THE NUMBER AND ARRANGEMENT OF THE FIBERS FORMING THE SPINAL NERVES OF THE FROG (RANA VIRESCENS).

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With Plates VI to XIII.

I. SUMMARY.

1. A gross examination of the different spinal nerves shows marked variations in their architecture (Plate VI).

2. The number of fibers in the ventral roots decreases from the spinal cord towards the spinal ganglion.

3. The number of fibers in the dorsal roots decreases from the spinal ganglion towards the spinal cord.

4. The section of the nerve trunk immediately distal to the spinal ganglion (dorsal branches excluded) contains a greater number of fibers than are fouud in a section of the trunk further distal.

5. The decrease in the number occurs among the smaller fibers of the nerve.

6. The general explanation of these relations is found in the fact that the fibers arising from the spinal ganglion grow, on the one hand, towards the spinal cord by way of the dorsal root and, on the other, towards the periphery by way of the nerve trunk; and, that the fibers of the ventral root grow from the spinal cord towards the periphery.

7. In frogs of increasing weight, the fibers of the dorsal root increase in number more rapidly than do those of the ventral root.

8. The sum of the fibers in the trunk and dorsal branches combined, exceeds in every case and by a considerable amount the sum of the fibers in the two roots.

9. The excess of fibers in the trunk and dorsal branches seems to be correlated both with the absolute and proportional size of the dorsal branches.

10. The method herein employed for the enumeration of nerve fibers possesses several features which commend it as more trustworthy than any of those previously employed.

II. INTRODUCTION.

The number, size and arrangement of the medullated nerve fibers constituting the peripheral nervous system in vertebrates presents many problems worthy of investigation.

The problem which forms the basis of this paper is a determination of the length of some of the nerve fibers arising from the cells of origin situated in the ventral horns of the spinal cord and in the spinal ganglia.

The region chosen for the study of this extension of the nerve fibers is, on the one hand, that formed by the roots of the spinal nerves, and on the other, that formed by the spinal nerve trunks as far as their union with the rami communicantes. The study involves first, an enumeration of the fibers contained in each nerve root at different levels, and second, a determination of the number of fibers contained in a section of the trunk and dorsal branches immediately distal to the spinal ganglion and in a section of the trunk further distal.

The animal examined was the common leopard frog, *Rana virescens*.

III. THE GROSS ANATOMY OF THE SPINAL NERVES.

The gross appearance of the different spinal nerves when dissected out and freed from adherent connective tissue is exhibited in Plate VI. The sketches there produced are drawn to scale.

Passing from the Xth to the Ist nerve, three chief features of variation are to be noted.

1. The most marked variation is exhibited in the abundance of the dorsal branches. It is seen that for the Xth these branches are few, for the IXth they are more abundant, while for the VIIIth there is seldom more than one, and this is relatively very small and arises directly out of the mass of the ganglion instead of springing from the nerve trunk at the distal end of the ganglion as it does in case of the IXth and Xth. For the VIIth, again, the condition is much as it is in the IXth. The dorsal divisions of the VIth, Vth, and IVth are both relatively and absolutely larger than those of any of the other nerves. For the IIIrd nerve the branches are less than for the three nerves caudad to it, and the larger branches of it usually arise out of the ganglion and at right angles to the long axis of the nerve trunk. The IInd gives off many very small branches but in a very irregular manner, while the Ist returns to the general type of those most caudad.

2. The dorsal and ventral roots vary both in length for the different nerves and in relative size for the same nerve. As a result of the difference between the rate of growth of the spinal cord and the canal in which it lies, the more caudal nerves possess the longer roots, since in the frog the spinal ganglia always remain in the intervertebral foramena. The length of the roots thus gradually decreases until, passing cephalad, the roots of the IInd and Ist nerves are merely long enough to reach at almost right angles their zones of exit and entrance upon the cord. The relation between the size of the two roots of a nerve is more constant than the relation between the size of the roots of any two successive nerves. The most striking and constant difference between the two roots is presented in the Ist or hypoglossal, the dorsal root being not only relatively but absolutely smaller than that of any other nerve.

3. The distance between the spinal ganglion and the ramus communicans (*c*, Plate VI) undergoes a very considerable variation for the different nerves. As shown in the plate, this distance decreases rapidly between the VIIth and IIIrd nerves, the ramus being given off much closer to the ganglion in case of the IVth, IIIrd, IInd and Ist nerves.

The relative size of any two or three successive nerves can often be observed as decidedly different in different frogs or indeed, on the two sides of the same individual. The VIIth, VIIIth and IXth have been more often noticed as varying in this respect. When the IXth is proportionately small, the VIIIth is generally large and when the VIIIth is smaller than usual, the VIIth and IXth appear more than ordinarily large. Quite often the VIth, VIIth, VIIIth and IXth nerves were noticeably larger on one side than on the other of the same individual. In these cases, the larger nerves were most often on the left side of the animal.

IV. THE NERVES WHICH WERE INVESTIGATED.

An enumeration of the fibers was made for all the spinal nerves except the IInd and Xth. The IInd was neglected for the time being, because of its large but very short roots, because numerous small branches are given off and received all along the short distance between its ganglion and its ramus communicans, and for the reason that, owing to its large size, it is penetrated by osmic acid with great difficulty.

The Xth, unlike the other nerves, passes out of the spinal canal through a long foramen in the *urostyle* (canalis coccygeus). This position renders its necessary exposure to the reagent and its removal without injury very difficult and justified its exclusion at this time also.

V. The Number and Arrangement of the Nerve Fibers in the Dorsal and Ventral Roots and in the Beginning Nerve Trunk.

1. The examination of the roots consisted in counting the fibers in sections taken from two or three different levels of each root; i. e., the fibers of each root were counted at different distances from their cells of origin. The points at which the sections were taken are indicated by the numbers 1, 2 and 3, Plate VI. The Plate shows also that in case of the longer roots three counts were made, while for the shorter, for obvious reasons, two counts were thought sufficient.

2. Counts were made of a section of the trunk taken immediately distal to the spinal ganglion and of a section of the dorsal branches made as close up to the ganglion as possible. (Sections 4 and 6, Plate VI).

3. Another enumeration was made of the fibers contained in the trunk at a point just central to its junction with the ramus communicans. (Section 5, Plate VI).

The results of such enumerations are presented in Tables I and II. These tables contain results obtained from two different specimens. They show (1) that, as indicated in the columns A, B and C, the number of fibers in the ventral root remains constant or decreases in the course of the root from the spinal cord towards the dorsal root ganglion; (2) that the number of fibers in the dorsal root remains constant or decreases in the course of the root from the ganglion towards the spinal cord. Leaving the roots and turning to the trunk, the tables show (3) that the section of the trunk taken next to the ganglion (dorsal branches excluded) contains more fibers than are contained in a section taken further distal (columns J and K). Further, Table I shows that the sum of the fibers in the trunk and dorsal branches taken immediately distal to the ganglion is always considerably greater than the sum of the fibers contained in the ventral and dorsal roots just distal to the ganglion. This fact of the excess of fibers in the trunk and branches is especially presented in Table III.

TABLE I.

FROG, FEMALE, WEIGHT 48.2 GRAMS.

		Roots							*Trunk*						
		A	B	C	D	E	F	G	H	I	J	K	L	M
		Sections of the Roots			Excess at	Excess at	Length between 1 and 3	Sum of Roots at	Excess of sum of trunk and branches at	Sum of trunk and branches at	Trunk and sum of branches at	Trunk at	Excess of trunk at	Length between 4 and 5
Level of section		1	2	3	3	1	mm	3	4	4	4	5	4	mm
I R.	D.	132		132	**0**		·75							
	V.	1046		1045		**1**	·75	1177	**78**	1255	1173 82	1168	**5**	1
II	--	----	----	----	---	---	---	----	----	----	----	----	---	---
III R.	D.	317		329	**12**		1							
	V.	397		379		**18**	1	708	**170**	878	653 225	572	**81**	1
IV L.	D.	367		371	**4**		2.5							
	V.	163		163		**0**	3	534	**103**	637	378 259	324	**54**	2
V L.	D.	288	290	299	**11**		3.5							
	V.	133	132	127		**6**	3.5	426	**145**	571	279 292	262	**17**	2.5
VI R.	D.	341	350	350	**9**		4							
	V.	261	259	251		**10**	4	601	**220**	845	475 370	469	**9**	4
VII L.	D.	1043	1102	1108	**65**		4.5							
	V.	398	391	377		**21**	5	1485	**212**	1697	1556 141	1530	**26**	6
VIII L.	D.	2051	2083	2108	**57**		6							
	V.	1316	1301	1295		**21**	6.5	3403	**217**	3620	3534 86	3501	**33**	8
IX L.	D.	1106	1167	1171	**65**		7							
	V.	757	752	721		**36**	7.5	1892	**257**	2149	1881 268	1854	**27**	7

TABLE II.

FROG, FEMALE, WEIGHT 59.5 GRAMS.

		Roots							*Trunk*					
		A	B	C	D	E	F	G	H	I	J	K	L	M
		Sections of the Roots			Excess at	Excess at	Length between 1 and 3	Sum of Roots at	Excess of sum of trunk and branches at	Sum of trunk and branches at	Trunk and sum of branches at	Trunk at	Excess of trunk at	Length between 4 and 5
Level of section		1	2	3	3	1	mm	3	4	4	4	5	4	mm
VI R.	D.	235	243	246	11		6							
	V.	134	132	129		5	6.5	357	138	513	227 286	223	4	2
VII R.	D.	791	798	814	23		7							
	V.	259	256	250		9	7	1064	199	1263	1108 155	1039	69	7
VIII R.	D.	1649	1714	1732	88		8							
	V.	1037	1023	1020		17	8.5	2752	86	2838	2789 49	2743	46	7.5
IX R.	D.	1437	1454	----	17		4.5							
	V.	991	973	955		36	9	2427	109	2536	2297 239	2285	12	8.5

EXPLANATION OF TABLES I AND II.—These tables contain the records of the enumeration of the fibers at different levels of the ventral and dorsal roots and of the nerve trunk of the spinal nerves of the frog. Table I contains counts made upon eight of the spinal nerves of a frog weighing 48.2 grams. Table II, counts of four nerves of a 59.5 gram frog. The figures at the head of the columns indicate the level of the section at which a count was made. For convenience of reference, each column is designated by a letter.

The point of principal interest which is claimed for these results lies in the change in the number of fibers to be found in the course of the dorsal and ventral roots and in the course of the trunk between the limits chosen.

It is first desired to determine whether there is any regularity in these variations such as would be exhibited by a percentage change in the number of fibers for a unit of length. Table I gives the absolute amount of variation in the number of fibers found between the different levels at which the counts were made and thus does not take into account the length of the root nor the distance between the points at which the sections of the trunk were taken. Plate VI will show that these distances through which the changes occur vary greatly for the different nerves. In order therefore to determine the variations more exactly it was necessary to compute for each nerve, the average amount of variation occurring in a unit of length and convert the variations thus obtained into percentage values. To do this the total amount of variation in a given case was divided by the number of millimeters through which it occurs. In a ventral root, for example, the number of fibers found in the section nearest the ganglion was subtracted from the number found in the most proximal, or section nearest the cord. The difference thus obtained was divided by the number of millimeters between these points (sections 1 and 3, Plate VI). This average variation per millimeter having been found, its percentage value of the entire number of fibers contained in the *most proximal* section was determined. For the dorsal root, of course, since the variation occurs in the opposite direction, the number found nearest the ganglion (section 3) was used as that on which to compute the percentage of variation; and for the trunk, the number in section 4, the section containing the largest number of fibers.

The percentages of the average amounts of variation per millimeter of length obtained for the several spinal nerves included in Tables I and II are as follows:

TABLE III.

FROG, FEMALE, WEIGHT 48.2 GRAMS.

The nerve	*Average percentage decrease per mm.* In ventral root	In dorsal root	In trunk
I	0.12%	0.00	0.42%
II	------	------	------
III	4.60%	3.60%	12.04%
IV	0.00%	0.43%	7.10%
V	1.30%	1.05%	2.44%
VI	0.96%	0.64%	0.48%
VII	1.05%	1.30%	0.28%
VIII	0.24%	0.45%	0.12%
IX	0.63%	0.79%	0.20%

FROG, FEMALE, WEIGHT 59.5 GRAMS.

	Average percentage decrease per mm.		
VI	0.57%	0.70%	0.88%
VII	0.50%	0.38%	0.79%
VIII	0.19%	0.60%	0.22%
IX	0.40%	0.26%	0.06%

These conditions of average variation per millimeter in the number of fibers found at different distances from their cells of origin, are illustrated by curves in Chart I.

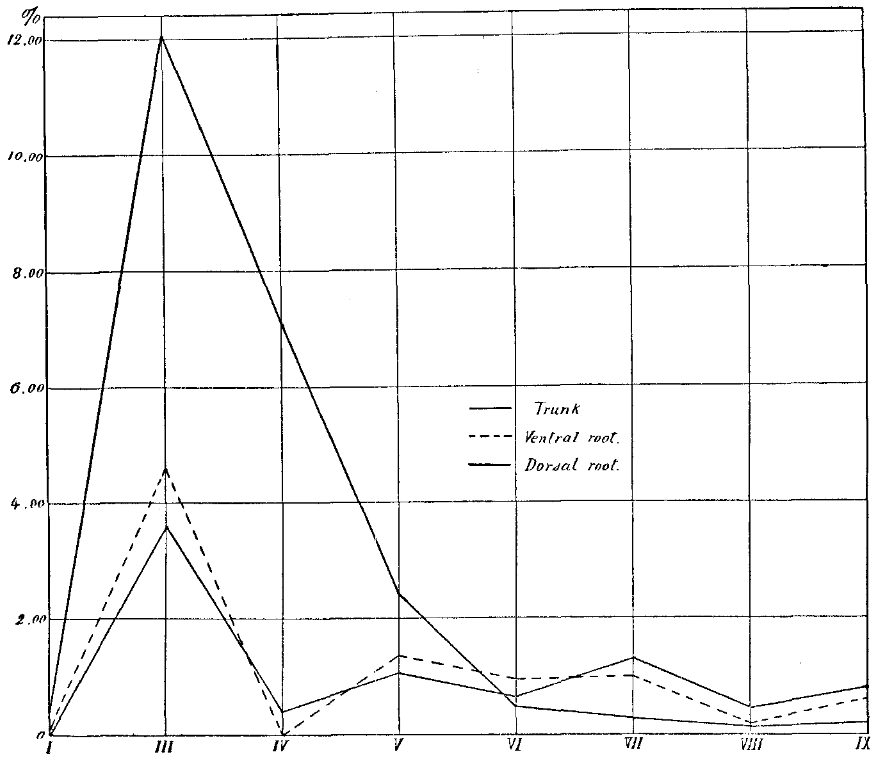


CHART I, showing, in the different spinal nerves of one specimen, the relations of the percentage values of the average change per millimeter in the number of fibers in the dorsal and ventral roots and in the trunk. The distances in which the changes occur and the numbers from which the percentages are computed are given in Table I.

The curves of this chart, constructed from the values in Table III, show more readily than does Table I, (1) that the changes in *the two roots* of a given nerve do not differ very considerably from each other; (2) that in the several nerves there is no regularity in the amount of variation occurring in a given root, the curves for the two roots crossing each other repeatedly; (3) that the percentage variation per millimeter in the trunk is greater than that in the roots in case of the Ist, IIIrd, IVth and Vth, and below that in the roots in case of the VIth, VIIth, VIIIth and IXth. Values obtained from the second specimen, however, show that this cannot be considered at all as a general condition. As a whole, (4) the greatest variations are seen to occur in the IIIrd, IVth and Vth, while the VIth, VIIth, VIIIth and IXth are more similar to one another.

The absence of data from nerves II and X is to be deplored. The abrupt rise from the Ist to the IIIrd nerves might be broken by data from the IInd. The explanation of why the changes in the IIIrd rank so much higher than those in the other nerves must be deferred till further investigation of this nerve can be made. As is well known, the IIIrd is the spinal nerve which, in the frog, contains the greatest percentage of fibers which pass to the sympathetic system, and therefore the nerve contains a greater percentage of small fibers than any other nerve. But, at present, no adequate explanation based upon this fact can be suggested.

To explain the decrease in the number of fibers of the ventral root as it passes from the spinal cord toward the point of its junction with the dorsal root, we assume that those fibers, which grow out from the ventral horn cells have extended unequal distances from the cord. In the dorsal root, the variation in the number of fibers is to be explained in the same way. Here the decrease is in the opposite direction from that in the ventral root, and naturally so since, as is now known, very nearly all the fibers comprising the dorsal root are the central prolongations of the outgrowths of the spinal ganglion cells.

This explanation is also applied to the decrease in the number of fibers found in the trunk between the section next the ganglion and that most distal. In the trunk the great mass of the fibers, at least, being of the same origin as those composing the roots, the changes in the roots demand that the decrease in the trunk should occur, as it does, towards the periphery. As is well known, no branches are given off from the roots, nor, with the exception of the IInd nerve, are any branches given off from the trunk between the spinal ganglion and the ramus communicans. This explanation of the changes as due to growth will be discussed later.

VI. THE RÔLE PLAYED BY THE SMALLER FIBERS.

It was suggested that it would be well worth while to determine whether the decrease in the number of fibers in the roots and trunk occurs through variations in the number of the large or the small fibers. It is known from previous descriptions that the two roots differ from each other in the general appearance of their transverse sections. The ventral root shows much more uniformity in the diameter of its fibers than does the dorsal. The ventral roots of the spinal nerves of the frog, while not possessing the very largest fibers, yet, with the exception of the ventral root of the IIIrd nerve, have the great majority of their fibers uniformly large. This excess of large fibers is interspersed with fibers distinctly small and with a very few intermediate in size. Plates VIII, X, and XII represent sections of the ventral root of the VIIIth nerve which is quite typical.

The dorsal root, on the other hand, shows no uniformity as to the diameter of its fibers. It is rather a mixture of fibers of all sizes from the very small up to a few larger than any found in the ventral root.

In order, therefore, to find if possible, in fibers of what general diameter the above changes in number occur, a second count was made of ten of the sections of the roots. Sections of the smaller roots were chosen since the labor of counting would be less and the results obtained would be fully as significant as in case of larger ones.

As will be explained in that part of this paper which deals with the methods employed, each count was made from a photograph of the section and controlled by having the section at at the same time under the microscope and highly magnified. Thus the use of the photograph guarded against any fibers being overlooked or recounted, and all doubtful cases could be settled at once and accurately by an appeal to the microscope. In the investigation with regard to the small fibers, all those having a diameter of 5 micra and under were considered as small fibers, and in order to have but two classes, all those above 5 micra were considered as large. With a combination of lenses producing a magnification of 800 diameters (Zeiss–obj. 8 mm, oc. 12), each division of the occular micrometer used was equivalent to exactly 5 micra. By the aid of a mechanical stage any fiber the diameter of which was questionable could be quickly tested. Each section was counted by fields marked out on the photograph. In case of a section containing fewer small than large fibers, the section was first examined by counting the small fibers. Then the large fibers were counted as a matter of control. In every case, when the numbers for large and small fibers were added, the sum was the same as that obtained in the previous counts and recorded in Table I.

The enumerations thus made were as follows in Table IV:

TABLE IV.

			Counts made for small fibers.			Counts recorded in Table I.		
			Sec. 1	Sec. 3	Dif.	Dif.	Sec. 3	Sec. 1
IIIrd N.	Dorsal root	small	155	164	9	12	329	317
		large	162	165	3			
	Ventral root	small	306	289	17	18	379	397
		large	91	90	1			
Vth N.	Dorsal root	small	193	203	10	11	299	288
		large	95	96	1			
	Ventral root	small	45	39	6	6	127	133
		large	88	88	0			
VIIth N.	Ventral root	small	107*	95	12	14	377	391*
		large	284	282	2			

TABLE IV, containing the results of a separate enumeration of the large and small fibers in the various dorsal and ventral roots indicated. In order to compare the results of these counts with those previously obtained in counts of the same sections and recorded in Table I, the corresponding numbers of that table are placed in parallel columns. The numbers of nerve VII, indicated by a star, are those found for section 2, Plate I, instead of section 1, as the column reads.

While in most of the cases, the differences in the number of small fibers do not accord with the differences between the entire numbers previously found, yet it is seen that by far the greater portion of a difference does occur among the small fibers; i. e., those 5 micra or less in diameter. The lack of complete accordance is perhaps due to fibers whose diameters were so near the border line between large and small that a slight distortion, or the lack of it, resulted in their being classed in the one category at one level and in the other at another.

These counts for small fibers were of dorsal and ventral roots exclusively. If the variations in the number of fibers in the roots can be shown to take place in the smaller fibers, it is reasonable to assume that the variations found in the trunk, occur among the small fibers also.

Birge ('82) made counts of the fibers at a single level of the two roots and of the trunk of frogs of different weights or ages. In regard to the fibers of the ventral roots, he found upon summing up the entire number of motor fibers determined for one side of each animal, that there existed such proportions between the numbers of these fibers and the weights of the frogs, that, given the one he could compute the other approximately. Assuming the number of ventral root fibers to be the same for the two sides of the animal, he found that an increase in the weight of the frog was accompanied by an increase of about 55 ventral root fibers for each gram of body weight.

This author also determined that the average diameter of the fibers in the roots of the younger specimens was less than that in the older and expressed the opinion that, during growth, small fibers thicken into larger ones.

Korybutt-Daskiewicz ('78) as early as 1878 described the ingrowing of new fibers in the sciatic nerve of the frog. He pictures the first formation of the medullary sheath about the outgrowing neuraxone, either as fine varicosities which blacken with osmic acid, or as first a thin layer of myelin which gradually thickens. These changes were noted near the end of the growing fiber. The sheath was observed to cease, not gradually, but more as the wood portion of a sharpened lead pencil, the axis cylinder preceeding the myelin sheath by short but varying distances.

Kolster ('93) in studying the regeneration of fibers following the section of the nerve, pictures the myelin sheath as being reformed a node at a time, the regenerating axis cylinder preceding the development of the myelin. Accepting the above description that the sheath forms, node by node, the sheath would necessarily cease bluntly and transverse sections stained with osmic acid alone would only show the growing fiber appearing or disappearing suddenly, the axis cylinder portion being invisible.

Whatever the source of the medullary sheath, it was thought highly probable that among the small fibers of the transverse sections here counted, there might be observed some fibers cut at the point at which this sheath was just being acquired. In examing the preparations with this object in view, certain difficulties had to be recognized.

1. The preparations not having been made with this point in mind, in the mounting, good sections rather than serial sections had been chosen.

2. Since it was desired that the section chosen for counting should be one from within a fraction of a millimeter of a certain point along the course of a root or trunk, but few sections were usually mounted.

3. The average of the decreases in number even in the roots being about 1 fiber to each 100 in a millimeter of length, it is evident that in the necessarily very thin sections, the opportunities of observing a disappearing fiber are rare. For these reasons it may be, very few satisfactory cases of a disappearing fiber have been observed. One of these is represented in Figs. 1-6, Plate VII. In the search for disappearing fibers, sections were now and then observed which were evidently cut so as to contain only the node of Ranvier. These of course when followed in the series showed in both directions a reappearance of the myelin sheath.

VII. THE RELATIONS OF THE VENTRAL AND DORSAL ROOTS TO EACH OTHER.

The absolute numbers of the fibers enumerated in the ventral and in the dorsal roots and in the trunks and dorsal branches are represented in the curves of Chart II. This chart is constructed directly from Table I, and is but a charted expression of the numbers given there. In order to complete the chart, the data for the IInd and Xth nerves are computed from the results obtained by Birge in the work above cited.

This interpolation in the curves was made on the basis of the following calculation. The total number of fibers found by Birge in the spinal nerves of a 63 gram frog was taken and the proportional values of the numbers for the nerves in question was determined. Then the same proportional values were found of the numbers recorded in Table I. The amounts thus determined for the ventral and dorsal roots and for the trunk of the IInd and Xth nerves were inserted to complete the chart. The number of fibers in the dorsal branches of these two nerves could only be roughly estimated, since Birge did not deal with with them at all.

Birge used a different species of frog (*esculenta*) from the one used in this work and, that the proportion of fibers contained in a given nerve is even approximately the same for the two species, can only be assumed. From observations made by Professor Donaldson in this laboratory, it appears that the European *Rana esculenta* has a central nervous system of proportionally smaller weight than the American *Rana virescens*. This relation is also indicated by comparing Birge's results with those mentioned in this paper.

Birge counted in a frog of 63 grams, a much smaller number of fibers than were here found for one of 48 grams, as recorded in Table I. For the ten spinal nerves of one side of a 63 gram frog, Birge found in the two roots a total of 9,618 fibers. Of the species here used, a frog weighing 48 grams was found to possess 14,783 fibers in the roots of the ten spinal nerves of one side. While the two sides of the same specimen may vary considerably, even in the total number of fibers, it is evidently not reasonable to suppose the variation can account for this difference which amounts to 5,175 fibers.

Chart II, more readily than Table I, conveys an idea of the absolute relations existing between the numbers of fibers in the trunk and dorsal branches of the ten spinal nerves and the numbers of fibers in the dorsal and in the ventral roots. It shows (1) the abrupt rise in the number of fibers at those regions of the spinal system from which the limbs are supplied; (2) as might be expected, the curves for the roots follow that for the trunk and dorsal branches fairly closely, and (3) that the curves for the two roots follow each other but roughly, crossing between the Ist and IInd nerves, between the IInd and IIIrd, IIIrd and IVth, and between the IXth and Xth. The dorsal root of the IInd contains the greatest number of root fibers and the dorsal root of the Xth the least number, both of which however are interpolations.

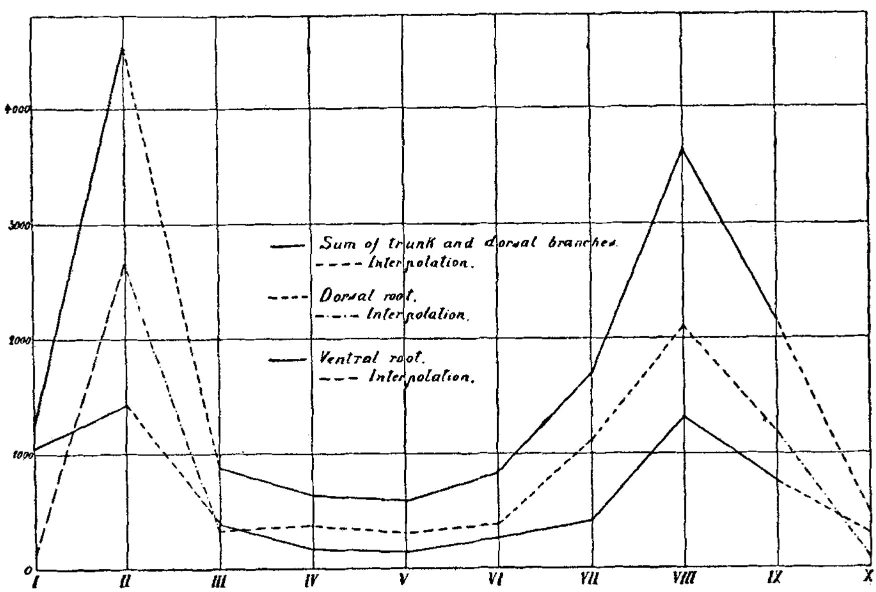


CHART II, showing the number of fibers in the dorsal roots, and in the ventral roots and the sum of those in the trunk and dorsal branches of the different spinal nerves. The parts of the curves in broken and dotted lines which represent the IInd and Xth nerves are interpolations from the records of Birge.

The relations existing between the two roots, as shown here, correspond in the main with those found by Birge.

1. The number of dorsal root fibers in the same animal is greater than that of ventral root fibers. A frog of 48 grams has for one side approximately 8572 dorsal root fibers against 6211 in the ventral roots. This is an excess of 38%. This excess in the dorsal roots however is greater than that obtained by Birge in a frog of 63 grams.

2. The apportionment of fibers to the different roots is widely different. Only in case of the Ist, IIIrd and Xth nerves, do ventral root fibers exceed those of the dorsal root. In the remaining nerves, dorsal root fibers predominate, but in very unequal degrees. Of all the nerves here investigated, the greatest percentage of dorsal root fibers in excess of those of the ventral root is found in case of the VIIth nerve. In Table I this nerve possesses an excess of 178% and in Table II an excess of 214%.

The percentage values of the excess of the larger root of each nerve represented in Chart II are given in Table V. For contrast the cases in which the ventral root contains the greater number of fibers are placed in a separate column.

TABLE V.

FROG, WEIGHT 48.2 GRAMS.

Number of the Nerve	Excess	
	Occurring in dorsal root	Occurring in ventral root
I		692%
II	83%	
III		20%
IV	127%	
V	125%	
VI	34%	
VII	178%	
VIII	60%	
IX	54%	
X		370%

The percentage values given in Table V are presented in the form of a curve in Chart III. In the Chart the cases of an excess of dorsal root fibers are recorded above the base line and those of an excess of ventral root fibers below it. It is seen that the curve begins far below the line of the Ist nerve, rises well above it for the IVth and Vth, falls again for the VIth, rises highest for the VIIth and ends below the line for the Xth.

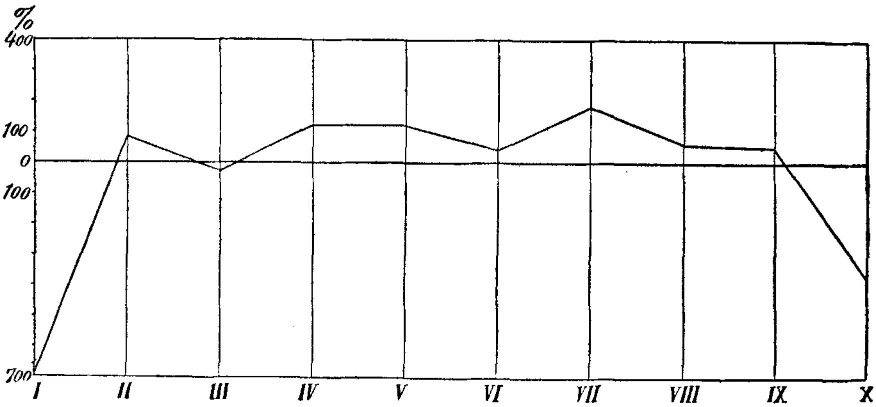


CHART III, exhibiting the excess in the larger root of the several spinal nerves. The records show the percentage value of the excess of the larger root. The records for the dorsal root appear above the base line and those for the ventral root, below it.

3. In regard to the relations existing between the two roots, it can also be shown that during growth the dorsal root fibers increase in number more rapidly than do those of the ventral root [Birge ('82)]. This is seen to be true even for slight differences in weight. Table II contains a record of counts made of four nerves from a specimen of somewhat greater weight than that entered in Table I. A comparison of the percentage values of the excess of dorsal root fibers in these four nerves as recorded in the two tables is give in Table VI.

TABLE VI.

Number of the nerve	Percentage of excess of dorsal root fibers from records of	
	Table I, frog wt. 48.2 grams	Table II, frog wt. 59.52 grams
VI	34%	83%
VII	178%	214%
VIII	60%	67%
IX	54%	49%

Table shows at a glance that for the heavier frog, there is a decided gain in the excess of the dorsal root fibers. That the percentage of the excess in the IXth nerve fails to show an increase is probably due to an unequal distribution of root fibers in the VIIIth, VIIth and IXth nerves. The gain in the VII is more than ample to balance the deficiency.

To determine whether the relation exhibited by these four nerves is true for the entire ten, we must again draw upon the records of Birge. To determine the increase in the number of fibers with increase in size of the animal, Birge counted the ten nerves of several specimens chosen with reference to their weight. Using his figures for the total numbers of dorsal root fibers and of ventral root fibers found for one side of the specimen, and computing the percentage of the excesss of dorsal root fibers in each case, we get

TABLE VII.

From Birge—*Rana esculenta.*

Weight of frog	Total number of dorsal root fibers	Total number of ventral root fibers	Excess of dorsal root fibers	%
23 grams	3781	3524	257	7.3
63 grams	5335	4283	1052	24.5

Taking the total number of fibers in each root of the VIth, VIIth, VIIIth and IXth nerve of the same specimens used in Table VII we get,

TABLE VIII.

From Birge—*Rana esculenta.*

Wt. of frog.	Total number fibers in Dorsal Roots.	Total number fibers in Ventral Roots.	Excess of Dorsal Root fibers.	Percentage.
23 grams.	1930	1225	705	55.5
63 grams.	2840	1612	1228	76.1

Thus it is seen that there is an increase in the excess of dorsal root fibers for the larger specimen when we consider the VIth, VIIth, VIIIth and IXth nerves as well as when we consider the entire ten nerves.

Then taking the sums of the dorsal root fibers and the sums of the ventral root fibers found in the VIth, VIIth, VIIIth and IXth nerves of the two specimens employed in Tables I and II, and determining the percentage of the excess of the fibers of the dorsal roots indicated by these sums we get

TABLE IX.

Rana virescens.

Wt. of frog.	Total number fibers in Dorsal Roots.	Total number fibers in Ventral Roots.	Excess of Dorsal Root fibers.	Percentage.
48.2 grams.	4737	2732	2005	73.7
59.52 grams.	4246	2421	1825	75 4

Thus, an increase in the excess of fibers in the dorsal root is found not only for an increase of 40 grams in weight but also for an increase of only 11 grams in weight. Also the increase in weight being slight, the increase in the excess of dorsal root fibers is correspondingly slight. So, it is hoped that some evidence is added to that brought forth by Birge to the effect that as the animal increases in size, the number of dorsal root fibers increases more rapidly than that of the ventral root.

It may be fairly asserted that by far the greater part of the sensory or dorsal root fibers of the frog go to innervate the skin. As the animal grows the skin of course increases in area, but exactly what relation this increase in the excess of sensory over motor fibers has to the increase in the area of the skin, is yet to be investigated.

A very interesting relation is found to exist between the numbers of the dorsal and ventral root fibers present in those nerves which contribute their fibers to the inervation of the leg. It is known that, in the frog, the VIIth, VIIIth and IXth nerves give nearly all their trunk fibers to the formation of the sciatic nerve. If the sums of the dorsal and ventral root fibers of these three nerves be taken and the one divided by the other, there is obtained for each of the above specimens the proportions represented in Table X.

TABLE X.

The proportion of ventral root to dorsal root fibers contained in the VIIth, VIIIth, and IXth nerves of four specimens.

Wt. of frog, grams.	Ventral root fibers.	Dorsal root fibers.	Proportion.	
23	1088	1754	1—1.7	*R. esculenta.*
63	1453	2656	1—1.8	
48	2471	4387	1-1.77	*R. virescens.*
59	2287	4000	1-1.75	

These proportions are interesting because they seem to indicate that, whatever the size of the animal, there exists an almost fixed relation between the inervation of the tissue of the leg by sensory and motor fibers. This similarity in the proportion of ventral to dorsal root fibers fails if there be included in the sums, the ventral and dorsal root fibers of other nerves than those which go to form the sciatic. Even when the VIth nerve alone is included the proportions become more varied.

Another point may be noted with regard to the roots. The average diameter of the fibers of the same root differs for the different nerves. Birge noted for the European frog that the greatest average diameter for ventral root fibers is possessed by the VIIth nerve and the least average diameter of ventral root fibers by the IIIrd. This is found to be true also for the specimens here investigated. In addition it may be added that the least average diameter of dorsal root fibers is to be found in the dorsal root of the Ist nerve and usually the largest of those very large dorsal root fibers before mentioned are to be found in the dorsal root of the VIIth. For the ventral root of the IIIrd, it may be said that the few fibers it contains of the ordinary ventral root type are as large as the average of those of the ventral roots of the other nerves. Its fibers however are sharply divided into two types and the average diameter of its fibers is reduced because of the presence of a very large number of that type of small fibers which Gaskell ('89) and others have described as destined to pass to the sympathetic system and which, in the preparations here used, can be seen to largely compose the rami communicantes.

Birge obtained the average diameter of the fibers of a root by dividing the area of the transverse section of the root by the number of fibers contained in it.

Schwalbe ('82) made some determinations of the diameter of the fibers of the two nerve roots of the frog by actually measuring a few fibers of each root and taking the average diameter of the fibers thus measured. Schwalbe, like Birge, obtains the least diameter for the motor root of the IIIrd nerve, but unlike Birge, he finds the greatest average diameter for dorsal as well as ventral root fibers possessed by the roots of the VIIIth and IXth nerves. The species of frog used by Schwalbe was the same as that used by Birge.

VIII. THE RELATION OF THE ROOTS TO THE TRUNK.

In presenting Tables I and II, attention was called to the fact that in every case the sum of the fibers in the trunk and dorsal branches taken just distal to the spinal ganglion, exceeds by a considerable amount the sum of the fibers in the two roots

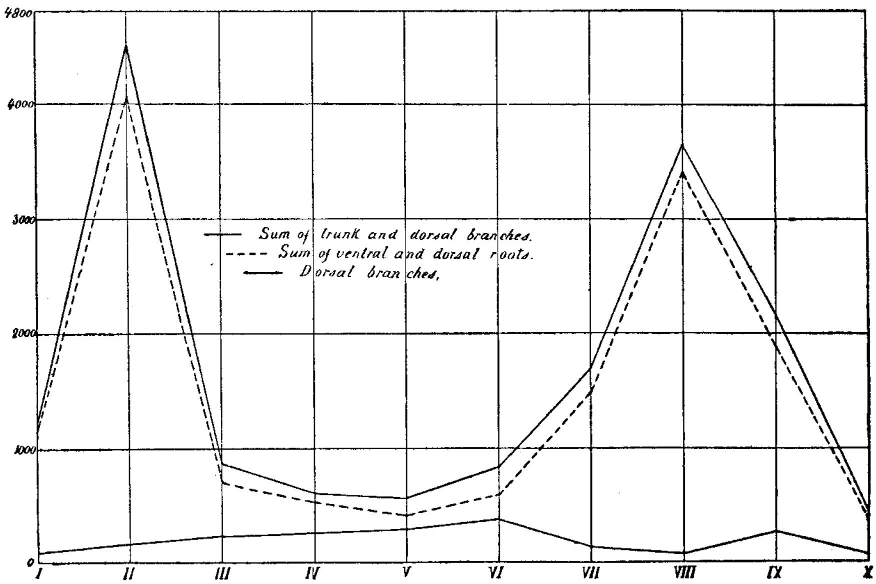


CHART IV. Curves showing the relation existing between the sum of the fibers in the two roots taken close up to the spinal ganglia and the sum of the trunk and dorsal branches taken just distal to the spinal ganglion. The lowest curve represents the sum of the dorsal branches alone.

taken just central to the ganglion, or at any point along the course of the roots. This relation is especially presented in Chart IV. This chart is constructed from the data employed for Chart II. It differs from the latter by the fact that the sum of the fibers in the two roots taken just central to the ganglion, is employed instead of plotting the roots separately. The relations presented, therefore, are those of absolute values. The lower curve represents the number of fibers separately contained in the dorsal branches of each nerve.

Attention is called to the chart as showing (1) the fact that the sum of the roots is always below the sum of the trunk and dorsal branches; (2) that the curve for the two roots runs nearly parallel with that of the sum of the trunk and branches; (3) that the greatest amount of deviation between the two curves does not occur in the larger nerves; and (4) that the dorsal branches attain their highest absolute values in the smaller nerves. Also, it is to be noted, that, though the value of the dorsal branches does not always coincide with the amount of the excess of fibers which these branches together with the trunk contain above the sum contained in the two roots, yet the curve for the branches suggests that there may exist some relation between the number of fibers contained in them and the amount of the excess. On comparing the percentage values of this excess of the trunk and branches over the sum of the roots for the different nerves, this probability presents itself still more strongly.

Table XI, here given, is derived from the numbers in Table I and is designed to show first, the amount of the excess of this sum of the trunk and branches over the sum of the roots and the percentage values of the excess based upon the sum of the roots; and second, the relation or coincidence between this excess and the absolute number of fibers in the dorsal branches.

TABLE XI.

FROG, WEIGHT 48.2 GRAMS.

	A	B	C	D
Number of the nerve	Excess of fibers in trunk and dorsal branches over sum of roots	Percentage of the excess	Percentage of dorsal branches	The number of fibers in dorsal branches
I	78	6.6	6.9	82
II				
III	170	24.2	31.7	225
IV	103	19.3	48.5	259
V	145	34.0	68.5	292
VI	220	38.3	61.4	370
VII	212	14.3	9.5	141
VIII	217	6.37	2.5	86
IX	257	13.6	14.1	268

TABLE XI, giving the percentages of the amount by which the sum of the trunk and dorsal branches taken just distal to the spinal ganglion, exceeds the sum of the ventral and dorsal roots taken just central to the ganglion. Column A contains the absolute number of the excess of fibers found in the trunk and dorsal branches, column B the percentage of this excess based upon the sum of the dorsal and ventral roots. In column C the percentage of the absolute number of fibers contained in dorsal branches is given based upon the sum of the fibers in the ventral and dorsal roots, and column D contains the absolute number of these fibers composing the dorsal branches.

From the table it is at once seen that those nerves which have the greatest amount of excess in the number of fibers distal to the ganglion, have also the greatest number of fibers in their dorsal branches. The VIIIth nerve has the lowest percentage of excess and has also the smallest dorsal branch, while both the greatest amount of excess and the greatest number of fibers in their dorsal branches are possessed by the VIth. This nerve shows an excess of 38.3% and its dorsal branches amount to 370 fibers.

Again, if column C of the table is brought in, it is seen that those nerves which have not only absolutely but proportionally the largest dorsal branches, have also the greatest percentage of an excess of fibers in the trunk and dorsal branches. The dorsal branches of the IVth, Vth and VIth are 48%, 68% and 61% respectively of the sum of the root fibers of the nerves to which they belong. These are the nerves which show the greatest excesses of fibers distal to their spinal ganglia. In the IXth the excess is very nearly the same as the dorsal branches; that is, 257 against 268 fibers or 13% against 14%.

The specimen used for the four sets of counts recorded in Table II, while being heavier, gives excesses in the trunk and dorsal branches absolutely less than those obtained in Table I, although the number of fibers contained in the four nerves involved is somewhat less. However, while the excesses are less, the dorsal branches are also both absolutely and proportionally less. The values obtained for the four nerves recorded in Table II, are given in Table XII.

TABLE XII.

FROG, WEIGHT 59.52 GRAMS.

Number of nerve	Excess of fibers in trunk and dorsal branches over sum of roots	Percentage of the excess	Number of fibers in dorsal branches	Percentage of dorsal branches
VI	138	36.7	286	76.2
VII	199	18.7	155	14.5
VIII	86	3.1	49	2.1
IX	109	4.4	239	9.8

On comparing Table XII with Table XI it is seen that the two specimens, differing in weight and in the number of fibers, agree in the general relation existing between the excess of fibers in the trunk and dorsal branches and the amount of the dorsal branches.

This approach to a conformity or correlation between the excess of fibers distal to the spinal ganglion and the number of fibers which do not enter into the formation of the trunk, may be a mere coincidence and entirely without significance. Indeed, in the VIIIth nerve (Table I), for example, as many as 131 fibers of the excess, are necessarily contained in the trunk; and, in case of the VIth, on the other hand, the trunk lacks 126 fibers of containing as many as there are in the two roots.

IX. DISCUSSION OF THE OBSERVATIONS AND COMPARISONS WITH THE RESULTS OF OTHER OBSERVERS.

1. The excess.

That there does occur a greater number of fibers distal to the spinal ganglion than is contained in the two roots central to it, is very evident. In several instances an excess has been obtained by previous observes.

Holl ('75) counted the fibers in the two roots and in the trunk of three of the lumbar nerves of the frog. In every case his counts showed a slight excess in favor of the trunk. This excess, however, never exceeded 2% and the author concluded that the number of fibers on the two sides of the spinal ganglion was the same. He explained the excess as due to errors in counting.

Freud ('78), in investigating the spinal ganglia of Petromyzon, reached the general conclusion that the number of dorsal root fibers was equivalent to the number of spinal ganglion cells. However, he barely mentions on page 79 that, in Petromyzon, he found an increase of fibers in the trunk. This increase he regards as occurring within the ganglion and considers it of little importance, thinking it due to a splitting of the processes of the spinal ganglion cells. Figures illustrating this splitting are give in his plates. Freud's counts were made from teased preparations.

Stienon ('80) in studying the relation of the dorsal root fibers to the cells of the spinal ganglion, made two counts of the fibers in the two roots and in the trunk. In one of these counts, a cervical nerve of the dog was used and in the other a lumbar nerve of the frog. In both he obtained a slight excess in the trunk (1.2% for the frog) and, like Holl, explained the excess as due to errors in technique.

Birge ('82) who, as before mentioned, made counts of the several spinal nerves of the frog, obtained for two of the nerves quite an appreciable excess of fibers in the trunk. In one (IInd) there were found nearly 16% more fibers in the trunk than in the two roots, and in the other (IXth) an excess of nearly 14%. Birge is the first to attach a significance to the excess found. While admitting that the differences obtained might lie within the bounds of error in technique, yet he did not think this probable and suggests that the matter be further investigated.

Gaule and Lewin ('96) in a preliminary paper, give some enumerations upon the nerves of the rabbit. At the time of publication counts had been made of the fibers contained in the two roots and in the trunk and dorsal branches of three of the sacral nerves. These counts revealed respectively, 19%, 11% and 15% more fibers in the trunk and branches combined, than there were in the sums of the two roots. They are the first to mention having included the dorsal branches in the enumeration.

Bühle ('98), in a study of the spinal ganglia of the frog, counted the fibers to be found on the central and distal sides of the spinal ganglion. The original of this paper of Bühle has unfortunately not yet been obtainable. According to a review by Lenhossék ('97), Bühle obtained an excess of fibers on the distal side of the ganglion which in one case amounted to as much as 25.5%. Even this, however, is less than the excess here found for the Vth and VIth nerves of the American frog (*R. virescens*).

To explain the excess of fibers on the distal side of the spinal ganglion, Gaule ('96) suggested the only two possibilities: (1) Fibers coming from the periphery may end in the ganglion and thus not pass over into the dorsal root; or, (2) fibers may arise in the ganglion and pass toward the periphery without there being a corresponding central process.

Since the excess can be looked upon as well established, the two possibilities suggested by Gaule may be presented in some detail.

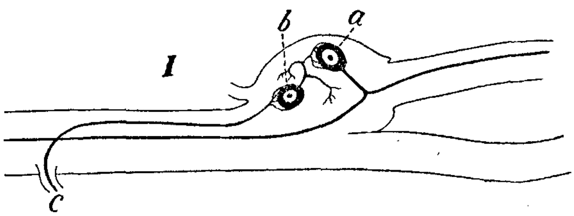
1. Medullated fibers arising from the cells of the sympathetic ganglia pass, by way of the ramus communicans and the nerve trunk, to the spinal ganglion and end there. For the existence of such fibers the evidence is as follows:

Bidder and Volkmann ('42), noting the great number of small medullated fibers present in the peripheral nerves of the frog and especially in the sympathetic system, arrived at the conclusion that these small medullated fibers originated in the cells of the sympathetic ganglia, and they suggested the small diameter as the characteristic by which the fibers of the sympathetic system could be distinguished from those of the spinal system.

Kölliker ('45), opposing this idea of Bidder and Volkmann, undertook a series of investigations which resulted in his being forced to agree with them to the extent that, in addition to the many non-medullated nerve fibers having their origin in the sympathetic system there do arise from the cells of the sympathetic ganglia quite a number of medullated fibers also. Kölliker found this to be true, not only for the frog but also for other vertebrates. In a later paper, Kölliker ('94) calls especial attention to these medullated sympathetic fibers. He describes them as "dunkle randige Fasern"—fibers possessing a thinner myelin sheath than that of the cerebro-spinal fibers, which sheath stains less black with osmic acid. Kölliker also states that, while the great majority of sympathetic fibers are "motor" in function, it is possible that there are also among them "sensory" fibers which may play a rôle in reflexes occurring in the domain of the sympathetic system. Since Kölliker ('94), medullated fibers of sympathetic origin have been often observed by Dogiel ('95 and '96) and others. There is no doubt that for both mammals and the frog, some sympathetic fibers are medullated.

Some fibers from the sympathetic ganglia pass by way of the nerve trunk to the spinal ganglia. By the observations of Cajal ('93), Retzius ('94), Huber ('96), Dogiel ('96 and '97), Cajal and Olóriz ('98) and others, it has been established that for the frog and certain mammals at least, fibers having their origin in the cells of the sympathetic ganglia, pass by way of the ramus communicans and nerve trunk to the spinal ganglia and end in pericellular plexuses about the cells there. Dogiel's observations on medullated sympathetic fibers have been made especially upon certain of these fibers going to and found in the spinal ganglion.

If sympathetic fibers pass to the spinal ganglia and end there and if some of these fibers are medullated, then an enumeration of the medullated fibers on the distal side of the spinal ganglion would include these medullated fibers which do not pass through it, and an excess in the trunk would be the natural result. This explanation of the excess is illustrated in scheme I.

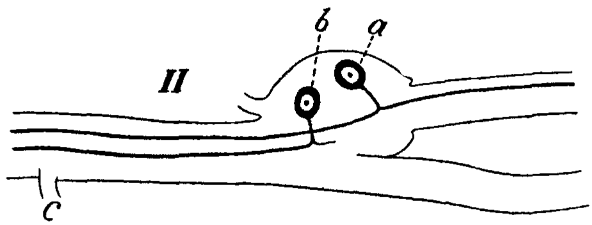


According to this scheme the medullated sympathetic fiber enters the ramus (*c*) and passes to the ganglion where it terminates in an end-brush about the body (*b*) of a Dogiel spinal ganglion cell of type II. Thus it is seen that a section taken immediately distal to the ganglion would contain two fibers, whereas one taken central to the ganglion would include only the central process of the ordinary spinal ganglion cell, or Dogiel's cell of type I, (*a*).

How numerous these medullated sympathetic fibers are, which are present only on the distal side of the ganglion would be difficult to determine. None of the observations so far made have been attempts to determine their number. But none of the descriptions of these fibers, nor Dogiel's ('96) description of what he considers sensory cells in the sympathetic ganglia, seem to justify the assumption that they are numerous enough to account for the excesses which have been found. It seems necessary therefore, to assume that, in addition to the fibers arising in the sympathetic ganglia, there are also other fibers present in the nerve trunk which do not occur in the roots. The other possibility is the second one suggested by Gaule; namely, that fibers may arise in the spinal ganglion and pass to the periphery without there being corresponding central processes in the dorsal root.

Three possible ways suggest themselves by which an excess in the trunk may occur according to this second suggestion by Gaule. These possibilities can be illustrated by three schemes.

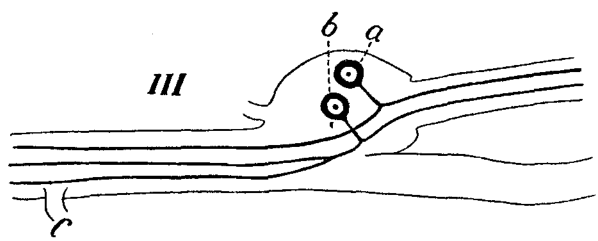
1. Scheme II is intended to illustrate a probability that on approaching maturity the peripheral process of a spinal ganglion cell (*b*) becomes medullated first. The mature cell (*a*) has sent a process in both directions and consequently in



that case a fiber would be counted on both sides of the ganglion. Direct histological evidence in favor of Scheme II is lacking. Some observations however may be considered as having an indirect bearing upon this point:

In addition to the work of Wagner ('48) on the spinal ganglia of fishes, the more recent observations of van Gehuchten ('91), Kölliker ('93), Cajal ('93 and '94), Lenhossék ('95) and Dogiel ('97), made chiefly upon foetal material, indicate that the central process of the T-fiber is often much thinner than the peripheral one. And the observations of Ambronn and Held ('96) seem to indicate that as a whole the peripheral system is medullated before the central and thus perhaps the peripheral process of the spinal ganglion cell acquires its medullary sheath before the central one which goes to form the dorsal root.

2. Scheme III illustrates a possibility of one source of the excess of fibers distal to the spinal ganglion. This sugges-



tion was offered by Bühle ('98). It is based upon the fact that in certain cases a splitting of the processes has been observed within the spinal ganglion.

A splitting of the peripheral process of the cell (*b*) would give two fibers on the distal side of the ganglion corresponding to one fiber on the central side. This splitting or division of the fiber has been observed by Stannius ('49) for fishes, Freud ('78) for Petromyzon, and by Dogiel ('97) for mammals. Dogiel's observations however lead him to think that the splitting more often occurs in the central rather than in the peripheral outgrowth of the spinal ganglion cell. Freud, on the other hand, positively states that for Petromyzon the splitting occurs in the peripheral process.

That a splitting or division of fibers may be one cause of the excess found on the distal side of the ganglion, is an explanation which may have a better foundation than at first appears. Dr. Elizabeth Dunn has just made, in this Laboratory, some enumerations of the fibers contained in the trunk of the sciatic nerve of the frog and also of the fibers contained in the several branches into which this nerve divides for the innervation of the leg. She kindly permits reference to be made to the fact that she finds an appreciable excess of fibers in the branches. In a frog (*Rana virescens*) of about 50 grams weight there were found in the trunk of the sciatic of one side, before any branches are given off, 4293 fibers, while the sum of the fibers found in the several muscular and cutaneous divisions to the thigh and in the *tibialis* and *peroneus* branches to the lower leg, amounted to 4511 fibers—an excess of 218. This excess of fibers in the branches was found to be very similar for the two sides of the same frog.

The only explanation which suggested itself for this greater number of fibers in the branches is that it was due to a splitting of the fibers of the trunk of the sciatic. This probable and evidently necessary splitting of the fibers called for by the results of Dr. Dunn suggested an examination of the sciatic nerve especially at the points at which the branches are given off, for it was thought that in these regions the splitting would most probably occur. This investigation is as yet incomplete. However at this stage abundant cases of divided fibers have been observed in the branches of the sciatic of the second and third order. They have been demonstrated both after treatment with osmic acid followed by maceration in a mixture of glycerine and hydrochloric acid and by means of methylene blue. In preparations pressed out under the cover glass rather than teased, numerous cases have been observed in which the fiber splits at the angle formed by the division of the nerve branch, one branch of the divided fiber passing into each branch of the divided nerve.

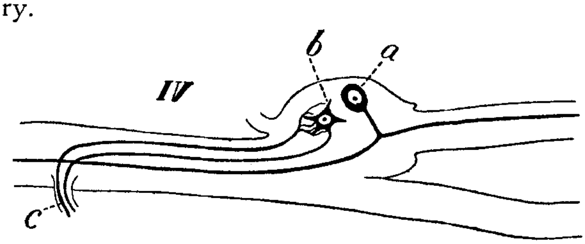
The splitting of the fiber occurs, as would be expected, at a node, and there is no diminution in the diameter of the fibers which result from the splitting. Each of the fibers—sometimes three in number—which result from the division possesses a diameter about the same as that of the fiber which divided to form them.

It has already been noted in this paper (Table XI) that there is a marked correlation between the actual number of fibers in the dorsal branches of the spinal nerves and the amount of the excess of fibers in the sum of the trunk and these dorsal branches above the sum of the fibers contained in the two roots. In those nerves in which the excess is greater the number of fibers in the dorsal branches is both absolutely and proportionately greater. The dorsal branches going to the dorsal muscles and to the skin of the back may be looked upon as branches of the spinal nerve just as well as the branches to the muscles and skin of the leg are branches of the sciatic nerve, and if some of the fibers in the branches of the sciatic are the result of splitting then some of the fibers in the dorsal branches of the spinal nerves may be the result of splitting and hence a splitting of fibers in and on the distal side of the spinal ganglion may be one cause for the excess of fibers present on the distal side of the spinal ganglion.

In order to determine the truth of this assumption an examination of the fibers in the region of the spinal ganglion is now in progress. At the present stage of this investigation a statement may be here inserted to the effect that divided fibers have been frequently noted in the dorsal branches well outside the limits of the ganglion but so far, such cases have been much less frequently observed within the boundaries of the ganglion. The fibers which pass to the dorsal branches from the ventral root cross the greater mass of fibers which go to form the nerve trunk and this interweaving results in a tangle which renders a sufficiently disassociated preparation quite difficult to obtain. A few cases however have been found in which a ventral root fiber was seen to split into two. That one of these passed to the dorsal branches could not be clearly determined owing to injury in the disassociation; that they were ventral root fibers, however, there is no question. This splitting of ventral root fibers is not included in the possibilities suggested by Gaule.

As to the splitting of fibers arising from the cells of the spinal ganglion, while many divided fibers have been observed, none have yet been seen concerning which it can satisfactorily be asserted that both the products of the division pass to the periphery. Unless each can be followed separately either into the nerve trunk or into the dorsal branches there is nothing to show that the structure is not the characteristic T-fiber, or one of the numerous divisions which Dogiel claims for the processes of his ganglion cell of the IInd type. However it must be remembered that the investigation is only just begun. But it should also be remembered that an occasional division of the peripheral process of the spinal ganglion cell has already been noted by previous observers.

3. The possibility to be suggested by Scheme IV is that among the spinal ganglion cells which never send processes to the dorsal root, there are some which send processes to the periphery.



There are no histological observations which directly support this scheme. The investigations of Hodge ('89) and

Bühle ('98) show that in the spinal ganglia of the frog there are from two to five times as many cells in a spinal ganglion as there are fibers in the dorsal root. Of this excess of cells many are no doubt latent ganglion cells, others are of the Dogiel spinal ganglion cell of Type II, but still others may be multipolar cells. Multipolar cells have been observed in spinal ganglia by Kölliker ('93), Disse ('93), Lenhossék ('94), Spirlas ('96), Dogiel ('97) and Cajal and Olóriz ('98), and the possibility presented by Scheme IV is that some of these multipolar cells are of the sympathetic type which have developed within the spinal ganglion. Such a cell (*b*) is assumed to be under the control of one of the medullated sympathetic fibers (*c*) which have been observed to pass from the sympathetic system to end in the spinal ganglia—an arrangement which would give an excess of at least two fibers in the nerve trunk.

Another barely possible arrangement might be noted: Although histological evidence for the frog is lacking, certain experimental evidence brought forth chiefly by Steinach ('95) and Horton-Smith ('97) seems to indicate that there are in the dorsal root certain fibers of intra-spinal origin which when stimulated produce disturbances in the domain of the sympathetic system. This evidence might be interpreted to mean that some of these fibers instead of passing through the spinal ganglion, terminate in the spinal ganglion and control some of the multipolar cells there of presupposed sympathetic type. Such a fiber might break up into a number of branches and thus control a number of these cells each of which would send a process to the periphery and thus an excess of fibers in the nerve trunk would be produced.

Against the probability that the multipolar spinal ganglion cells play the rôle above assigned them, it must be stated that Dogiel ('97) who has made a careful study of these cells in mammals, in the maze of fibers present, was unable to follow any of their processes outside the bounds of the ganglion. He suggests that they are either cells of a sympathetic type or are modifications of his spinal ganglion cells of type II.

2. THE VARIATIONS IN THE NUMBER OF FIBERS FOUND AT DIFFERENT LEVELS IN THE ROOTS AND TRUNK.

As before mentioned, these differences are explained on the supposition that fibers growing into the roots and into the trunk have proceeded unequal distances from their cells of origin.

Ever since the investigations of His ('86 and '89) it has been admitted that the medullated fibers forming the spinal nerves are the outgrowths, on the one hand, of the cells of the dorsal root ganglia, and on the other, of those situated in the gray matter of the spinal cord.

Kaiser ('91) has shown that the number of mature nerve cells in the cord increases with the growth of the animal, and the work of Birge ('82) is sufficient to show that the fibers of a nerve increase in number during the growth of the frog. Birge found for the frog that the increase in the number of fibers in the spinal nerves was quite proportional to the increase in the weight of the animal.

Donaldson ('97) determines the fact that the entire neurone, the nerve fiber as well as the cell body, increases in size during the growth of the animal.

Korybutt-Daskiewicz ('78) observed growing fibers along the sciatic nerve of the frog. This investigator also described the myelin sheath in its formation about the growing axis cylinder as tapering bluntly and ceasing a short distance behind the growing tip.

Kolster ('93) in a study of the regeneration of fibers following the section of the nerve, pictures the sheath as being acquired a node at a time and consequently as ending bluntly —the axis cylinder preceding the formation of the sheath. Both observers describe the myelin as first appearing either as a thin layer blackening with osmic acid or as fine irregularly accumulated droplets. Stroebe ('93) observed the sheath as making its appearance in much the same way.

These descriptions accord fairly well with the results of searches made here for growing nerve fibers, one of which is represented in Plate VII, figs. 1 to 6.

It is concluded therefore, that the variations found to occur in the number of fibers at different levels of the dorsal and ventral roots and in the nerve trunk as recorded in Tables I and II are but the natural result of the growth of the nerve fibers.

X. METHODS AND TECHNIQUE.

If a detailed account were given of the difficulties experienced before obtaining satisfactory preparations, this division of the paper would be much more lengthy than it is intended to make it.

The first and prime object was not only to obtain preparations in which medullated nerve fibers were so sharply differentiated that each one stained could be readily distinguished as such, but also to obtain preparations in which it could be satisfactorily asserted that all the medullated fibers were distinguishable.

Little can be said concerning the effect that the general nutritive condition of the animal, the season of the year etc., may have upon the staining properties of its medullated fibers. It is reasonable to suppose that pathological or even certain natural conditions may influence to some extent at least, the stain reactions of nerve fibers as well as those of other tissues. Gaule ('89) recognized season as having an influence and recommended *Rana temporaria* in May and *Rana esculenta* in June for the clearness with which their nerve fibers might be stained by the Weigert method. Little more can be said here however, than that the best results were obtained with freshly caught specimens, and that while the experiments for a satisfactory staining method extended through several seasons, both the specimens from which the results recorded in this paper were obtained were killed in October.

For bringing out medullated nerve fibers, treatment with osmic acid was of course, the first that suggested itself. Osmic acid however was found to possess several defects. These defects were principally due to the well known slowness with which osmic acid penetrates and to the distortion of the nerve fibers which it often produces either by a swelling effect upon the myelin sheaths or by a shrinkage of the perineuric investment of connective tissue. The myelin sheath seems to undergo some form of disintegration shortly after death and unless the osmic acid penetrates the sheath before this occurs, the reduction of the osmium is incomplete and indefinite structures are the result. To avoid this the nerve must be exposed to the action of the reagent as quickly as possible. For the entire ten spinal nerves, it was found difficult to accomplish this, especially in case of the intervertebral portions of the nerves, without some pulling or crushing or the loss of some of the smaller dorsal branches. It is well known that the action of osmic acid upon the medullary sheaths of fibers which have been injured results in very abnormal appearances.

Little difficulty was met in obtaining with osmic acid well stained sections from those portions of the nerve roots lying free in the spinal canal, or in obtaining fairly satisfactory sections from that part of the nerve trunk lying exposed in the body cavity. The great difficulty was experienced in getting sections well blackened and intact of those portions of the nerve immediately central and distal to the spinal ganglion. Even if removed from the intervertebral foramen without injury or loss of some of the branches, there still remained the periganglionic capsule of connective tissue and the "periganglionic gland." If this was left on, it interfered with the penetration of the osmic acid and the contraction of the capsule produced by the reagent resulted in an injurious condensation of the nerve fibers which it enclosed. If removed, its removal in the fresh state was well nigh impossible without injury to the nerve. The use of osmic acid being thus beset with difficulties, other methods of treatment were resorted to. However, after trying both the Pal-Weigert method and a modification of the iron haematoxylin method, neither of which gave preparations so suitable for photographing nor, after the required decolorization, preparations in which the small fibers appeared satisfactorily distinct, it became necessary to return to osmic acid as offering the greatest possibilities of obtaining satisfactory results.

In order to obviate the difficulties attending its use, several osmic acid mixtures were tried. These again had to be abandoned as inefficient. Where one was an advantage in one respect it was a drawback in another. Various mixtures of osmic acid and formalin were tried, but, while giving cylindrical and well blackened fibers, had to be abandoned because formalin was found not only to accelerate the reduction of the osmium but, what was not desired, also to produce a brittleness which made it more difficult to obtain sections thin enough for photographing and reliable counting.

The most promising combinations with osmic acid were found in the application of a reducing agent after the exposure to the action of the acid. After trying several of the reducing reagents used in the ordinary photographic developers, the best results were obtained with a 0.5 % water solution of pyrogallic acid. If the nerve be washed for two and a half or three hours in distilled water after removal from the osmic acid and then placed for an hour or so in this solution of pyrogallic acid, a decided increase is noticeable in the intensity with which the medullary sheath is blackened, without any indication of a reduction of the osmium in the other structures of the nerve. Some of the nerves used in making the counts of fibers recorded in this paper had been treated in this way.

The method of procedure which proved the most satisfactory is the following.

DISSECTION.

The animal was killed and weighed and, if female, the weight of the ovaries deducted. Then the viscera, the skin, the limbs and a portion of the abdominal and thoracic muscles were removed. This left only the head and the more dorsal portion of the body. From the ventral side, the spinal cord was now laid bare by carefully severing the pedicles of each vertebra and thus removing the centra of the entire vertebral column. This also lays open the intervertebral foramena. Then with small scissors or fine bone forceps incisions were made in the dorsal muscles just under the position of each spinal ganglion in order to allow the reagent to reach the dorsal branches more freely.

The head was next severed well above the origin of the first spinal nerve and, with a fine pipette, 1 % osmic acid was forced under the cord washing out the cerebro-spinal fluid and replacing it with osmic acid. The cord was left in position at this stage lest its removal should disturb the normal tension of the nerve roots or otherwise injure them. The whole was then placed for 10 or 15 minutes in a vial containing a one-fifth one per cent. solution of osmic acid and this was frequently agitated. The idea in using the weaker solution was to insure a slower fixation of the connective tissue investments. It was thought that a slower fixation would result in a less violent contraction and consequent condensation of the nerve bundles and thus also allow a more rapid penetration of the stronger solution which follows.

At the end of the 15 minutes the specimen together with the weaker osmic solution was transferred to a dish under a dissecting microscope. With the eyes and nose of the observer protected from the fumes of the acid, each nerve root was gently severed from its connection with the spinal cord and the cord removed. Next each nerve trunk was severed a few millimeters distal to its connection with the ramus communicans and the ramus itself cut. Then with the aid of a fine tentaculum and small spring scissors (under an enlargement of 16 diameters) each nerve was detached and removed from the intervertebral foramen, especial care being taken not to injure nor lose any of the dorsal branches. The periganglionic capsule was now opened and partially teased away and the nerve transferred to a 1 % solution of osmic acid.

At the end of from 12 to 24 hours the nerves were removed from the osmic acid solution to distilled water. Again under a dissecting lens, the teasing away of the periganglionic capsule and other loosely attached connective tissue was carefully completed and camera outlines were made of the dissected nerve and roots under a known magnification. The length of the roots and trunk was then determined in millimeters and indicated on the drawings.

The washing in water was continued from 4 to 12 hours, or long enough to remove all the surplus osmic acid. The material was then transferred to 70 % alcohol, dehydrated in alcohol of about 97 %, cleared in a mixture of 3 parts pure xylol and one part carbolic acid and embedded in very hard paraffin (melting point about 56°). Absolute alcohol was usually avoided, since in the previous experiments there were occasional indications of the myelin having been slightly dissolved. Clove and cedar oils were also avoided for the same reason.

Sections 3 micra in thickness were taken at each of the prescribed levels of the roots and nerve trunk. Those from each level were mounted on separate slides as soon as cut. The sections were fastened to the slide by means of the "albumen water method." For photographic purposes it is absolutely necessary that the sections be flat on the slide. The flattening was accomplished by placing the slide with the sections floating upon it, upon a water bath the temperature of which was just below the melting point of the paraffin. Here, the sections having flattened out, the slide remained with the paraffin in a plastic state till the water had practically dried out from under the sections. Then the slide was placed aside for several hours longer to allow the drying to become complete.

After mounting in balsam, the sections on each slide were examined as to their fitness for photographing and counting and those chosen were marked by a small square on the cover glass.

THE PHOTOGRAPHY.

As can be readily seen, the object striven for in the photography was to get the greatest amount of contrast and at the same time precision as to outline. Of the three brands of photographic plates tried, that made by the Cramer Dry Plate Works of St. Louis, Mo., and sold under the name of "Cramer's Contrast Plate," was by far the best for the purpose.

Also, after trying several formulae, the developer which gave the best satisfaction was made according to the "Bromo-hydro-chinon" formula which accompanies these plates.

The apparatus used was the large photographic and projection outfit manufactured by Zeiss. This was mounted on a large stone pillar which insured the perfect steadiness of the apparatus.

The sections were photographed under a magnification of from 500 to 800 diameters and the exposure made according to the intensity of the light. A small diaphragm and long exposure gave the best results. The source of light was an ordinary Welsbach burner.

THE PRINTING.

Each negative of a section was labeled with the weight of the frog, the number of the nerve and with the region and level from which the section was taken. This label was cut through the film on one corner of the negative so that in the printing the label would print also.

That grade of velox paper sold under the name "Carbon Velox" was found best suited for reproduction, since pure blacks and whites can be obtained with it. Plates IX, XI, and XIII are reproductions of prints made with this paper. Blue prints were used almost exclusively in the counting procedure. Blue print paper is easier to manipulate and if a good quality be used, prints can be obtained possessing a high degree of sharpness and detail.

THE METHOD OF COUNTING.

The method employed was devised in this laboratory and has not been previously described. In order to count a given section, a photograph of it was fastened upon a small board of soft wood, the section itself placed under the microscope and the two oriented. With a sharp pointed pencil, the photograph was marked off into fields of small area the boundaries of which were determined by the grouping of the fibers.

The section, kept under the microscope for reference, was subjected to an enlargement of 640 diameters or an enlargement usually above that under which the photograph had been made.

After a short study of the section under the microscope, it was found suprisingly easy to identify any fiber in a field of the photograph with its original in the section. Thus, also, the boundaries of the fields marked off on the photograph could be readily determined in the microscope.

The counting itself was largely mechanical. An automatic registering machine, one common use of which is to count telegraph poles, was modified by attaching to its finger press a short steel rod. Into the end of this rod was inserted a needle so arranged with a set screw that the point could be protruded any distance required. Plate VII, Fig. 7, represents a photograph of the machine as it was here employed. In want of a better name it will be referred to as the "counter." When the rod is pushed up, the counter with an audible "click" registers one; the pressure removed, the rod springs back to its first position. The machine registers up to 1000 and repeats. By piercing with the needle the center of a fiber in the photograph, the rod is pushed up and the counter records. Thus when each fiber represented in a photograph has been punched, the counter has recorded on its register the total number of fibers in the section. When a section contained more than 1000 fibers, each thousand when indicated by the register, was noted and the counting continued. Since the counter began to repeat immediately upon reaching 1000, there was absolutely no danger of loss or mistake.

Each field as marked off on the photograph was counted separately. Not till all the fibers in one field had been counted, was the counting of those in an adjacent field begun. The aid of the microscope was most necessary in counting the small fibers; large blood vessels were distinguishable on the photograph as well as in the microscope. Every case which occasioned the least doubt was quickly and finally settled by an appeal to the microscope. Sometimes, among the smaller fibers, there appeared in the photograph what seemed to be two fibers lying in such close contact as to resemble the figure 8. These cases were always referred to the microscope which often showed them to be one fiber collapsed in such a way as to give its section this form.

In using the counter, the eye of the observer naturally follows the point of the needle. Since a puncture is made in the center of each fiber at the time it is counted, this puncture is readily seen and protects the fiber from being counted a second time.

After a photograph had been counted in this way, each field was examined a second time. This time the microscope was used even more frequently than the first and especial search was made for small fibers which might have been overlooked. This search seldom added more than 2% to the first count. Finally each field of the photograph was examined with a hand lens, search being made for unpunched large fibers. The hand lens was an advantage both in reducing the field of view and in so magnifying the fibers that one with an unpunctured center could be quickly discerned. Strange to say, if any of the larger fibers had been omitted in the previous counts, they were usually found to be the very large ones.

When a count had been completed the number of fibers found in the photograph was written below the label which had been printed on it and the photograph was filed. In order to more thoroughly guard against any personal equation, no two sections from consecutive levels were counted in succession. Sometimes the counting of two nerves was carried on simultaneously and thus in the large number of sections involved, the number of fibers contained in a section from a given level had passed out of mind before determining the number contained at an adjacent level.

In order to test the accuracy of the enumeration, in quite a number of cases a second photograph was counted. In almost every case the second number obtained was identical with that of the first count. If there did result a difference in the two counts it seldom amounted to 1% and the fibers causing the difference were always found.

The advantages claimed for this method of counting are notably three:

1. The method reduces the mental stain to a minimum. The ordinary "net" method used by earlier observers, and even as improved by Gaule ('96), involves many difficulties which are entirely avoided by the used of a photograph.

2. The method is largely mechanical. The work of carrying numbers in the mind and the final addition of the numbers so carried, is transferred to the machine.

3. The work of counting a section may be interrupted at any time without the least danger of loss in the accuracy of the number finally obtained. One who has ever attempted to count a section containing several thousand fibers will immediately realize this advantage.

By reason of the accuracy attainable with this method it is justifiable to attach great weight to the small numerical differences found to occur in the course of the nerve roots and trunk.

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XII. EXPLANATION OF PLATES.

PLATE VI.

The ten spinal nerves of *Rana virescens*. Left side. Drawn to scale and enlarged six diameters.

The Roman numbers indicate the number of the nerve.

The Arabic numbers indicate the levels at which the sections were taken for the enumeration of fibers recorded in Tables I and II. The Arabic number 6 indicates the region at which the sections of the dorsal branches were taken. In case of the VIIIth and IXth nerves the branches are here so arranged that the transverse section of the trunk may also involve a transverse section of the branches.

C.—Ramus communicans.

D. r.—Dorsal root.

V. r.—Ventral root.

PLATE VII.

Figs. 1-6. A series of transverse sections which show a growing fiber. Taken from ventral root of VIth nerve of frog used in Table I. The series, beginning at Fig. 1, passes toward the spinal cord and includes a length of about 30 micra.

The smallest fiber of the group, the growing fiber, makes its first appearance as a brown ring and soon acquires its medullary sheath.

Camera drawings. Magnification, 466 diameters.

Fig. 7. Automatic registering apparatus used in counting technique. When rod containing the needle is pressed inward the machine records.

½ natural size.

PLATES VIII TO XIII.

General explanation :

Plates IX, XI and XIII represent sections from the ventral root of the VIIIth nerve of a frog of 48.2 grams. The plates are made from the identical photographs employed for the enumeration of the fibers of this root as recorded in Table I and correspond respectively to columns A, B and C of that table or to levels 1, 2 and 3, Plate VI.

Plates IX and XI contain two sections each as the root is divided in the same manner as the ventral root of IXth nerve, Plate VI.

All the figures are reduced to about ½ the size of the photographs from which they were made.

Plates VIII, X and XII are companions to Plates IX, XI and XIII respectively. They show the fields as marked out on the photographs during the counting and also the number of fibers determined for each field. The boundaries of each figure coincide with those of the companion photograph. The total number of fibers determined for each section is placed at the lower right hand of each figure.

The black dots in the center of each fiber represent the holes made by the needle of the "counter," Plate VII, Fig. 7. This dot is sometimes obscured in the reproduction, especially in case of some of the smallest fibers where the small white axis cylinder space is obliterated by the puncture which is reproduced in black like the medullary sheath.

Blood vessels are also reproduced in black.

A hand lens will facilitate the examination of the prints.

PLATE VIII.

Fig. 1. Companion figure to Fig. 1, Plate IX.

Fig. 2. Companion to Fig. 2, Plate IX.

PLATE IX.

Figs. 1 and 2. Transverse sections of ventral root, VIIIth nerve, from region corresponding to level 1, Plate VI.

Magnification: { Fig. 1. 300 diameters.
Fig. 2. 310 diameters.

PLATE X.

Fig. 1. Companion to Fig. 1, Plate XI.

Fig. 2. Companion to Fig. 2, Plate XI.

PLATE XI.

Figs. 1 and 2. Transverse sections of same ventral root but from region corresponding to Level 2, Plate VI.

Magnification: { Fig. 1. 284 diameters.
Fig. 2. 275 diameters.

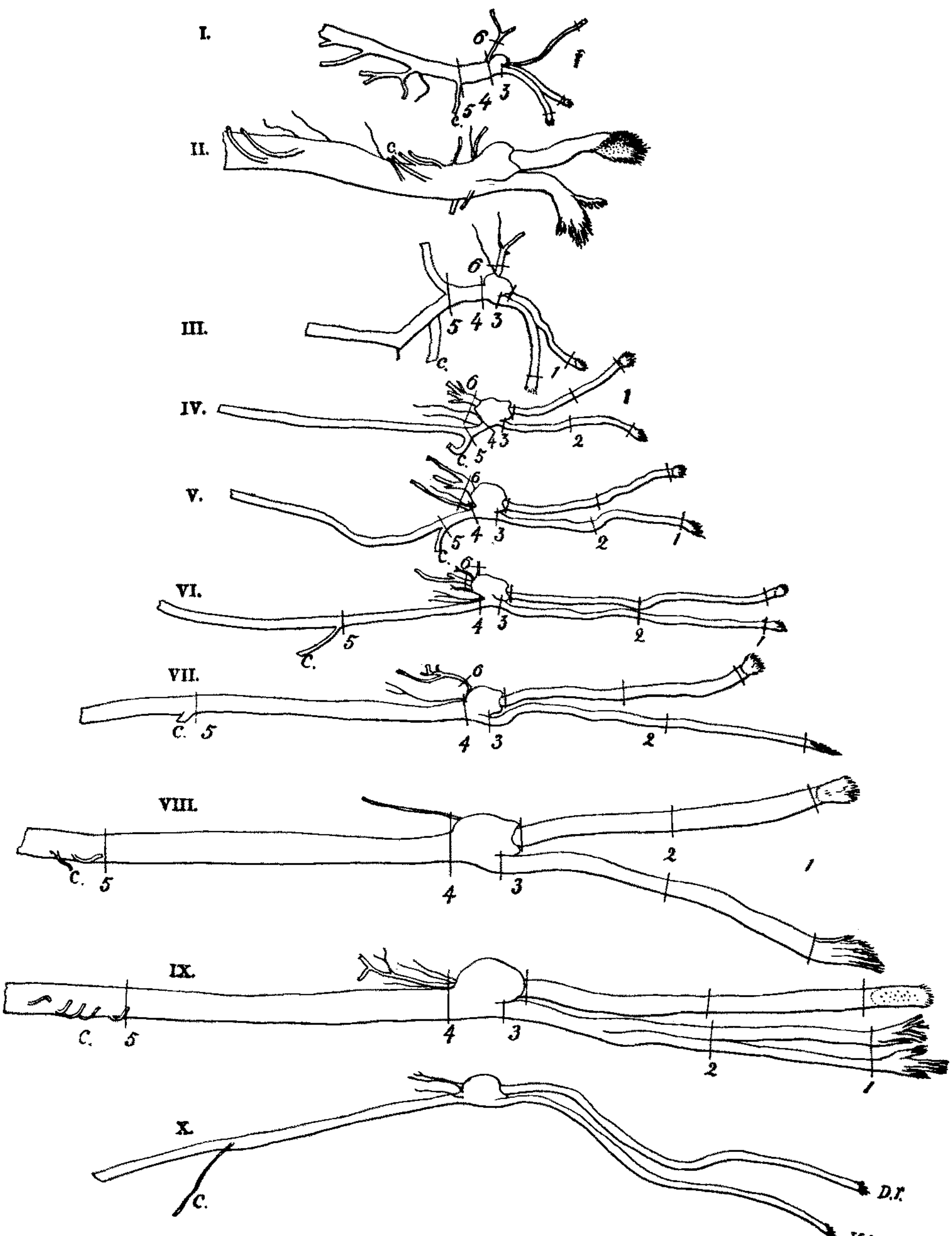
PLATE XII.

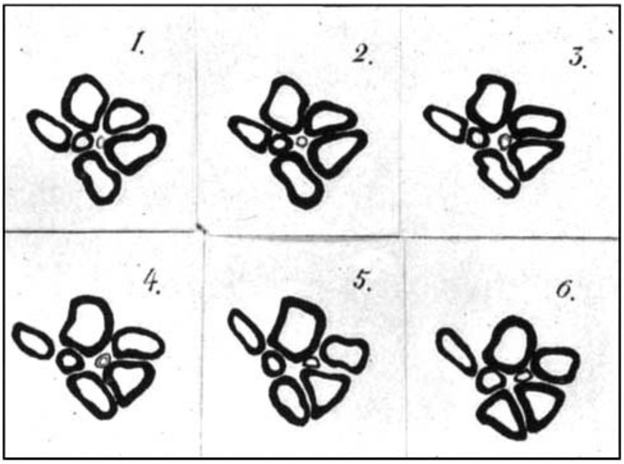
Companion to Plate XIII.

PLATE XIII.

Transverse section from same ventral root, taken from region corresponding to Level 3, Plate VI. The two divisions represented in Plates IX and XI have here fused.

Magnification, 307 diameters.


I.
II.
III.
IV.
V.
VI.
VII.
VIII.
IX.
X.
C.
D.r.
V.r.



7.

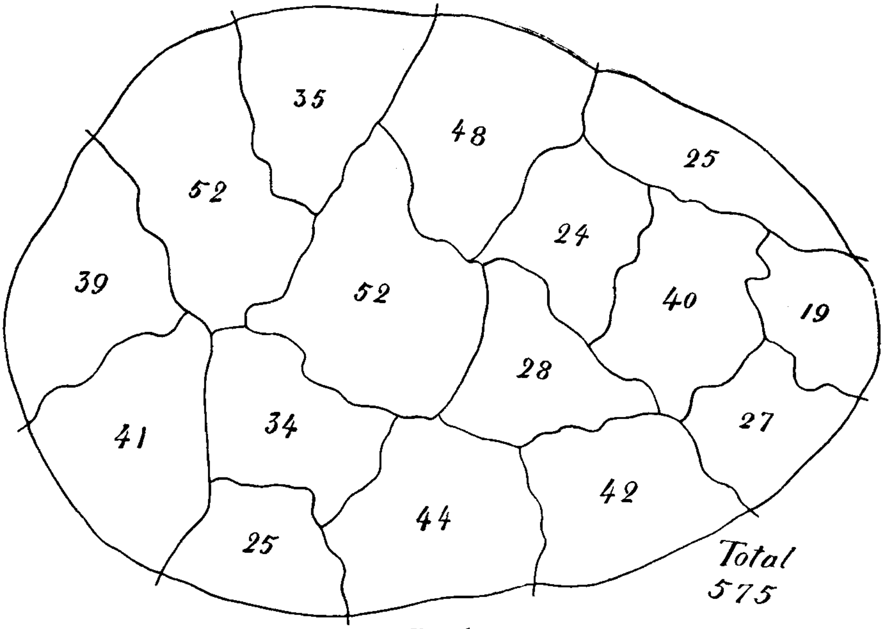
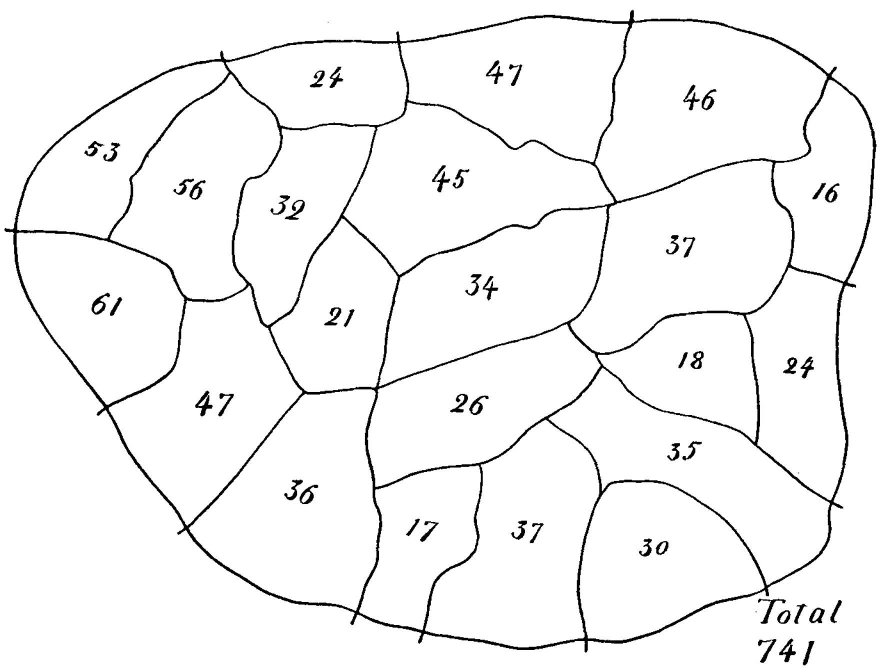

35
48
25
52
24
39
52
40
19
28
34
41
27
42
44
25
Total
575

FIG. 1.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47
46
53
56
32
45
16
37
61
34
21
18
24
47
26
35
36
17
37
30
Total
741

FIG. 2.

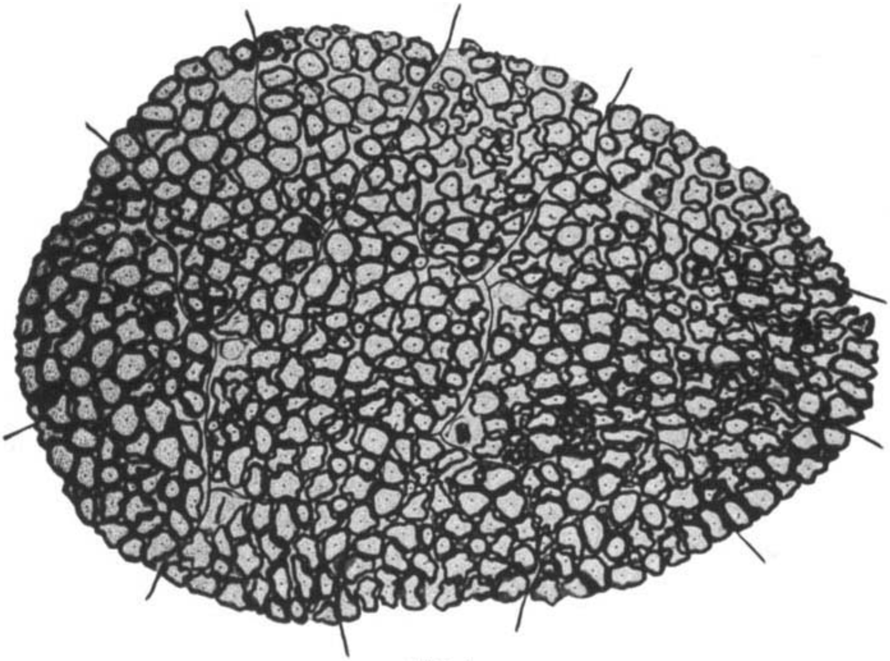


FIG. 1.

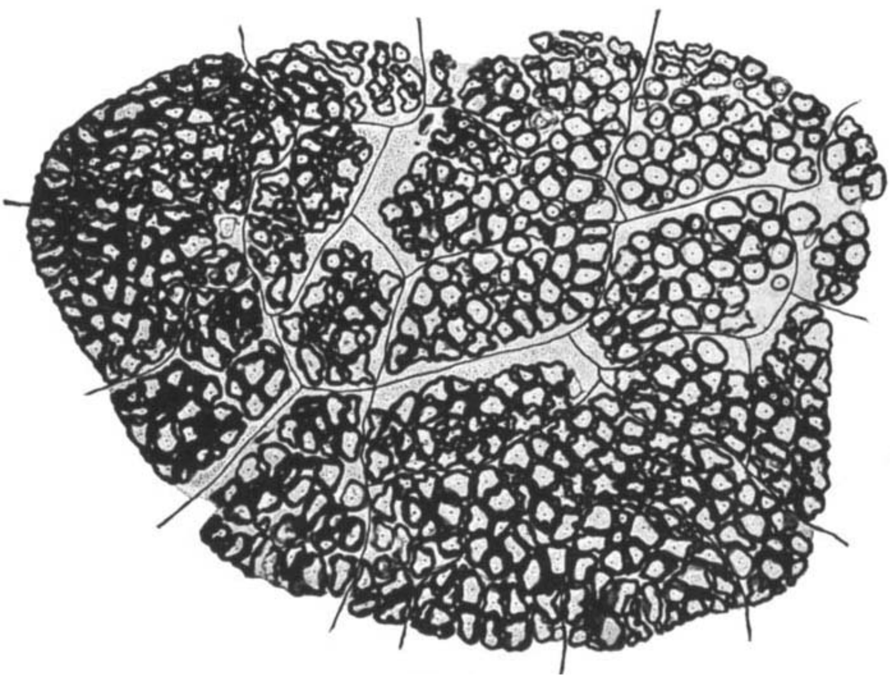
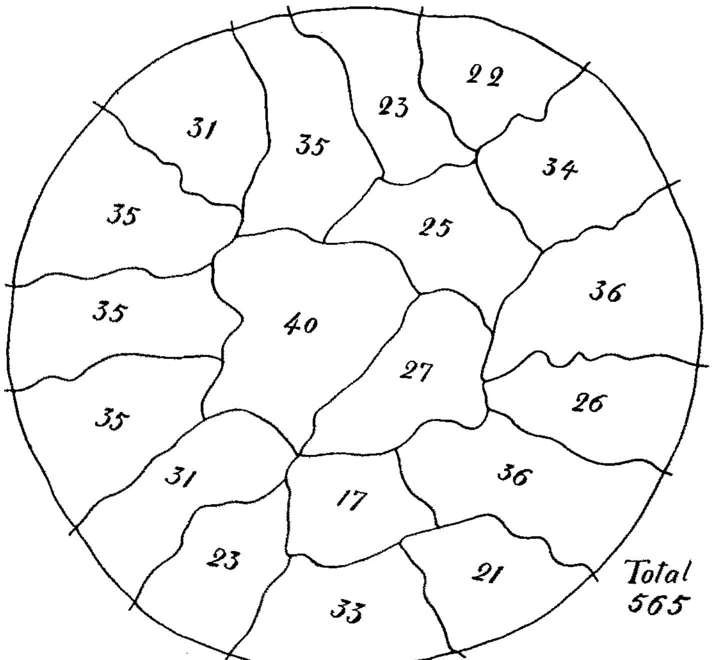


FIG. 2.


22
23
35
31
34
35
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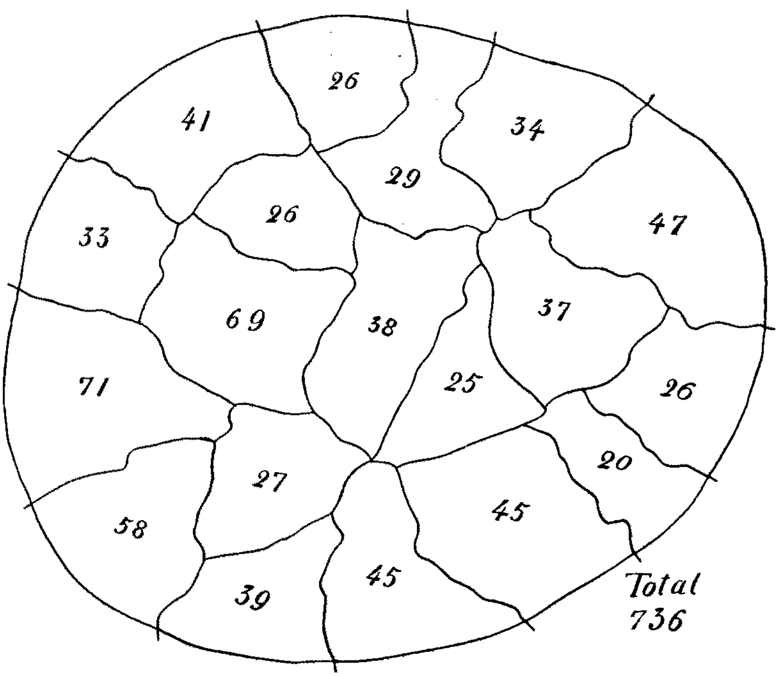
36
35
40
27
35
26
31
36
17
23
21
33
Total
565

FIG. 1.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29
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33
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20
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45
45
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Total
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FIG. 2.

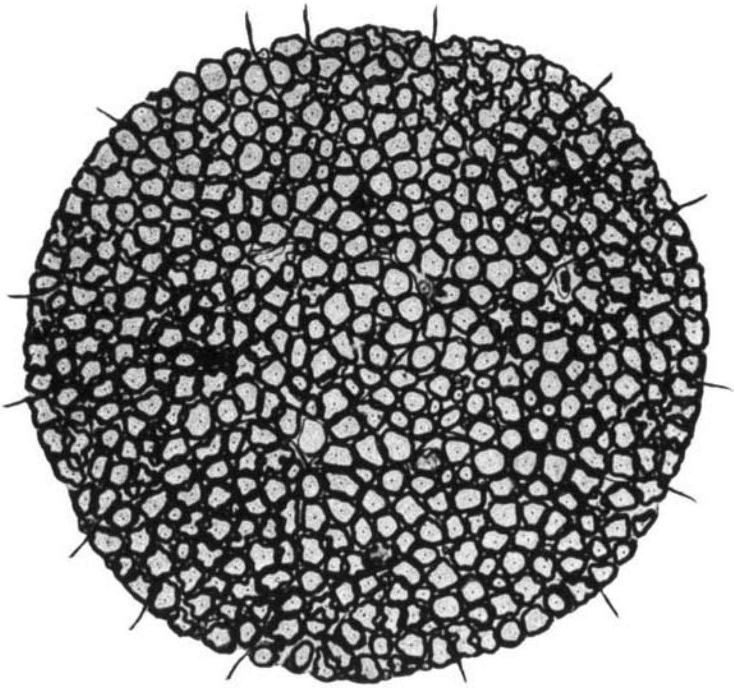


FIG. 1.

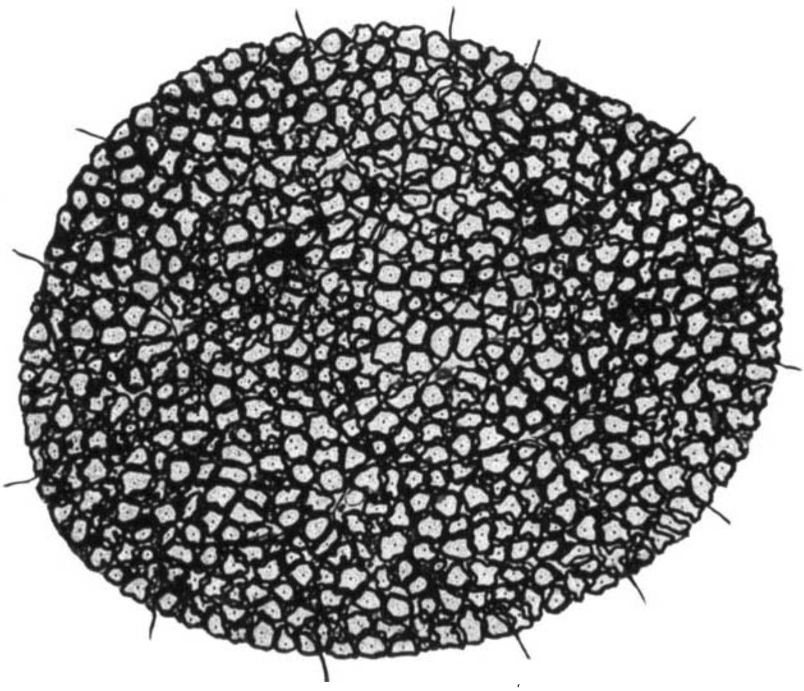
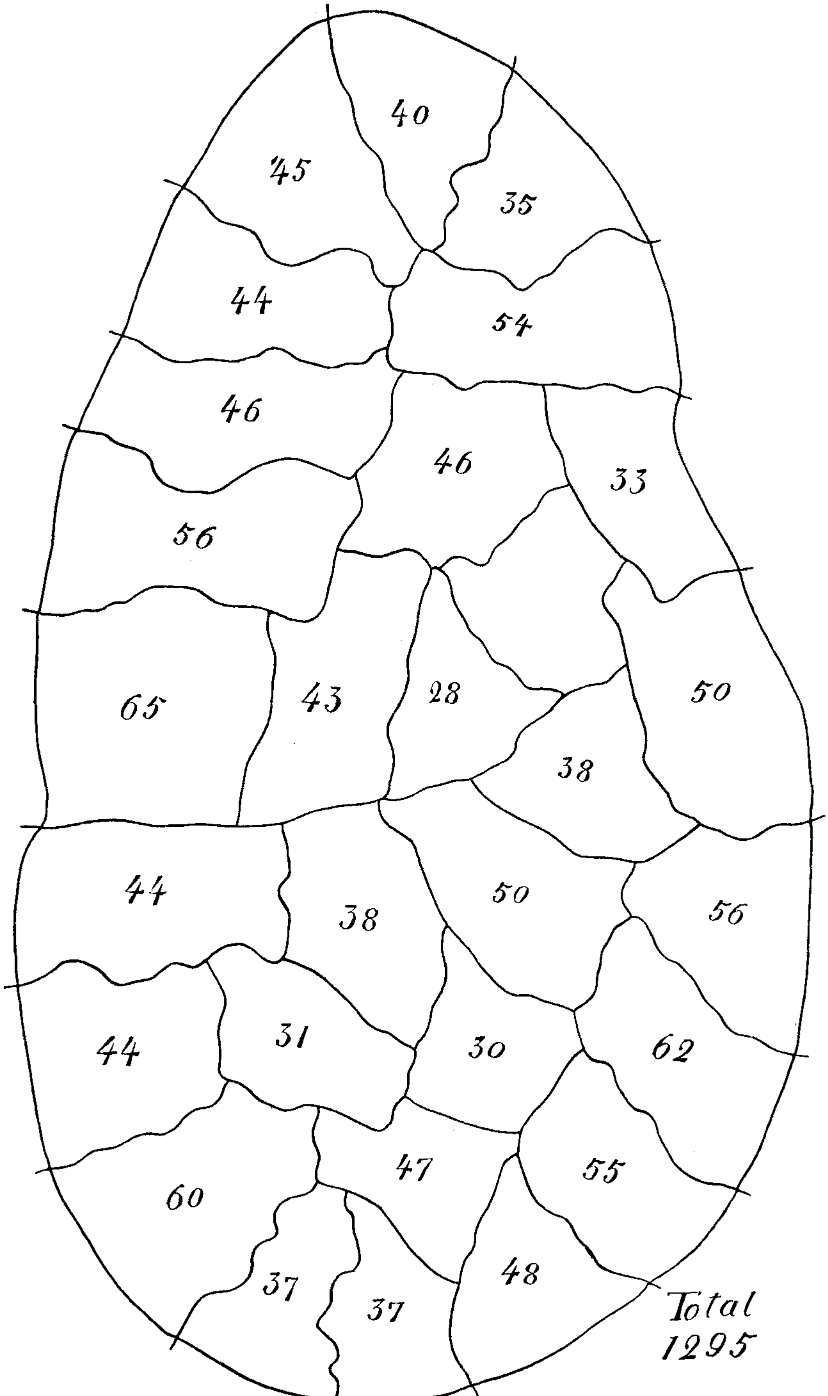


FIG. 2.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45
35
44
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46
46
33
56
65
43
28
50
38
44
38
50
56
44
31
30
62
60
47
55
37
37
48
Total
1295