

ON THE NUMBER AND ON THE RELATION BETWEEN DIAMETER AND DISTRIBUTION OF THE NERVE FIBERS INNERVATING THE LEG OF THE FROG, *RANA VIRESCENS* BRACHYCEPHALA, COPE.

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With two figures in the text.

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SUMMARY.

1. A comparison of the gross nerve supply to the leg shows no marked differences between *Rana esculenta* and *Rana virescens*.

2. In the branches both to the thigh and the shank of *Rana virescens* the number of fibers observed exceeds the number calculated. This excess seems to be due to dividing fibers. In teased preparations such dividing fibers have been observed occurring near the points where branches are to be given off.

3. A constant increase in the number of fibers at successive levels of the sciatic where no branches are given off indicates that fibers divide within the nerve trunk.

4. The proportional number of muscular and cutaneous fibers varies quite widely in different frogs, while for the two legs of the same frog the proportions are similar.

5. The diameter of the largest fibers going to the different segments of the leg diminishes in regular order from thigh to foot. Hence, for the leg of *Rana virescens* at least, it is true that the largest fibers run the shortest course. The conclusion of SCHWALBE that the largest fibers run the longest course has been shown to be unsupported by his observations.

6. A confirmation of the theory of the conical diminu-

tion of the nerve fiber in its course is found in the constantly decreasing average area for the nerve fibers at successive levels in a region where no branches arise.

7. In this series of observations it has always been found that in a cross section of a nerve the area of the axis cylinder was approximately equal to the area of the medullary sheath—therefore all the observations made upon the area of the fiber as a whole can be expressed in terms of the more functionally active portion—the axis cylinder.

INTRODUCTION.

The innervation of the thigh in the frog, *Rana virescens* brachycephala, Cope, has been quite fully discussed in a paper, DUNN, 1900, which embodied the results of a study carried on in this laboratory. The present study is an attempt to verify the results then obtained by a repetition of the investigation for the thigh branches and a further determination of the number and diameter of the fibers innervating the shank and the foot.

This present investigation contains data based upon a study of the right and left hind extremities of a single frog. The results obtained from the two sides, while acting as mutual controls, are of further value in illustrating the similarity of innervation for the right and left sides of the same individual.

SECTION I. Gross Anatomy of the Muscular and Cutaneous Nerves Supplying the Thigh and the Shank.

The VIIth, VIIIth and IXth¹ spinal nerves unite to form the plexus lumbo-sacralis from which the skin and muscles of the hind extremity of *Rana virescens* receive their innervation. From this plexus the fibers pass to their destination by way of two main nerve trunks, the crural and the sciatic nerves. The crural nerve, the smaller of these trunks, sends branches only to the thigh. The sciatic nerve, the larger of the trunks, con-

¹ GAUPP designates these nerves as the VIIth, IXth and Xth, numbering the spinal nerves from II to XI, inclusive. See GAUPP's edition, ECKER's und WIEDERSHEIM's *Anatomie des Frosches*, Part II, p. 156.

veys the chief mass of fibers to innervate the hind extremity, and innervates the thigh, shank and foot. After giving off its branches to the thigh it divides in the lowest third of the thigh into the tibial and the peroneal nerves. The tibial nerve divides just below the knee to form what are known as the rami superficialis et profundus of the tibial nerve. The peroneal nerve divides near the middle of the shank into the nervus peroneus lateralis and the nervus peroneus medialis.

The skin and muscles of the thigh, therefore, are innervated by branches from the crural and the sciatic nerves; the muscles and skin of the shank by branches from the tibial and peroneal nerves and their divisions; and the tissues of the foot by the terminal branches from the first and second subdivisions of the tibial and peroneal nerves.

A. Comparison of Rana virescens with Rana esculenta and Rana temporaria.

In the previous study, DUNN, 1900, of the innervation of the thigh, adoption was made of the nomenclature used by GAUPP in his late edition of ECKER and WIEDERSHEIM'S Anatomy of the Frog, to identify the muscles and the nerve branches. The same authority will be followed in the anatomical nomenclature used in this paper.

A comparison of the gross innervation of the thigh in *Rana virescens* with that of *Rana esculenta* and *Rana temporaria* revealed a few minor variations. A similar comparison of the gross innervation for the shank reveals a correspondingly small number of differences.

Tables I and II are presentations in brief of the nerve branches to the thigh and to the shank.

Table I contains only the main branches of the crural and sciatic nerves to the thigh, and the designations by which they may be identified in Figure 1. A more complete tabulation with an accompanying figure may be found on pp. 220 and 221 of the preceding study, DUNN, 1900.

TABLE I.

Tabulation of the chief Nerve Branches passing to the Skin and Muscles of the Thigh in *Rana virescens brachycephala*, Cope.

- C. Nervus cruralis s. N. femoralis anterior.
 - a) Ramus cutaneus femoris lateralis.
- S. Nervus ischiadicus s. N. femoralis posterior.
 - 2. R. cutaneus femoris posterior.
 - 3. R. muscularis to the M. pyriformis.
 - 4 and 5. Rr. musculares to the M. gemellus and the M. obturator internus.
 - 6. R. profundus posterior.
 - 7. R. muscularis to the M. ileo-femoralis.
 - 8. R. profundus anterior.
 - [x. R. muscularis to the M. ileo-fibularis.]

Table II is a presentation of all the nerve branches to the shank. GAUPP's designations are used for these tables and for Figure 1.

Figure 1 is a dorsal view of the right plexus lumbosacralis of *Rana virescens*, and includes all the ramifications from the point where the spinal nerves emerge from the vertebral foramina, to a point opposite the ankle. The attempt has been made to represent in this figure the relative sizes of the trunks and branches, and the points and order of branching.

TABLE II.

Tabulation of the Nerve Branches passing to the Skin and Muscles of the Shank in *Rana virescens brachycephala*, Cope.

- T. Nervus tibialis.
 - α. Ramus cutaneus cruris posterior.
 - β. R. muscularis for the M. plantaris longus.
- 1. R. superficialis of the N. tibialis.
 - a. R. muscularis to the M. plantaris longus.
 - b. R. cutaneus cruris medialis inferior.
- 2. R. profundus of the N. tibialis.
 - a. R. cutaneus cruris medialis superior.
 - b. Rr. musculares for the M. tibialis posticus.
- P. Nervus peroneus.
 - a. R. articularis genu et pedis.
 - α. R. articularis genu.
 - β. R. articularis pedis.
 - b. R. cutaneus cruris lateralis.
 - c. R. muscularis to the M. extensor cruris brevis.
 - d. Rr. musculares to the M. peroneus.
- 1. N. peroneus lateralis.
 - a. Rr. musculares to the M. tibialis anticus longus.
- 2. N. peroneus medialis.
 - a. R. muscularis to the M. tibialis anticus longus.
 - b. Rr. musculares to the M. tibialis anticus brevis.

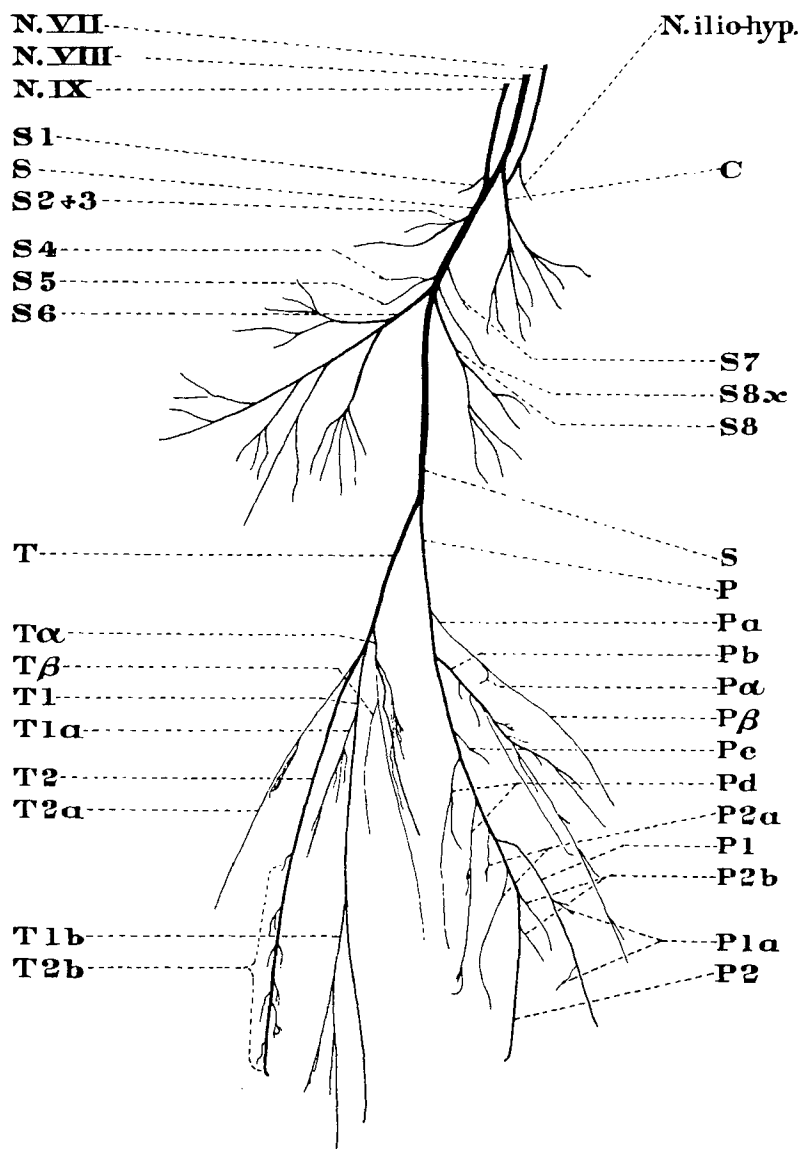


FIGURE I.

The musculature of the shank is so much more simple than that of the thigh that a comparison of the different accounts of the innervation of the shank as found in successive editions of ECKER is not deemed necessary. But as GAUPP has introduced new names for two of the six muscles of the shank, a brief tabulation of the muscles as named by ECKER and GAUPP is here appended.

TABLE III.

GAUPP's Edition of ECKER. 1896—1901	ECKER's <i>Anatomie des Froches</i> , 1864 HASLAM's translation, 1889.
Muscles of the calf.	
M. plantaris longus.	M. gastrocnemius.
M. tibialis posticus.	M. tibialis posticus.
Muscles of the extensor aspect.	
M. peroneus.	M. peroneus.
M. tibialis anticus longus.	M. tibialis anticus.
M. extensor cruris brevis.	M. extensor cruris brevis.
M. tibialis anticus brevis.	M. flexor tarsi anterior.

I. Variations appearing in successive dissections of *Rana virescens*.

In both the thigh and the shank the order in which the branches leave the main trunk presents little variation in different individuals, but the number and size of the branches vary more frequently in the shank than in the thigh. This condition is probably due to the fact that, while in the thigh a small number of large branches is given off within a short distance and these large branches subdivide to innervate many muscles, the branches to the shank separate at many points along the course of the nerve trunks and supply single muscles or send numerous branches to one muscle.

The large number of branches sometimes given off to single muscles is indicated in Table IV, which is a tabulation of the muscular and cutaneous nerve branches to the shank, as found in the frog designated as Frog II B, whose peripheral nervous system furnished material for the data discussed at a further point in this study.

TABLE IV.

Tabulation of the muscular, cutaneous and articular nerve branches to the shank in Frog II B.

Muscular branches.

From the Tibial nerve.	No. of branches.
Plantaris longus.	2.
Tibialis posticus.	8.
From the Peroneal nerve.	
Extensor cruris brevis.	2.
Peroneus.	2.
Tibialis anticus longus.	6.
Tibialis anticus brevis.	2.

Cutaneous branches.

From the Tibial nerve.
R. cutaneus cruris posterior.
From R. superficialis of the Tibial nerve.
R. cutaneus cruris medialis inferior.
From R. profundus of the Tibial nerve.
R. cutaneus cruris medialis superior.
From the Peroneal nerve.
R. cutaneus cruris lateralis.

Articular branches.

From the Peroneal nerve.
R. articularis genu et pedis.

2. Differences between *Rana virescens* and the standard furnished by *Rana esculenta*.

As has already been stated, the method of ramification of the shank branches varies greatly in the different frogs. This being the case, it has been difficult, even after a large number of dissections, to fix a standard for comparison with the standard adopted by GAUPP for *Rana esculenta*. The conditions most frequently present in the ten dissections already made have been indicated in Figure 1. Further dissections may reveal that some of the differences which seem to be constant are only accidentally coincident in these dissections. While this possibility of error is annoying in an attempt to make a statement as to fact, yet in the pursuance of an investigation such as the one here undertaken a variation of this character has no importance. Before any study was made of the number of fibers of any nerve branch, that branch was identified as supplying a certain muscle

or a certain cutaneous region, irrespective of its method or point of separation.

The differences in the innervation of the shank between *Rana virescens* and *Rana esculenta* are in detail as follows.

The branch designated in Figures 1 and 2 and Table II as T_{β} which in *Rana esculenta* is given off directly from the tibial nerve, in *Rana virescens* is given off with T_{α} , the ramus cutaneus cruris posterior. This branch T_{β} is one of the branches innervating the M. plantaris longus. A similar joining of a muscular and a cutaneous branch is found in the thigh in the instance of S_2 and S_3 which in *Rana virescens* are frequently given off together.

2. One branch to the M. tibialis anticus longus is given off from the main trunk of the N. peroneus in addition to those branches arising from the N. peroneus lateralis and the N. peroneus medialis. In *Rana esculenta* all the fibers supplying the M. tibialis anticus longus pass by way of the N. peroneus lateralis or the N. peroneus medialis.

SECTION II. Number and Diameter of the Nerve Fibers Innervating the Thigh, Shank and Foot.

A. Introduction.

The results obtained from a series of observations undertaken to ascertain the number and diameter of the fibers innervating the thigh made probable the deduction that, contrary to the long accepted statement of SCHWALBE, 1882, the largest nerve fibers do not run the longest course and so innervate the foot, but pass at a comparatively high level to innervate the tissues of the thigh.

This deduction rests upon two ascertained facts. The first is that the average area for the nerve fibers at the level just above the thigh branches, S_1 Figure 2, is greater than that for the fibers at a level just below the thigh branches, S_2 Figure 2, and is less than the average area for the fibers of the thigh branches. The second fact is that by actual measurements of the ten largest fibers in each of these three regions it was ascertained that the very large fibers that are present above the

thigh branches cannot be found in the sciatic nerve at a level below the thigh branches, but do appear among the fibers passing by the branches to the thigh.

These two facts pointed so strongly to the necessity for a revision of our beliefs regarding the destination of the largest nerve fibers and the interpretation of the variations in the size of nerve fibers that a more complete study of the number and diameter of the nerve fibers at stated levels, increasing in distance from the spinal cord, seemed very desirable. A study of the fibers in the branches to the thigh and to the shank was also fortunately completed upon the same frog, thus giving data for comparison which were all obtained from a single specimen.

So many very small branches required to be sectioned in this examination that a frog of the maximum size was selected.

The choice in this second series of observations fell upon a frog, designated as Frog II B, female, corrected weight 61.5 grams, length 234 mm. Both the enumeration of the fibers and the calculation of the areas were carried through for the right and left hind extremities.

1. Levels at which observations were made.

The exact points at which observations were made are indicated in Figure 2 by the breaks in the continuity of the nerve trunks and branches. Figure 2 is a reproduction of the main branches shown in Figure 1. The designations are those used for Tables I and II and Figure 1, with the addition of S_1 , S_2 , and $T + P$, to indicate the levels on the main trunks at which sections were made.

The levels on the main trunks chosen for comparison were four; the first at the point just above the branches to the thigh, indicated by C and S_1 ; the second at a point just below the thigh branches at S_2 . The data furnished by the section at S_2 , just above the point of division of the sciatic nerve, acted as a control upon the results obtained at S_1 . The third level was at a point just above the branches to the shank, cutting the tibial and peroneal nerves at T and P. The section at $T + P$, the immediate point of division of the sciatic nerve, fur-

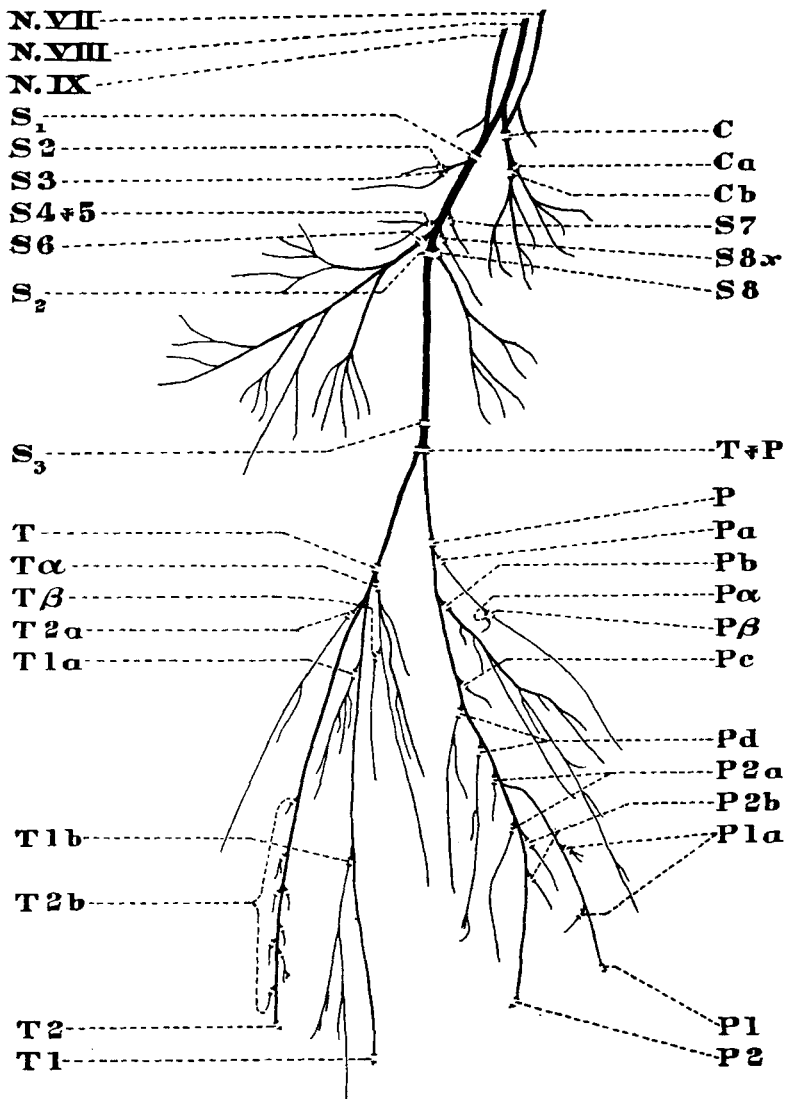


FIGURE 2.

nished control results for this third set of observations. The fourth level was at the ankle. At this point four trunks, T₁, T₂, P₁, and P₂, the subdivisions of the tibial and peroneal nerves, were sectioned.

The branches to the thigh and to the shank were sectioned as close as possible to their points of separation from the main trunks, as is indicated in Figure 2.

2. Methods of observation.

The methods of observation followed in this work varied but slightly from those used in the preceding study, DUNN, 1900. As soon as possible after the frog was chloroformed and its weight and length ascertained, the nerve tissue at the chosen levels was laid bare, the overlying tissues being so carefully separated that the nerve tissue was undisturbed. Into the cup-like cavity of the surrounding tissues was dropped a small portion of a one per cent. solution of osmic acid. This hardening agent fixed the undisturbed tissue, and made possible a later removal and further fixation of it. After this preliminary fixation of from fifteen to thirty minutes the tissues were carefully lifted into a capsule containing one per cent. osmic acid solution and the capsule placed in a darkened chamber for twenty-four hours. After the expiration of this period and an additional three hours of washing in distilled water, the material was carried through increasing percentages of alcohol, cleared in xylol and embedded in paraffin.

The sections were cut in ribbons by a MINOT microtome to a thickness of three and one-third micra, and, after being spread by gentle heat, were fastened to the slides with albumen fixative. Thorough drying was followed by a second clearing in xylol, after which the sections were mounted in colophonium under thin cover glasses.

a. Enumeration. The fibers in the sections of the main trunks were enumerated by the "photographic method" which has been used and described by Dr. HARDESTY, 1899.

For the thigh and shank branches the "net method" was adopted.

In the employment of either method special effort was made to include the very small medullated fibers which, under ordinary circumstances, elude observation.

b. Determination of average areas for¹ each fiber. The determination of areas was made by the aid of camera lucida drawings of the selected sections. In the execution of these drawings all of the nerve sheath was excluded and the outline was made to follow the periphery of the nerve fibers, thus including only the fibers themselves and the spaces lying between them. The area included by the outline of this camera drawing was determined by planimetric measurement and the diameter by a millimeter scale. The accuracy of this diameter measurement was tested by actual measurement upon the section itself. From these data the true area of the section was computed.

The average area for each nerve fiber was determined by dividing this true area expressed in square micra by the number of fibers contained in the section.

For any section in which spreading had occurred, the percentage of added space was determined by a special method. The section was photographed and on the print the additional space was lightly outlined in pencil. By carrying the planimeter along the pencil line the added area was measured. The portion of the entire area of the photographed nerve which this included area covered gave the percentage to be deducted from the former computed area of the nerve.

c. Determination of the average area of the largest fibers. Confirmatory measurements were made upon the largest fibers at the several levels. For each of these fibers the mean of two diameters was obtained, and from this mean the area of the selected fibers was computed.

¹ The area for the fiber is an average area determined by dividing the area of the nerve trunk by the number of nerve fibers of which it is composed, while the area of the fiber is determined by measurements made upon the fiber alone. The distinction made in this and the following section between the area for the fiber and the area of the fiber is maintained throughout the discussion, and the italicised words require this difference in interpretation wherever they are introduced.

The number of fibers selected at each level varied according to the proportion of fibers present at that level. In measuring the largest fibers in the branches, a number of fibers equal to the number of fibers measured at a level just above the branches was selected and their average area computed. A second computation for the proportional number of fibers in the branches was then undertaken. Both results are included in the tables.

B. Number of Nerve Fibers.

1. Enumerations at the various levels of the sciatic and crural nerves; and of the thigh and shank branches at their points of separation from the main trunks.

2. In ascertaining the number of nerve fibers at different levels for this frog, the first count was made at a point in the sciatic and crural nerves above their branches to the thigh, at the points C and S_1 , Figure 2. The next level selected was in the sciatic nerve immediately below its branches to the thigh, at S_2 . As the crural nerve sends all its fibers to the thigh tissues, the sum of the numbers at the first level gave the total number of fibers which innervate the thigh, shank and foot. That at the second level indicated the number passing on to the shank and foot, while the difference between the numbers at the two levels gave the calculated number of fibers innervating the thigh.

Reference to Table V gives the desired counts. The crural nerve for the left side supplies 1630 fibers, for the right side 1627 fibers to the thigh. The sciatic nerve above its branches to the thigh shows for the left side 5499 fibers, for the right side 5480 fibers. This gives a total count of 7129 fibers for the left side, 7107 fibers for the right side. The number of fibers at S_2 , below the branches to the thigh is for the left side 3962 fibers, for the right side 3942 fibers. By process of subtraction the probable number of fibers innervating the thigh is found to be for the left side 3167 fibers, for the right side 3165 fibers.

TABLE V

Showing the calculated number of fibers innervating the thigh.

Frog II B.			
Levels ¹		L.	R.
C	Observed in crural nerve,	1630	1627
S ₁	Observed in sciatic nerve above its branches,	5499	5480
C+S ₁	Observed total to thigh, shank and foot,	7129	7107
S ₂	Observed in sciatic nerve below its branches to the thigh,	3962	3942
(C+S ₁)-S ₂	Calculated number innervating the thigh,	3167	3165

¹The designations of levels in this and the succeeding tables are those of Tables I or II, and Figures 1 and 2.

Table VI shows the actual number of nerve fibers by count passing to innervate the thigh. The number of fibers to the left side is 3481, to the right side 3508 fibers. These thigh fibers innervate on each side, the cutaneous covering of the thigh and twenty thigh muscles.

TABLE VI.

Showing the observed number of nerve fibers innervating the muscles and skin of the thigh.

Frog II B			
Designations of levels.	No. of muscles.	No. of fibers.	
		L.	R.
S ₂ and 3	1	398	407
S ₄ and 5	2	150	167
S ₆	8	852	846
S ₇	1	67	65
S ₈	2	311	318
S _{8x}	1	73	78
Sciatic nerve,	15	1851	1881
Crural nerve,	5	1630	1627
Totals	20	3481	3508

Table VII indicates the observed number of fibers to the shank and the number of muscles, six, which they innervate. The proportion of muscular to cutaneous fibers in the thigh and the shank will be discussed in another section. The ob-

served number of fibers to the shank, as shown in Table VII, is **2108 fibers** for the left side, **2130 fibers** for the right side.

TABLE VII

Showing the observed number of fibers innervating the muscles and skin of the shank.

Frog II B.

Levels.	No. of muscles.	L.	R.
T. α .	0	20	31
T. β .	}	322	26
T. i. a.		147	142
T. i. b.	0	260	347
T. z. a.	0	126	125
T. z. b.	1	136	134
P. a. α .	0	9	10
P. a. β .	0	8	8
P. b.	0	480	482
P. c.	1	42	46
P. d.	1	75	52
P. i. a.	}	2	47
P. z. a.		457	464
P. z. b.	1	24	26
Total to shank	6	2108	2130

Table VIII contains the enumeration of the nerve fibers innervating the foot. The number here entered is the result of a count at the fourth level which corresponds with the ankle. The count shows 2486 fibers for the left side, 2497 fibers for the right side.

TABLE VIII.

Showing the observed number of fibers innervating the foot.

Frog II B.

Levels.	L.	R.
T. 1	480	529
T. 2	840	833
P. 1	821	802
P. 2	345	333
Total to foot	2486	2497

For the enumerations contained in Table IX the levels selected were two in number, the ones that have been described as the third and fourth levels. The third level sections the tibial and peroneal nerves just above their branches to the

shank. The fourth level cuts, at a point opposite the ankle; the four subdivisions of the tibial and peroneal nerves which supply the tissues of the foot. This level lies only slightly below the point at which the last branches to the shank are given off.

The total number of fibers at the third level is for the left side 4146 fibers, for the right side 4152 fibers. The total number of fibers at the fourth level is, as has been shown in Table VIII, for the left side 2486, for the right side 2497.

The total number at the third level less the total number at the fourth level gives the calculated number of fibers to the shank, which is 1660 for the left side, 1655 for the right side.

TABLE IX.

Showing the calculated number of nerve fibers innervating the shank.

Frog II B.

Levels.		L.	R.
P.	Observed in peroneal nerve above branches to shank.	1922	1873
T.	Observed in tibial nerve above branches to shank.	2224	2279
P.+T.	Total observed to shank and foot	4146	4152
P. 1+P. 2+ T. 1+T. 2	Observed to foot.	2486	2497
(P+T) - (P ₁ +P ₂ +T ₁ +T ₂)	Calculated to shank.	1660	55

2. Significance of the excess of the observed over the calculated number of fibers to both thigh and shank.

A comparison of Tables V and VI, relating to the fibers innervating the thigh, with Tables VII and IX, relating to the fibers for the shank, reveals a surprising discrepancy between the observed and the calculated number of fibers in each instance.

Table X, exhibiting the differences for the thigh, shows for the left side an excess of 314 fibers or 9% of the number of fibers concerned in innervating the thigh, for the right side 343 fibers or nearly 10% of the observed number of fibers innervating the thigh.

In the preceding study, DUNN, 1900, p. 233, the excess was from 6 to 8% of the observed number of fibers to the thigh.

TABLE X.

Showing the excess of the observed over the calculated fibers innervating the thigh.

Frog II B.

	L.	R.
Observed to thigh.	3481	3508
Calculated to thigh.	3167	3165
Excess of observed over calculated.	314	343
Percentage excess.	9%	10%

Table XI, a similar table for the shank, shows for the left side an excess of 448 fibers or 21% of the observed number of fibers and for the right side 465 fibers or 22% of the number of fibers found in the shank branches close to their points of separation from the main trunks.

TABLE XI.

Showing the excess of the observed over the calculated fibers to the shank.

Frog II B.

	L.	R.
Observed to the shank	2108	2130
Calculated to the shank	1660	1665
Excess of observed over calculated	448	465
Percentage excess	21%	22%

This disparity between the observed and the calculated numbers to the thigh, existing in each of the three frogs on which observations have been made, can hardly be due to an error in counting. The probable explanation, as pointed out in the earlier discussion of this disparity, DUNN, 1900, p. 233, is that of a branching of certain of the nerve fibers at some point between the levels at which they were counted. In several experimental teasings a number of large fibers were found in the sciatic trunk dividing near the departure of the thigh branches.

Another point of interest is the greater percentage of dividing fibers present in the shank. Their presence is ex-

plained by the fact that the point of section for these fibers was much nearer to the periphery than that for the fibers to the thigh. As has been indicated earlier in this study, the branches to the shank are given off to single muscles or frequently several branches pass to one muscle, while in the thigh the larger branches furnish fibers to several muscles, in one instance, the *ramus profundus posterior*, to eight muscles.

3. Discussion of the increase in the number of fibers at successive levels.

Enumerations of the fibers at two levels other than those already mentioned were made that they might serve as controls to the results obtained at the levels just below the branches to the thigh and just above the branches to the shank. At no point between these four levels are nerve branches given off with the exception of a few very fine branches of four or five fibers each, which cannot be traced by fine dissection but seem to pass to surrounding tissues other than muscular tissue.

From the level in the sciatic nerve just below the thigh branches, indicated in Figure 2 by S_2 , through the levels indicated in the same figure by S_3 and $T+P$, to the level just above the branches to the shank, level T and P , the number of fibers shows a gradual increase. Reference to Table XII reveals the exact amount of this increase from one level to the next one. This increase must be due to fibers dividing in the main trunk.

TABLE XII.

Showing gradual increase in the number of fibers at successive levels between the separation of the last branch to the thigh and that of the first branch to the shank.

Frog II B.

Levels		L.	R.
S_2	In sciatic nerve at a level below branches to thigh.	3962	3942
S_3	In sciatic nerve above its division into peroneal and tibial.	3988	4083
$T+P$	In peroneal and tibial just after their separation.	3996	4103
T and P	In tibial and peroneal above branches to shank.	4146	4152

The average increase in number for the two sides shows for the distance S_1 to S_2 (see Figure 2) an increase of 85 fibers, for the distance from S_2 to T+P of 14 fibers, and from T+P to T and P of 100 fibers. While this increase in the number of fibers is not proportional to the distances between the various levels, nevertheless the shortest distance, S_1 to T+P, gives the least increase.

4. Proportion of the number of muscular to the number of cutaneous fibers.

The proportion of the number of muscular to the number of cutaneous fibers will be of more definite interest when further data concerning the weight of the muscles and the area of the skin innervated have been obtained. As, however, the cutaneous and the muscular fibers were distinguished in this series of enumerations the results are recorded.

Tables XIII and XIV give in detail the number of muscular and of cutaneous fibers for the thigh and for the shank. Table XIII gives as the totals for the thigh 1805 muscular, 1676 cutaneous fibers for the left side, 1830 muscular, 1678 cutaneous fibers for the right side. In this instance the muscular fibers, while surpassing in number the cutaneous fibers, are not so much in excess as in the two frogs which furnished the data for the preceding study (DUNN, 1900, pp. 233, 234). The proportional differences in the several frogs seem to be matters of individual variation.

TABLE XIII.

Showing the number of muscular and of cutaneous fibers innervating the thigh.

Frog II B.	Muscular.		Cutaneous.	
	L.	R.	L.	R.
$S_2 \& 3$	23	55	375	352
$S_4 \& 5$	150	167		
S_6	710	705	142	141
S_7	67	65		
S_8	311	318		
S_{8x}	73	78		
Sciatic.	1334	1388	517	493
Crural.	471	442	1159	1185
Totals.	1805	1830	1676	1678

Table XIV, for the shank, shows also a greater number of muscular than of cutaneous fibers. The absolute numbers are, for the left side, 1205 muscular, 903 cutaneous fibers, for the right side, 1127 muscular, 1003 cutaneous fibers. There are at present no data for comparison with this enumeration for the shank.

TABLE XIV.

Showing the number of muscular and of cutaneous fibers innervating the shank.

Frog II B. Levels.	Muscular.		Cutaneous.	
	L.	R.	L.	R.
T. α			20	31
T. β	322	216		
T. 1. a.	147	142		
T. 1. b.			260	347
T. 2. a.			126	125
T. 2. b.	136	134		
P. a. α			9*	10*
P. a. β			8*	8*
P. b.			480	482
P. c.	42	46		
P. d.	75	52		
P. 1. a.	2	47		
P. 2. a.	457	464		
P. 2. b.	24	26		
Totals.	1205	1127	903	1003

*Articular.

5. Comparison of the number of nerve fibers for the two sides.

Even the most casual examination of the tabulations already presented reveals a striking accordance between the number of fibers for the two sides. This confirms the previous observation. There appears however to be a slightly greater variation for the branches to the shank. This irregularity may be a matter of individual variation, or, if it be found present in other specimens, may be one of several irregularities of innervation which the shank seems to exhibit.

No interpretation of the uniformity in the number of fibers for the two sides seems called for. The fact of symmetry in the number of fibers is what might naturally be expected from the corresponding uniformity for the two sides of the mass of tissue innervated and from their physiological similarity.

C. Diameter of the Nerve Fibers.

1. Introduction.

We propose to consider at this point whether the destination of a nerve fiber in the frog's leg can be inferred from its caliber. The law of SCHWALBE (SCHWALBE 1882), to which reference has already been made, sets forth the view that the largest fibers run the longest distance. The researches upon which this theory is based were carried on some twenty years ago, having been published in 1882. SCHWALBE based his observations on a study of the dorsal and ventral roots of the spinal nerves in *Rana esculenta*. His technique consisted of macerating the entire nerve root in 20% nitric acid at 40° C, and washing once in a large amount of water, or of hardening and staining in 1% osmic acid for twenty-four hours, washing and macerating in glycerine, acidulated with 1% of hydrochloric acid commercial strength, for 24 hours at 40° C (DUNN, 1900, p. 13). The nerve tissue thus prepared was then teased, and transverse measurements were made of the selected fibers. The average number of measurements for each root was twenty (SCHWALBE, p. 11). From these measurements he constructs a table showing first the average thickness, and next the thickness of fibers of greatest frequency. Now, when the corresponding curves from the ventral and dorsal roots are plotted it appears that in the cervical region the fibers of the second nerve have the largest average diameter, and in the lumbar region those of the eighth nerve. Finding these largest fibers present in the roots distributed to the fore and the hind leg respectively, he asks the question on page twenty, "Was liegt nun aber näher, als die stärkeren Kaliber in den Wurzeln der Extremitätennerven mit der grösseren Länge der betreffenden Nervenstrecke in Zusammenhang zu bringen! Wollte man diese natürlichste Auffassung nicht acceptiren, so wüsste ich nur eine Möglichkeit der Deutung jener Unterschiede. Man könnte nämlich der Meinung sein, dass die Extremitätennerven häufiger innervirt würden, als die Nerven des Brust- und Bauchumfanges, und in Folge dieses häufigeren Gebrauches sich stärker entwickelt hätten."

As SCHWALBE finds no evidence that the nerves to the extremities are more frequently innervated than those to the thorax and abdomen he is by exclusion compelled to accept the first hypothesis. This he proceeds to test. In the three frogs chosen he finds the length of the brachial nerve to its extremity as compared to the length of the ischiadicus to be approximately 1:2.35 (SCHWALBE, 1882, p. 21); that is, if there were a direct relation, the ratio between the squares of the diameters of the nerve fibers in these localities should correspond to that for their length and be 1:2.35, but their ratio is much less, being in the most favorable case, 1:1.5.

Instead of admitting, therefore, that the relation does not hold, SCHWALBE prefers to assume that the general relation is true but is modified by some other factor. He assumes, then, that some other influence is working to render the caliber of the nerve fibers in the brachial nerve greater than it should be in proportion to the length of the nerve.

Moreover he emphasizes the fact (p. 24) that these remarks refer to the sections of the spinal nerves nearest the spinal cord and that in their further course these fibers are modified, that is to say, reduced in diameter.

He then proceeds to explain in accordance with his theory what must be the meaning of a nerve containing fibers differing greatly in diameter, and states that the smallest fibers must be given off in the branches nearest the spinal cord. It is to be noted that SCHWALBE has no observations to confirm this view.

During the course of the present study the attempt was made to ascertain the destination of the very large fibers in the sciatic nerve. To this end measurements of the nerve fibers were taken at the levels at which the fibers had been enumerated. These levels are shown in Figure 2 and fully explained in Section B, Number of Nerve Fibers, page 310.

These two lines of investigation were followed: first, a determination of the average area *for each* nerve fiber at the various levels; second, a determination of the absolute measurements *of* the largest nerve fibers at the same levels, and in the branches.

2. Average areas *for* the nerve fibers above and below the branches to the thigh and shank.

The average area *for* each nerve fiber at the selected levels is shown in Table XV.

TABLE XV.

Showing the average area *for* each nerve fiber of the sciatic and crural nerves and their subdivisions at the levels above and below the thigh branches and above and below the shank branches.

Frog II B.		L.	R.
S ₁ & C.	Sciatic and crural nerves above branches to thigh.	97.2 □ μ	87.2 □ μ
S ₁	Sciatic nerve above branches to thigh.	100.9	91.2
S ₂ .	Sciatic nerve below branches to thigh.	76.6	81.9
T. & P.	Tibial and peroneal nerves above branches to the shank.	62.2	62.6
P ₁ . T ₁ .	Tibial and peroneal nerves below branches		
P ₂ . T ₂ .	to shank.	51.5	52.3

Looking at the left side alone, the average area for each fiber above the branches to the thigh is 97.2 square micra, when the average of both the sciatic and crural nerves is taken, 100.9 square micra, when the attention is confined to the sciatic nerve alone. The computation for the crural nerve should be disregarded in the discussion of changing caliber, since the crural nerve sends all its fibers to the thigh and hence furnishes but the one level for measurement.

The comparison of the average area mentioned with the area of 76.6 square micra for each fiber at a level below the branches to the thigh, S₂, shows a considerable lessening in the average area for each nerve fiber. By comparing the average area above the shank branches, T & P, 62.2 square micra, with that below the shank branches T₁, T₂, P₁, P₂, 51.5 square micra, we find a similar if not proportionate lessening in the average area *for* each nerve fiber. We find then in both the thigh and the shank a greatly decreased average area at the level below the branches. This decrease is in part due to the absence at the second level, below the branches, of the largest fibers which appeared at the level above the branches,

and the departure of these large fibers to the thigh tissues, as we shall show by our measurements upon the largest fibers at the two levels and in the branches. A second factor influencing this decrease is the diminution in caliber in the course of the individual nerve fibers.

That the second factor is subsidiary to the first is shown by the fact that the distance between the first and second levels is a comparatively short one, while the decrease in average area is very marked. We proceed to discuss these factors in detail.

3. Average areas of the largest fibers at the various levels.

We have suggested that the largest fibers at a level above the branches to the thigh and shank respectively are not present at a level below the branches. To verify this observation and to ascertain absolutely the destination of these fibers, measurements were made of a selected number of the largest fibers at these levels and also in the branches themselves. The number to be measured at each level was made proportionate to the total number of fibers at that level.

Table XVI indicates for the sciatic nerve and its subdivisions, the number of fibers measured, the level, its designation, and the average area of the fibers expressed in square micra, while Table XVII records the measurements for the branches. The measurements recorded in Table XVI are for the levels above and below the branches to the thigh and the shank, and control measurements, as the third and fourth records, which were made at levels lying between the level below the thigh branches and that above the shank branches. In Table XVII two sets of measurements are recorded; the first is the average area of the number of fibers corresponding to that measured in the section at a level above the branches; the second shows the average of the number which correspond to the proportion which the number of fibers in the branches bears to the number in the trunk. The tabulated measurements are for the left side of Frog II B.

TABLE XVI.

Showing the average areas of the largest nerve fibers at the various levels of the sciatic nerve and its subdivisions at which the areas for the nerve fibers had been computed. The number of fibers measured at each level is proportionate to the entire number at that level.

No. of fibers	Nerve	Level selected	Designation	L.
22	Sciatic	Above br. to thigh.	S ₁	229.6□
16	Sciatic	Below br. to thigh.	S ₂	173.0
16	Sciatic	Above division into peroneal & tibial.	S ₃	167.4
16	Tibial & peroneal	At separation.	T-P	166.0
16	Tibial & peroneal	Above br. to shank.	T and P	164.7
10	Tibial & peroneal	Below branches to shank	T ₁ , T ₂ , P ₁ , P ₂	106.0

TABLE XVII.

Showing the average area of the largest fibers in the thigh and shank branches. The first computation in each instance is for the number of fibers corresponding to that measured at a level just above the separation of the branches. The smaller number of fibers is proportional to the total number in the branches.

No. of fibers.	Level selected	Averages areas
22	Branches to left thigh.	212.8□ μ.
8	Branches to left thigh	232.9
16	Branches to left shank	127.9
6	Branches to left shank	144.9

We have, then, in Table XVI, the average areas for the same relative number of fibers at various distances from the spinal cord. These largest fibers at each level are highly uniform in size and hence the average represents also the individual fiber.

If the largest fibers in the lumbo-sacral plexus run the longest distance, the largest fibers at the highest level should appear again and again with at least only slightly decreasing diameter at the successive levels. By reference to Table XVI, the largest fibers at S₁, and S₂, points separated by not more than one centimeter, do not show this equality, but reveal at the second level a marked diminution in the average area, one of 46.7 square micra or 20%. Conical diminution cannot

furnish an explanation for so marked a variation in diameter at these adjoining levels. We must consider the possibility of the disappearance from the second level of the largest fibers, and the consequent measurement at that point of a set of fibers of less though still large caliber. The largest fibers may have passed out by way of the thigh branches to supply the thigh tissues, so their presence in those branches must be determined. Reference to Table XVII shows that the average area of the twenty-two largest fibers in the branches approximates that of the twenty-two largest fibers at a level above the branches, while the measurements of the eight largest fibers in the branches, the number proportional to the total number of fibers in the branches, show an average slightly greater than that recorded for the largest fibers at the level above the branches.

With these measurements for our consideration, we can arrive at but one conclusion, namely, that the largest fibers of the lumbo-sacral plexus, formed from the VIIth, VIIIth, and IXth spinal nerves, pass off in the branches to the thigh and therefore run a comparatively short course to terminate in the tissues which lie above the knee.

Fixing our attention upon the results of the measurements below the knee, we find that the difference between the average areas above and below the shank branches is 58.6 square micra or 36%, a greater difference than exists in the thigh. At the same time we find that the distance between the levels at which the measurements were made is much greater than that between the corresponding levels in the thigh, so that conical diminution may here exert a more decided influence than in our results for the thigh.

Comparing for the shank the average area for the largest fibers in the trunks, Table XVI, with the average for the branches, Table XVII, we find that the average for the largest fibers at a level above the branches is nearly equal to the average for the largest fibers in the branches and is much larger than the average area for the largest fibers at the level just below the branches.

We find, then, from a study of Tables XVI and XVII,

that the area of the largest fibers at the ankle is less than one-half that of the largest fibers in the sciatic nerve above the level at which the first branches pass off to the thigh. While there may be present a conical diminution of the nerve fibers in their course, yet the actual presence of fibers in the branches equal in their area to the area of the largest fibers in the trunk above the branches, and the absence of fibers of equal diameter below the level of the branches seems to justify the definite statement that the largest fibers at each level of the sciatic nerve run the shorter course, while the largest fibers which innervate tissues more remote from the spinal cord are of less diameter.

4. Possibility of conical diminution in the extent of the nerve fiber.

Although the foregoing explanation seems the correct one for the great decrease in diameter when the largest fibers in a section above the branches are compared with the largest fibers in a section immediately below the branches, it is possible that conical diminution of the nerve fiber in its course may be present and exert some influence upon the results obtained.

By comparison of the averages for the measurements at successive levels between the first and last of which no fibers are given off, we obtain certain interesting results. The levels considered are those of S_1 , S_2 , T+P, and T and P, Table XVI. Measurements are here obtained at levels of more than four centimeters distance from S_1 which show a gradual diminution in the average areas of the largest fibers at successive levels, with a total diminution, from the level below the branches to the thigh, to that above the branches to the shank, of 8.2 square micra.

This gradual, though slight, decrease seems to point to a confirmation of the theory of the conical diminution of the nerve fiber in its course.

STILLING (1869, pp. 931 to 935) in his classical researches upon the spinal cord, during the course of an historical and critical survey of the question of the decrease in diameter or the conical diminution of the nerve fiber, states that in the

white substance of the spinal cord certain fibers occur which decrease in diameter at lower levels. His conclusions, however, are vitiated, as are those of other of the earlier histological investigators, by the crudity of the technique of that period.

SCHWALBE, (1882, p. 40) in the paper already quoted touches upon the question of conical diminution in the peripheral nervous system. After referring to the findings of HENLE, KÖLLIKER and others, in which the smaller size of the motor and sensory nerve fibers near their terminations was recognized, SCHWALBE states the conclusions at which he had arrived from his own observations. These observations consisted of the measurement of fibers in the trunks of nerves destined for the muscles or the skin and again upon the corresponding fibers near their destinations.

His conclusion is in this sentence, p. 44: "Beide zeigen Verfeinerungen nach der Peripherie, die motorischen aber erst an der zahlreichen Theilfasern unter Querschnittszunahme der gesammten motorischen Nervenbahn; die sensiblen Fasern verschmälern sich dagegen schon vor der Theilung unter bedeutender Querschnittsabnahme der sensiblen Bahn!"

Of the later authorities KÖLLIKER, 1896, in his condensation of the subject of nerve fiber diameter states that some fibers are smaller near the nerve cell than at a greater distance and that certain fibers, notably the sensory fibers, diminish in diameter near the periphery. He reaches the conclusion that the entire subject of nerve fiber diameter has not been sufficiently worked over to make possible any definite statements regarding it.

Since, then, the observation of SCHWALBE regarding the lessening diameter of nerve fibers at the periphery is the only one based upon observations made upon fibers from mixed nerves between the joining of the spinal roots and the periphery, the present observations are offered in partial confirmation of the theory of conical diminution of peripheral nerve fibers in their course.

The special interest of these findings centers in the fact

that the diminution in the diameter of the fiber occurs in a large nerve trunk and at a considerable distance from any peripheral branches.

5. Area of the axis cylinder substance proportional to the area of the section.

In the discussion of the innervation of the various portions of the hind extremity of *Rana virescens* we have necessarily included both the medullary sheath and the axis cylinder in the determination of the average areas of the nerve fibers.

In attempting to eliminate as completely as possible all sources of error, there arose the question of a possible change from the normal proportion of the medullary sheath to the axis cylinder in these sections. Measurements were therefore made at the three chief levels to ascertain the existing proportion of the average area of the ten largest fibers at each level to the average area of their axis cylinders.

The results are embodied in Table XVIII.

TABLE XVIII.

Showing the average areas for the ten largest fibers and for their axis cylinders.

Frog II B. Left Side.	A.	B.	
Level selected.	Average area ten largest fibers.	Av. area of their axis cylinders.	Ratio B. to A.
Sciatic above branches	222.73 $\square \mu$	108.62 $\square \mu$	1:2.05
Sections at knee	130.69	62.49	1:2.09
Sections at ankle	89.20	43.01	1:2.07

Reference to this tabulation shows that at each of the three levels the average area of the axis cylinders of the ten fibers measured is approximately one-half that of the average areas of the entire fibers. Hence the 1:1 relation (DONALDSON, 1895) of the medullary sheath to the axis cylinder prevails here also.

The results at each level are so nearly uniform that we are justified in the conclusion that, in all the measurements, not only the areas of the entire fibers bear certain relations to one another, but that their most actively functional portions, the

axis cylinders, stand in the same relations—a fact that increases somewhat the value of this series of observations.

6. Comparison of the average areas *for* the fibers with those determined by previous measurements.

While the general relations of the results of these measurements are much the same as those obtained by earlier investigations, so far as the thigh is concerned, yet the resulting figures are considerably lower in value than those heretofore obtained. This may be due to one of several causes.

First, the proportion of fibers of various sizes may be differently adjusted in the various frogs. Concerning the numbers and proportions of the fibers of varying sizes in the peripheral nervous system we have no definite information. A fact that seems to negative the probability of changed proportions is the observation that the ten largest fibers at any level are in this series of observations less in diameter than the ten largest fibers at the same level in any previous series of observations.

Again, some change may have taken place in the individual nerve fibers during the process of preparation of the sections, but if a shrinkage of the fiber in its entirety had taken place the sections would exhibit increased spaces between the fibers. No such separation of fibers appears in these sections. Or, if the change were in the axis cylinder alone, or in the medullary sheath alone, the usual relation of 1:1 existing between the areas of these two portions would be lost.

Table XIX, showing the average areas of the nerve fibers and of their axis cylinders in Frog B of the first series and Frog B of the second series, shows but slight deviation in either frog from the usual 2:1 relation of the fiber to its axis cylinder or of the 1:1 relation between the axis cylinder and the medullary sheath. The smaller diameter of these ten fibers in Frog II B, the subject of the present study, is also shown in this table.

TABLE XIX.

Showing the average areas of the ten largest fibers and of their axis cylinders in Frog B and Frog II B.

Frog B. Sex. F. Weight 49.7 grams. Length 205 mm.
Frog II B. Sex. F. Weight 61.5 grams. Length 234 mm.

	A.	B.	
	Average area of fibers at level S ₁ .	Average area of their axis cylinders.	Ratio of B. to A.
Frog B.	290.13 $\square \mu$	156.14 $\square \mu$	1:1.85
Frog II B.	222.73	108.62	1:2.05

From these facts we seem justified in the conclusion that the relative smallness of the diameters of the fibers is a characteristic of this particular frog and is common to all the fibers.

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