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Components of the Vagus Nerve.

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The number of unmyelinated fibers in the cervical vagus trunk greatly exceeds the number of myelinated fibers. Kiss[1] regards the unmyelinated fibers as sympathetic fibers which join the vagus trunk mainly in the region of the superior cervical sympathetic ganglion. He denies the existence of unmyelinated fibers in the vagus rootlets. According to Ranson and Chase,[2] and others, the unmyelinated fibers in the vagus trunk arise from cells in the brain stem and emerge in the vagus rootlets.

In the present investigation, carried out on cats, anatomical and experimental studies were made of the relationships between the cervical sympathetic and vagus trunks and their myelinated and unmyelinated components.

The superior cervical sympathetic and nodose ganglia were removed together from several cats. Threads were then tied to each of the ends of the respective trunks and the 2 ganglia were tied upon a glass frame with sufficient tension to draw them slightly apart, to provide an opportunity for the demonstration of any possible connections between them when the preparation was later sectioned. In several instances the cervical vago-sympathetic trunk was removed and sectioned transversely at intervals of less than 0.5 cm. Some of these preparations were stained with osmic acid and some with pyridine silver.

Study of the sections showed that the 2 ganglia are connected by several small rami which contain both myelinated and unmyelinated fibers. These small rami contribute no more than a small fraction of the unmyelinated fibers of the vagus trunk. Sections of the vago-sympathetic trunk taken at short intervals show no interconnecting branches between the vagus and sympathetic trunks distal to the nodose ganglion.

The vagus trunk is joined by rami from the inferior cervical sympathetic ganglion. These are more apparent on the right than on the left side. Schinozaki[3] also finds this to be true in man. The sum total of all the fibers which join the vagus from the inferior

[1] Kiss, F., *Arch. du Museum National d'Historie Naturelle*, 1931, **6**, 7.

[2] Ranson, S. W., and Chase, M., *J. Comp. Neur.*, 1914, **24**, 31.

[3] Schinozaki, S., *Folio Anatomica Japonica*, **6**, 599.

cervical sympathetic ganglion is relatively small as compared with the total numbers of fibers in the vagus trunk.

Eight cats were subjected to extirpation of the superior and inferior cervical sympathetic ganglia on the same side. In 4 the ganglia were removed from the right, and in 4 from the left side. The inferior ganglion usually was removed first and the superior ganglion 4 to 7 days later, and the cats were autopsied 10 to 21 days later. Portions of both vagi were taken at various levels, 1 to 2 cm. above the origin of the recurrent nerve, 1 to 2 cm. below this point, and 1 to 2 cm. below the heart. The tissues from 4 cats were stained with osmic acid and those from the other 4 with pyridine silver. Sections of the vagus on the operated side were compared with those on the unoperated side.

The operations had but little effect on the vagus trunk at either of the 3 levels described. Pyridine silver sections of the vagus nerve of 2 cats just distal to the recurrent nerve show a very small area of degeneration on the operated side. This probably represents a small contribution of fibers from the inferior cervical sympathetic ganglion.

Various workers have called attention to an apparent increase in the number of unmyelinated fibers in the vagus trunk in the region of the nodose ganglion. The results of recent work indicate that there is no increase in the number of unmyelinated fibers in the trunk below the nodose ganglion, *i. e.,* myelinated fibers do not lose their myelin sheaths as they are traced distally. Careful study of sections of the vagus rootlets reveals unmyelinated fibers in considerable abundance, but in much smaller numbers than the numbers of unmyelinated fibers present in the vagus trunk distal to the nodose ganglion. The vagus trunk proximal to the nodose ganglion contains unmyelinated fibers in approximately the same numbers as the vagus rootlets.

Comparison of the vagus trunk proximal and distal to the nodose ganglion shows a great difference in the ratio of myelinated to unmyelinated fibers. Proximal to the ganglion the greater part of the cross-sectional area of the trunk is occupied by myelinated fibers, while distal to it the greater part of the cross-sectional area is occupied by unmyelinated fibers. Serial sections through the nodose ganglion show that this change is effected by a gradual increase downwards through the ganglion in the number of unmyelinated fibers. Another factor in the change is the deviation from the vagus trunk of the fibers which make up the superior laryngeal nerve. Counts made of the fibers in the vagus trunk proximal and distal to

the nodose ganglion and in the superior laryngeal nerve are recorded in Table I. These counts indicate quite clearly that the nodose ganglion is the site of origin of the majority of the unmyelinated fibers in the vagus trunk.

TABLE I.

	Cat No. 40, Osmic Acid No. myelinated fibers	Cat No. 25, Pyridine Silver No. unmyelinated and myelinated fibers
Left vagus above nodose	5,377	7,877
Left superior laryngeal	2,182	2,808
Left vagus below nodose	2,848	11,872

The large size of the nodose ganglion, as expressed especially by its length, might indicate that it probably is more than an ordinary sensory ganglion. If the cells in the nodose ganglion were associated only with afferent fibers, there should be fewer ganglion cells in this ganglion than there are fibers above it. Counts of the cells in the nodose ganglion and the myelinated fibers in the vagus trunk proximal to the ganglion are recorded in Table II.

TABLE II.
Cat No. 34—Osmic Acid.

No. of cells in nodose ganglion	14,384
Myelinated fibers in vagus trunk above ganglion	4,263

The number of unmyelinated fibers in this trunk could not be determined since the stain used was osmic acid. The data of Table I, which were obtained from a cat of the same size, however, show that the number of cells in the nodose ganglion far exceeds the total number of myelinated and unmyelinated fibers in the vagus trunk proximal to the ganglion. The number of cells counted closely approximates the number of fibers counted in the vagus trunk distal to the ganglion. The greater number of the cells of the nodose ganglion, therefore, must be regarded as the cells of origin of the unmyelinated fibers.

The vagus trunk was severed above the nodose ganglion on the right side in one cat, and on the left side in another. After a degeneration period of 15 days, these cats were autopsied and pieces of the vagus nerves distal to the ganglia were obtained and prepared for study. These preparations showed only partial degeneration of the unmyelinated fibers, indicating that the major portion of the unmyelinated fibers arise from cells in the nodose ganglion. The degenerated fibers in these preparations presumably represent pre-

ganglionic components of the vagus which terminate in ganglia located farther distally. These results confirm the physiological evidence of the existence of efferent cells in the nodose ganglion recently reported by Morgan and Goland.[4]

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Estimation of Hemoglobin on a Basis of Protein Iron.

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There have been several micro-methods in the recent literature for the determination of total iron in blood,[1, 2, 3] and it has been suggested that these determinations be used for the calculation of hemoglobin. On account of the constant breakdown of hemoglobin to bilirubin there is a variable quantity of liberated inorganic iron which may amount in certain anemias to more than 5% of the hemoglobin iron.[4] Consequently it would seem advisable to make a preliminary separation of inorganic iron from hemoglobin. Riecker[4] has observed that such a separation may be accomplished by means of a trichloracetic acid precipitation.

In the present study hemoglobin has been calculated from the iron determined in the trichloracetic precipitate from whole blood according to the following technique: A 1 cc. sample of blood is transferred to a 50 cc. centrifuge tube, baked with N/10 HCl and precipitated with trichloracetic acid. After centrifugation the supernatant fluid is poured off, the precipitate dissolved in a minimal quantity of NaOH, transferred to an Erlenmeyer flask, and digested with 5 cc. of nitric acid and 0.5 cc. of 60% perchloric acid. The digest is dissolved in 5 N H_2SO_4, transferred to a 50 cc. volumetric flask and the prussian blue color developed, using gum ghatti as a protective colloid. A standard of known iron content for comparison is prepared at the same time. This technique for determination of protein iron has been used to standardize a dry hemoglobin preparation which is used for the determination of hemo-

[4] Morgan, L. O., and Goland, P. P., *Anat. Rec.*, 1932, **52**, 26.

[1] Wong, S. Y., *J. Biol. Chem.*, 1923, **55**, 421.

[2] Kennedy, R. P., *J. Biol. Chem.*, 1927, **74**, 385.

[3] Reis, Frederick, and Chakmakjian, H. H., *J. Biol. Chem.*, 1931, **92**, 59.

[4] Riecker, Herman H., and Winters, Mary E., *Am. J. Physiol.*, 1930, **92**, 197.