THE EMBRYOLOGICAL DEVELOPMENT OF THE ELEVENTH CRANIAL NERVE OF THE CHICK

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TABLE OF CONTENTS.

PREVIOUS INVESTIGATION.

No previous investigation along this line is known to me. A commissure extending posteriorly from the tenth cranial nerve along the dorso-lateral side of the hind brain and cord has been mentioned in the literature.

Marshall (1878) guessed this to be part of a commissure running along the spinal cord and brain.

Van Wijhe (1886) suggests that this is the anlage of the eleventh cranial nerve.

Lillie (1927) calls attention to this commissure.

Adult chicken heads were dissected in order to determine the position of the eleventh cranial nerve. This was facilitated by immersing the heads for two weeks in 4% formaldehyde to which hydrochloric acid had been added to dissolve the lime in the bones. The spinal accessory was found to be a very small nerve lying on the dorso-lateral side of the spinal cord to which it is connected by many small fibers. It enters the brain cavity at the foramen magnum and runs down besidethe ganglion jugulare to the ganglion nodosum of the vagus with which it seemingly joins.

Since the commissure spoken of above has the same position and no other trace of a nerve has been found, it is maintained that this, as suggested by van Wijhe, is the anlage of the eleventh cranial nerve. The purpose of this investigation is to determine the embryonic development of this anlage,

MATERIALS AND METHODS.

Two general methods were employed in studying the embryos.

(1) Ordinary embryological staining methods.

The embryos were removed from the eggs in physiological salt solution and the somite number or age carefully recorded. They were then fixed in Bouin's fluid for from three to several hours.

Washed in 30% alcohol.

50% alcohol---30 minutes.

70% alcohol--- over night or until ready to proceed.

The embryos were then carried back to distilled water and some were stained in Delafield's hematoxylon for from 45 minutes to three hours depending on the size of the embryos. Other embryos were stained from several hours to over night in alum-cochineal.

The stained embryos were then washed in water and carried up through 30% and 50% alcohol to 70% alcohol to which a drop or two of hydrochloric acid had been added in which they were kept until no color washed away freely. Alkaline 70% alcohol was used to restore the blue color if hematoxylon were used as a stain.

95% alcohol--- one hour.

100% alcohol--- two hours.

Imbedded in paraffin. A student electric lamp over a series of tumblers in oat meal boxes proved to be the most successful method of imbedding because the embryos could be left from one

to twelve hours without harm. Sections were made seven to thirty microns thick. They were mounted on slides with Mayer's albumen fixative and the slides dried over night in the 37.5 degree incubator. The paraffin was then removed with xylol and the sections mounted in balsam.

(2) Nerve fiber staining methods.

The above method proved effective in tracing the nerve from the ganglion of the third cervical nerve to the ganglion nodosum of the vagus but did not prove adequate in following the individual fibers. As a consequence, a fiber staining process following the Lane modification of the Bielschowski-Paton method was used. The technique is as follows:

Neutral formol is made up as follows: Ordinary 40% formalin is neutralized or made slightly alkaline by an excess of magnesium carbonate. One part of this stock solution is added to nine parts of water, making a 4% solution of neutral formol. The somite number of the embryos is carefully recorded and then they are placed in this neutral formol for four days to several months. They are then subjected to the following procedure using the Lane technique.

(1) Upon removing from the neutral formol, the specimen is washed over night in running water, then rinsed three or four times in distilled water and placed in three fourths of one percent (0.75%) solution of silver nitrate in the dark. In this, it is left a varying number of days, depending on the room

temperature, until it acquires a light brown color.

(2) The specimen is again rinsed in distilled water and put for two hours in the dark into a solution made according to the following formula:

0.75% silver nitrate--- 20 cc.

40% sodium hydroxide---4 drops.

Concentrated ammonium hydroxide--- 12 drops.

The addition of the ammonium hydroxide to the silver solution produces a dark brown precipitate which is dissolved by the ammonium hydroxide. In this solution the specimens become mahogany colored and more or less translucent.

(3) The specimen is again rinsed in distilled water and placed for fifteen minutes in the following solution to dissolve any connective tissue that may be prsent.

Distilled water--- 20cc.

Glacial acetic acid---10 drops.

In this it becomes yellowish brown in color.

(4) Again the specimen is rinsed in distilled water and put for from twelve to twenty hours in the dark in a solution composed of:

1% aqueous solution of hydroquinone---20cc.

4% neutral formol----------2 cc.

The time in this solution is determined by the size of the specimen.

(5) Once more the specimen is rinsed in distilled water,

gradually dehydrated in alcohol, cleared in benzol or chloroform, imbedded in paraffin and sectioned. Serial sections of four to eight microns are best for most purposes. The slides were then treated as in the first method.

It was later discovered that the slides were better dehydrated in anilin oil than alcohol because it prevents them from getting hard. The following procedure was used after the specimens were taken from the hydroquinone solution:

Rinse in distilled water.

30% alcohol--- one hour.

50% alcohol--- one hour.

Two thirds 50% alcohol and one third anilin oil--two to four hours.

One third 95% alcohol and two thirds anilin oil-- two to four hours.

Pure anilin oil-- over night or longer.

Xylol is gradually added to the anilin oil and the specimens allowed to stand in pure xylol at least one hour.

Imbed in paraffin and section.

Later in the research, the Esaki method was used and found to be quite a sure method for this very uncertain type of staining. The technique is as follows:

(1) Fix one to two hours in a solution of

Chloral hydrate-- 15 grams.

95% alcohol-- 30cc.

Distilled water --70cc.

Add two to three drops of 2% silver nitrate solution for every 100cc. of the above just before using.

(2) Transfer directly to ammonium alcohol for 18 to 36 hours (Not over 48 hours)

100% alcohol---50cc.

Liquor ammonia caustic--- 2 to 4 drops.

(3) Transfer to 75% alcohol--- 3 to 5 minutes.

(4) Wash in distilled water for a few minutes.

(5) Place in 39 degree incubator in 2% aqueous silver nitrate solution for 14-28 days.

(6) Wash in distilled water a few seconds.

(7) Reduce two hours in pyrogallic acid.

1 gram pyrogallic acid.

90 cc. distilled water.

10 cc. formol.

(8) Wash in running water for 30 minutes.

(9) 75% alcohol.

(10) 95% alcohol.

(11)100% alcohol.

(12) Xylol.

(13) Imbed in the usual way.

It was found that dehydrating in anilin oil was just as effective as in alcohol with this method.

Camera lucida and microprojector drawings were made of desirable sections. It was found that a series of camera lucida drawings could be traced on cellophane and upon placing the drawings of a series upon each other, a reconstruction of the

entire course of the nerve could be made. These reconstructions proved very effective in showing the exact position and angle of the nerve. Photomicrographs were also made of the sections used for the drawings in order to confirm the accuracy of the drawings.

The work was completed under the guidance of Dr. H.H. Lane, Department of Zoology, University of Kansas. His interest and helpful suggestions are very gratefully acknowledged by the author.

DISCUSSION AND RESULTS.

As has been stated, the spinal accessory nerve of the adult hen is a small nerve running from the ganglion of the third cervical nerve anteriorly along the dorso-lateral side of the spinal cord with which it is attached through many small fibers. It enters the cranial cavity through the foramen magnum of the skull and leaves the cranial cavity with the vagus with which it seemingly unites. Kaupp (1918) states that it sends a fine branch to the sub-cutaneous colli artery. It was found from this embryological study, as will be brought out later, that it contributes to a small nerve innervating the neck musculature. The purpose of this investigation was to discover the embryological development of this nerve.

Although to my knowledge no work has been done on the embryological development of the eleventh cranial or accessorius nerve of the chick, a commissure extending posteriorly from the vagus in the region of the first five somites has been mentioned in embryological studies of the chick. Marshall (1878) considered this to be an anterior extension of a longitudinal commissure which connected together the posterior roots of the spinal nerves. Marshall states that this commissure was first described in elasmobranchs by Balfour but that he, himself, did not follow it late enough in the chick to know its ultimate fate. He concludes " that it gradually atrophies and finally vanishes without leaving a trace." Van Wijhe (1886) in speaking of this

commissure states: "Bekanntlich ist der Vagus mit der dorsalen Wurzel des ersten Halsnerven durch eine Commissure welche wahrscheinlich die Anlage des N. Accessorius bildet, verbunden." Lillie (1927) calls attention to this commissure extending from the hinder portion of the spreading vagus nerve roots but describes it as near the base of the neural tube, running parallel with it and later uniting with the main sympathetic trunk. He mentions that is provided with three ganglion-like swellings.

In my own work, the commissure was easily observed in 34- and 35-somite embryos when they were sectioned after having been stained in hematoxylon and eosin but it was found to have the position described and illustrated by the first two authors. That is, it is higher on the cord than suggested by Lillie. Plate XII is a drawing of a five-day embryo showing at "a" the posterior end of the commissure which has the appearance of a ribbon-like band extending forward from the ganglion of the third cervical nerve designated at "c". The ventral or motor roots of the first and second cervical nerves are shown at "d" and "e". It will be seen that the commissure is not at the base of the cord where it would be near these ventral roots but on a level with the spinal ganglia of the cervical nerves. Plate XVII-3 is a photomicrograph of this same section and shows how the structure actually appears when stained with hematoxylon and eosin as the clearly distinguishable band designated at "b". Plate XVII-2 is a photomicrograph of a section ten microns

lateral to the preceding section and shows the band "a" running anteriorly, keeping its same general position in relation to the cord. Plate XVIII-2, "a" shows this commissure as it reaches the posterior roots of the vagus proper and turns ventrally. This is a 46-somite embryo stained by the nerve fiber staining process and the commissure appears as compact groups of posteriorly projecting fibers instead of the ribbon-like band characteristic of the hematoxylon and eosin process. Plate XIV in the region marked "eleventh nerve" shows diagrammatically the position of the entire commissure. This diagram was sketched from a cellophane reconstruction of a five-day embryo and shows the position of the nerve on the cord and the path which it follows in comparison with the ganglia of the spinal and cranial nerves. Embryos up to eleven days were stained and sectioned and the "commissure" or nerve was found to be present in all of them. It then becomes apparent that this structure, as suggested by Van Wijhe, must be the anlage of the eleventh cranial nerve because (1) it has the same position as that nerve in the adult, (2) is constantly present and (3) no trace of any other nerve can be found. The problem then becomes one of tracing the exact path and embryological development of this so-called commissure.

Young embryos of 28-35 somites were stained by the nerve fiber staining methods and sagittal, frontal and cross sections were made. They were then examined carefully in the region which

had previously been determined to be that of the eleventh nerve as stated above. In 29-somite embryos, slightly developed neural crest cells lying on each side of the spinal cord were observed in this region. This corroborates the statement of Lillie (1927) that the neural crest is slightly developed in the region of the first five somites correlated with the fact that in the chick the first two spinal nerves are devoid of ganglia. Plate I-"c" shows the position of these cells on the left side of the cord in a 30-somite embryo where in this early stage, they resemble the mesenchyme cells except that they are somewhat more compactly arranged and seem to have fewer protoplasmic processes. The nuclei are rather large in comparison to the size of the cells and stain a deep brown by the nerve fiber staining methods. They range about three or four cells deep along the cord and are easily picked out after one gets used to seeing them. Plate XV-1 is a photomicrograph of a 30-somite embryo which shows these cells in the region marked "a". It can be seen that they escape detection at first glance but can be picked out by close inspection. Their appearance and comparison with the mesenchyme cells is perhaps better illustrated in the photomicrograph of the section of the 32-somite embryo shown in PlateXV-3, "a" where they stand out prominently in their regularity as compared with the mesenchyme cells near them. This is especially well illustrated on the right side of the picture. Plate XV-5 shows the depth of these cells in a 33-somite embryo where four cells can be picked

out on the right side in the region marked "a". Three cells are near the cord while the fourth is more lateral in position.

At the 30 to 31-somite stages, these neural crest cells in this anterior region of the first five somites are found to establish connections with the cord through definite fibers. Plate II is a drawing of a cross section of a 31-somite embryo showing the fibers connecting the cord and the neural crest cells on the left side. Since this is the region of the cervical flexure of the chick, it is impossible to show the cells all along the anterior part of the cord in one section. However, four cells are shown in this section with definite fibers connecting them to the cord. Plate III shows these fibers from the cells to the cord in a 32-somite embryo. The connections here are more numerous than in 30 and 31-somite embryos. It was observed that the fibers seem to wind around in the protoplasm of the cell before leaving. This can be seen somewhat on the left side of the high power drawing of the 32-somite embryo shown on this plate. This is characteristic of fibers of dorsal root ganglia cells. In 33-somite embryos, most of the cells have established connections with the cord as is shown in Plate IV where numerous connections are seen on the right side. With embryos stained and cut in this way, the cells and their fibers somewhat resemble rows of black-headed pins stuck into the cord.

By cutting true cross sections of this region of the embryo, the path of these fibers in the cord can be followed as well

as a better view of the small groups of ganglion cells obtained. Plate V shows the path of these fibers in a 35 somite embryo. The fibers are seen here to run ventrally in the cord. It was observed in a few cases that short fibers run dorsally and in many cases the fibers branch in the cord. A more posterior section in the region of the true spinal ganglia of the same embryo shows exactly the same arrangement of fibers from the neuroblasts to the cord as is the case here. Plate VII shows the fibers connected with the cord at the region of the third cervical nerve ganglion. A comparison with the two preceding plates will show the similarity of arrangement of neuroblasts and fibers in all three plates. Tissue culture seems to have proved that fibers grow from the neuroblasts of the neural crest to the cord instead of from the cord to the neuroblast. It is assumed that the same process is true in the anterior neural crest cells as in the posterior. It will be noticed from the high power drawing in PlateV that the cells in the cord are not yet well differentiated when connections are established. However, motor root fibers can be seen at this time or at least their path is marked out by the chains of cells which presumably form their sheaths. This may be observed from the same plate. Plate XVI-3 is a photomicrograph of a semi-sagittal section of a 37-somite embryo stained by the nerve fiber staining process. This photograph shows four or five fibers running into the cord at "d" from neuroblasts "c". The fibers are seen to be quite stout ones,

a characteristic which was noticed frequently throughout the work.

During this time peripheral fibers are produced from these same neural crest cells but instead of growing ventrally as is the case of the neural crest cells posterior to this region, they turn anteriorly and run forward, the more posterior fibers lateral to the more anterior. Enough of them are developed so that they can be seen as a nerve in 34-somite embryos as is shown by the photomicrograph of an embryo of this age on Plate XV-6 "a". Here the fibers photograph as a black line. One difficulty of the nerve fiber staining process is that the mesenchyme usually shrinks away from the nervous tissue breaking the connections of the neural crest neuroblasts to the cord. This has happened here so that the fibers usually lie closer to the cord than is shown in the picture. The detailed structure of the arrangement of the peripheral fibers is better demonstrated in Plates VIII and IX showing drawings of 44 and 46-somite embryos which represent little groups of neural crest cells as remaining close to the cord and the peripheral fibers running laterally for a space before they turn anteriorly. A photomicrograph of the 44-somite embryo shown on Plate VIII is shown on Plate XVI-4. The neuroblasts are seen as black dots "a" on the left side from which the small fibers "b" are running out to become part of the eleventh nerve. The photomicrograph of the 44-somite embryo on Plate XV-5 is a section which was

cut obliquely enough so that the cells can be seen standing out from the cord, showing the neuroblasts at a better advantage. Since the cord continues to enlarge laterally as the age of the embryo increases, the cells in question seem to lie on the cord in these cases when ordinary cross sections are made which because of the cervical flexure cut the cord longitudinally in this region.

These peripheral fibers run anteriorly and then turn as a group beside the ganglion jugulare of the vagus keeping their identity. This is illustrated in Plate XI "a" where the fibers show as a distinct band. The cells of the ganglion jugulare are represented at "b". This embryo was stained with hematoxylon and eosin. The photomicrograph of this section on Plate XVII-1 perhaps shows the actual appearance of the nerve more clearly than the drawing. It stains as the clear streak shown at "a" while the cells of the ganglion jugulare shown at "b" stain more darkly. This picture also shows that the fibers are on the same level as the ganglion jugulare cells and not ventral to them. Plate XVIII-2 shows a photomicrograph of the fibers in a 44-somite embryo stained by the nerve fiber staining process. Being near the color of the background, the fibers do not stand out sharply but can be seen at "a". After they pass the ganglion jugulare, they mingle with the fibers of the vagus as it enters the ganglion nodosum , very poorly shown at "b", where they are lost to view. The position of these peripheral fibers just

described is the same as that of the commissure spoken of above and also of the adult eleventh cranial nerve with its numerous connections to the cord and medulla as it runs anteriorly. They are, therefore one and the same thing.

It was mentioned above that the neural crest cells remain close to the cord in the anterior region. In this they differ from the crest cells of the true spinal ganglia where the neuroblasts collect in a group and move ventrally making the posterior root ganglion. However, these anterior crest cells do collect in groups which were found to be about 24 microns apart in a 35-somite embryo. This must be correlated with the growth of the cord in this region which would separate the cells originally quite close together. It was found that even in five day embryos the anterior fibers of the fan-shaped dorsal roots of the spinal nerves were quite contiguous with the posterior fibers of the nerve anterior to it. This suggests that the neural crest cells making the dorsal root ganglia are drawn into groups by the lengthening of the fibers connecting them to the cord and that not many are converted into mesenchyme as often suggested in embryological literature. It was commonly observed in this work as shown in the drawing previously referred to on Plate VII that the neural crest cells are near the cord when relationship is established between the cells and the cord through fibers. As will be noticed from the drawings on Plates V, VI, VIII and IX, these anterior neural crest cells lose their appearance of undifferentiated cells in older embryos and the

cell bodies stain dark as do most nerve cell bodies with the fiber staining process.

The vagus-accessory group below the ganglion jugulare was carefully observed in older embryos. It was found that a small nerve leaves this group at five days just at the point where the vagus crosses the anterior cardinal vein and before it enters the ganglion nodosum. This is whoen on Plate X at "a" and Plate XVIII-5 "a" in a drawing and photomicrograph of a section of a five-day embryo. The nerve shows as a slender projection almost escaping detection, which starts dorso-laterally in the embryo. The vagus-accessory group remains lateral to the anterior cardinal vein which is shown at "b" while the glossopharyngeal fibers shown at "c" become medial to it. Consequently, the vein separates the ninth and tenth cranial nerves at this region. By cutting cross sections of this vagus-accessory group, it was discovered that the small nerve branch is composed of elements which come both from the region of the accessory component and from the region of the vagus component. Plate XIII-A shows the cross section of an eight-day embryo stained with the nerve fiber staining process. The accessory part of the vagus-accessory region was carefully followed and is shown at "b". It will be noticed that some fibers come from that region as well as from the region of the vagus proper shown at "c". The nerve can be followed in the same embryo to the neck myotome which is shown in Plate XIII-B. The myotome is shown at "a" and the nerve fibers spreading out on it at "b". In this embryo

four little branches on the myotome were observed, three being shown in the above mentioned drawing. Photographs of the sections used for the drawings are shown on Plate XVIII-4 and 6.

Briefly summarizing, the eleventh nerve is derived, then, from neural crest cells above the first five somites of the embryo and extends anteriorly from the region of the ganglion of the third cervical nerve, along the dorso-lateral side of the spinal cord with which it is connected at each small group of crest cells. It runs on the level with the dorsal roots of the spinal nerves and the vagus. It turns ventrally beside the ganglion jugulare of the vagus and then contributes with the vagus to a small nerve to the neck myotome before it enters the ganglion nodosum with the vagus.

As has been stated, the first two cervical nerves of the chick are devoid of ganglia. Chiarugi is reported by Lillie to have observed transitory ganglia in the second, third and fourth somites. The commissure reported earlier in this discussion is reported to have three swellings. One of these is undoubtedly the ganglion of the third cervical nerve to which the commissure seemingly runs and the other two were found to be in the regions above the ventral roots of the first and second cervical nerves and to consist of somewhat larger groups of neural crest cells which send out more fibers at that region, a few of which run ventrally. Plate XII-"b" shows a swelling at the region of the second cervical nerve. It will

be noticed that the fibers from the neuroblasts of the eleventh nerve posterior to the swelling are shown at the region as well as the enlarged group of ganglion cells with their few fibers running ventrally. These few fibers are evidently transitory because they were not seen in eight day embryos or adults. Plate XVII-3 is a photomicrograph of the same section and shows the condition as clearly as the drawing. Plate XVIII-1 is a sagittal section of a six day embryo showing two swellings "a" and "b" at the eleventh nerve, "a" being the third cervical nerve ganglion. These embryos were stained with hematoxylon and eosin and do not show individual fibers. Plate XVIII-3 shows the nerve starting forward from the ganglion of the third cervical nerve in a five day embryo in a section cut lengthwise of the cord. All of these sections show that the nerve is a comparatively large one in the embryo.

It becomes apparent as a result of this investigation, that the fibers of the so-called eleventh nerve in the chick originate from the neural crest cells and are, therefore, sensory fibers as are all fibers originating from the neural crest cells along the spinal cord, Although the literature concerning the eleventh nerve in birds is very vague, it is generally considered a motor nerve. The first two cervical nerves of the chick and the twelfth cranial nerves are devoid of sensory elements. The twelfth cranial nerve fibers are considered in the literature to be homologous with the ventral roots of the spinal nerves.

The ventral roots of the first and second cervical nerves come out along the ventral part of the cord in the form of many small, almost continuous bundles which finally unite in the common trunks of the nerves. It seems scarcely logical that in addition to all these motor roots, the same region should send out other motor roots from the more dorsal part of the cord. The observations recorded here clearly indicate that the so-called apinal accessory nerve of the chick represents the sensory elements of these nerves. It is true that the motor elements of the fifth, seventh, ninth and tenth cranial nerves come out of the medulla higher than do the motor roots of the spinal nerves but since it has been found that the fibers of the eleventh nerve come from neuroblasts outside of the cord, these fibers cannot be homologous with the motor fibers of these cranial nerves.

Why the peripheral fibers of these anterior neural crest cells run anteriorly instead of connecting with motor elements at the same level may be explained by the fact that since the vagus is a composite nerve and probably represents several spinal nerves, it has captured the sensory elements of this region of the chick.

Since it was found that the vagus-accessory complex gives off a small nerve to the myotome of the nect region from the regions of the vagus and the accessorius, it is presumed that

this small nerve receives a motor complex, if such be present, from the motor fibers of the vagus. Except for the fact that the fibers of the spinal accessory keep their identity past the ganglion jugulare of the vagus, we would scarcely be justified in calling this a separate cranial nerve. Even so it seems that "vagus-accessory" or "spinal accessory of the vagus" might be better terms.

RECAPITULATION AND CONCLUSIONS.

Chick embryos from 28 somites to eleven days were studied to determine the embryological development of the eleventh cranial nerve which was found to be as follows:

The neural crest cells opposite the first five somites are only slightly developed (Lillie) because no spinal ganglia are generally developed from them. However, Chiarugi is cited by Lillie to have observed that ganglia are transiently developed at the regions of the first and second cervical nerves. These anterior neural crest cells are ill-defined from the mesenchyme cells but are somewhat more compact and lined up along each side of the spinal cord. Plates I and XV-1 show these neural crest cells in cross sections of 30-somite embryos. In general, they extend about three or four cells deep as shown in Plate XV-5. Toward the end of the third day of incubation, at the 30 to 32-somite stages, these neural crest cells establish connections with the cord through definite fibers. Since tissue culture seems to have proved that fibers grow to the cord from the neuroblasts of the spinal ganglia and since these particular fibers have exactley the same position as those in the true spinal ganglia (Plates VI and VIII) and since no cells were found in the cord directly connected with the fibers, it is assumed that the same condition holds true in the anterior neural crest cells as in the posterior. Plates II and XV-2

show the fibers beginning to attach themselves to the cord in a 31-somite embryo. Plates III, IV and XV-3, 4 show 32- and 33-somite stages with the neuroblasts and their fibers.

The above condition prevails along the cord and medulla from the ganglion of the third cervical nerve to the roots of the vagus nerve. Plates V and XVI-1, 2 show the group of neuroblasts and fibers running into the cord in a 35-somite embryo at the region of the second cervical nerve. Plate VI shows a section of the same embryo ten microns posterior to the section in Plate V. The fibers which enter the cord run ventrally mainly and sometimes branch. In a few cases, short fibers were observed to run dorsally in the cord.

The peripheral fibers of these neural crest cells instead of running ventrally as in the case of the fibers of the spinal ganglia grow anteriorly along the cord, the posterior ones lateral to the more anterior. (See Plates VIII and IX). They begin to be definitely seen in 33- and 34-somite embryos. Plate XV-6 shows the nerve in a photomicrograph of a cross section of a 34-somite embryo. The fibers run forward as a group, turn ventrally beside the ganglion jugulare of the vagus as independent fibers and then mingle with the fibers of the vagus entering the ganglion nodosum with them. Here the fibers lose their indentity. Plates XI and XVII-1 show a camera lucida drawing and a photomicrograph of the fibers of the eleventh nerve running along the ganglion jugulare of the vagus. Plate

XVIII-2 shows a photomicrograph of a 44-somite embryo showing the fibers of the eleventh nerve running into the ganglion nodosum. At five days, a branch comes from the vagus-spinal accessory group just as it crosses the anterior cardinal vein and before it enters the ganglion nodosum and runs out to the myotome of the cervical region where it spreads out in several branches. This small nerve takes fibers from the group both from the region of the spinal accessory and the region of the vagus. Plates X and XVIII-5 show this small nerve leaving the group in a five-day embryo. Plates XIII-A and XVIII-4 show the nerve leaving the group in an eight day embryo. Plates XIII-B and XVIII-6 show the nerve spreading out on the myotome in an eight day embryo.

The neuroblasts giving rise to the eleventh nerve fibers were observed in all the embryos studied from 28 somites to eleven days, becoming progressively smaller in comparison to the size of the cord as the embryos became progressively older. They remain comparatively close to the cord instead of grouping themselves in a more ventral position as is the case of the true spinal ganglia. Plates VIII and XVI-4,5 show camera lucida drawings and a photomicrograph of a 44 somite embryo showing the neuroblasts and the lateral fibers running anteriorly. Plate IX shows a camera lucida drawing of a 46 somite embryo showing the neuroblasts.

Three swellings or enlargements on the commissure running posteriorly from the vagus are reported in the literature.

One of these, the posterior, is undoubtedly the ganglion of the third cervical nerve because the fibers of the eleventh cranial nerve run posteriorly to this ganglion where the peripheral fibers of the neuroblasts instead of growing anteriorly grow ventrally to meet the ventral root fibers. The first and second swellings are at the regions above the ventral roots of the first and second cervical nerves where there is a slight enlargement or grouping of the neural crest cells. A few fibers transitorily run ventrally at this region. Plates X11, XV11-3 and XV111-1 show the swelling at the second cervical nerve region and the spinal accessory fibers running from the region of the third cervical nerve ganglion in five and six day embryos. These swellings are probably the transitory ganglia reported by Chiarugi. In the adult, ganglia are lacking in the first and second cervical nerves.

In later development, the first four somites contribute to the formation of the skull and the ventral roots of the first cervical nerve come out through the foramen magnum of the skull. The ventral roots of the second cervical nerve come out through the foramen between the atlas and the axis and the third cervical nerve comes out between the axis and the third certebra. The spinal accessory then runs from the posterior end of the axis, through the foramen magnum of the skull and out of the cranial cavity in the vagus foramen and runs to the ganglion nodosum which lies at the base of the skull on the ventral side, the

vagus and accessory each contributing to a small nerve in the neck region just before entering the ganglion at the five day stage. Plate XlV shows a diagramatic representation drawn from a cellophane reconstruction of the posterior cranial and anterior cervical nerves in an embryo of five days.

The conclusions reached by this research show that the so called spinal accessory of the chick is in itself undoubtedly a sensory nerve representing the sensory elements of the first and second cervical and twelfth cranial nerves which lack sensory elements. The muscles innervated by this nerve must receive their motor components from the vagus.

SUMMARY

(1) The development of the eleventh cranial nerve of the chick was studied in 28-somite to eleven day chick embryos.

(2) The nerve fibers were stained by a modification of the Lane adaptation of the Bielschowski-Paton method and by the Esaki method.

(3) The eleventh cranial nerve of the chick runs on the dorso-lateral side of the spinal cord and medulla running anteriorly with numerous attachments to the cord from the ganglion of the third cervical nerve through the foramen magnum of the skull and turning ventrally beside the ganglion jugulare of the vagus, leaves the cranial cavity with the vagus and after contributing with the vagus to a small nerve innervating the neck muscles enters the ganglion nodosum where its fibers are lost to view.

(4) The fibers of the eleventh nerve are developed from the neural crest cells opposite the first five somites of the embryo at the 30 to 32- somite stage. Medial fibers of these neuroblasts establish relationship to the cord and peripheral fibers grow anteriorly, the more posterior lateral to the more anterior.

(5)The peripheral fibers of the eleventh nerve run as a unit beside the ganglion jugulare and some fibers at five days contribute with the vagus to a small nerve branch innervating the myotome at the cervical region.

(6) The spinal accessory nerve of the chick is fundamentally a

sensory nerve representing the sensory elements of the first and second cervical and the twelfth cranial nerves and the structures innervated by it receive their motor innervation, if such, from the contributions of the vagus.

(7) The nerve appears large in comparison to the spinal cord in the embryo but becomes a small, thread-like nerve in the adult.

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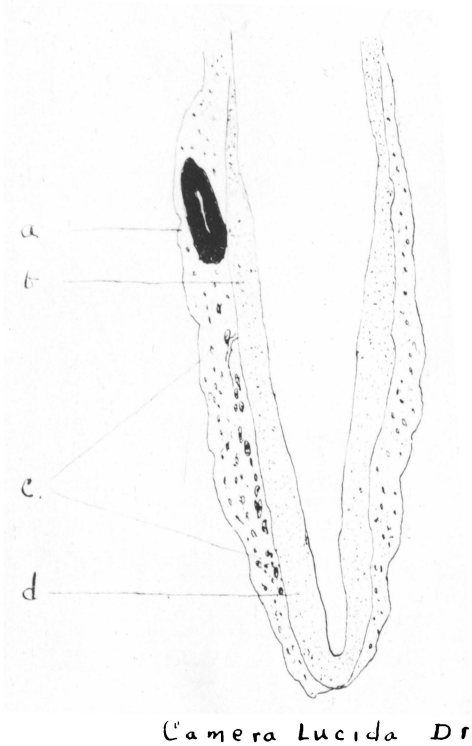
30 Somite Chick Embryo
Showing Neural Crest Cells

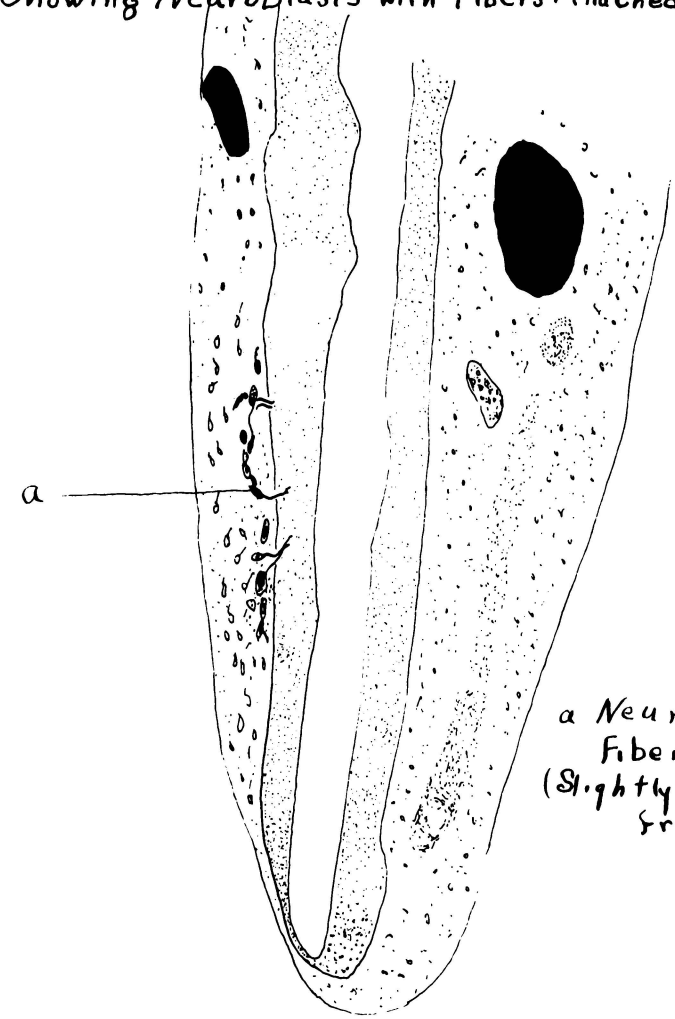


Camera Lucida Drawing

a. Otic capsule
b. Myeloncephalon
c. Neural Crest Cells
d Spinal Cord

31 Somite Chick Embryo

Showing Neuroblasts with Fibers Attached to Cord

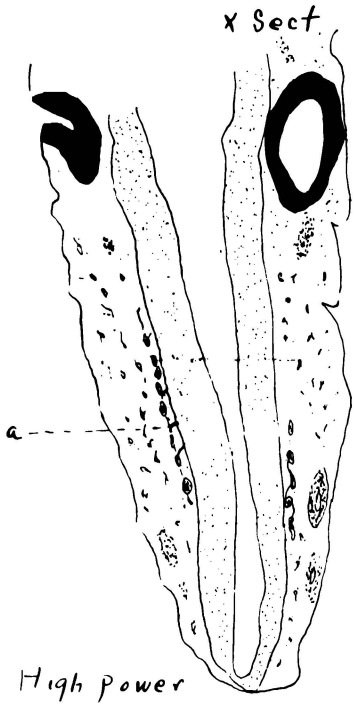


a Neuroblast with Fibers. High power (Slightly pulled away from cord)

Camera lucida Drawing

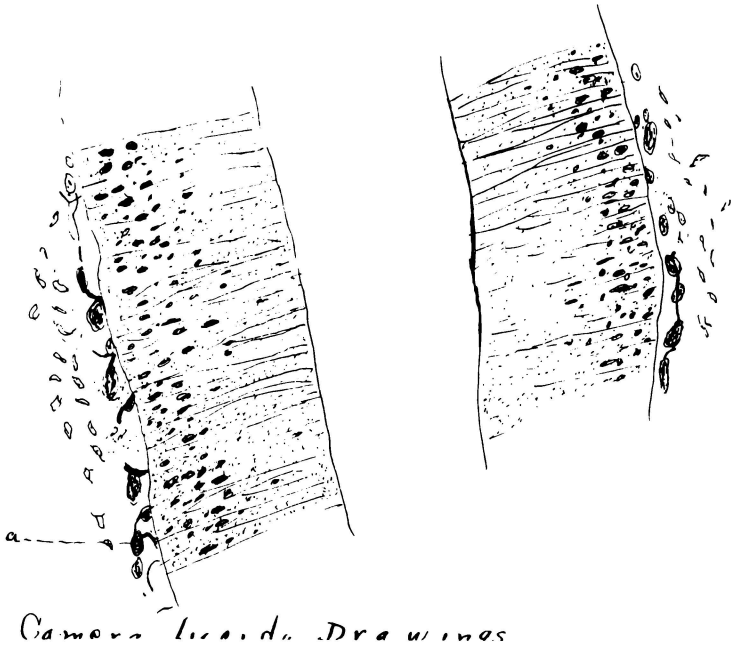
Plate III

32 Somite Chick Embryo



a Neuroblast cells with connections established to cord

High power

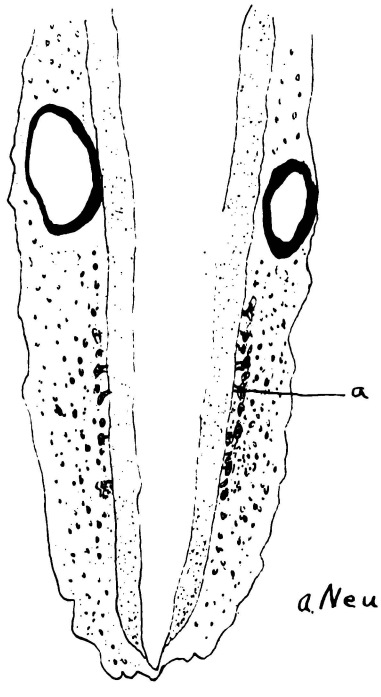


Camera Lucida Drawings

Plate IV

33 Somite Chick Embryo

Showing Neuroblasts with Fibers Attached to Spinal Cord



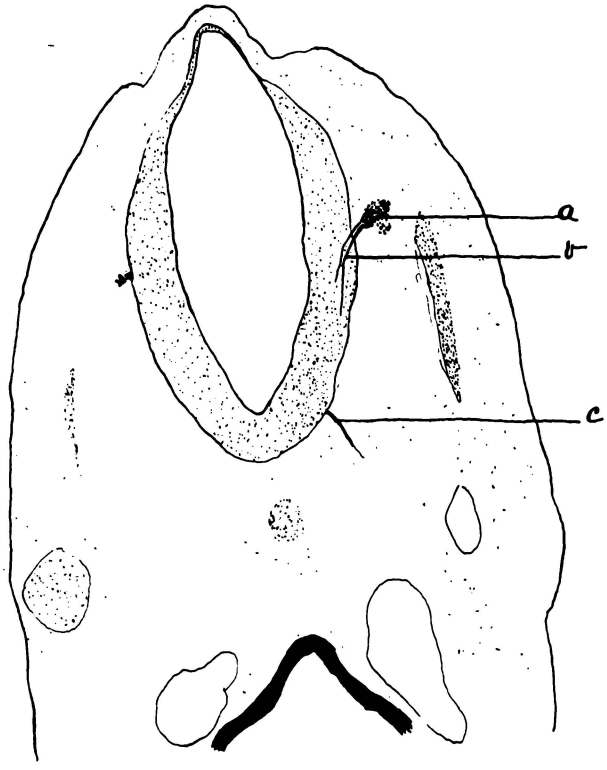
a. Neuroblast with Fiber

Microprojector Drawing

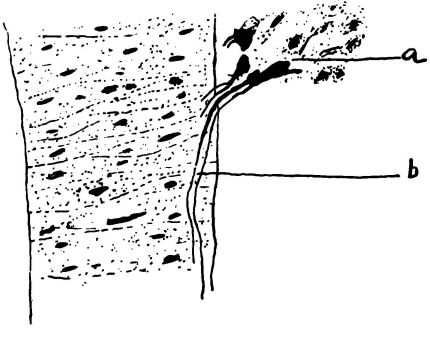
Plate V

35 Somite Embryo

x sect. thru 2nd Cervical Nerve Region



High Power

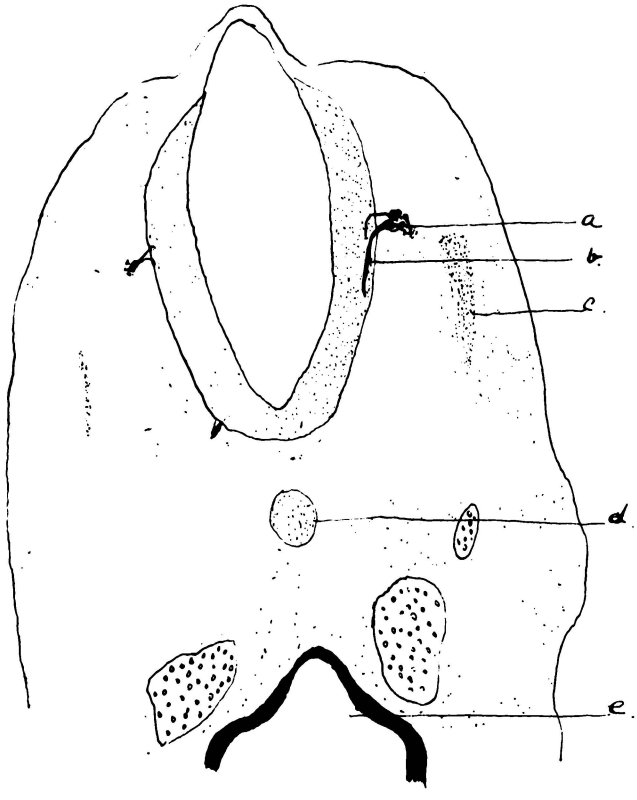


a Neuroblasts
b. Fibers to Cord
c. 2nd Cervical Nerve (Ventral root)

Camera Lucida Drawings

35 Somite Embryo

x sect 10 μ posterior to the second cervical nerve



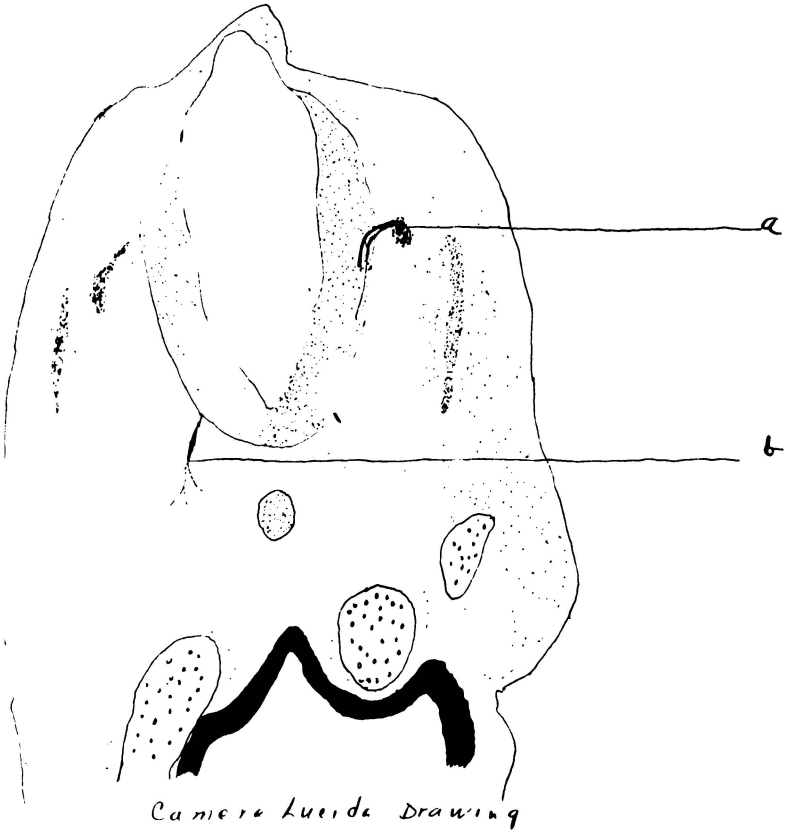
Camera Lucida Drawing

a. Neuroblast
b. Fiber from Neuroblast
c. Somite
d Notochord
e Gut

Plate VII

35 Somite Chick Embryo

x Sect. through 3rd cervical Nerve



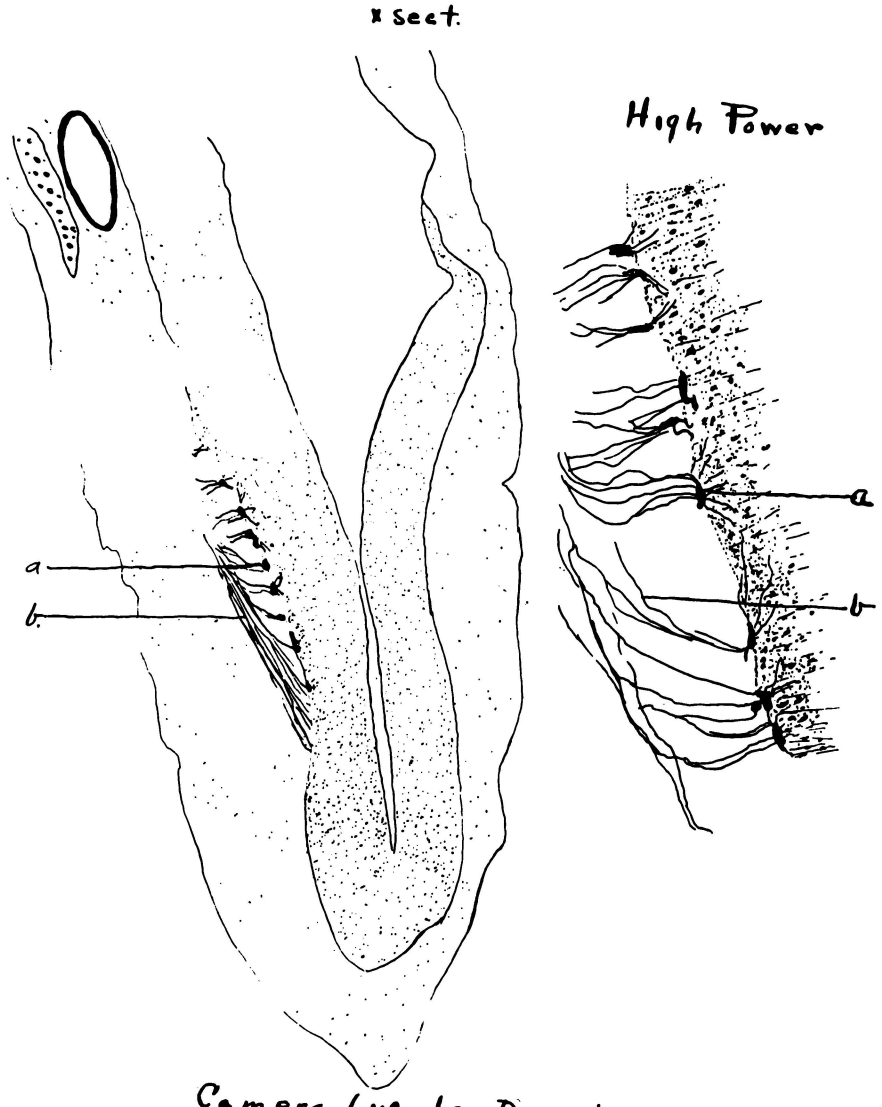
Camera Lucida Drawing

a Neuroblast cells of 3rd cervical Nerve Ganglion

b Ventral Root of 3rd cervical Nerve

Plate VIII

44 Somite Chick Embryo



Camera Lucida Drawing

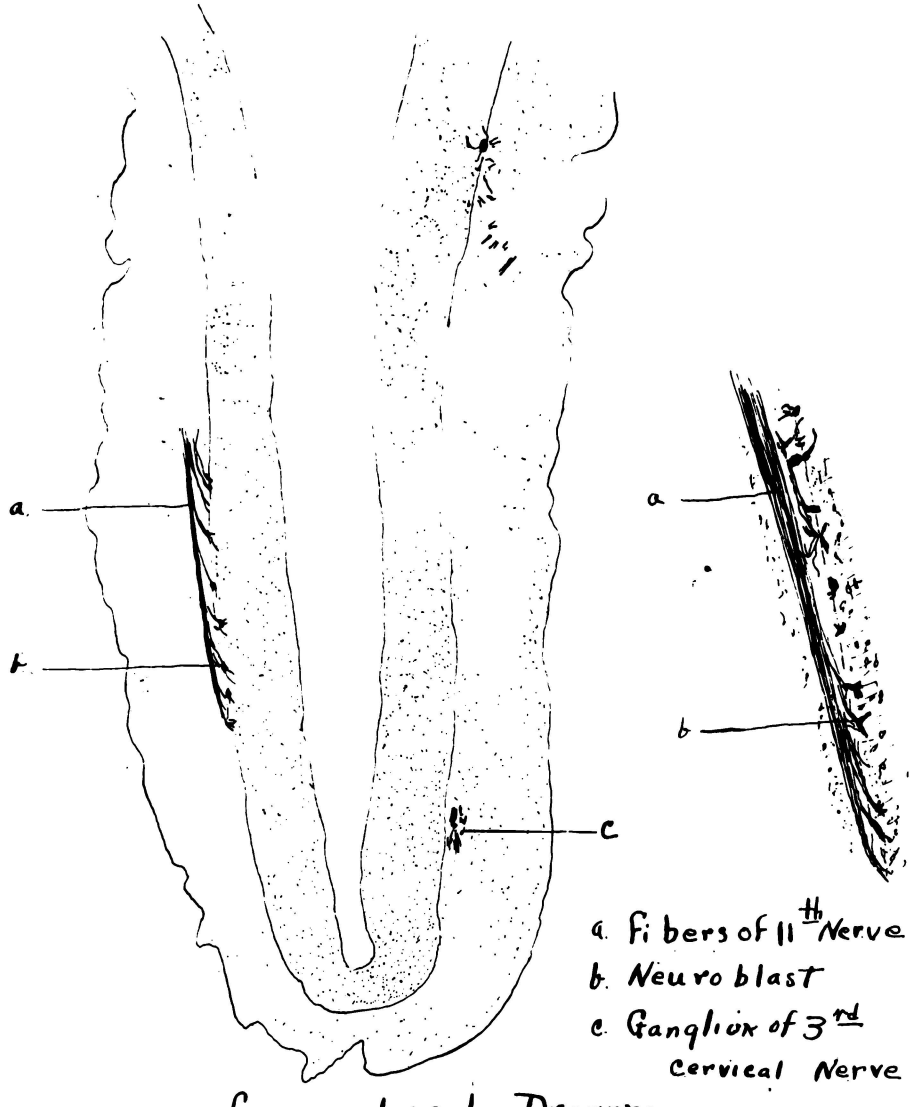
a. Neuroblast

b Fibers of 11th Nerve

Plate IX

46 Somite Chick Embryo

x seet

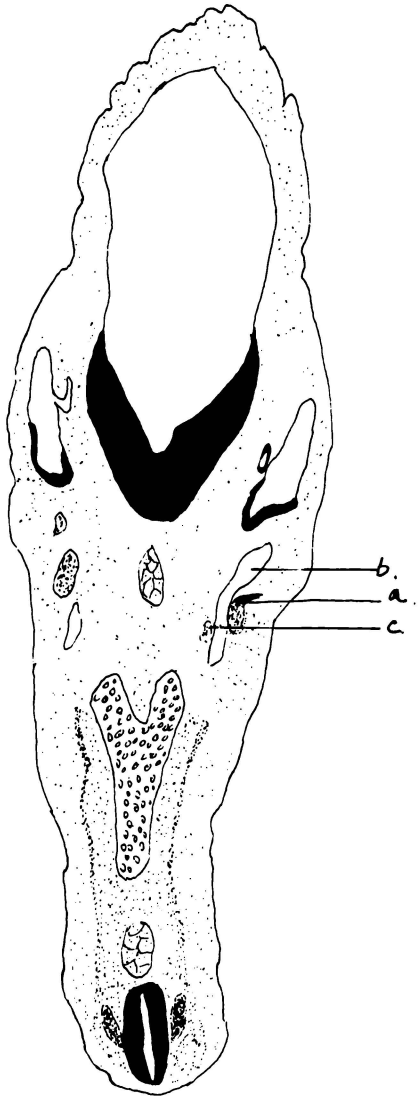


a. Fibers of 11th Nerve
b. Neuro blast
c. Ganglion of 3rd Cervical Nerve

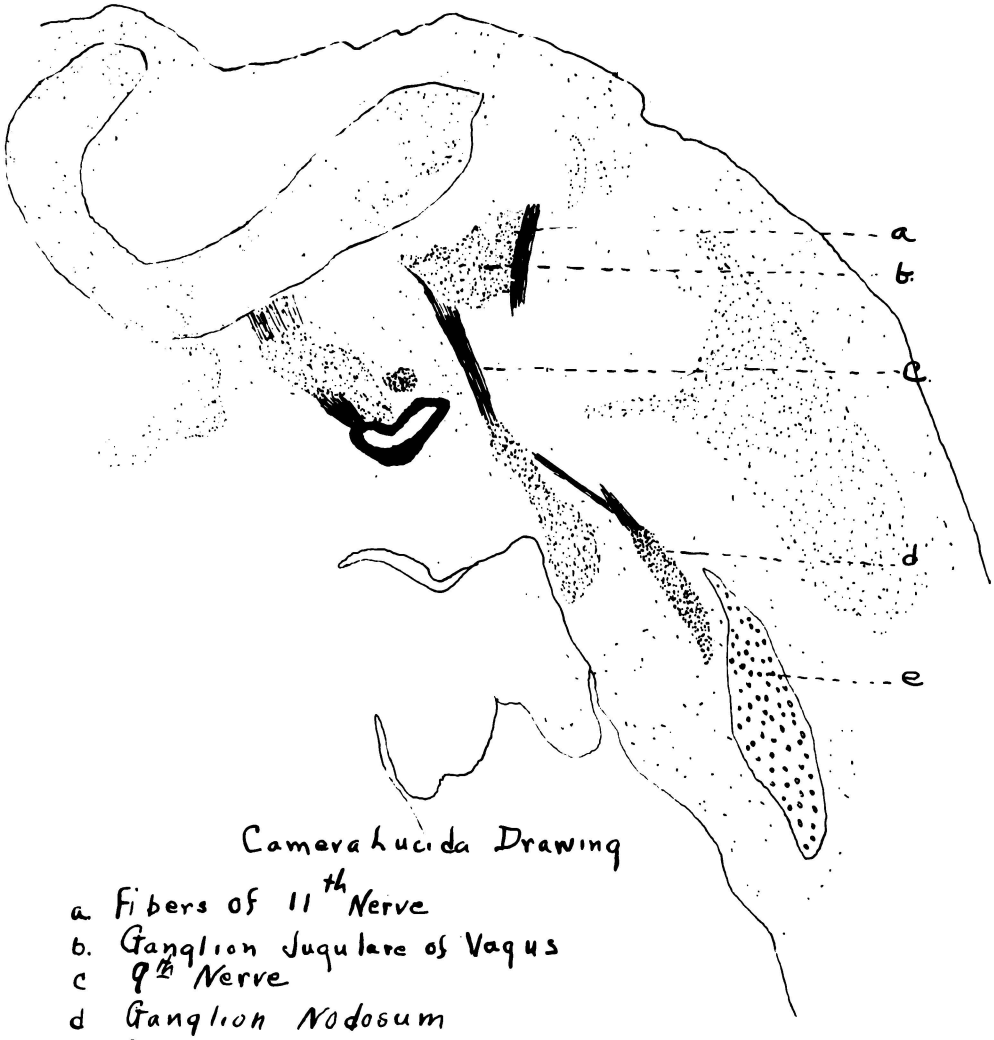
Camera Lucida Drawing

Plate X

Five Day Embryo Showing
Small Nerve Leaving Vagus-Accessory
Group at a



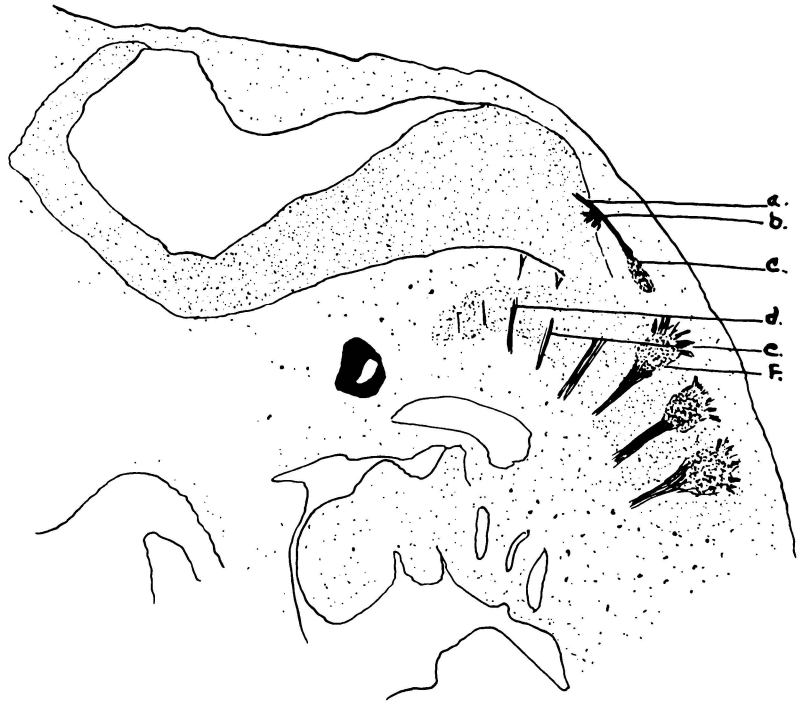
Sagittal Section of 5 da. Chick Embryo Showing Fibers of 11th Nerve Running Beside Ganglion Jugulare



Camera lucida Drawing

a. Fibers of 11th Nerve
b. Ganglion Jugulare of Vagus
c 9th Nerve
d Ganglion Nodosum
e Anterior Cardinal vein

Five Day Chick Embryo Showing Fibers of 11th Nerve Running into 3rd Cervical Nerve Ganglion



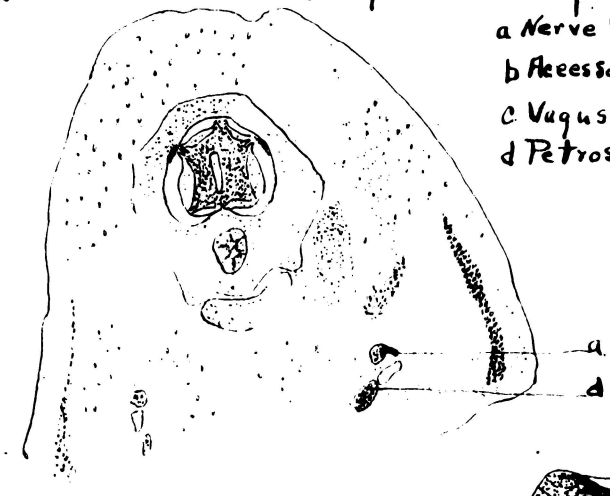
Microprodector Drawing

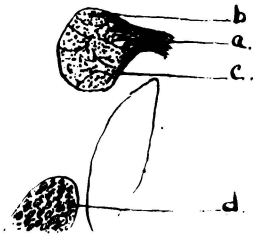
a. Fibers of 11th Nerve
b Rudimentary Ganglion of 2nd Cervical Nerve
c. Ganglion of 3rd Cervical Nerve
d. Fibers of 1st. Cervical Nerve
e. Fibers of 2nd Cervical Nerve
f. Ganglion of 4th Cervical Nerve

Eight Day Embryo

A Showing Small Nerve Leaving Vagus-Accessory Group

a Nerve Leaving Group
b Accessory Component
c Vagus Component
d Petrosal Ganglion





B Showing Above Nerve Reaching the Myotome

Microprojector Drawings

a Myotome
b Nerve Innervating Myotome

FINAL EXAMINATION FOR PH.D

Clara Hartley
B.S.Educ. 1923, Miami University
A.M. 1926, University of Michigan

Major: Zoology
Minor: Botany

Exam. to be held:
April 27, 1935
9:30 a.m. Room 206, S.
Committee: Lane
Baumgartner
Taylor
Mix
Stevens

GRADUATE RECORD--University of Michigan
SS 1924, 1925, Fall, 1925, SS-1930-

Zoology	4	B	Botany	1	A
Zoology	4	A	Botany	2	B
			Botany	2	A
			Botany	4	A
			Botany	2	A
			Botany	5	A
			Botany	3	B

University of Wisconsin--SS 1930

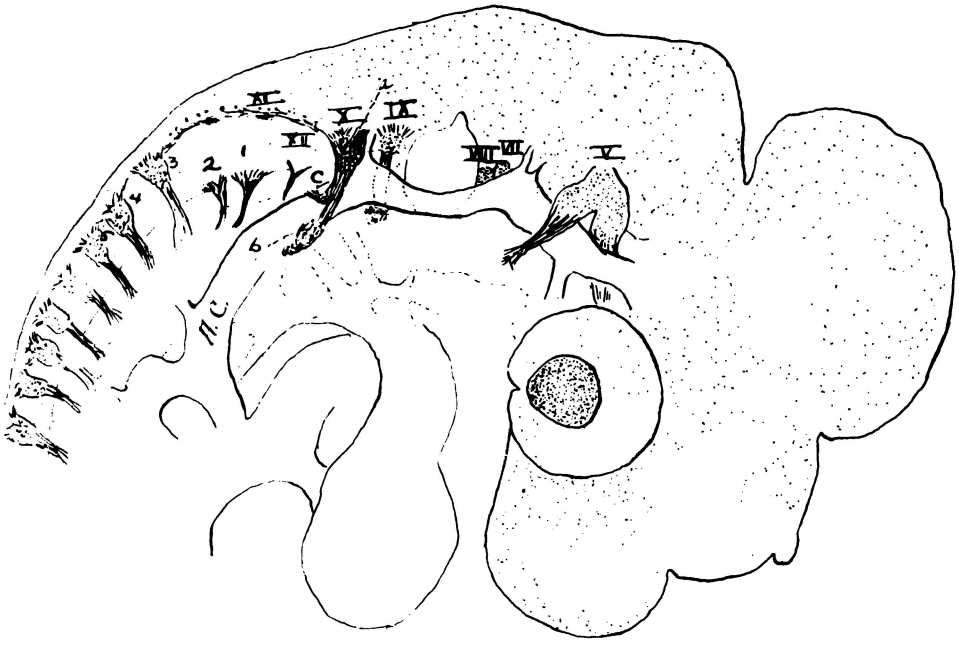
Zoology	3	A	Botany	1	A
Zoology	3	A			

University of Kansas--SS-1931,
1931-32, SS 1932, 1932-33,
SS 1933, 1933-34, SS-1934,
1934-35

160 Parasitology	3	A	304 Conference	1	
325 Research	1	A			
325 Research	4	A	**Bacteriology**		
325 Research	4	A	150M Pathogenic Bact.	5	A
325 Research	4	A	151 Medical Immunol.	3	A
325 Research	4	A	162 Journals	1	S
325 Research	4	A	261 Special Problems	4	S
154 Cytology	3	S			
325 Research	4	A			
325 Research	4	S			
325 Research	S	A			
300 Thesis	4	A			
301 Fund. Concepts	2	S			
374 Conference	1	S			
301 Fund. Concepts	2				
300 Thesis	2				

Plate XIV

Diagramatic Representation of the Position of the Last Cranial and First Cervical Nerves



V - XII : Cranial Nerves
1 - 5 : Cervical Nerves
a = Ganglion Jugulare
b = Ganglion Nodosum
c = Small branch of V and VI
A.C. = Anterior Cardinal vein

EXPLANATION OF PLATE XV

1. Photomicrograph of a 30 somite embryo. The slightly differentiated neural crest cells lined up along the myelencephalon and spinal cord are shown at (a).

2. Photomicrograph of the 31 somite embryo drawn in Plate 11. The neural crest cells have fibers running to the cord and a few fibers started anteriorly. (a) is the cell designated in Plate 11.

3. Photomicrograph of the 32 somite embryo drawn in Plate 111. Anterior neural crest cells are shown on each side of the cord.

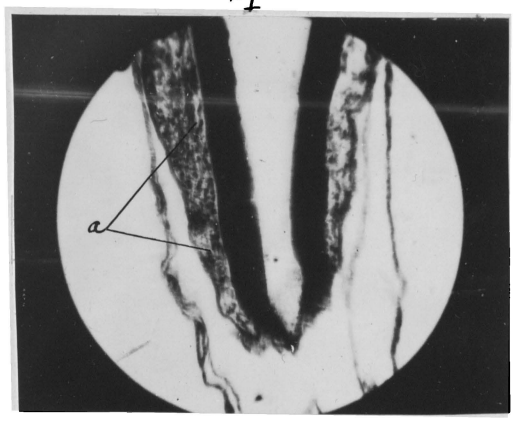
4. Photomicrograph of the 33 somite embryo drawn in Plate 1V showing attachment of fibers to the cord.

5. Photomicrograph of the 33 somite embryo stage showing several layers of neuroblasts in the anterior neural crest at (a).

6. Photomicrograph of a 34 somite embryo showing the fibers of the eleventh cranial nerve at (a).

Plate XV

-1-



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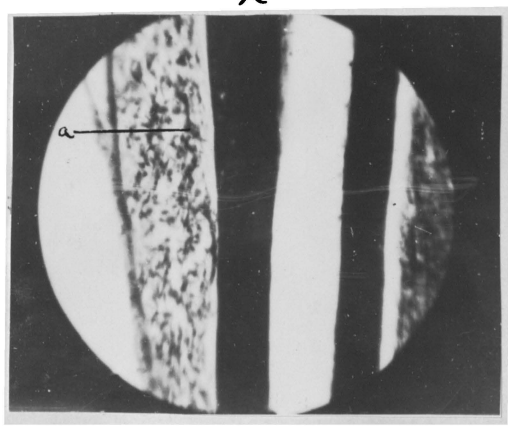

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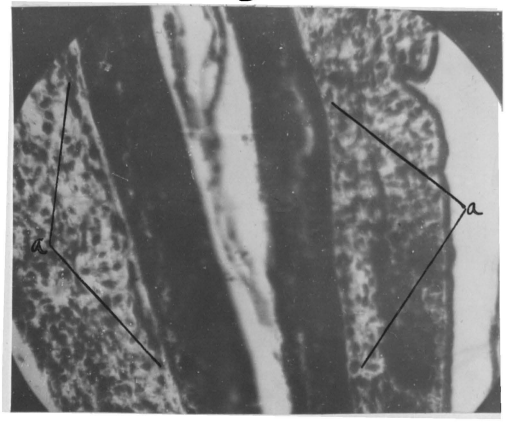

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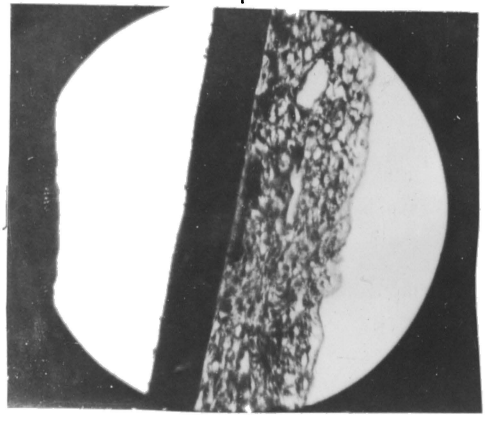

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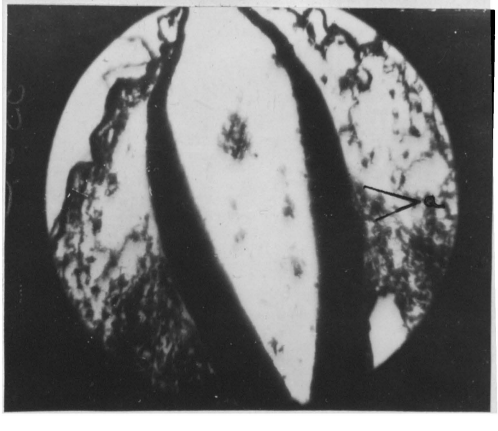

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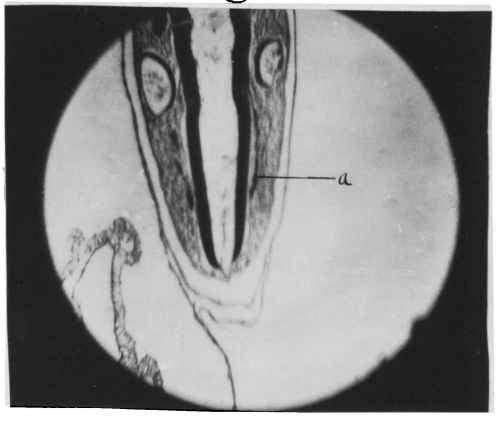


EXPLANATION OF PLATE XVl

1. Photomicrograph of the cross section of the 35 somite embryo drawn in Plate V. The small group of neural crest cells are shown at (a) and the root of the second cervical nerve at (b).

2. Photomicrograph of the same embryo under high power showing the neuroblasts with the fibers running into the cord at(a)

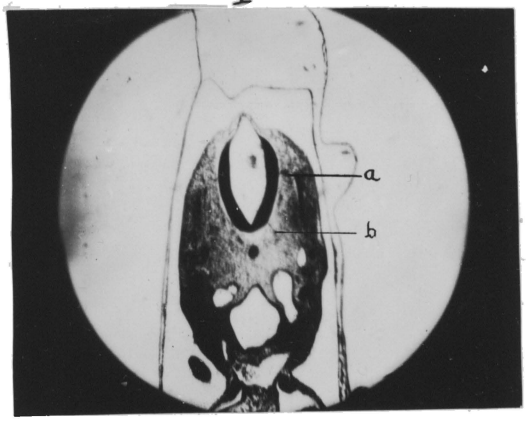
3. Photomicrograph of a sagittal section of a 37 somite embryo showing the ganglion jugulare of the vagus at (a), the fibers of the eleventh nerve at (b), a neuroblast at (c) and a fiber running into the cord at (d)

4. Photomicrograph of the 44 somite embryo drawn in Plate Vlll. Groups of neuroblasts are shown at (a) and peripheral fibers running anteriorly at (b).

5. Photomicrograph of a 44 somite embryo cut somewhat obliquely so that the neuroblasts (a) stand out prominently from the cord. Peripheral fibers are shown at (b).

Plate XVI

-1-



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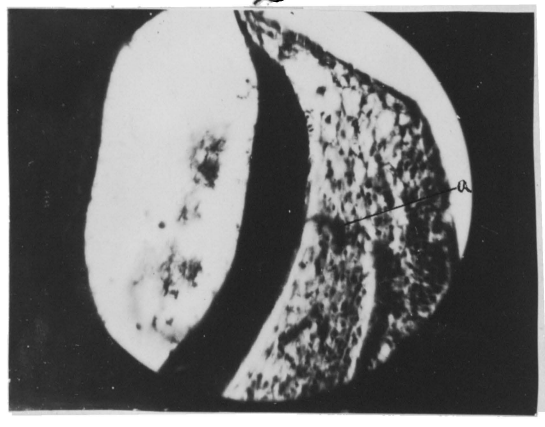

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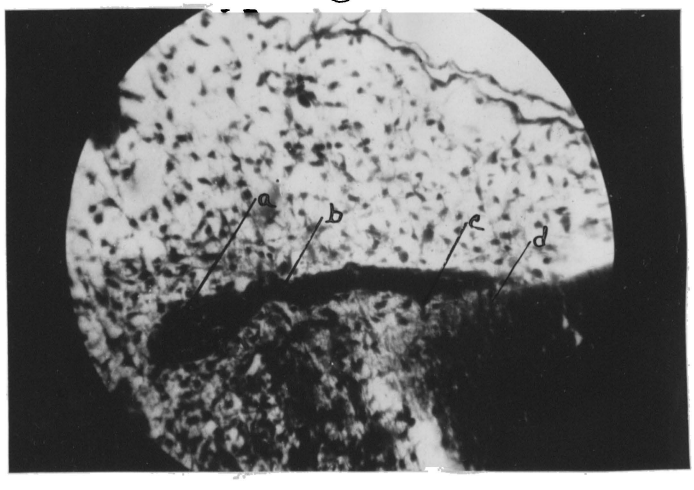

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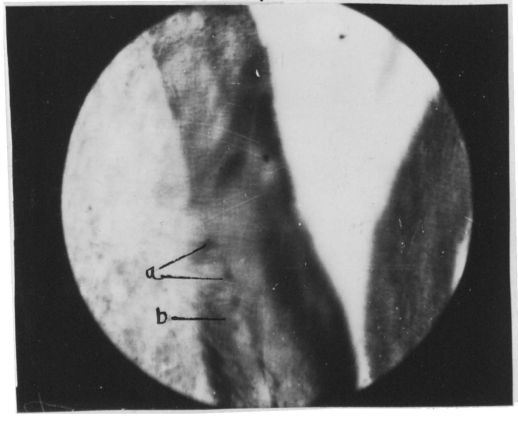

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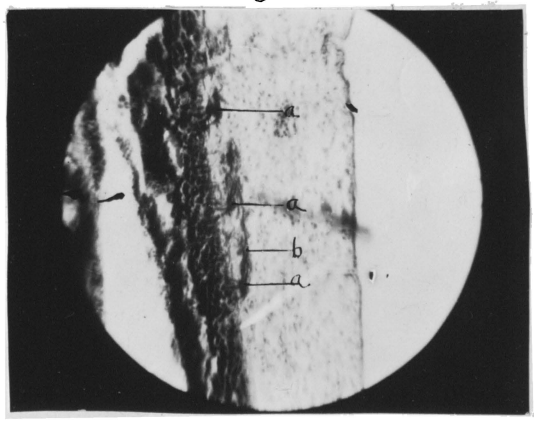


EXPLANATION OF PLATE XVll

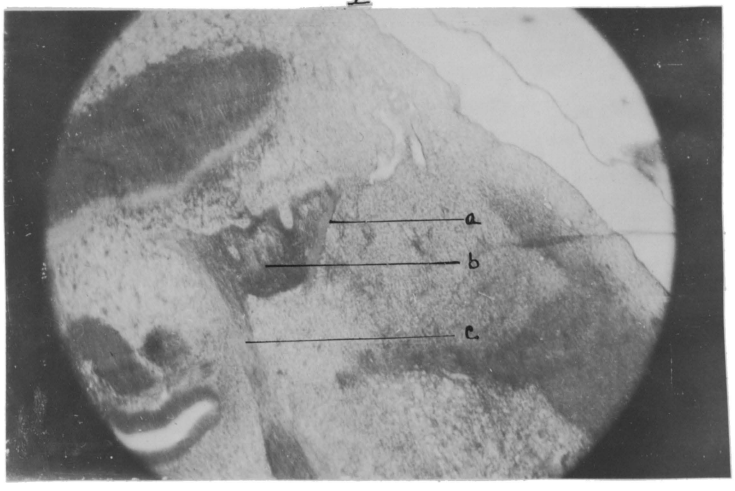
1. Photomicrograph of the sagittal section of the five day embryo drawn in Plate Xl. (Hematoxylon and eosin stain). The eleventh cranial nerve (a) is shown running as a unit beside the ganglion jugulare of the vagus (b). The glossopharyngeal nerve is shown at (c).

2. Photomicrograph of another section of the same embryo. The fibers of the spinal-accessory are shown at (a) and the ganglion of the third cervical nerve at (b).

3. Photomicrograph of a section of the same embryo ten microns medial to 2. (Plate Xll) The transitory ganglion opposite the second cervical nerve is shown at (a), the fibers of the eleventh nerve at (b), the ganglion of the third cervical nerve at (c) and of the fourth at (d).

Plate XVII

-1-



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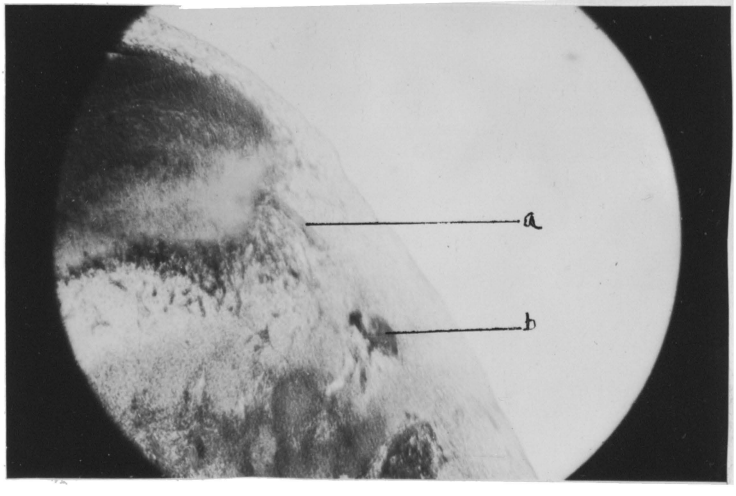

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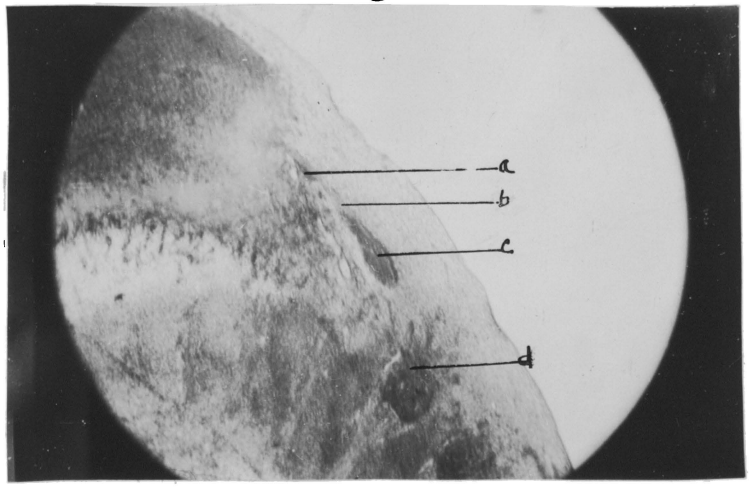


EXPLANATION OF PLATE XVlll

1. Photomicrograph of a sagittal section of a six day embryo showing the transitory enlergement of the eleventh nerve at (b) and the third cervical nerve ganglion at (a). (Hematoxylon and eosin stain).

2. Photomicrograph of a sagittal section of a 44-somite embryo showing the fibers of the eleventh nerve (a) running into the ganglion nodosum at (b).

3. Photomicrograph of the cross section along the spinal cord of a five day embryo showing the ganglion of the third cervical nerve at (a) and the fibers of the eleventh nerve running along the cord at (b). (Hematoxylon and eosin stain)

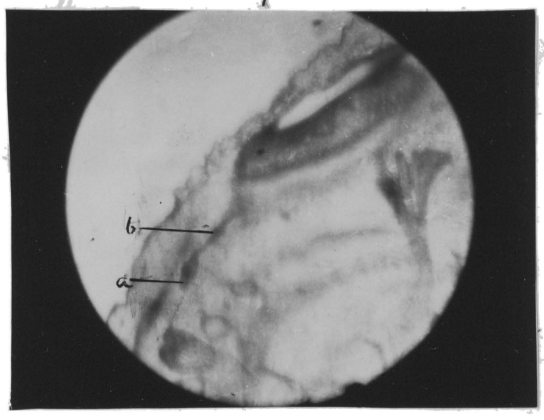
4. Photomicrograph of the cross section of the eight day embryo drawn in Plate Xlll-A showing the small nerve leaving the vagus-spinal accessory group at (a).

5. Photomicrograph of the cross section of the five day embryo drawn in Plate X showing the small nerve leaving the vagus-spinal accessory group at (a) and the anterior cardinal vein at (b).

6. Photomicrograph of the cross section of the eight day embryo drawn in Plate Xlll-B showing the small nerve reaching the myotome at (a).

Plate XVIII

-1-



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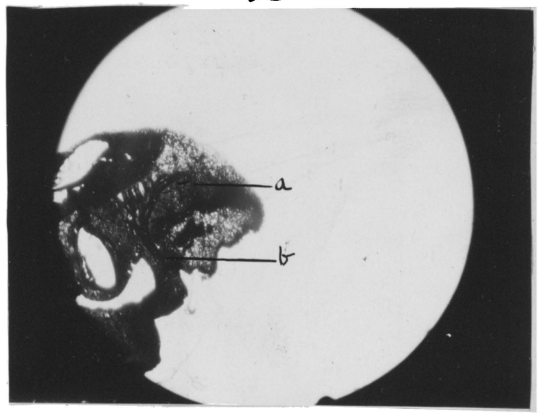

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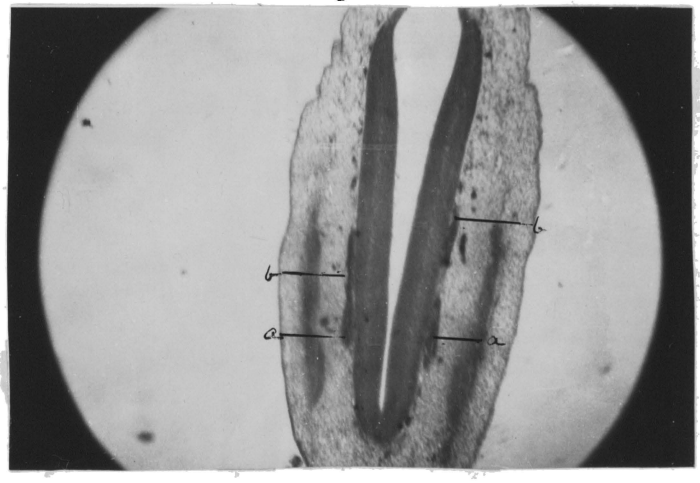

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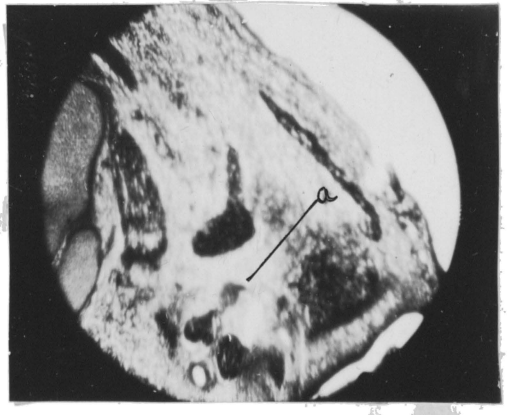

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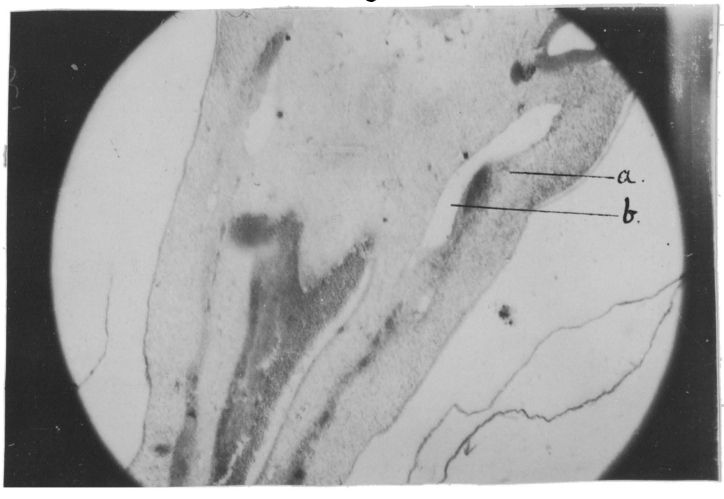

-6-