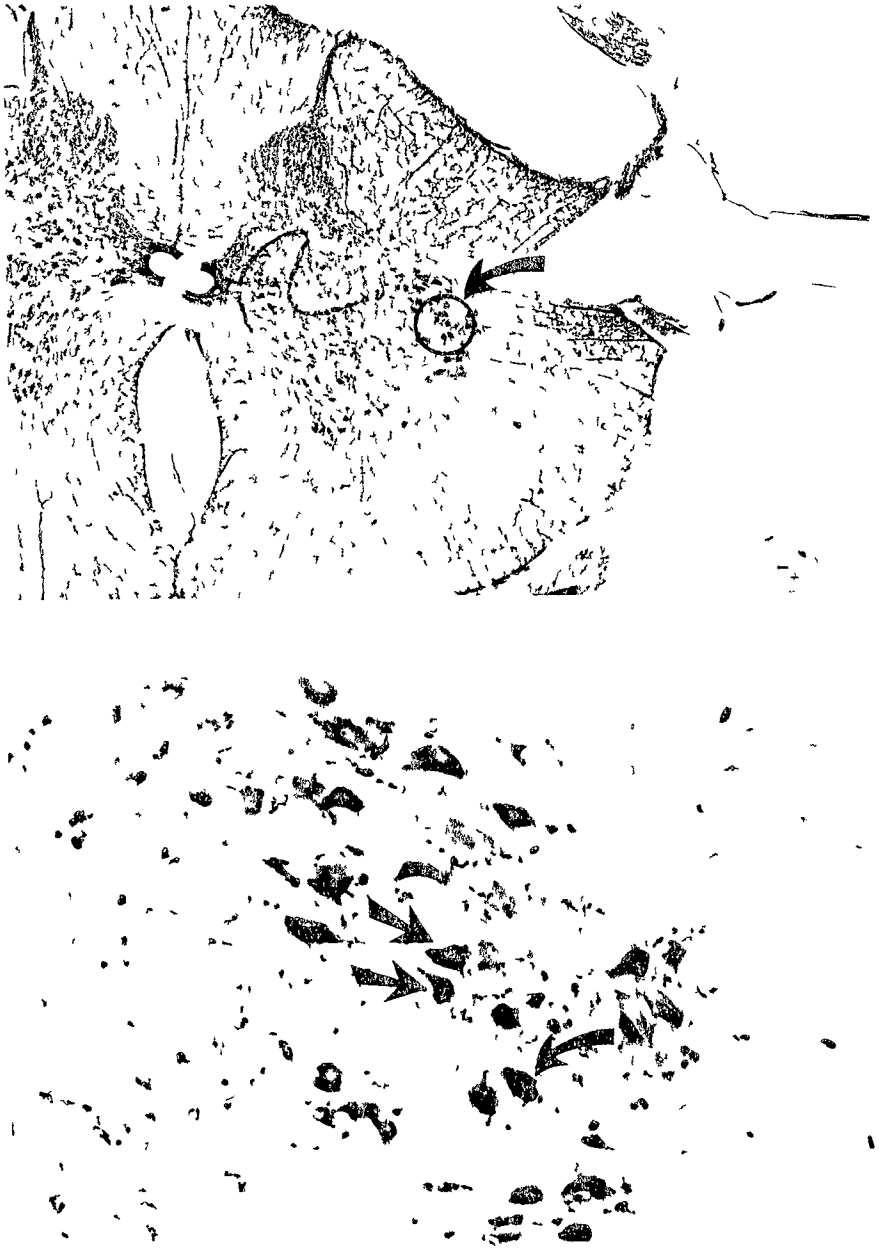

Figure 10b Superficial Digital Flexor
Upper photomicrograph shows a LPF demonstrating the location of labled neurons in the ventral horn
Lower photomicrograph shows a HPF of these same labled neurons

Flexor Carpi Radialis

Forty-seven neurons were labeled after isolation and perfusion of the flexor carpi radials muscle with horseradish peroxidase in this dog Thirty-eight (80%) of these neurons were found in spinal cord segment C6 and nine (20%) in segment C7 These labeled neurons form bilateral columns within the gray matter of the spinal cord These columns formed by the neurons supplying the flexor carpi radialis are intermingled with the columns formed by the neurons supplying the other muscles of the antebrachium The columns begin dorsal laterally in the ventral horn gray matter of segment C6 but move medially in segment C7 ventral horn The columns extended from the middle of C6 to the first one-fourth of C7 as depicted in figures 11, 11a, and 11b. As shown by the above figures, the columns of the flexor carpi radials differ from the other columns formed by the neurons of the muscles of the antebrachium by occupying so much of spinal cord segment C6. The only other column that extended into segment C6 was that of the common digital extensor This column of neurons (like that of the other columns formed by the neurons of the flexor muscles) tends to move medially on its course through the spinal cord ventral horn.

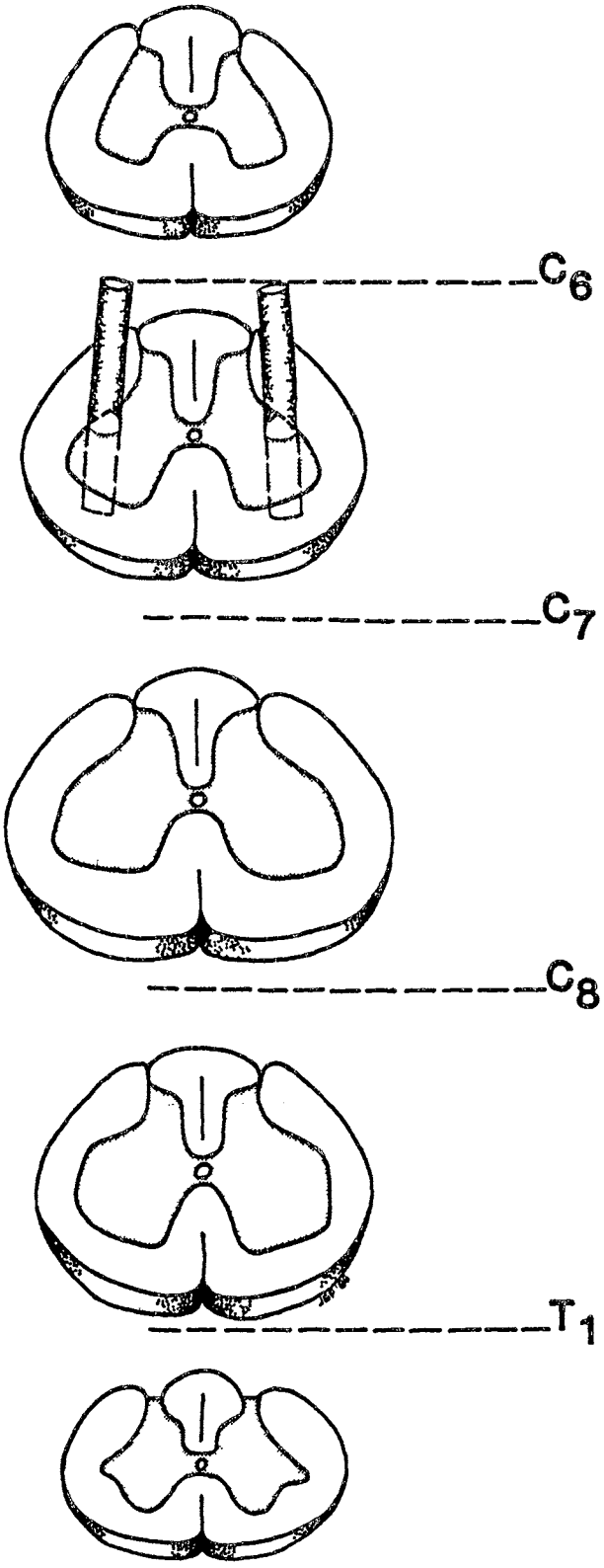

Figure 11. Flexor Carpi Radialis

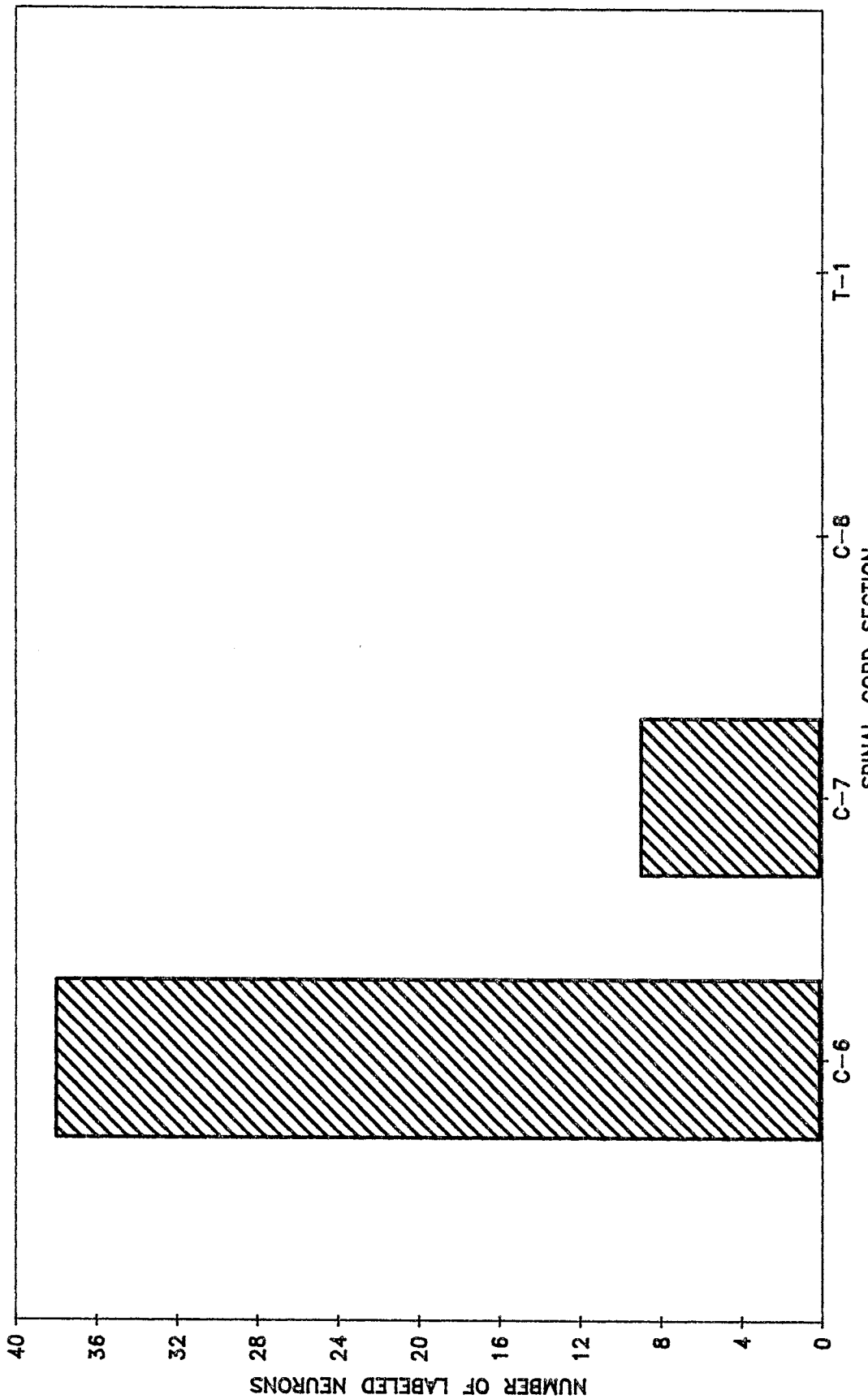

Figure 11a. Flexor Carpi Radialis

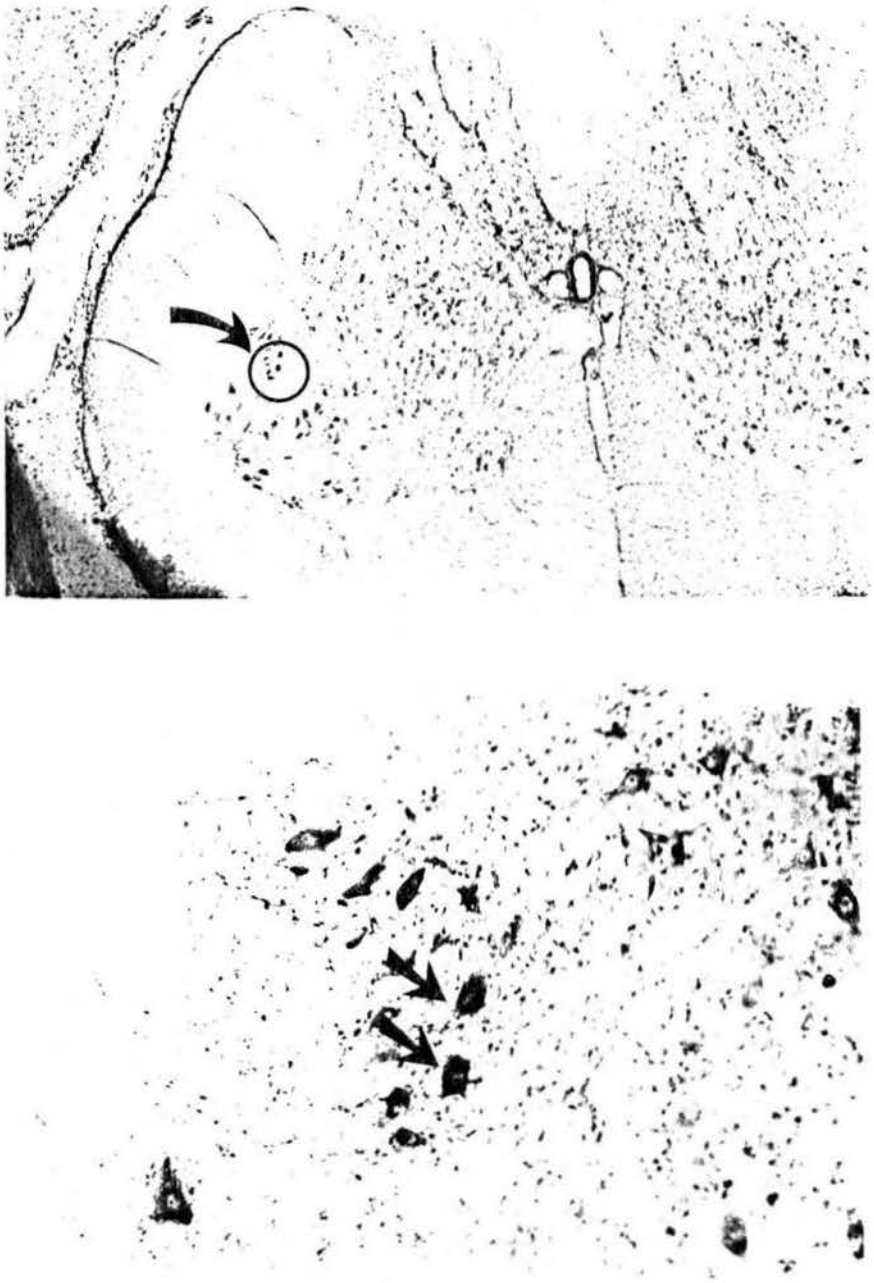

Figure 11b. Flexor Carpi Radialis
Upper photomicrograph shows a LPF demonstrating the location of labled neurons in the ventral horn. Lower photomicrograph shows a HPF of these same labled neurons.

Pronator Teres

Neurons were labeled in bilateral columns in the ventral gray of the spinal cord after isolation and perfusion of the pronator teres muscle with horseradish peroxidase. This procedure resulted in fifty-one labeled neurons. Thirty-six (71%) of these neurons were found in spinal cord segment C7 and fifteen (30%) in segment C8. The columns formed by the neurons supplying the pronator teres were intermingled with the columns formed by the neurons supplying the other muscles of the antebrachium. These columns were located more medially in segment C7 and appeared to move laterally and dorsally in segment C8 extending from the beginning of C7 to the first one-eigth of C8 as depicted in figures 12, 12a, and 12b The columns formed by the neurons of the pronator teres muscle differed from the columns formed by the neurons of the other muscles of the antebrachium in that the columns of the pronator teres appeared to move dorso-laterally on their course through the spinal cord ventral horn and for the most part occupy only segment C7.

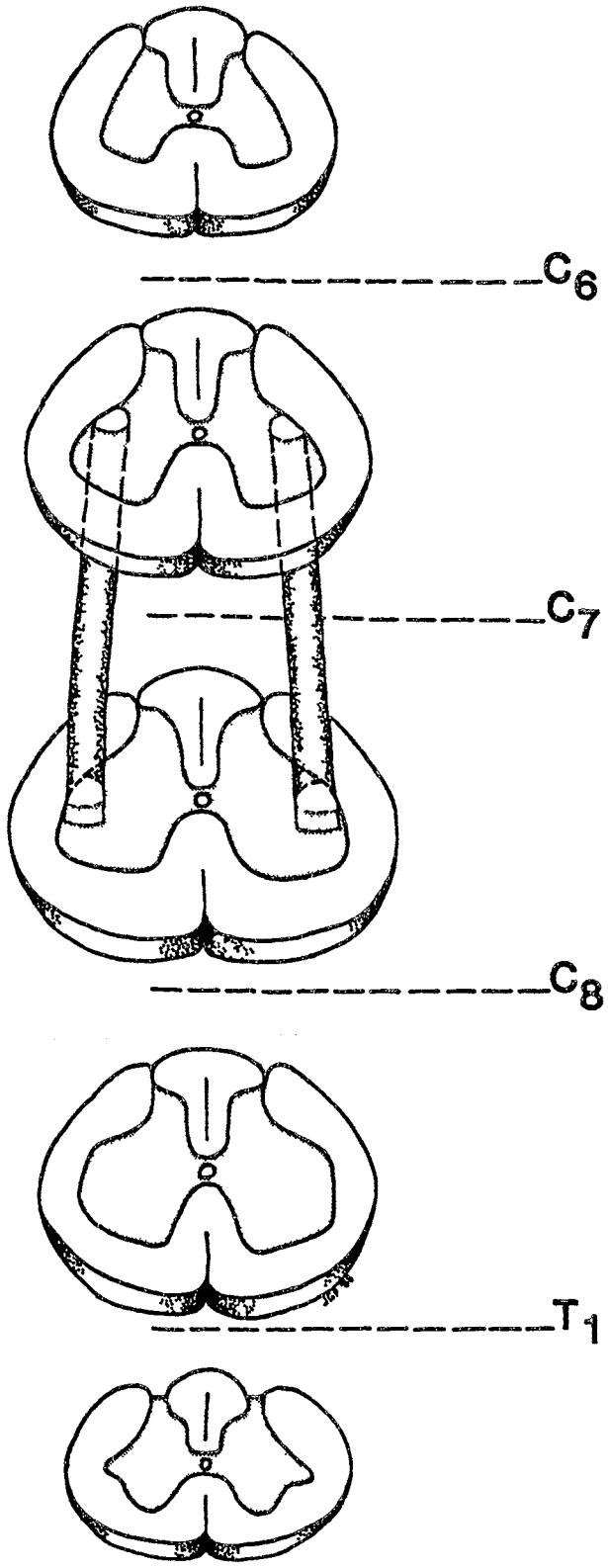

Figure 12. Pronator Teres

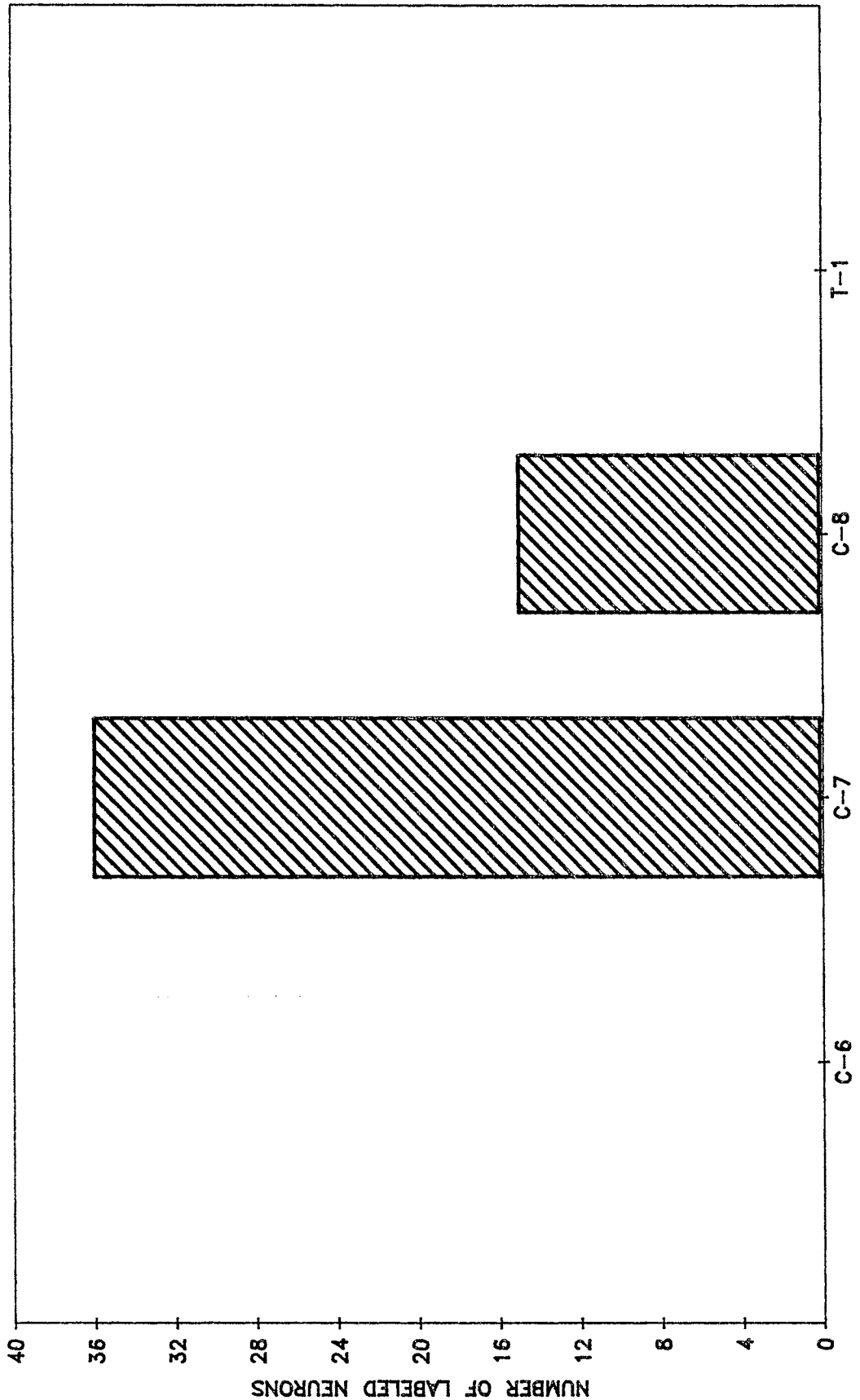

Figure 12a. Pronator Teres

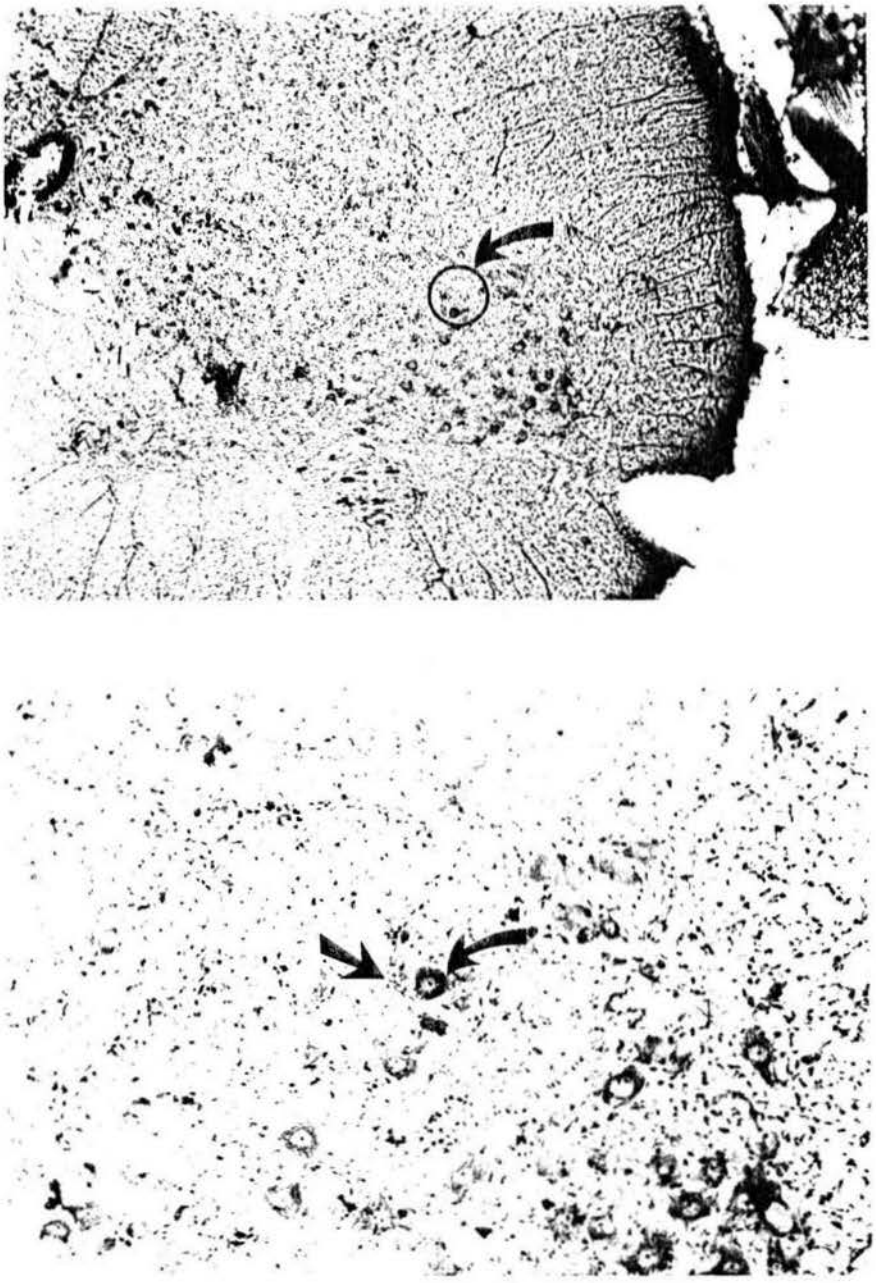

Figure 12b. Pronator Teres
Upper photomicrograph shows a LPF demonstrating the location of labled neurons in the ventral horn. Lower photomicrograph shows a HPF of these same labled neurons.

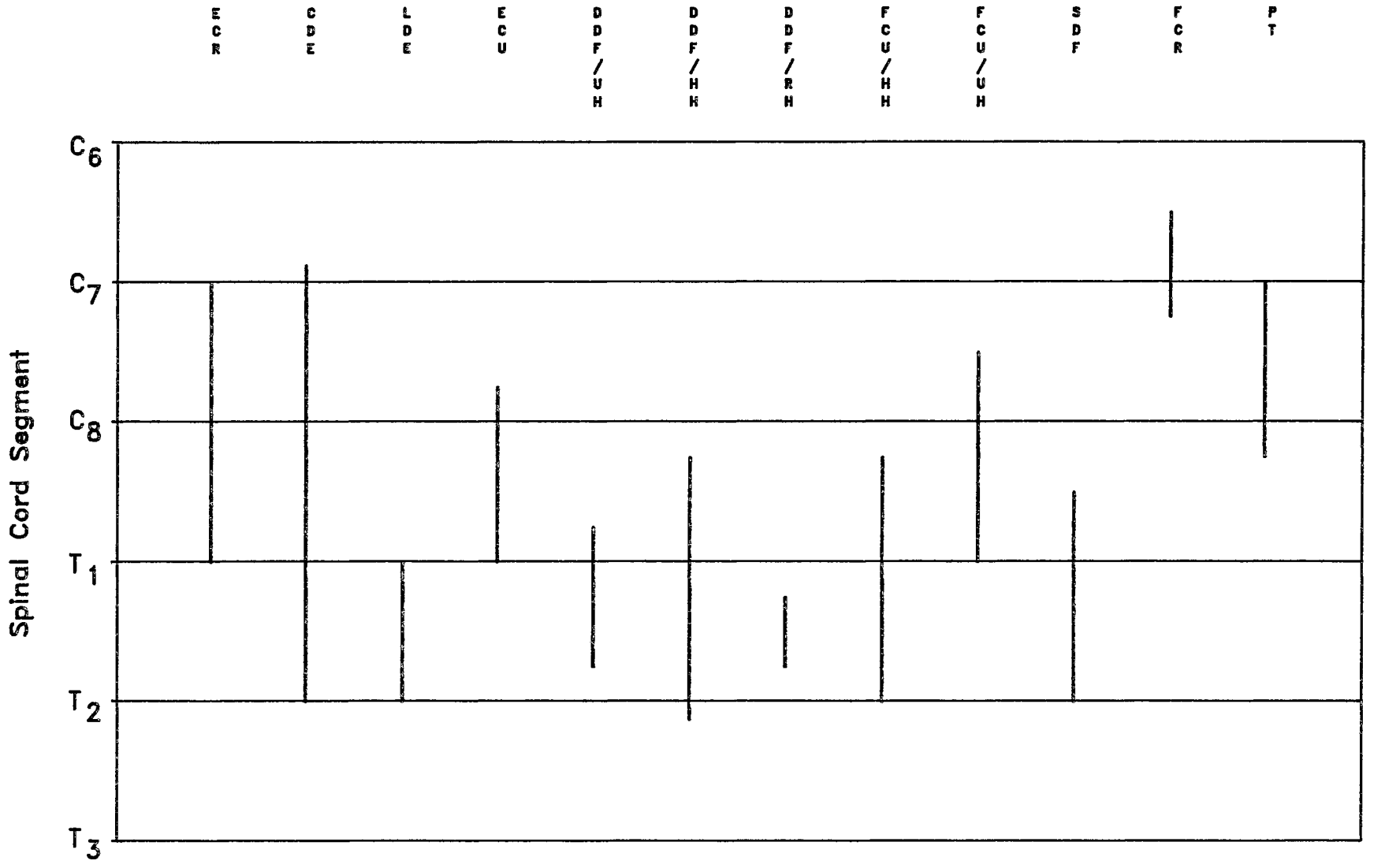

Figure 13. Extent of Neuronal Columns Supplying Each Muscle Studied

CHAPTER V

DISCUSSION

The literature, beginning with Sherrington's paper (92) indicates that motor neurons of the spinal cord are organized into discrete pools or columns Sherrington identified only two columns, a ventral lateral column, which he thought provided nerve cells that supply the more distal limb muscles and a dorso-lateral column which innervated the more proximal muscles of the limbs Goering's (38) observations, in his work with rats, increased the number of columns to five He assigned each column a distinct function but his work was never substantiated Romanes (88), working with cats, made a more definitive study of the columnar organization of the spinal cord, demonstrating that different columns supplied different muscles depending on which segment of the cord was examined. Romanes' studies were limited by the techniques available to him (ie chromatolytic studies) Romanes also demonstrated that the cells in the most dorsal columns innervate the most distal part of the limb and cell groups innervating the extensor muscles lie in the more lateral portions of the ventral horn, while flexors are supplied by more medially placed cells He also stated that a discrete nucleus for each muscle is not found Landmesser (57), using the more sensitive HRP techniques in chickens, substantiated Romanes' earlier works and concluded that all muscles arising from the dorsal muscle mass (extensors) were innervated by laterally situated motoneurons, and all

muscles arising from the ventral muscle mass (flexors) by medially situated motoneurons Ohmori et al , (80) in an even more recent work, reported that in domestic fowl laterally situated motoneurons project their axons to the hindlimb muscles derived from the dorsal muscle mass, while medially located motoneurons send axons to the hindlimb muscles differentiated from the ventral mass

Allam et al ,(4) and Miller (75), in their anatomical studies of the brachial plexus and origins of the peripheral nerves supplying the antebrachium of the dog, made significant contributions to the understanding of the innervation of the canine forelimb When this anatomical data is combined with the knowledge derived form Sherrington, Romanes, Landmesser, Ohmori, and others (11,15,22,23,27,29,33,38,43,and 90) a clear organization of the structure and function of the spinal cord is obtained.

The dorsolateral group (in relation to the appendicular skeleton) of the antebrachial muscles consist chiefly of the extensors of the carpus and digits These muscles are the extensor carpi radialis, the common digital extensor and the lateral digital extensor

The extensor carpi radialis muscle arises on the lateral epicondylar crest of the humerus and has two tendons of insertion One of these tendons inserts on a small tuberosity of metacarpal II and the other inserts on metacarpal III. The primary action of the muscle is extension of the carpal joint and is combined with a secondary action of flexion of the elbow The muscle is innervated by the radial nerve As reviewed in the results section, the neurons innervating this muscle form bilateral columns localized in the most dorsal lateral portion of the spinal cord ventral horn, extending from the beginning of C7 to the

end of C8 The very accurate picture of the neuronal cell columns obtained using the HRP process substantiates the work done in other species by the researchers mentioned above and provides a clear picture of the spinal cord neuronal organization for the extensor carpi radialis According to Miller (75) the radial nerve in the dog arises from the seventh and eighth cervical and first and second thoracic nerves. The work reported in this paper substantiates Miller's finding by demonstrating innervation of the extensor carpi radialis from the seventh and eighth cervical nerves.

The common digital extensor arises on the lateral epicondyle of the humerus, somewhat in front of and above the attachment of the ulnar collateral ligament of the elbow joint. After the tendons of insertion cross the extensor surface of the carpal joint, the individual tendons separate from each other and pass on the extensor surface of the corresponding metacarpal bones and phalanges to the distal phalanges of digits II to V. The action of this muscle is the extension of the four principle digits The neurons supplying this muscle form bilateral columns that are slightly less dorsal but more lateral than the columns formed by the neurons supplying the extensor carpi radialis These columns extend form the last one-eighth of C6 to the end of T1 The results reported for this muscle also substantiate the prior work in other species and show columns supplying extensors to be dorsal and laterally placed within the ventral horn The common digital extensor is innervated by the radial nerve which is reported to be made up of spinal nerves C8 to T1 (75). The more accurate mapping provided by the HRP technique demonstrates contributions form spinal nerves C6 to T1 to this muscle This result appears to indicate that the radial nerve (at

least in this case) receives more cranial contributions than those reported by Miller.

The lateral digital extensor arises on the cranial edge of the ulnar collateral ligament of the elbow joint, and on the head and lateral tuberosity of the radius The tendons of insertion terminate on the proximal and distal phalanges of digits III, IV, and V. The action of the muscle is extension of the joints of digits III, IV, and V Innervation is via the radial nerve The labeled neurons of this muscle form bilateral columns located dorso-laterally This is the area of the ventral horn where extensor motor neurons were found by previous investigators The contribution to the radial nerve supplying the lateral digital extensor comes exclusively from spinal nerve T1, confirming in part the literature regarding the origins of the radial nerve.

The extensor carpi ulnaris arises from the lateral epicondyle of the humerus and inserts on the proximal end of metacarpal V Miller states that the action of the muscle is extension of the carpal joint with weak lateral rotation, but Sisson and Grossman (1953) state its action as abduction In the study reported here the columns formed by the neurons supplying the muscle are more medial and ventral than those of the extensor muscles described above This location would conform more to the location of flexor muscle neuron columns reported for other species (57,80,88). Innervation of the extensor carpi ulnaris is via the radial nerve. This paper demonstrates innervation via spinal segments C7 and C8 which is in accordance with the origin of the radial nerve

The muscles of the antebrachium located caudally (both lateral and medial) consist primarily of flexors of the carpus and digits. These muscles are the deep digital flexor (ulnar head, humeral head, and radial head), the flexor carpi ulnaris (humeral and ulnar heads), superficial digital flexor, flexor carpi radialis, and pronator teres

The deep digital flexor (humeral head) arises on the medial epicondyle of the humerus and inserts as individual tendons on digits II to V The radial head arises from the medial border of the proximal three-fifths of the radius and inserts on digit I The ulnar head arises on the caudal border of the ulna from the olecranon to the distal fourth of the ulna and inserts as individual tendons on digits II to V This muscle is the flexor of the forepaw (carpus and digital joints) Innervation is via the median and ulnar nerve. The neurons supplying this muscle form three sets of bilateral columns in the ventral horn of the spinal cord Those for the ulnar head lie in the most dorso-lateral part of the ventral horn and therefore do not coincide with the position of columns supplying flexors as reported previously for other species (57,80,88) The columns of the humeral head are in the dorsal middle portion of the ventral horn This more medial position indicates a flexor muscle neuron column (88). The motor columns for the radial head are lateral but not as dorsal as for the other columns of the deep digital flexor This more ventral position again indicates a flexor motor neuron pool The median and ulnar nerves are reported to be formed by contributions from spinal nerves C8 to T2 (75). This paper reports columns in spinal cord segments C8, T1, and T2, thus conforming to the data reported in the literature.

The flexor carpi ulnaris (humeral head) arises on the medial epicondyle of the humerus and inserts on the accessory carpal bone The ulnar head arises medially on the palmar border of the proximal end of the ulna and ends independently on the accessory carpal bone. The action of both heads is flexion of the forepaw with abduction Innervation is via the ulnar nerve(75) The neuronal columns for the humeral head are dorsal and somewhat medial in C8 and move to the center of the ventral horn in T1 The columns of the ulnar head begin in the dorsal lateral part of C7 but move medially in segment C8 The more medial and ventral positions of the terminations of these neuronal columns conforms more to the location of flexor neurons reported previously for other species (57,80,88). The ulnar nerve is reported to be composed of contributions from C8 to T2 (75) but the more accurate methods used in this study have demonstrated contributions to the ulnar head of the flexor carpi ulnaris arising from spinal cord segment C7. This would appear to indicate a more cranial contribution to the ulnar nerve than was previously reported

The superficial digital flexor arises by a short strong tendon on the medial epicondyle of the humerus and inserts at the metacarpophalangeal joints of the second to fifth digits. The action of this muscle is flexion of the proximal and middle digital joints of the four principal digits Innervation is via the median nerve(75) The bilateral columns formed by the neurons of this muscle are located dorsally and somewhat medially in the spinal cord ventral horn and occupy the gray matter from the middle of C8 to the end of T1. The medial position of the columns conforms with the area occupied by flexor neurons in other species The median nerve is reported to be composed

of contributions from segments C8 to T2 The columns in this study were found in segments C8 to T1 thereby conforming to data presented in the literature for other species (57,80,88)

The flexor carpi radialis arises on the medial epicondyle of the humerus At the metacarpus, it splits into two strong tendons which end on the palmar side of metacarpals II and III The action of this muscle is flexion of the carpal joint. Innervation is via the median nerve The neuronal columns supplying this muscle begin in the dorsal lateral ventral gray of segment C6 but move medially in segment C7 The medial position taken by the columns in segment C7 conforms more to the published location of flexor neurons in the ventral horn of other species The column of neurons begins in segment C6 (80% of the neurons supplying the muscle) This finding indicates a more cranial contribution to the median that was reported by Miller (75) Miller attributes the median nerve to spinal cord segments C8 To T2

The pronator teres arises form the medial epicondyle of the humerus and ends on the medial border of the radius as far as its middle The action of the pronator teres is to rotate the forearm so that the dorsal surface tends to become medial, but it may function only as a flexor of the elbow joint (75). Innervation is supplied by the median nerve The bilateral neuronal columns supplying this muscle are located more medially in segment C7 and appear to move laterally and dorsally in segment C8 These columns extend from the beginning of C7 to the first one-eighth of C8. The original medial position of the columns tend to indicate a flexor muscle. The contribution of neurons from spinal cord segment C7 again indicates a more cranial contribution to the median

nerve than reported by Miller (75), as Miller attributes the median nerve to spinal segments C8 to T2

CHAPTER VI

SUMMARY AND CONCLUSIONS

The use of the extremely sensitive horseradish peroxidase technique described in this work resulted in a detailed and accurate map of the location of the motor neuron pools supplying the muscles of the canine antebrachium

When the details of this map were compared to the existing literature for other species some correlations and some discrepancies were found The flexor muscles (deep digital flexor ulnar, humeral and radial heads, flexor carpi ulnaris humeral and ulnar heads, superficial digital flexor and flexor carpi radials) are all (except for the deep digital flexor ulnar head and the flexor carpi ulnaris ulnar head) innervated by neuronal cell columns that are located somewhat medially and ventrally in the spinal cord ventral gray. The columns for the deep digital flexor, ulnar head and flexor carpi ulnaris, ulnar head are located in the most dorso-lateral part of the spinal cord ventral horn The reason for this discrepancy is not clear but may be attributable to the fact that both muscles have their origin on the ulna The extensor muscles (extensor carpi radialis, the common digital extensor and the later digital extensor) are all innervated by neuronal cell columns that are located laterally and somewhat dorsally

There are two muscles in this study which are not easily classified as either flexors or extensors The extensor carpi ulnaris is one of

these Miller (75) states that the action of this muscle is extension of the carpal joint with weak lateral rotation, but Sisson and Grossman state its action as abduction The neuronal columns supplying this muscle are more medial and ventral than those of the extensor muscles The other muscle is the pronator teres The neuronal columns supplying this muscle are located medial in spinal cord segment C7 but move laterally in C8 The action of the pronator teres is rotation of the forearm so that the dorsal surface becomes medial, but it may only function as a flexor of the elbow joint (75) If the work done with other species and this work done in the dog are accurate, it appears that neuronal columns are organized in regard to their function (ie. flexion or extension). If this is the true, then it could be conceivable that muscles that are not explicitly flexors or extensors could have their neuronal columns located somewhat between the lateral columns of extensor muscles and the more medial columns of flexor muscles This appears to be the case with the pronator teres and the extensor carpi ulnaris, as their neuronal columns do not lie in the domain of either strictly flexors or extensors

This paper demonstrates the accuracy the HRP technique provides when determining the location of spinal cord motoneurons via retrograde tracing There are many muscles of both the canine forelimb and hindlimb for which neuronal maps are not available The technique used in this paper is the ideal method for pursuing and finishing this work

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APPENDIX

PREPARATION OF SOLUTIONS

Below are the steps for preparing the solutions used in the materials and methods section

1) Phosphate buffer (0 2 M at pH 7.4)
   a) Dissolve 6 9 gm of monobasic sodium phosphate (NaH_2PO_4-H_2O mol. wt. 137.99) in 250 ml of distilled water
   b) Dissolve 28 2 gm of dibasic sodium phosphate ($NaHPO_4$ mol wt 141 98) in 1000 ml of distilled water
   c) Mix the two solutions, giving 1250 ml of 0 2 M phosphate buffer at pH 7 4
   d) The working buffer is made by mixing equal volumes of the phosphate buffer and distilled water, giving a 0 1 M phosphate buffer at pH 7 4

2) Buffered saline solution (pH 7 4)

   Add 100ml of the phosphate buffer to one liter of 0 09% NaCl giving a 0 02 M phosphate buffered saline solution pH 7.4

3) Sucrose solution 5%

   Dissolve 50 gm of sucrose in one liter of the working phosphate buffer

4) Sucrose solution 30%

Dissolve 300 gm of sucrose in one liter of the working phosphate buffer.

5) Gluteraldehyde solution 2 5%

Add 100 ml of 25% gluteraldehyde solution to 900 ml of the working phosphate buffer

6) DMSO solution 2%

Dissolve 2 ml of DMSO in 98 ml of the buffered saline solution

7) Acetate buffer pH 3 3

To 200 ml of 1 0 M sodium acetate add 200 ml of distilled water and to these add 190 ml of 1.0 M HCL, make to a volume of 1000 ml with distilled water. Titrate to pH 3 3 with concentrated acetic acid or sodium hydroxide.

8) Postreaction storage and rinsing solution

Add 50 ml of the pH 3.3 buffer to 950 ml of distilled water.

9) Incubation solution

a) Solution A

Mix 92.5 ml of distilled water with 5 ml of the pH 3.3 buffer and dissolve in this 100mg of sodium nitroferricyanide

b) Solution B

Dissolve 5 mg of 3,3',5,5'-tetramethylbenzidine dihydrochloride (TMB,Sigma) in 2 5mls of distilled water.

c) Mix the two solutions (neither more than 2 hrs old) in the incubation vessel just before the sections are introduced.

10) Peroxide solution

Add one ml of 30% hydrogen peroxide solution to 99 ml of distilled water. This sould be a fresh solution, mixed just before use.

11) Acetate buffer pH 4 8

Add 16 mls of 0 1 N acetic acid to 24 mls of 0 1 N sodium acetate solution Titrate to pH 4.8 with concentrated acetic acid or sodium hydroxide.

12) Neutral red solution

Add 40 ml of pH 4 8 acetate buffer to each liter of a 1% aqueous neutral red solution (10 gm neutral red in each liter of distilled water)

VITA

Richard H Jessen

Candidate for the Degree of

Doctor of Philosophy

Thesis AN ANATOMIC STUDY OF THE MYOTOMAL ORGANIZATION OF THE ANTEBRACHIUM OF THE DOG (CANIS FAMILIARUS)· A RETROGRADE HORSERADISH PEROXIDASE STUDY

Major Field. Physiological Sciences

Biographical·

Personal Data Born Milwaukee, Wisconsin, the son of Richard L. and Mary Jane Jessen

Education. Received Bachelor of Science Degree in Pharmacy from the University of Wisconsin, Madison Wisconsin in June, 1970, completed requirements for the Doctor of Philosophy Degree at Oklahoma State University, December 1988.

Professional Experience. The United States Army; Pharmacist, Instructor Spartan School of Aeronautics, Teaching Assistant, Department of Physiology, Oklahoma State University.