AN INVESTIGATION OF THE LIMITS OF THE THORACO-LUMBAR OUTFLOW AND THE DEVELOPMENT OF THE RAMI COMMUNICANTES IN HUMAN EMBRYOS

by

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II. INTRODUCTION

This thesis deals with two aspects of the embryology of the sympathetic nervous system. One problem was to define the upper and lower limits of the white rami communicantes in human embryos and to compare these results with other studies on man. While examining the limits of the white rami, it was noted that as the embryo matures, both white and grey rami communicantes apparently make their appearance in increasing numbers according to a specific pattern. The pattern of development of the grey rami has been investigated and is presented as the second part of this report. The data on the developmental pattern of the white rami is less complete due to the lack of certain crucial stages of early human development.

III. HISTORY

One of the earliest references to the sympathetic nervous system was by Galen[1] in the second century A.D.. Further knowledge, limited mainly to the gross anatomy of the thoraco-lumbar portion of the autonomic nervous system, appeared in writings of the seventeenth and eighteenth centuries. It was not until the nineteenth century, however, that advancements in scientific research made it possible to investigate the developmental and functional aspects and establish the present knowledge of the anatomical structure of the sympathetic nervous system.

During the fourth decade of the nineteenth century, two important discoveries occurred. In 1836 Valentin[2] observed the difference between the white and grey rami communicantes and noted that the white rami came from the spinal cord. In 1838 Remak[3] described the unmyelinated nerve fibers in the grey rami communicantes. He also reported that each sympathetic ganglion was connected with the corresponding spinal nerve by at least two branches. These were outstanding contributions that provided a basis for subsequent investigations of the sympathetic nervous system.

Midway through the nineteenth century, Bidder and Volkmann[4] measured the caliber of nerve fibers in the sympathetic trunks and in the dorsal and ventral spinal roots of frogs and distinguished between fine and large myelinated nerve fibers. This observation helped lay the groundwork for Gaskell's monumental research 50 years later.

In 1880 Schenk and Birdsall [5] examined human embryos and recognized that short branches from the spinal nerves terminated in small irregular cell masses. They noted that these cell masses were not connected to each other at first, but later developed fibrous interconnections, forming ganglionic chains.

During the latter part of the nineteenth century, Gaskell[6,7] made several important discoveries that ushered in the modern concepts of the sympathetic nervous system. He examined osmic acid preparations of the anterior roots of spinal nerves cervical one through lumbar four in dogs for the presence of small medullated nerve fibers less than three microns in diameter. At the level of thoracic two, there was a sudden appearance of large numbers of these small fibers, and they remained in abundance throughout the thoracic region. In the lumbar region there was a gradual disappearance of these small nerves, being decidedly fewer in number at lumbar one, sparse at lumbar two, and absent at lumbar three. He traced these fine nerve fibers to the ganglia of the sympathetic trunks and noted that some nerve fibers passed through certain ganglia into the splanchnic nerves. Gaskell concluded that only the thoracic and upper lumbar spinal nerves have white rami passing to the sympathetic trunks and grouped them under the term "thoracic outflow".

In 1898 Harman[8] dissected six human fetuses and examined each ramus connecting a spinal nerve to the sympathetic trunk, from thoracic twelve through lumbar four, inclusive. He counted the number of medullated nerve fibers that were less than three microns in diameter in each ramus, noting a significant decrease in these small fibers below the level of lumbar two. Therefore, he concluded that the caudal limit of the lumbar efferent visceral outflow in man was usually at the level of the twenty-second spinal nerve (lumbar two). In 1900 Harman[9] made a similar study on the upper limit of the visceral efferent outflow in human fetuses. He reported that the upper limit was usually at the level of the ninth spinal nerve (thoracic one).

Near the turn of the century, Langley[10,11,12] made important contributions to the anatomy, physiology, and terminology of the visceral nervous system. He introduced the three words "autonomic", "preganglionic", and "postganglionic", which have become standard terminology regarding this portion of the nervous system. Although Langley's studies were performed on lower vertebrates, he estimated that thoracic one and lumbar two were the upper and lower limits, respectively, of the thoracolumbar outflow in humans. By observing pilomotor activity induced by electrical stimulation of the white and grey rami in cats, after using nicotine to paralyze the cells in the sympathetic ganglia, Langley was able to determine the cell stations and areas of distribution of many of the white and grey rami communicantes.

Most of the articles on the embryology of the sympathetic nervous system have been devoted to the development of the sympathetic trunks and chain ganglia.

In 1877 Balfour[13] studied the development of the peripheral nervous system in elasmobranchs. He wrote that the cells composing the anlagen of the sympathetic trunks were derived from the dorsal root ganglia. His theory was supported by Harrison[14], Streeter[15], and others and remained dominant for many years.

One of the earliest descriptions of the modern concept of the development of the sympathetic trunks was written by Kuntz[16] in 1910. According to his investigation, the primordia of the sympathetic trunks first appeared in the thoracic region of pig embryos about six millimeters in length. Cells from the neural tube migrated along the ventral roots and accompanied cells from the dorsal root ganglia in a peripheral migration along the spinal nerves. Some of these cells left the course of the spinal nerves and migrated to regions dorso-lateral to the aorta, giving rise to the sympathetic trunks. These migrating cells formed cellular bridges between the spinal nerves and the sympathetic trunks, representing the future pathways of the white rami communicantes. The sympathetic trunks appeared first as continuous cell columns without distinct segmentation. Through proliferation and migration of these cells in the thoracic and upper lumbar regions, the columns were extended cranially into the cervical region and caudally into the lumbo-sacral region.

Kuntz noted that fibrous rami communicantes were not present in six millimeter embryos. He stated that in nine millimeter embryos fibers were present in the communicating rami, but that they did not extend into the primordia of the sympathetic trunks until the embryos were 10 millimeters, or more, in length. The theory that neuroblasts migrate from the neural tube by way of the ventral roots and contribute to the formation of the sympathetic trunks has been supported by the experiments of Jones[17,18], Kuntz[19,20], Brizzee and Kuntz[21], and others.

Embryological research has disclosed the following information regarding the development of the grey and white rami communicantes. Small nerve fibers from the neural tube leave the anterior primary rami in the thoracic and upper lumbar regions, terminating in the sympathetic trunks. These nerve fibers first appear during the time the sympathetic neuroblasts undergo their migration to form the sympathetic trunks and later become myelinated. They are the white rami communicantes in the adult. After further development of the embryo, the neuroblasts in the sympathetic ganglia send nerve fibers back to the spinal nerves. These fibers remain unmyelinated and form the grey rami communicantes in the adult.

IV. MATERIALS AND METHODS

A group of 45 serially sectioned human embryos, ranging in crown-rump length from 4 to 35 millimeters, was available for this investigation. Two of these embryos (numbers 241 and 3216) were borrowed from the collection of the Department of Embryology of the Carnegie Institution of Washington at Baltimore, through the courtesy of Dr. G. W. Bartelmez and Dr. J. D. Ebert. The other embryos used in this study are in the collection of Dr. A. A. Pearson at the University of Oregon Medical School. A preliminary examination revealed that 14 of the 45 embryos were sectioned in a plane unsuitable for following the course of the communicating rami, as will be explained later in this paper.

The staining method and the quality of the stain are important factors in identifying and tracing the communicating rami. The quality of a stain depends upon the method used, the skill of the technician, and the type and quality of the fixation. With human material the latter is difficult to control.

The list of embryos used in this study and the method of staining each embryo are shown in Table 1 (pages 12 and 13). Embryos stained with the activated protargol method of Bodian[22] and the silver-gelatin method of Pearson and O'Neill[23] were found to be most useful. The above methods stained the axis cylinders a dark blue color, causing them to become distinct for microscopic observation. The other stains used were more general in their coloring of animal tissues and were not selective for nerve fibers. Where general stains were used, nerve bundles could be distinguished; however, individual nerve fibers were not as readily observed.

The serial sections of each embryo were examined microscopically, and sections containing rami communicantes were carefully evaluated and pertinent data recorded.

Photomicrographs of sections of 15 human embryos used to study the white and grey rami communicantes are included in Chapter X. It should be pointed out that many of the distinguishing features of histologic slides are lost in black and white photomicrographs, due to the absence of contrasting colors.

In young human embryos the white and grey rami were closely approximated and their anatomical position was useful in distinguishing one from the other. It was observed early in this study that the grey rami lay medial or proximal to the white rami and pass between the white rami and the vertebral bodies.

It should be noted before evaluating the results of this project that the only criterion applied in determining the age of most of these embryos was the crown-rump length measurement. Streeter[24] has shown that size alone is not always a reliable gauge for estimating the developmental age of an embryo. He has devised several other criteria that facilitate a more accurate determination of over-all organ development.

Streeter postulated that if tissues and organs depend on one another for their origin and form, it should be possible to know from the developmental status of one organ, the status of all other organs in that particular embryo. He examined a large collection of human embryos 2 to 32 millimeters in length and reported that a definite and invariable schedule of organ correlation did occur. He grouped this correlation of the stages of organ development into what he called "developmental horizons".

In order to utilize Streeter's "developmental horizons" accurately, a detailed analysis of each embryo would be necessary, which was not within the scope of this project. In considering the data of two embryos, however, it was desirable to use Streeter's method. The reason for this is explained later in the text. It should be pointed out that Streeter's method of age determination is not widely applied, the crown-rump length measurement being more generally used.

V. OBSERVATIONS

A study of the upper and lower limits of the thoraco-lumbar outflow was made on 31 human embryos, 4 to 35 millimeters crown-rump length. A search for white rami communicantes was made on both sides of each embryo between spinal nerves cervical seven and lumbar five, inclusive.

Wherever possible a white ramus was identified by observing its passage from a spinal nerve to a sympathetic ganglion in a single section. This pathway, however, more often had to be followed through several serial sections. Figures 9 and 14 in the section on Illustrations (Chapter X) show nerve fibers leaving a spinal nerve to form a ramus communicans and entering a sympathetic ganglion.

The smallest embryos in which white rami could be found in this study were six and six and one-half millimeters crown-rump length. This observation was in accord with those of Kuntz(25), who has reported finding communicating rami in embryos six millimeters long. The white rami communicantes were present in the six millimeter embryo from spinal nerves thoracic three through thoracic eight, inclusive, and in the six and one-half millimeter embryo between thoracic two through thoracic nine, inclusive.

Two embryos that were seven millimeters and eight millimeters crown-rump length did not possess any white rami. Since white rami were found in two smaller embryos six millimeters and six and one-half millimeters crown-rump length, an attempt was made to determine more accurately the developmental age of the seven millimeter and eight millimeter embryos.

One of Streeter's(24) criteria, applicable to serially sectioned human embryos, was used. According to Streeter the development of the lens vesicle is closely correlated with the over-all organ development of the embryo. By comparing the stage of lens vesicle formation in the seven millimeter and eight millimeter embryos with Streeter's scheme of "developmental horizons", it was discovered that both embryos were longer than their actual organ development would indicate. This is believed to account for the absence of white rami in these embryos.

The upper limit of the white rami communicantes was at the level of the first thoracic nerve on both sides of all embryos nine millimeters long or longer. In 38 out of 46 sides of these embryos the lower limit was the second lumbar nerve, and on the remaining eight sides the lower limit was lumbar three, (See Table I, pages 12 and 13).

Table II (page 14) indicates the results of observations made on the developmental pattern of the grey rami communicantes in 23 embryos, ranging from 4 to 35 millimeters in length. Each embryo was examined bilaterally to determine the number of grey rami present and their cranial and caudal limits.

In young embryos the white and grey rami communicantes tended to run in a transverse plane, and for this reason the rami were more easily studied in embryos sectioned transversely. Although the grey rami communicantes in human adults are considered to be present at every spinal nerve level from cervical one to the coccygeal nerve, inclusive, it was not possible to identify grey rami at all these nerve levels in small embryos. The cervical and caudal flexures of the bodies of young embryos made it impossible to section them transversely throughout. When good transverse sections were obtained through the thoracic and lumbar regions, sections through the upper cervical and lower sacral regions were oblique or frontal. Therefore, it was necessary to limit this investigation to those grey rami present between spinal nerves cervical seven and sacral five, inclusive. Twenty three of the forty five available embryos were

Explanation of Tables 1 and 2

1. C-R -- Crown-rump length in millimeters
2. SG -- Silver gelatin method of Pearson and O'Neill
3. H-CR -- Hematoxylin and congo red
4. AC -- Alum cochineal
5. HE -- Hematoxylin and eosin
6. B -- Activated protargol method of Bodian
7. + -- From the Carnegie collection

TABLE I

Thoraco-lumbar outflow limitations

Series number	Staining method	C-R length (mm)	Side: right or left	Upper limit	Lower limit
152	SG	4	L	--	--
			R	--	--
178	SG	5	L	--	--
			R	--	--
137	SG	5	L	--	--
			R	--	--
100	SG	5	L	--	--
			R	--	--
241 +	H-CR	6	L	T 3	T 8
			R	T 3	T 8
3216 +	AC	6.5	L	T 2	T 9
			R	T 2	T 9
182	B	7	L	--	--
			R	--	--
129	SG	8	L	--	--
			R	--	--
124	HE	9	L	T 1	L 2
			R	T 1	L 2
125	SG	9	L	T 1	L 2
			R	T 1	L 2
59	SG	10	L	T 1	L 3
			R	T 1	L 3
173	SG	10	L	T 1	L 2
			R	T 1	L 2
179	SG	12	L	T 1	L 2
			R	T 1	L 2
248	B	13	L	T 1	L 3
			R	T 1	L 3

TABLE I (Continued)

Series number	Staining method	C-R length (mm)	Side: right or left	Upper limit	Lower limit
82	B	14	L	T 1	L 3
			R	T 1	L 2
249	B	15	L	T 1	L 2
			R	T 1	L 2
175	SG	15	L	T 1	L 2
			R	T 1	L 2
140	SG	16	L	T 1	L 2
			R	T 1	L 2
171a	SG	17	L	T 1	L 3
			R	T 1	L 3
87	SG	18	L	T 1	L 2
			R	T 1	L 2
180	SG	20	L	T 1	L 2
			R	T 1	L 2
143	SG	20	L	T 1	L 2
			R	T 1	L 2
103	SG	20	L	T 1	L 2
			R	T 1	L 2
234	B	20	L	T 1	L 2
			R	T 1	L 2
207	B	21	L	T 1	L 3
			R	T 1	L 2
165	SG	22	L	T 1	L 2
			R	T 1	L 2
246	B	24	L	T 1	L 2
			R	T 1	L 2
218	B	25	L	T 1	L 2
			R	T 1	L 2
27	B	29	L	T 1	L 2
			R	T 1	L 2
174	SG	30	L	T 1	L 2
			R	T 1	L 2
166	SG	35	L	T 1	L 2
			R	T 1	L 2

TABLE II

Grey Rami Communicantes

Series	C-R length (mm)	Limits of grey rami communicantes	
		right	left
152	4	none found	none found
178	5	none found	none found
137	5	none found	none found
100	5	none found	none found
241 +	6	none found	none found
3216+	6.5	none found	none found
182	7	none found	none found
129	8	none found	none found
124	9	none found	none found
125	9	none found	none found
59	10	C8 to T1	C8 to T1
179	12	C8 to T3	C8 to T3
82	14	C7 to T4	C7 to T4
249	15	C7 to T4	C7 to T5
87	18	C7 to T9	C7 to T8
180	20	C7 to L3	C7 to L3
234	20	C7 to L3	C7 to L3
165	22	C7 to L4	C7 to L4
246	24	C7 to L4	C7 to S1
218	25	C7 to S3	C7 to S3
27	29	C7 to S5	C7 to S5
174	30	C7 to S5	C7 to S5
166	35	C7 to S5	C7 to S5

considered satisfactory for this study because they were sectioned in a transverse plane throughout most of this region.

To facilitate an understanding of the conclusions drawn from this investigation, a detailed report of the grey rami communicantes in each embryo follows. Photomicrographs of typical sections of each embryo used in this study are presented in Chapter X, Illustrations.

Seven human embryos with crown-rump lengths of less than nine millimeters were examined for grey rami, but none were found.

Series 124 and 125: In the two nine millimeter embryos that were examined, white rami were seen at every spinal nerve level from thoracic one through lumbar two, inclusive. No grey rami were present in either embryo between cervical seven and lumbar three, inclusive. In series 124 (Figure 1) the white rami were large, and although lightly stained, could be satisfactorily observed. The white rami in series 125 (Figure 2) were made up of fine nerve filaments that were difficult to observe against the darkly stained cellular background of the embryo.

Series 59: Four embryos of 10 millimeters crown-rump length were examined, but only one was sectioned transversely and was satisfactory for studying grey rami. At the levels of spinal nerves cervical eight and thoracic one, bilaterally, fine strands of tissue were noted in a location normally occupied by grey rami and were identified as nerve fibers by their staining qualities. Spinal nerves cervical seven through lumbar three, inclusive, were investigated for grey rami. White and grey rami are shown at the level of the first thoracic nerve on the right side of Figure 3.

Series 179: One embryo of 12 millimeters crown-rump length was suitable for study. At each level from cervical eight through thoracic three, bilaterally, there were bundles of nerve fibers which could be identified by their anatomical position as grey rami communicantes. The embryo was studied for grey rami between cervical seven and lumbar three, inclusive. On the left side of figure 4, a portion of a grey ramus can be seen extending dorsalward from a sympathetic ganglion toward the second thoracic spinal nerve.

Series 82: One embryo 14 millimeters long was examined. In this embryo grey rami could be identified on both sides from cervical seven through thoracic four, inclusive. The grey rami were difficult to follow, because they were made up of loose fiber bundles or merely fine strands of nerve fibers as shown on the right side of figure 5. The embryo was examined for grey rami on both sides between cervical seven and lumbar three, inclusive.

Series 249: One 15 millimeter embryo was studied. Between cervical seven and thoracic four, inclusive, grey rami were present, bilaterally. At the level of the fifth thoracic nerve a grey ramus was noted on the left side, but none could be found on the opposite side. Below thoracic five no evidence of grey rami was present. The embryo was examined from cervical seven to lumbar three, inclusive. Figure 6 shows portions of both a white and a grey ramus at the level of the second thoracic nerve. It can be seen in this photomicrograph that, although the nerve fibers are lightly stained, the rami are well formed and are in their characteristic positions.

Series 87: One embryo 18 millimeters in length was studied. Grey rami were present on both sides of this embryo from cervical seven through thoracic eight, inclusive. At the level of thoracic nine, a grey ramus was seen on the right side of the embryo, but on the left side none could be identified. A search for grey rami was made from cervical seven through lumbar three, inclusive, but none were noted below thoracic nine on either side. The grey rami were small and often indistinct against the cellular background of this embryo. Figure 7 shows part of a large white ramus separated from a portion of a small grey ramus by a vein at the level of the fifth thoracic spinal nerve.

Series 180 and 234: Two 20 millimeter embryos were satisfactory for investigation. On both sides of each embryo well differentiated grey rami were present at each spinal nerve level from cervical seven through lumbar three, inclusive. Although both embryos were carefully examined through sacral one, no further grey rami were found. White and grey rami are shown on both sides of figure 8 at the level of the fourth thoracic spinal nerve. Figure 9 clearly depicts a white and a grey ramus at the level of the eighth thoracic nerve.

Series 165: One 22 millimeter embryo was studied. The rami communicantes were well formed and the sections were well stained. Grey rami were present on both sides of this embryo from cervical seven through lumbar four, inclusive. Below lumbar four no grey rami were visible. Portions of two grey rami, lying close together, and the proximal end of one white ramus are shown in figure 10. Two or more grey rami were not uncommonly seen growing back from a sympathetic ganglion in older embryos.

Series 246: One 24 millimeter embryo was examined. Grey rami were seen on both sides between cervical seven and lumbar four, inclusive. On the left side grey rami were found through sacral one, but on the right side none were seen below lumbar four. It was difficult to be certain of the exact locations of grey rami in the lower lumbar and upper sacral regions, because of the oblique plane of the sections. This embryo was studied from cervical seven through sacral five, inclusive. Figure 11 shows the distal end of a grey ramus at the level of the fifth thoracic spinal nerve.

Series 218: In this 25 millimeter human embryo grey rami were present on both sides from cervical seven through sacral three, inclusive. The nerve fiber bundles were well differentiated and easily followed from the chain ganglia to the spinal nerves. The embryo was studied from cervical seven through sacral five. The close approximation of white and grey rami as shown in figure 12, makes it seem probable that they would have fused into a single bundle in the adult.

Series 27: The next embryo suitable for examination was 29 millimeters in length. Grey rami communicantes were present bilaterally, from cervical seven through sacral five, inclusive. These levels were the cranial and caudal limits studied for this project. All grey rami were present as well-formed nerve bundles and were followed without difficulty. Grey rami are shown bilaterally at the level of the fourth lumbar nerve in figure 13. These rami were more clearly visible under the microscope than they are in the photomicrograph.

Series 174 and 166: Two larger human embryos were examined. The former was 30 millimeters and the latter was 35 millimeters in length. A complete series of grey rami was found on both sides of each embryo from cervical seven through sacral five, inclusive. Further investigation of larger embryos was beyond the scope of this project. Figure 14 is one of the better illustrations of both white and grey rami at the same spinal level. Some of the nerve fibers in the white ramus could be traced into the eighth thoracic spinal nerve and centrally toward the ventral and dorsal roots of that nerve. Both white and grey rami are also well shown in figure 15. The proximal end of the grey ramus fans out into several bundles; one joins the white ramus close to its attachment to the spinal nerve, one joins the ventral root of the spinal nerve, and the third bundle lies between the other two.

VI. DISCUSSION

Although a vast amount of research has been done on the sympathetic nervous system, relatively few investigators have specifically attempted to determine the limits of the thoraco-lumbar outflow in man. Gaskell(7) and Langley(11) performed their classical studies on dogs and cats, respectively, and as Sheehan(26) has mentioned, these results have often been transferred directly to man without taking into account species variation. Harman(8,9) dissected human fetuses, and was one of the first to study these limits using human material. In 1941 Sheehan(26) pointed out that there was still no general agreement as to the extent of the thoraco-lumbar visceral efferent outflow in man. He attempted to remedy this situation, and by examining the sympathetic nervous system in cadavers, he established what are now generally accepted to be the limits of the thoraco-lumbar outflow in man.

Sheehan(26) investigated the limits of the thoraco-lumbar outflow in nine adult cadavers. He examined the ventral spinal roots from both sides of each cadaver, searching for small medullated nerve fibers less than three microns in diameter. In five cases the ventral roots from both sides were studied from cervical one to the coccygeal nerve, inclusive. In the other four cadavers the ventral roots from the upper and lower limits of the thoraco-lumbar outflow were studied; namely, cervical seven to thoracic three, inclusive, and thoracic twelve to sacral five, inclusive.

His material was prepared by removing the spinal cords at autopsy and treating them with a ten percent formol saline solution. Later the ventral roots were dissected off the cords and stained in bulk with osmic acid, and then cut transversely and longitudinally. Measurements and counts of the nerve fibers were made with an ocular micrometer.

The results of Sheehan's investigation indicate that the thoraco-lumbar outflow in man is fairly constant despite the relatively small number of cases he examined. He found the upper limit to be at thoracic one in every instance, 18 out of 18 sides. The lower limit varied from lumbar one to lumbar three, being at lumbar two on 15 out of 18 sides, at lumbar one on two sides, and at lumbar three on the remaining side.

A subsequent examination of human cadavers by Pick and Sheehan[27] in 1946 confirmed Sheehan's earlier observations in 1941 that the limits of the thoraco-lumbar outflow in man were usually thoracic one and lumbar two.

The results of this study on human embryos showed that the upper limit of the white rami was at the level of thoracic one on both sides of all 23 embryos nine millimeters crown-rump length or longer; namely, on 46 out of 46 sides. The lower limit showed some variability, being at lumbar two on 38 out of 46 sides, and at lumbar three on the remaining eight sides.

A close correlation between the results of the studies of cadavers and human embryos appears evident. In every instance there is complete agreement that the upper limit of the white rami communicantes is at thoracic one. Although there are variations in the lower limit, it is apparent that lumbar two predominates in both instances.

Sheehan's[26] results on cadavers placed the lower limit of the white rami at lumbar two in 83.3% of the sides examined. In human embryos the lower limit was at lumbar two in 82.6% of the sides. From this comparison it can be concluded that there is probably no change in the limits of the thoraco-lumbar outflow once they have become established in young embryos.

While investigating the spinal autonomic outflow in cadavers, Sheehan examined the cervical and lower lumbar ventral roots for the presence of small medullated nerve fibers. He found these fibers to be present throughout the cervical and lower lumbar regions, but they were not grouped into nerve bundles and were greatly reduced in quantity as compared to their number within the usual limits of the thoraco-lumbar outflow. He stated that these small fibers resembled preganglionic sympathetic nerves, but that their significance was not clear.

Although the results of this study on human embryos and that of Sheehan on cadavers indicate that the limits of the thoraco-lumbar outflow are usually at thoracic one and lumbar two, there is a possibility that the white rami communicantes may be present outside of these limits.

In the series of embryos available for this study, white rami were first observed in two embryos measuring six and six and one-half millimeters crown-rump length. These rami were present in the six millimeter embryo from spinal nerves thoracic three through thoracic eight, inclusive, and in the six and one-half millimeter embryo from thoracic two through thoracic nine, inclusive. In the two nine millimeter embryos examined, the white rami had reached the limits of the thoraco-lumbar outflow, namely thoracic one and lumbar two.

Even from these limited data on the early appearance of white rami (Table 1), certain assumptions are suggested concerning their developmental pattern. It is probable that the earliest white rami appear in the mid-thoracic region. As the embryo matures, additional white rami are formed by the outgrowth of nerve fibers from the spinal nerves to the sympathetic trunks, and these rami develop both cranially and caudally from this region until the limits of the thoraco-lumbar outflow are reached. This growth pattern of the white rami closely resembles that of the sympathetic trunks previously outlined in this paper.

While examining these human embryos to determine the limits of the thoraco-lumbar outflow, it was observed that the grey rami also made their appearance in sequence at successive spinal nerve levels as the embryos increased in length. As sufficient embryos were available in the age group during which the grey rami appeared, a study was made to determine their pattern of development.

The grey rami made their initial appearance at the levels of cervical eight and thoracic one in a 10 millimeter embryo. In the 14 millimeter embryo that was examined, grey rami were found at cervical seven. This was the highest level investigated in this project.

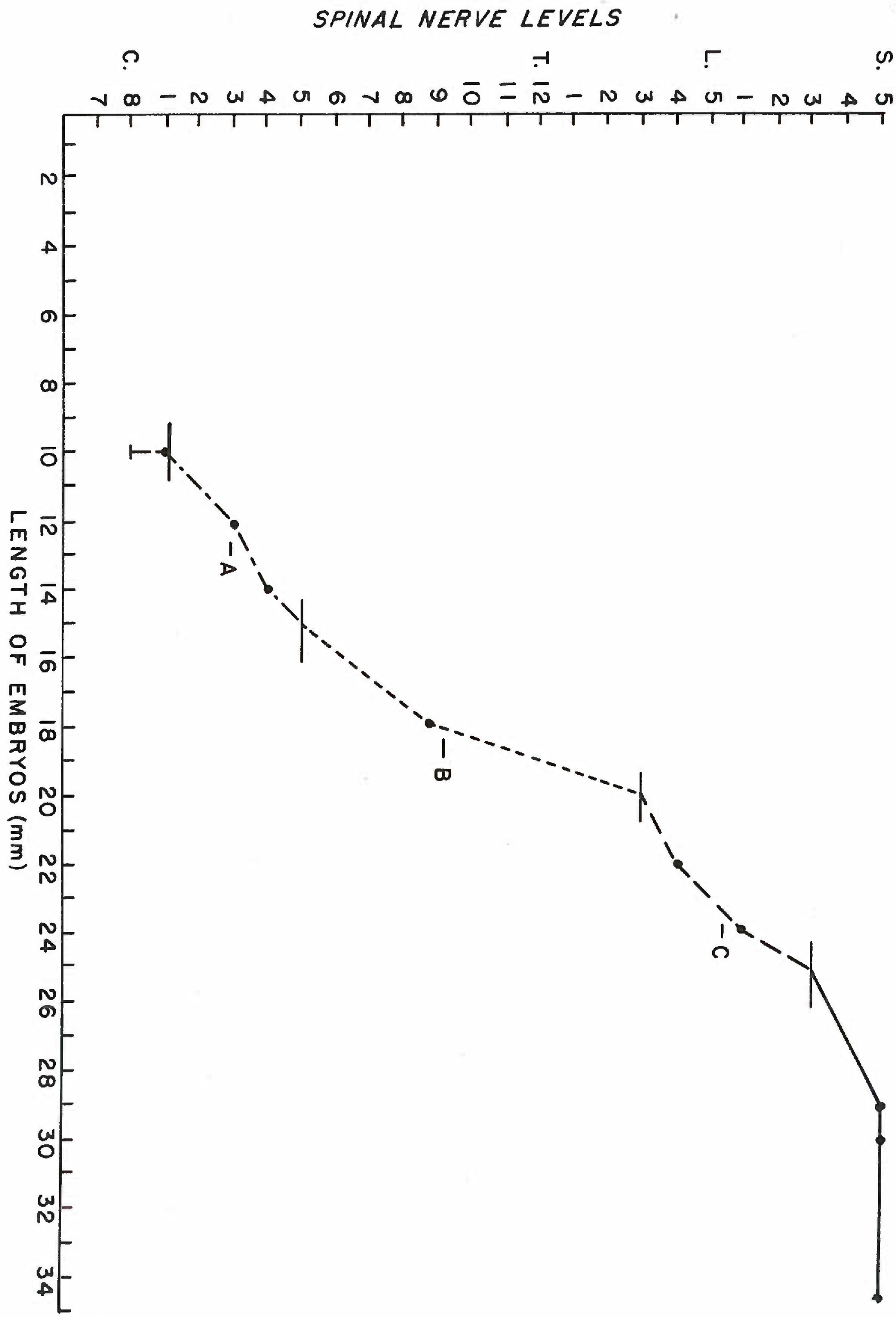
In the early stages of development, the grey rami increased in number slowly, extending caudally only as far as thoracic five in an embryo of 15 millimeters. At about this stage of embryonic maturation, a marked change occurred. The number of grey rami increased rapidly as the embryos increased in length to 20 millimeters. In an 18 millimeter embryo, a grey ramus was present as far caudally as thoracic nine; and in both 20 millimeter embryos the grey rami extended as far as lumbar three. In embryos longer than 20 millimeters, the rate of appearance of the grey rami again decreased. In the 25 millimeter embryo, the most caudal grey rami were at sacral three, and, in the 29 millimeter embryo, at sacral five.

An investigation of two embryos longer than 29 millimeters crown-rump length revealed that grey rami could be observed between cervical seven and sacral five on both sides of each embryo. These levels encompassed the upper and lower limits of this study.

Although it was not possible to examine embryos at every millimeter stage between 9 and 35 millimeters crown-rump length, certain aspects of the appearance of the grey rami communicantes formed a pattern. It was noted that not all of the grey rami grew back from the sympathetic ganglia to the spinal nerves at one time. The earliest grey rami, composed of a few loose nerve fibers, were found growing back from immature sympathetic ganglia opposite the eighth cervical and first thoracic spinal nerves in an embryo 10 millimeters in length. As the length of the embryos increased, the caudal extension of the grey rami appeared to occur at successive spinal nerve levels, reaching sacral five, the caudal limit of this study, in a 29 millimeter embryo.

It was also observed that the caudal extension of the grey rami communicantes did not take place at equally spaced intervals. This observation can be best illustrated by a graph (page 24), which depicts the relationship between the crown-rump length of an embryo, measured in millimeters and the progressive appearance of the grey rami communicantes at specific spinal nerve levels.

The point at which the graph begins shows the simultaneous appearance of grey rami at cervical eight and thoracic one in a 10 millimeter embryo. After the graph was plotted, it was noted that the curve could be divided into three segments, each representing a five millimeter increase in embryonic length: 11 through 15 millimeters, 16 through 20 millimeters, and 21 through 25 millimeters. These segments are indicated on the graph by the letters A, B, and C, respectively.


SPINAL NERVE LEVELS
C. 7 8 1 2 3 4 5 6 7 8 9 10 11
T. 12 1 2 3 4
L. 5 1 2 3 4
S. 5
2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34
LENGTH OF EMBRYOS (mm)
A
B
C

It may be seen that in the 11 through 15 millimeter section there are four new grey rami. The next five millimeter division contains 10 new grey rami. In the third five millimeter division, five more grey rami are present.

From these results it is apparent that the caudal extension of the grey rami communicantes occurred more than twice as rapidly during the period when the embryos increased in length from 16 through 20 millimeters as it did during the period when the embryos increased in length from 11 through 15 millimeters and from 21 through 25 millimeters.

If a larger series of human embryos could be examined, a more complete story of the developmental pattern of the communicating rami could be told.

VII. SUMMARY AND CONCLUSIONS

A microscopic examination of the cranial and caudal limits of the white rami communicantes was made bilaterally of serial sections of 31 human embryos ranging from 4 to 35 millimeters crown-rump length. The white rami were first noted to be present from thoracic one to lumbar two, inclusive, in two nine millimeter embryos. In the 23 embryos nine millimeters long or longer, the upper limit of the white rami was thoracic one in every instance. The lower limit varied between lumbar two and lumbar three, being at lumbar two, however, in the large majority of embryos.

The results of this investigation were compared with a similar study made by Sheehan(26) of the limits of the thoraco-lumbar outflow in nine cadavers, and a close correlation of the two studies was demonstrated. It appears, therefore, that the limits of the thoraco-lumbar outflow do not change once they have become established in young embryos.

Two embryos were available that were young enough to show the white rami communicantes during their process of development. Even though the data are limited, it may be postulated that white rami probably first appear in the mid-thoracic region and develop both caudally and cranially from this region until the limits of the thoraco-lumbar outflow are reached. Further observations on certain crucial stages of early human development are needed to substantiate these conclusions.

An analysis of the developmental pattern of the grey rami communicantes on both sides of 23 human embryos, 4 to 35 millimeters long, revealed two significant findings:

1. The grey rami appeared caudally at successive spinal nerve levels from thoracic one to sacral five, as the

embryos increased in length from 10 to 29 millimeters;

2. The caudal extension of the grey rami did not progress at an even rate. More than twice as many grey rami developed during the period in which the embryos increased from 16 through 20 millimeters in length as appeared during the 11 through 15 millimeter increase and the 21 through 25 millimeter increase in embryonic length.

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IX. TABLE OF ABBREVIATIONS

1. WR -- White rami communicantes
2. GR -- Grey rami communicantes
3. SG -- Sympathetic ganglia
4. SN -- Spinal nerve

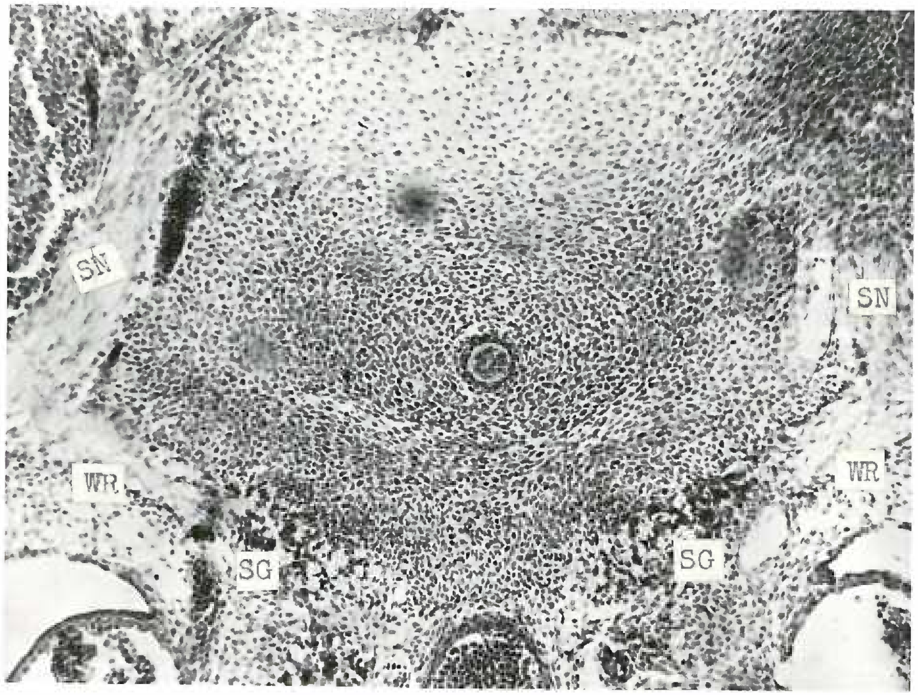
X. ILLUSTRATIONS

Photomicrographs of transverse sections of human embryos studied in this research, showing development of the grey rami communicantes

PLATE I.

Figure 1. Series 124. A low-power photomicrograph of a transverse section through a nine millimeter embryo showing white rami (WR) passing from the fifth thoracic spinal nerves (SN) to immature sympathetic ganglia (SG) bilaterally. No grey rami are present in this embryo.

Figure 2. Series 125. A low-power magnification of a transverse section through a nine millimeter embryo at the level of thoracic three showing immature white rami (WR) entering clusters of sympathetic neuroblasts that constitute the sympathetic ganglia (SG). No grey rami are seen in this embryo.



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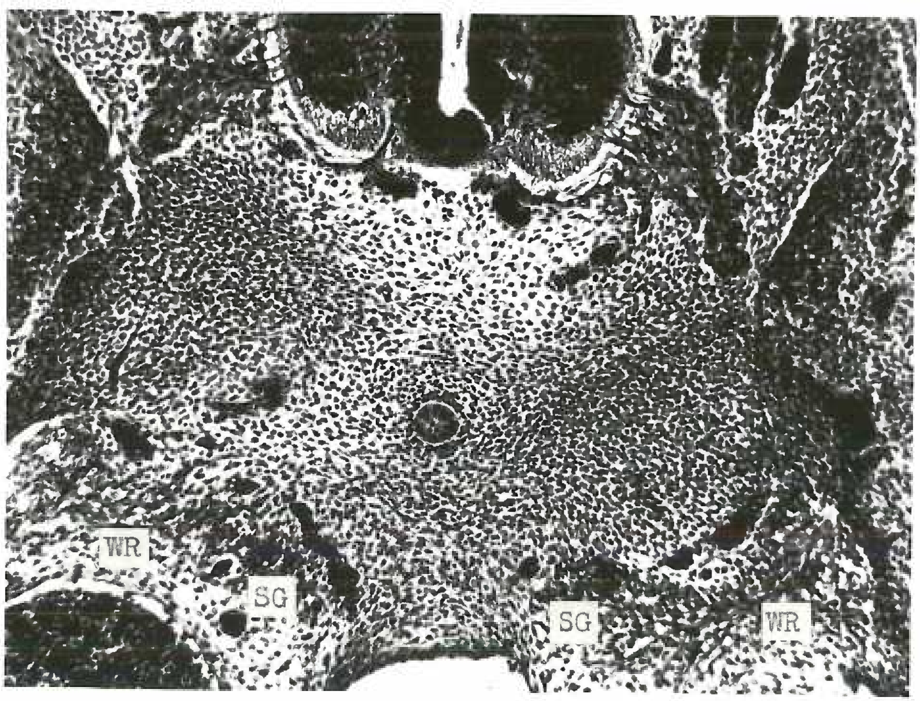

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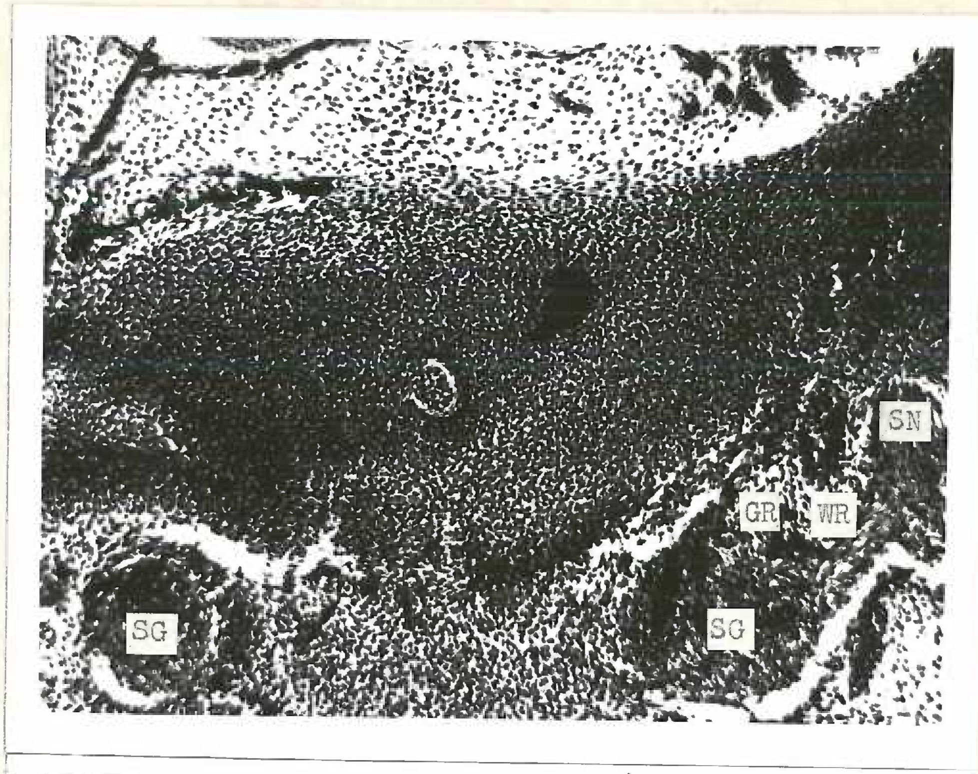
PLATE II.

Figure 3. Series 59. A view of a transverse section from a 10 millimeter embryo showing a white ramus (WR) passing between the first thoracic spinal nerve (SN) and a sympathetic ganglion (SG). What are believed to be a few nerve fibers of a grey ramus (GR) can be seen growing back from the ganglion.

Figure 4. Series 179. A photomicrograph of a section from a 12 millimeter embryo showing a white ramus (WR) passing from the second thoracic spinal nerve (SN) to a sympathetic ganglion (SG), and a definite grey ramus (GR) emerging from the ganglion.



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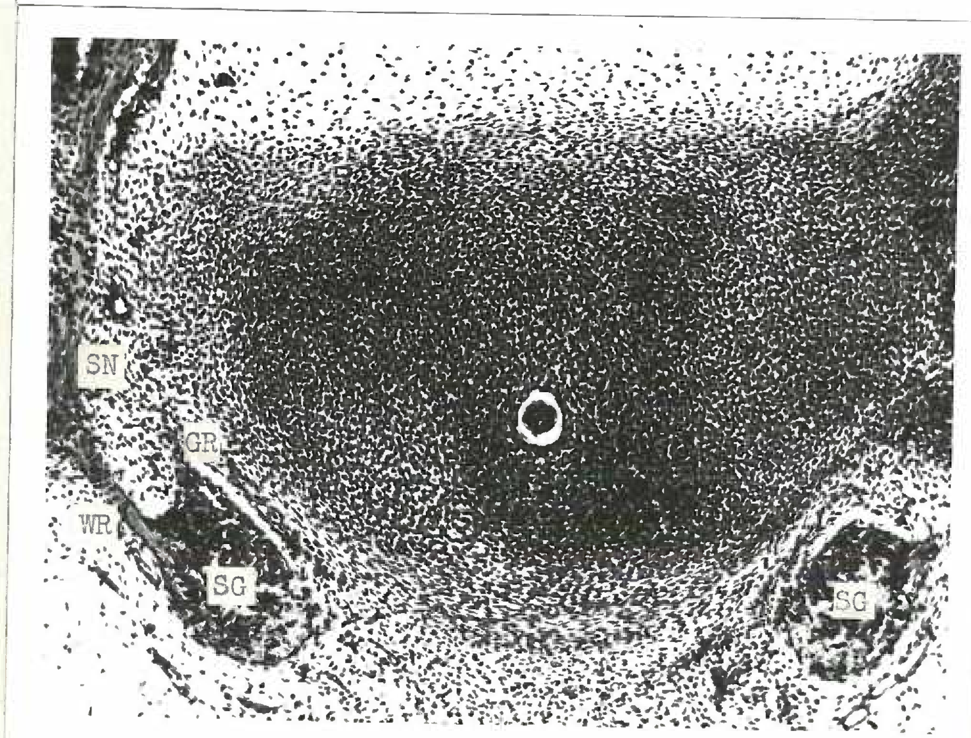

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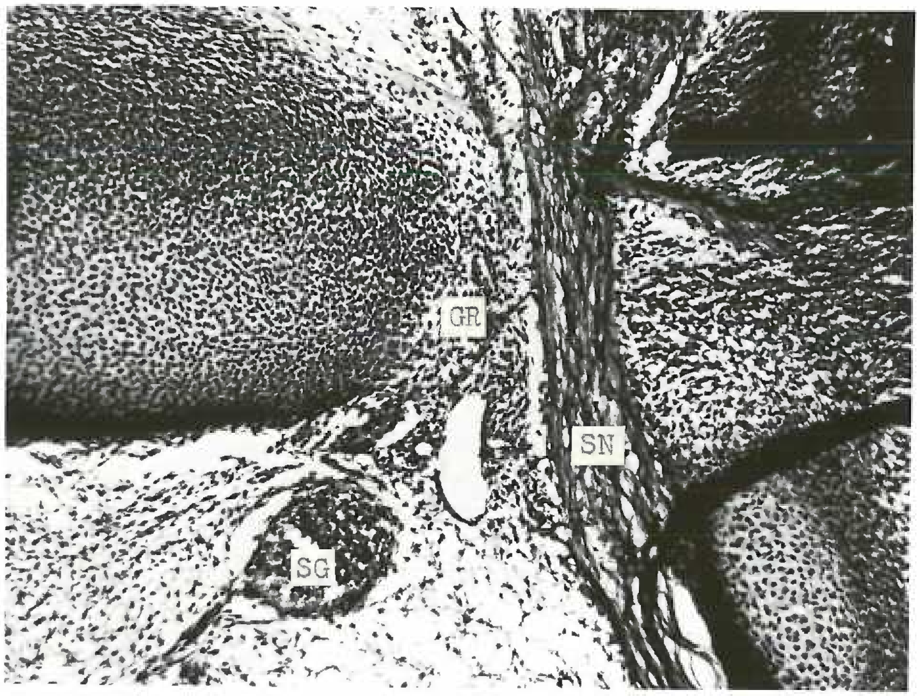
PLATE III.

Figure 5. Series 82. A low-power magnification of a section through a 14 millimeter embryo showing a few fibers of a grey ramus (GR) approaching the first thoracic spinal nerve (SN). This section does not show a white ramus.

Figure 6. Series 249. This photomicrograph depicts a white ramus (WR) leaving the second thoracic spinal nerve (SN), and a grey ramus growing back from the sympathetic ganglion (SG) in a 15 millimeter embryo.



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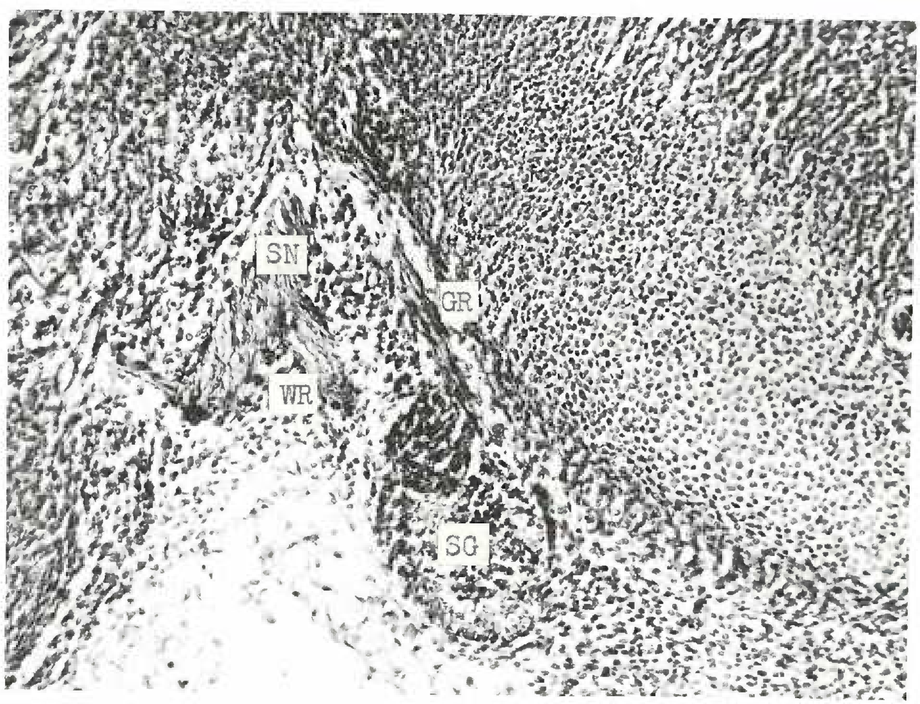

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PLATE IV.

Figure 7. Series 87. This section from an 18 millimeter embryo shows a white ramus (WR) leaving the fifth thoracic spinal nerve (SN), and a grey ramus (GR) originating from the sympathetic ganglion (SG).

Figure 8. Series 180. A low-power photomicrograph from a transversely sectioned 20 millimeter embryo illustrating the white rami (WR), grey rami (GR), and sympathetic ganglia (SG) bilaterally, at the level of the fourth thoracic spinal nerve.

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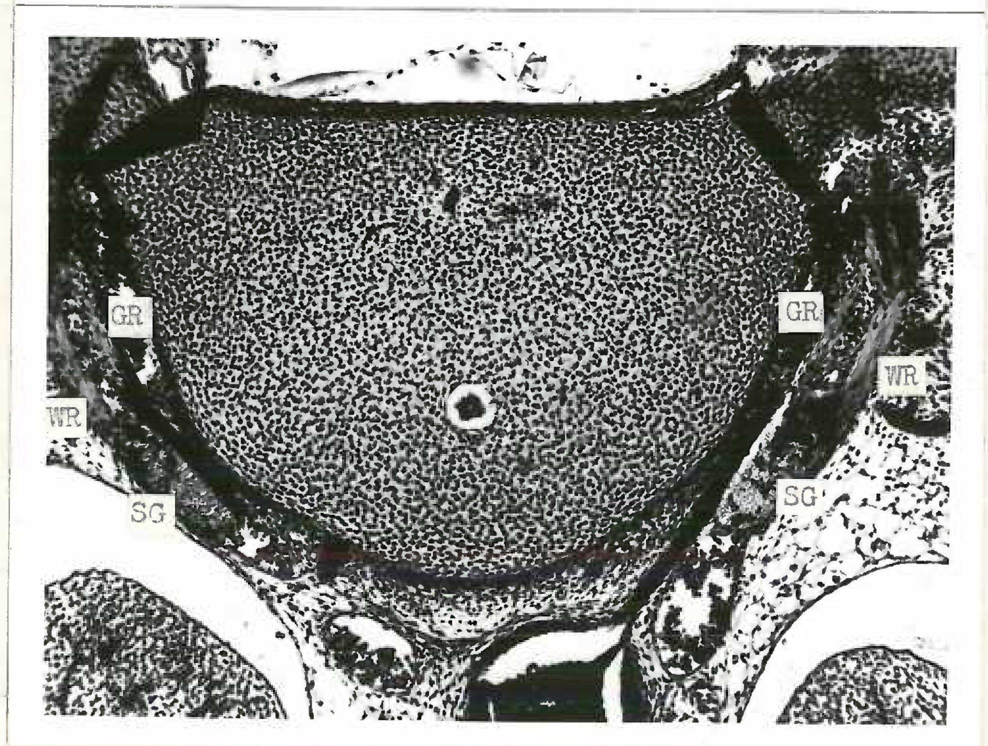

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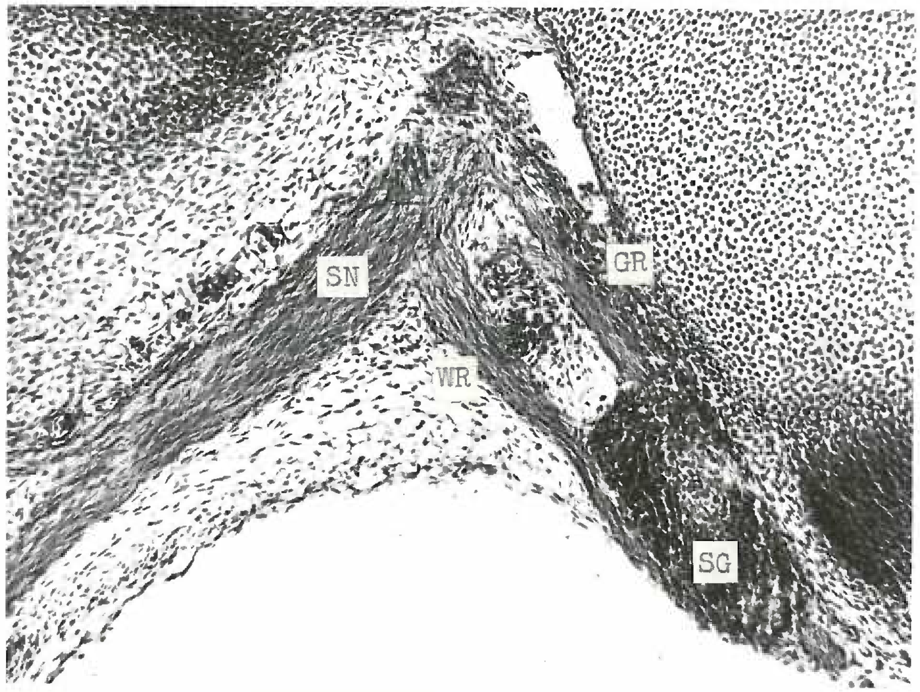
PLATE V.

Figure 9. Series 234. This section from a 20 millimeter embryo shows the white rami (WR) and the grey rami (GR) joining the eighth thoracic spinal nerve (SN) with a sympathetic ganglion (SG).

Figure 10. Series 165. A low-power photomicrograph of a section through a 22 millimeter embryo showing a white ramus (WR) leaving the second thoracic spinal nerve (SN), and a grey ramus (GR) growing back from the sympathetic ganglion (SG).



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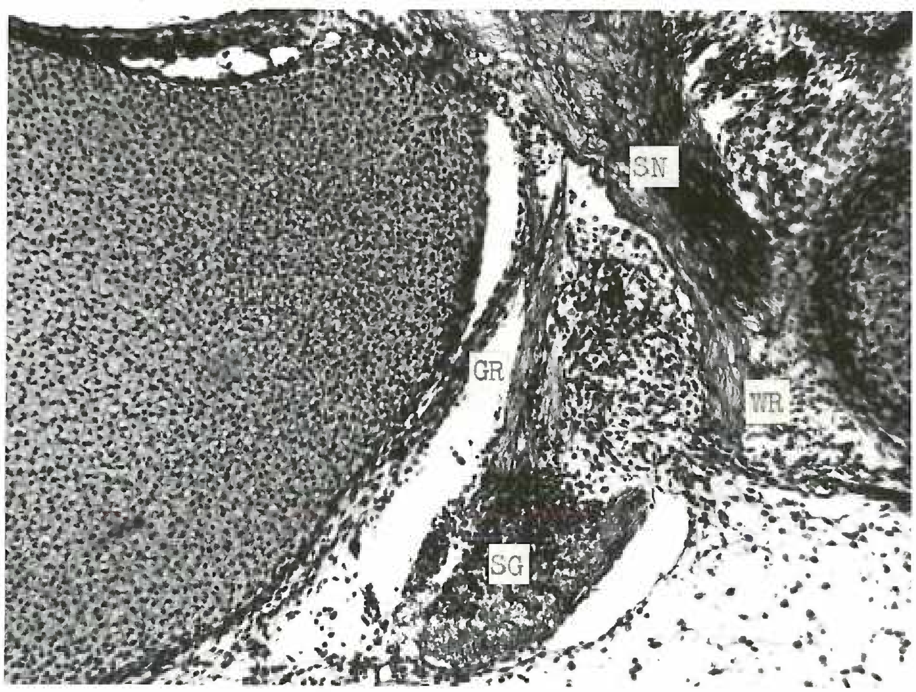

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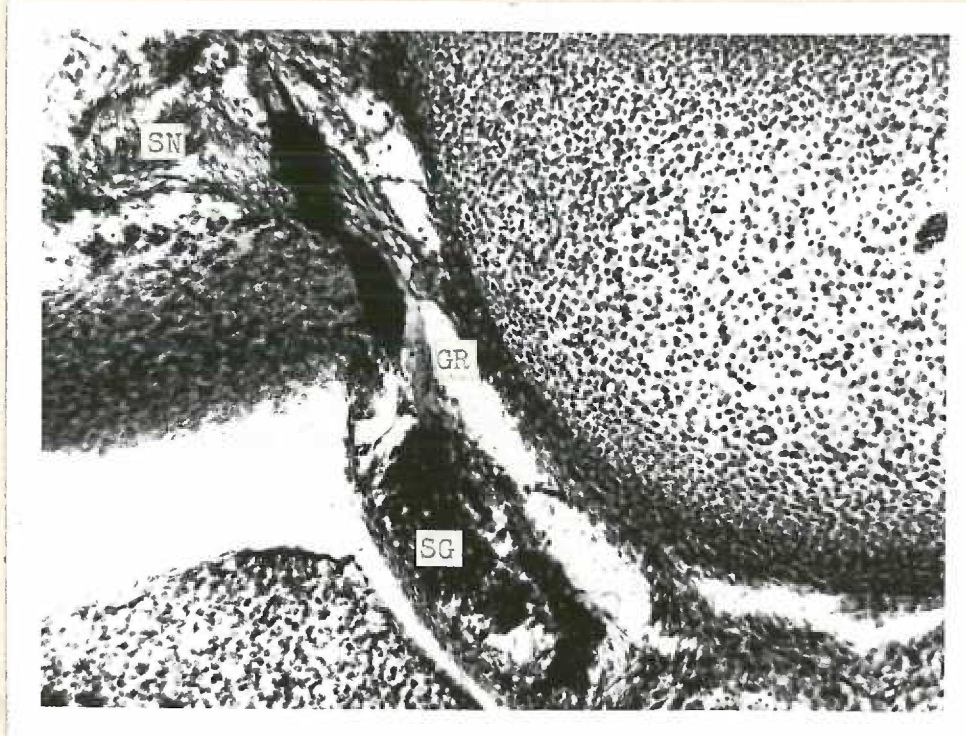
PLATE VI.

Figure 11. Series 246. A low-power magnification of a section from a 24 millimeter embryo depicting the fifth thoracic spinal nerve (SN), and a grey ramus (GR) leaving the sympathetic ganglion (SG).

Figure 12. Series 218. A section from the 25 millimeter embryo reveals a white ramus (WR) entering the sympathetic ganglion (SG), and a grey ramus (GR) approaching the ninth thoracic spinal nerve (SN).



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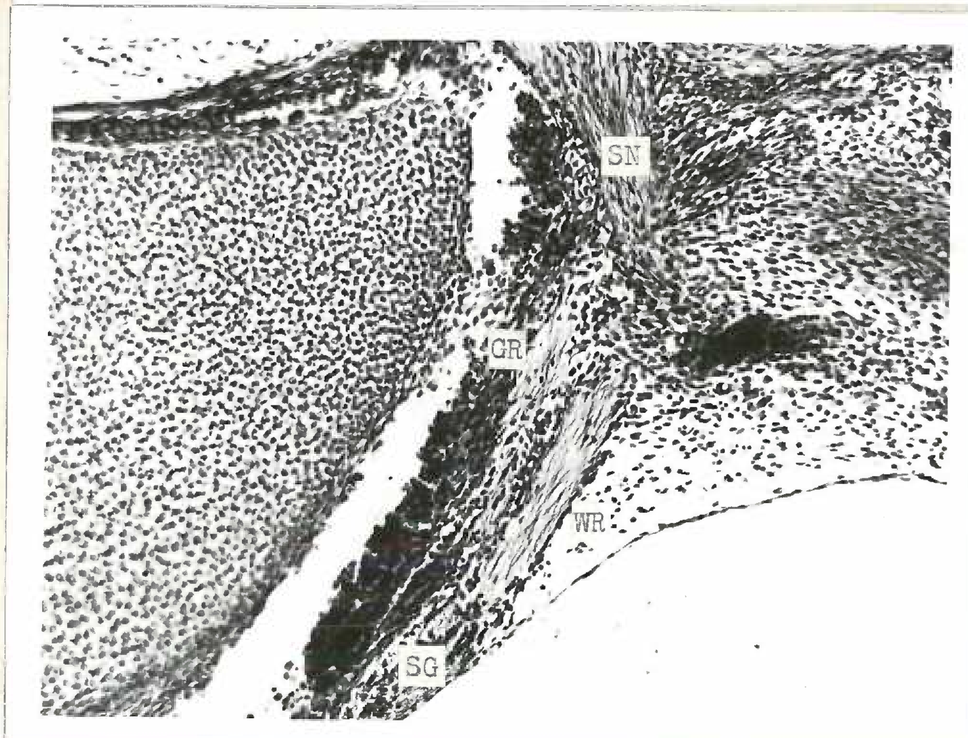

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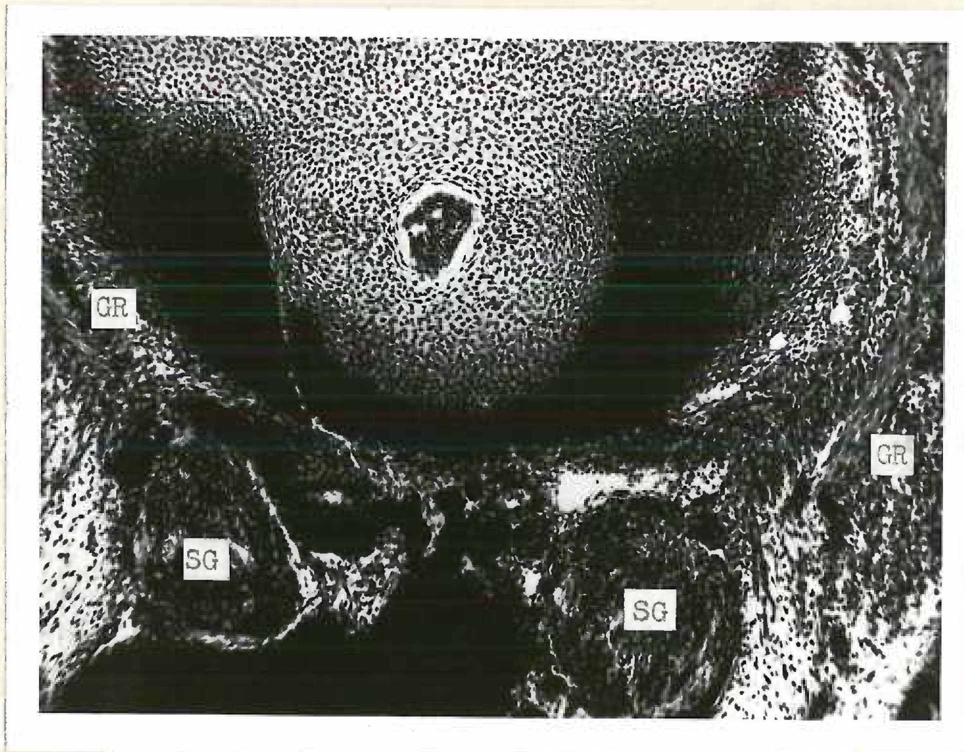
PLATE VII.

Figure 13. Series 27. A transverse section at the level of the fourth lumbar nerve from a 29 millimeter embryo demonstrates the presence of grey rami (GR) originating from sympathetic ganglia (SG).

Figure 14. Series 174. A white ramus (WR) can be observed leaving the eighth thoracic spinal nerve (SN), and a grey ramus (GR) can be seen passing from the spinal ganglion (SG) back to the spinal nerve in this transverse section of a 30 millimeter embryo.



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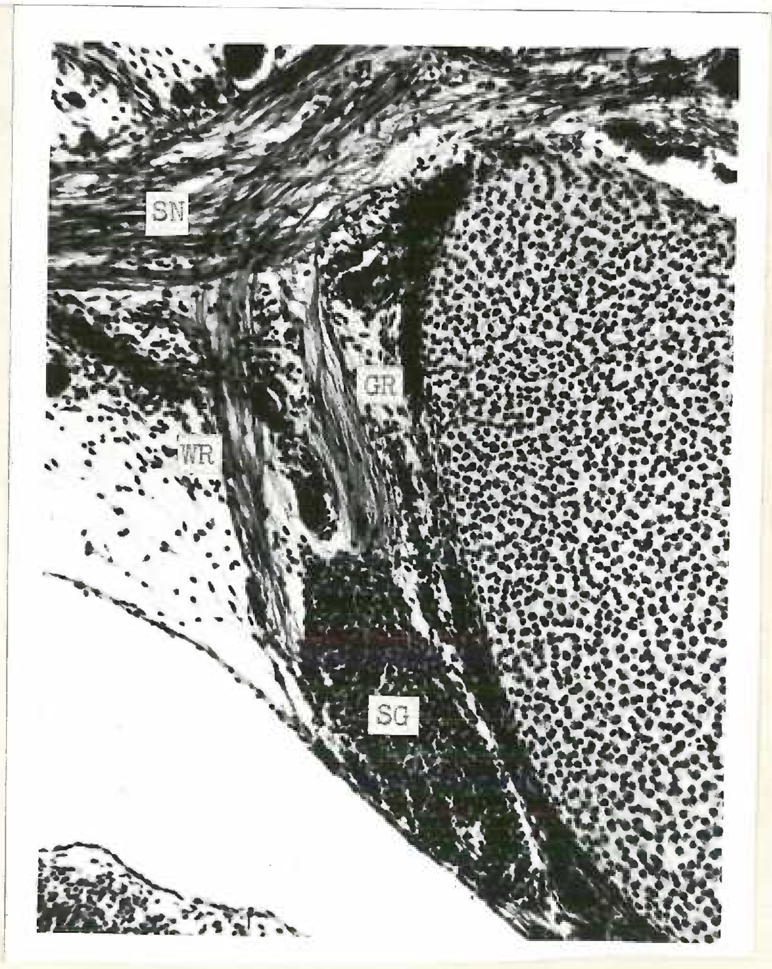

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PLATE VIII.

Figure 15. Series 166. A transverse section taken from a 35 millimeter embryo depicts the white rami (WR) and the grey rami (GR) passing between the third thoracic spinal nerve (SN) and the sympathetic ganglion.

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