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From The Wistar Institute of Anatomy and Biology, Philadelphia.

With Four Figures

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INTRODUCTION.

It is generally believed that the spinal ganglion contains several types of nerve cells which can be morphologically differentiated from one another. The varieties of cells whose existence in the ganglia have been repeatedly confirmed are: (1) cells with a T- or Y-shaped division of the processes. Such cells are considered to be most abundant and to be both large and small in size. (2) DOGIEL's cells of second type, multipolar cells, and (3) multipolar cells which resemble in shape and structure sympathetic ganglion cells. The most complete classification is based on the study of methylene blue preparations. In a general way the presence of the several varieties of cells in the ganglia may also be demonstrated in ordinary paraffine sections treated with any of the basic dyes followed by a counter-stain. In such preparations one can easily distinguish cells of different sizes as well as those exhibiting different arrangements of the stainable substance. These two characters, size and arrangement of stainable substance, have been used as a criterion by several investigators in order to classify these cells.

By this method LUGARO ('96) distinguishes in the dog five different varieties of the spinal ganglion cells, LENHOSSÉK ('96) in the human spinal ganglion distinguishes three varieties, COX ('98) in the spinal ganglion of the rabbit, two main varieties, and the author ('01) using the same criterion has distinguished three varieties in the spinal ganglion of the albino rat. It is my

intention later to analyse in detail all these classifications and at the present moment it is merely necessary to call attention to the fact that in the spinal ganglion several varieties of cells have been distinguished from one another. Can all these cells of different varieties be considered as belonging to a single class or are there really several types of cells composing the spinal ganglion? In other words, a frequency of distribution of all these cells based on their sizes[1] should give us more than one mode if there were more than one type of cell involved. If but one mode appears we have good ground to conclude that all these cells, though differing in size as well as in structure, may be considered from the standpoint of size, as members of a homogeneous population. The differences in structure are for the moment neglected and must form the subject of a special study.

MATERIAL AND TECHNIQUE.

For the present investigation the second cervical spinal ganglion of the adult albino rat was employed. The second cervical ganglion was purposely selected since through the investigation of RANSON ('06) we have already some numerical data in regard to this particular nerve.

The second nerve with ganglion was removed from right side of a healthy male having a body-weight of 194 grams and was fixed with osmic acid. Following the usual procedure the sections of the ganglion were cut 12 micra thick and mounted in series. Three sections from the middle of the entire series of 80 sections and three sections from midway between the middle and end on both sides, thus making altogether nine sections, were chosen. These nine sections were selected for the measurement of the cells and nuclei on the assumption that the cells of the different sizes were uniformly distributed and consequently that the nine sections would adequately represent the total cell "population" of the ganglion.

The measurements obtained from each cell and its nucleus were recorded on a separate card. In every case two maximum diameters at right angles to each other were determined for both cell-body and nucleus by means of the ocular micrometer. The

[1] Although this point could be tested also from the standpoint of the structure, nevertheless it is very difficult to obtain numerical data in terms of the structure, suitable for biometric treatment.

values of the two diameters thus obtained were multiplied together and the square root of the product was called "calculated diameter" of the cells and nuclei. Of course every section of a cell which possessed a distinct nucleus and nucleolus was measured from the nine sections and altogether 1108 such cells were found. The 1108 cells and nuclei thus measured were arranged according to the magnitude of the "calculated diameters" and the frequencies of the variates were determined as shown in the correlation table (see p. 490). For grouping the variates I have selected 2 micra as the unit for the cell-body and 0.65 of a micron for the nucleus. As will be seen later, small differences in the value of the unit do not produce any significant change in the final results, and therefore it is advisable to select some integral number for convenience in computation.

ANALYTICAL CONSTANTS AND FREQUENCY DISTRIBUTION.

I shall first discuss the frequency distributions of the cell-bodies and nuclei. The fundamental analytical constants necessary for such discussion are given in the following table:

TABLE I.

No. of measurement.	Cell-body.	Nucleus.	Skewness.	Cell-body.	Nucleus.
	1108	1108		.4081± .024	.1734± .024
μ_3	27.1627	9.5254	Modal divergence	1.3558± .006	.4918± .002
μ_4	446.9891	233.3303	Standard deviation	6.6448± .952	1.8457± .026
β_1	.5486	.1731	Mean	23.3356± 1.346	10.9523± .037
β_2	3.6687± .099	3.5889± .099	Mode	21.9798	10.4605
$\sqrt{\beta_1}$	.7407± .052	.4161± .028	Coef. of variation	28.4749±0.4398	16.8521±0.2482
β_2-3	.6687	.5889	Coef. of correlation	.8616± .006	
κ_1	−.3084	.6585	Lower and upper ends of ranges	7.5844 60.9693	
κ_2	−1.5179	.8248	Type of curve	I	IV

It has been shown by PEARSON ('05) that in order to fit a given distribution of frequency to a Gaussian probability curve

the following conditions, within the limit of random sampling, must be fulfilled:

$$\sqrt{\beta_1} = 0; \beta_2 - 3 = 0; \tfrac{1}{2}\frac{\sqrt{\beta_1}(\beta_2 + 3)}{5\beta_2 - 6\beta_1 - 9} = 0; \text{and } d, \text{modal divergence} = 0.$$

By examining the analytical constants given in Table I, it is seen that all those constants for both the cell-bodies and nuclei are considerably greater than zero even when their respective

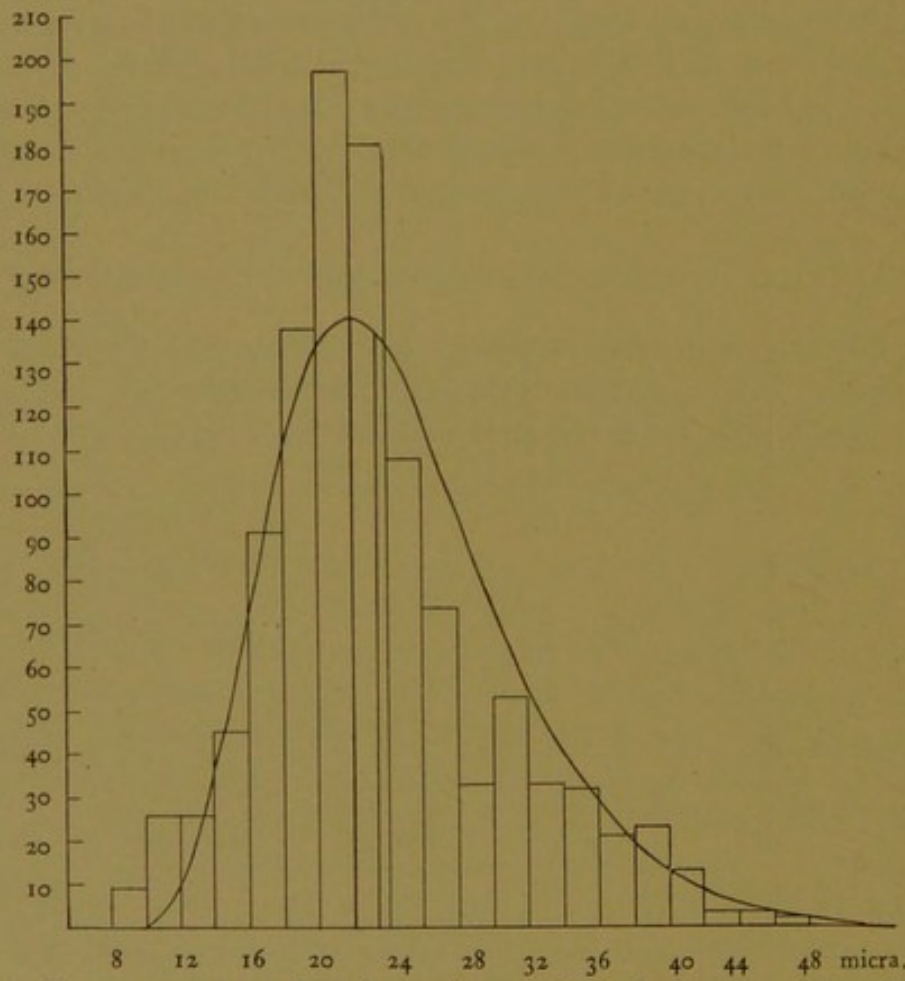

FIG. 1. Frequency polygon and fitted curve for variation in the diameters of the cell-bodies.

probable errors are considered. This at once leads to the conclusion that in both cases the frequency distribution can not be represented by the normal curve. Furthermore a considerable deviation of those constants from zero, *i. e.*, skewness, as well as modal divergence, indicates that they can never be represented by any other symmetrical curve since the deviation in excess and

defect are not equally probable. It is therefore evident that n order to represent the data in hand we must find a curve which is able to represent the odds against any given deviation.

It has also been shown by PEARSON ('95, '01) that the assignment of a given distribution of frequency to any one of the six

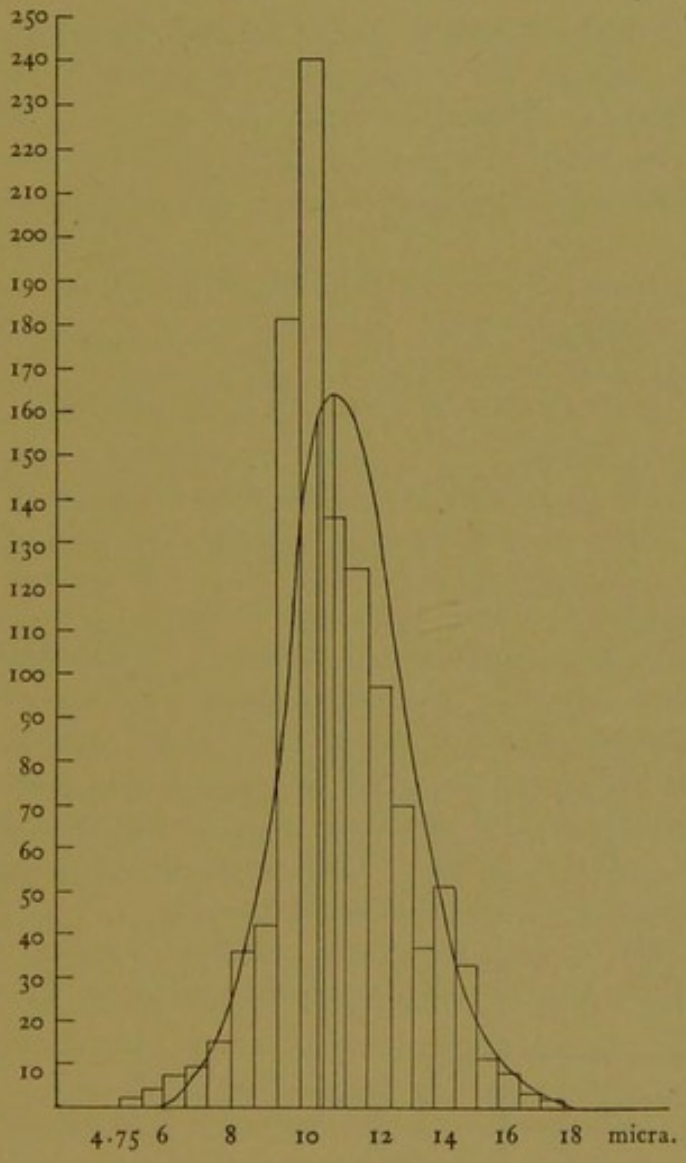

FIG. 2. Frequency polygon and fitted curve for variation in the diameters of the nuclei.

types of his skew curves depends on the value of the analytical constants κ_1; κ_2; β_1 and β_2. As is shown in Table I, in the case of the cell-body we have the following relations:

$$\kappa_1 < 0; \kappa_2 < 0 \text{ and } \beta_1 > 0$$

These three conditions satisfy PEARSON's skew frequency curve of Type 1, while for the nuclei we have

$$\kappa_1 > 0; \beta_1 > 0; \beta_2 > 3; \text{and } \kappa_2 > 0 \text{ and } < 1,$$

which calls for PEARSON's curve of Type 4.

The frequency distributions and their fitted curves are shown graphically in Figs. 1 and 2. The equations for the curves are:

For the cell-bodies (Type 1)[2]

$$y = 140.0657 \left(1 + \frac{x}{6.2286}\right)^{3.6107} \left(1 - \frac{x}{62.3049}\right)^{35.6365}$$

origin at mode.

For the nuclei (Type 4)

$$y = 13.0825 \left(Cos\,\theta\right)^{24.0118} \varepsilon^{11.2038\theta}$$

origin at 7.4341 micra,

$$x = 11.6002 \quad tan\,\theta$$

Examining Figs. 1 and 2 we see at once that the theory and observation do not agree at all well. The theoretical curve in both cases considerably underestimates the observed ordinates for the smaller values of variates x, and overestimates the same for the larger values of x. The degree of deviation between the observed and theoretical curves is most pronounced at or in the neighborhood of the mode. This unexpected results forced the writer to reinvestigate the following points:

1. Since the spinal ganglion contains various sized cells it may be possible that these cells are not uniformly distributed from

[2] Original formula of Type 1 is given by

$$y = y_0 \left(1 + \frac{x}{a_1}\right)^{m_1} \left(1 - \frac{x}{a_2}\right)^{m_2}, \text{ where } y_0 = \frac{\alpha}{b} \cdot \frac{m_1^{m_1} m_2^{m_2}}{(m_1 + m_2)^{m_1 + m_2}} \cdot \frac{\Gamma(m_1 + m_2 + 2)}{\Gamma(m_1 + 1)\,\Gamma(m_2 + 1)}.$$

and for Type 4

$$y = y_0 (Cos\,\theta)^{2m} \varepsilon^{-\nu\theta}, \text{ where } y_0 = \frac{\alpha}{a} \cdot \frac{\varepsilon^{\frac{1}{2}\nu\pi}}{\pi \int_0 Sin^{r}\theta\, \varepsilon^{\nu\theta}\, d\theta}.$$

section to section. In other words, some sections may contain relatively more cells having larger or smaller diameters, therefore the nine sections selected might not give a proper representation of the entire cell population and therefore the 1108 cells measured might not constitute a real random sampling of the entire population.

2. The disagreement between the theory and observation may be due to an improper selection of the unit for grouping the variates. If so, it may be improved by taking some other unit.

3. The two curves may agree more closely if the uncorrected or raw moments about the mean were used in determining various analytical constants.

4. The spinal ganglion cells may not represent a homogeneous population, but a mixture of various groups of elements. If so, dissection of the frequency curve into several components should give a better agreement.

The question contained in point 1 has been answered in the following way: the nine sections were divided into three series, each represented by three sections. For the series 1, one section was taken from the middle and one from the midway between the middle and the extremes on both sides, while for the series 2, the three sections which lie to the right of the three sections of the series 1 and for the series 3 those which lie toward left. The percentage values of these three series just mentioned were plotted separately, the same unit of course being used for each series. Comparison shows that these three curves agree with each other in every minor detail, and therefore with the original curve, too. This means that the cell-bodies of different sizes are uniformly distributed, otherwise the three curves should not agree so closely. Therefore the 1108 cells here measured can be regarded as giving a true representation of the entire cell-population. The disagreement found between the theoretical and observed curves is consequently not due to a lack of uniformity in distribution.

The question contained in point 2 was also answered by taking three different units for the cell-bodies; 1, 1.8, 2 micra; and one different unit for the nuclei, 1 micron, and comparing the new results with those already obtained. It was found that these variations in the units did not make any significant alteration in curve. This proves then the difficulty is not due to an improper choice of the unit.

As to point 3, I have treated the data for the cell-bodies, using the uncorrected moments, but without effecting any improvement on the results.

Finally I have tried to split the observed frequency curve (point 4) into two normal curves according to the method given by PEARSON ('94). After laborious calculations it was found that the present data can not be split. The reason for this conclusion is omitted since it needs an elaborate mathematical presentation. The result shows however that there is not the slightest indication of separate groups in the cell-population.

Therefore the cause of disagreement must depend on other conditions than those already enumerated.

After failing to obtain in this way a reasonable explanation for the considerable deviations between the observed and theoretical

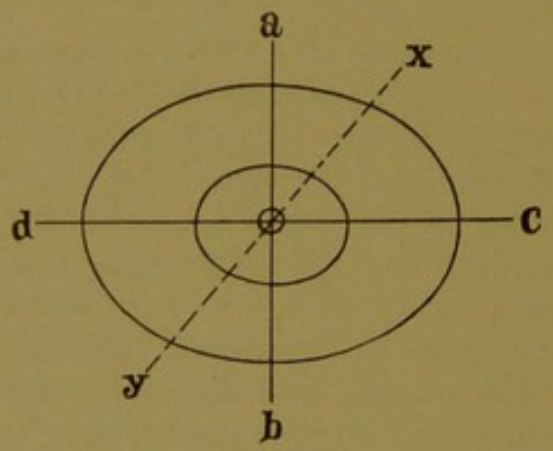

FIG. 3. Diagram of the spinal ganglion cell containing nucleus and nucleolus.

curves, it occurred to me that the explanation might be found in the method of sectioning and measurement. In order to make clear the relations existing within the ganglion let us suppose that 8000 spinal ganglion cells of various forms (from spherical to oblong) are thoroughly mixed in an ovoid receptacle. This is then cut into 80 slices of equal thickness. The entire series is sampled by taking three slices from the middle and three slices from the midway between the middle and extremes on both sides. Thus nine slices are selected for examination. The slices of the ganglion which we have examined in this way contained 1108 cells. Under these conditions the knife cuts the individual ganglion cell

in various planes. In some cases the knife makes a right angle with the longest axis (*dc*, Fig. 3) of the cell-body and some cases with the shortest axis (*ab*). In the remaining cases the angles will always be less than 90°. It must be remembered that the 1108 cells counted are those which contain both nucleus and nucleolus, and therefore it is assumed that the knife always passed through the approximate center of the cell-body. The chance that the knife will make a right angle with shortest axis[3] must however be very small compared with a failure. Whichever plane we cut, as long as the knife passes through the center, the diameter (*ab*) is constant and therefore the product of the diameters varies directly with the changes in the longer. But (*cd*) is the maximum diameter and its length diminishes as the axis moves from the original position toward the axis (*ab*). As soon as it reaches (*ab*) it becomes minimum. As was stated already, there are more chances for the knife to pass through somewhere between the two points (*a*) and (*c*) than to cut through (*c*) itself. If therefore we determine the two diameters of the cell from the cut surface and the square root of their product is taken as the mean or "calculated diameter" of the given cell, the final value thus obtained will often be less than it should really be, because $\sqrt{ab \times dc}$ is always $> \sqrt{ab \times xy}$, where (*xy*) is any arbitrary line between (*a*) and (*c*). But as was stated already, the sections of the smaller cells are more nearly circular in outline. Therefore the mean diameters thus obtained may represent nearly the true value in the case of the smaller cells but less nearly, in the case of the larger cells which have become ovoid. From this it will be clearly seen that the frequency curve of the diameters of the ganglion cells based on the square roots of the product of the observed diameters can not represent the true frequency.

As to the range of the diameters, the maximum "calculated diameters" found may be considered to be the true value since there is at least one chance that the knife could pass through the longest axis while on the other hand the observed minimum diameter may be somewhat less than the true minimum diameter since there is a tendency for the diameter of those cells which are in any degree ovoid to be made smaller. Consequently if the values

[3] The maximum size of the cell is obtained only when the knife makes 90° with the axis (*ab*), provided it also passes through the center of the cell.

found for the small cells were nearly right but those found for the large cells were less nearly right it is evident that we should expect to find more cells showing smaller diameters than are actually present in the nine slices examined. This leads at once to the conclusion that the ordinates representing the cells of the small diameters must be compounded of two heterogenous elements; *the true small cells plus those cells which are artificially made smaller* by the method of section. In the same way the ordinates for the larger cells will be compounded of the measurements on *the larger cells minus those which were artificially made smaller.* Thus we shall find an excess of cases towards the smaller value of x and a deficit towards the larger value of x. This is just what we have observed. It was found, when the observed polygon was compared with the theoretical curve that the latter considerably underestimated the observed ordinates which correspond to the smaller values of x and overestimated the observed ordinates which correspond to the larger values of x. The facts mentioned above indicate that the deviations of the observed polygon from the theoretical curve are mainly, if not entirely, due to the method of section. The greater observed excess found in the neighborhood of the mean or mode is interesting, since it may be assumed that up to the neighborhood of the mean or mode the cells remain nearly spherical while beyond this region the increase in size is accompanied by a change in shape.

If my argument is correct, then we should expect the greater percentage deviation of the two diameters to appear more frequently towards the larger abscissal values. Although it would seem at first easy to test such hypothesis by taking the percentage deviation from the averages corresponding to the different abscissal values, nevertheless in practice we meet considerable difficulties. Any one who is familiar with the sections of the spinal ganglia prepared by the usual fixation methods, will recall that there are present a number of *small* cells with very unequal diameters. Such cells are most abundant along the periphery of the sections. The general outline of such cells is either rectangular, instead of curved, or the opposite boundaries are represented by nearly parallel lines. The cause of the deformation may be attributed to a shrinkage of the capsule of the spinal ganglion itself. On account of the presence of such deformed cells a mere comparison of the

percentage deviations of the two diameters is unsatisfactory. Although it may not be a conclusive test, yet remembering that the spherical or nearly spherical cells should occur more frequently either towards the negative side or at the mode than towards the positive side, a determination of the relative frequency of such spherical cells, or the cells with nearly equal diameters, may be employed. Under the circumstances I think this is the only feasible method of testing this point. From an examination of original data, it has actually been found that such spherical or nearly spherical cells diminish with the increasing calculated diameter and increase with a diminishing calculated diameter. Even without any further test we cannot doubt from the theoretical standpoint that the method of section diminishes the diameter of the large cells, thus artificially increasing the frequencies of the small cells.

If this fact just mentioned is accepted, the conclusion follows that the theoretical curves may be considered as satisfactory representations under the circumstances and also may be considered much truer representations of the frequency distributions of the cell-bodies and nuclei than that shown by the actually observed data.

Since the curve of Type I has limited range in both directions, we find from the constants that

$$\text{lower limit of range} = 7.5844\mu$$
$$\text{and upper limit of range} = 60.9693\mu,$$

while the observed limits are 7.8 micra and 47.4 micra, respectively. We see therefore that the theoretical lower limit agrees very closely with the observed, while the upper limit in the theory is considerably higher than that of the observed. But that this upper value may not be entirely improbable is indicated by my previous work ('02) on the spinal ganglion cells where I find cells in the fourth cervical spinal ganglion of the adult albino rat as large as 52.7 micra. This figure just given is the average for the three largest cells observed, therefore one or two individual cells must be still larger. Nevertheless it is not necessary to assume that these cells are the largest which could be found. This fact indicates that there is a tendency at least to approximate the values given by the theoretical curve.

MEAN, STANDARD DEVIATION, AND COEFFICIENTS OF VARIATION.

In the equation $A = \frac{\Sigma (V. f)}{n.}$, where A represents the mean, it will be clearly seen that the absolute value of mean (A) varies directly according to the greater or smaller number of frequencies associated with the smaller or greater values of V, as long as "n," the total number of variates, is constant. We have demonstrated above that the number of the observed frequencies of V for both cells and their nuclei cannot be considered as the true frequency owing to the method of section. The true frequencies for the smaller values of V should be the observed frequency minus those cells which have been transferred from the group of large cells, while for the larger values of V it should be observed frequency plus those cells which have been thus transferred. Consequently the mean values actually found for the cell-bodies and nuclei must be considered as smaller than they should actually be. However we cannot determine at the present moment how large the true mean values should be, owing to the difficulty of determining the number of the cells and nuclei which are assumed to have been transferred. On the other hand, the values for the standard deviation and for the coefficients of variation in the present case should be smaller than those found, since following an increase in the frequencies towards the larger values of V the resulting frequency distribution would become more regular than they are shown to be by the observed polygons and consequently the mean square deviation would become smaller. Diminution in the mean square deviation causes a reduction in the value of the standard deviation and consequently in the value of the coefficients of variation. As a matter of fact, we found the value for the standard deviation as well as the coefficients of variation decidedly larger when compared with apparently more variable characters. For example PEARL ('05) found the coefficient of variation in Paramecium from 8 to 9 per cent and in Arcella 10 per cent (PEARL and DUNBAR, '03) while in the present case that of the cell-body is as high as 28 per cent and that of the nucleus 17 per cent. Although we have not as yet any available data with which directly to compare our own, nevertheless our own values appear too great when they are compared with the coefficients of variation obtained from the measurement of highly variable organs like the weight of the

liver (21 per cent, GREENWOOD, '04), weight of the body (10 per cent, PEARSON, '97), weight of the heart (18 per cent, GREENWOOD, '04), etc. I therefore corrected the values of the mean, standard deviation, and coefficients of variation, assuming that the theoretical curves represent more nearly the true distribution of frequencies. On employing the values of the theoretical ordinates there was found for cell-bodies, mean, 28.5948 micra, standard deviation, 14.8824 micra and coefficient of variation, 18.36 per cent; while for the nuclei, the mean was 13.0535 micra; and standard deviation, 1.7929 micra; the coefficient of variation being 13.73 per cent. When these corrected values are compared with uncorrected ones we find an increase of 3 micra for the mean in both the cells and the nuclei, and a reduction by 10 per cent in the coefficient of variation in the case of the cells and a reduction by 4 per cent in the case of nuclei. These corrected values appear to be the more probable, and are the best we can obtain until some further means of correcting the raw observations have been found.

CLASSIFICATION OF THE SPINAL GANGLION CELLS.

The unavoidable modification in the size of the spinal ganglion cells due to the method of sectioning as here described suggests a revision of the classification of the cells so far as it depends on their observed sizes. It has been mentioned already that using the size of the cells and the arrangement of the stainable masses as criteria, several investigators have attempted to classify the cells composing the spinal ganglion. Three such classifications proposed by LUGARO, LENHOSSÉK and COX will be presented in detail.

LUGARO ('96) distinguishes in the dog five different varieties of the spinal ganglion cells:

1. Large cells with delicate, closely packed stainable masses which are distributed uniformly throughout the cell-body. Around the nucleus are large stainable masses closely packed. The nucleus is large and clear and is provided with a nucleolus. These cells appear to be numerous.

2. Clear, medium-sized cells with irregularly formed small and large stainable masses which are large at the periphery. Even here we see that individual masses are not isolated but are united

together by fine processes. The nucleus is clear and possesses a nucleolus. These cells are most numerous.

3. Small, dark cells with numerous small stainable masses lying in the region of the nucleus. The ground substance becomes diffusely stained. The nucleus also stains diffusely and contains two or more nucleoli. These cells rank third in point of number.

4. Small or medium sized clear cells with large stainable masses which are present in small numbers and connected with each other by processes. The nucleus frequently possesses more than one nucleolus. These cells are not numerous.

5. Large clear cells with long drawn out masses which are continuous with one another and which arrange themselves in concentric lines around the nucleus. These last cells present a laminated appearance like the cross section of an onion. These cells are least numerous.

LENHOSSÉK ('96) in the human spinal ganglion distinguishes three varieties.

1. The first variety consists of cells with a light staining ground substance only. These, which are the largest cells, have a pale ground substance and less numerous, loosely arranged stainable masses, which are most dense around the nucleus.

2. To the second variety belongs coarsely granular cells (grobscholligen Zellen), the appearance of which depends on the arrangement of the stainable substance, and most of the cells in the ganglion belong to this variety. These cells are of medium size, but sometimes small and rarely very large.

3. The third variety contains small cells which have a peculiar internal structure. These cells stain darkly because of the density of the ground substance.

COX ('96) distinguishes in the spinal ganglion of the rabbit two main varieties.

1. One variety contains larger or smaller irregular masses of stainable substance, which do not show a distinct concentric arrangement. The cells of this variety may be either large or small.

2. The other variety contains large, irregular masses of stainable substance arranged concentrically.

It will be clearly seen from the description given by these authors that there exist some structural characters common to both large

and small cells. That is to say, some small cells have characters possessed by large cells and therefore size is the only means of distinguishing two forms. There is however another group of the small cells (third variety of both LUGARO and LENHOSSÉK) which exhibit still different structural characters. They are much darker in appearance owing to a strong affinity for staining reagents. The arrangement of the stainable substance is irregular and indistinct. The cell-outline is irregular. Thus there is no question as to the presence of the two kinds of the small cells which differ in both structure and shape from each other. The entire series of small cells which exhibit a resemblance to the large cells were considered by COX, LENHOSSÉK and LUGARO as early formed cell elements which persist in the spinal ganglion as such in small size. I have however just shown that a considerable number of the large cells are made smaller artificially by the method of sectioning. One would therefore expect to find a number of the small cells similar in structure to the large cells, except that the arrangement of the stainable substance may differ slightly according to plane of section. The cell-outline of the majority of the "artificial" small cells should be nearly spherical, unless they are distorted. Therefore the existence of small cells with the internal characters of the large cells can be explained readily on the assumption that they are in part if not entirely those large cells modified by the method of sectioning. I therefore conclude that a majority of these cells with the characters of the large cells do not preëxist as such and that consequently the conclusions of LUGARO, LENHOSSÉK and COX are to this extent misleading.

While the writer was engaged in the study of the structure of the spinal ganglion in the albino rat (HATAI '01) the following groups of cells were recognized and described. The one group is larger in size and stains lightly with eosin or erythrosin, while another group is smaller in size and stains deeply with eosin or erythrosin. Still a third group which, although it agrees in staining reaction as well as in an irregular outline with the small cells, nevertheless differs in the arrangement of the stainable substance and in size. The size is slightly larger on the average than that of the small deeply staining cells but much smaller than large cells. It now seems better to consider the group intermediate in size as a variety of the small cells rather than as a distinct type. The following are the reasons for this conclusion:

1. The intermediate sized cells agree with small cells in two important characters, the cell-body stains deeply with eosin or erythrosin and the cell-outline is irregular.

2. Since the arrangement of the stainable substance is rather unstable its difference has significance only when other characters also differ from any other given group under consideration. If the recently proposed hypothesis by SCOTT ('05) that the stainable substance is identical with the zymogen granules of the pancreas turns out to be true, then the size and form of the granules as well as their distribution may vary considerably according to the functional condition of the cell.

Consequently we have in the spinal ganglion two forms of cells; one which stains deeply and the cell-outline of which is irregular. Such cells are usually small in size. The other the cell-body of which stains lightly the cell-outline being regular. Such cells range from small to large in size. It must be remembered however that the entire cell-population when they are grouped according to their sizes grade from smallest to largest without showing any interruption. This means of course that there is no definite demarcation line to divide the large from the small cells or vice versa. As a matter of fact, the cells which stain lightly and which also exhibit regular outlines are by no means constant in size. This is also true for the group of the cells which stain deeply and which exhibit an irregular outline, although they are more uniformly small. For this reason, the size of the cell-body is not a proper criterion by which to classify them. The writer has adopted the staining reaction of the cell for classification because, as has been mentioned above, the ganglion cells fall readily into one of the two classes: that is (*a*) those with a deeply stained cell-body with irregular cell-outline and (*b*) lightly stained cell-body with regular cell-outline.

Although we should expect to find intermediate forms, which must always be present, nevertheless grouping by this method is more definite and practical than by the size and is much simpler than by those proposed by other investigators. In addition, these different histological characters are undoubtedly associated with different physiological states (HATAI '01).

I have chosen from NISSL's nomenclature two terms by which to designate the two groups of the ganglion cells with the idea that they may aid description.

1. Pycnomorphic cells, those cells which appear darker owing to a stronger affinity to the staining reagents. The cell-outlines are irregular. Such cells are usually small in size.

2. Apycnomorphic cells, those cells which appear pale owing to weaker affinity for the staining reagents. The cell-outlines are regular, being either spherical or oblong. Such cells range from small to large and include those which are made artificially smaller owing to the method of sectioning.

Attention is called to the fact that the above classification does not modify our views concerning the existence of three histological varieties of cells recognized in the spinal ganglion (see p. 1) but merely shows that these varieties do not distinguish themselves by their diameters in such a way as to form separate groups under this method of examination.

ON THE CORRELATION BETWEEN CELL-BODY AND NUCLEUS.

The intimate physiological relations existing between the cell-body and the nucleus suggest that there may also exist a definite size or mass relation between these two structures. Generally speaking in the growing spinal ganglion cells (HATAI '01), the cell-body grows much faster than the nucleus. It was my object to determine this mass relation, between the cells and nuclei and if possible to find some mathematical expressions by which such relation could be concisely stated.

The correlation table (Table II, p. 490) furnishes us all the data necessary to determine such a relation. The table shows the range of variates in one character corresponding to that in the other. The coefficient of correlation would be then a numerical expression of the occurrence of the several values of x in one character in association with the several values of y in the other. PEARSON gives the formula for obtaining the coefficient of correlation in the following form:

$$r = \frac{\Sigma\ (x.\ y.)}{n.\ \sigma_1\ \sigma_2}$$

Using the above formula, the value of r (coefficient of correlation) was found to be 0.8616 ± 0.0055. This shows that the size of the cell-body is highly as well as positively correlated with the size of the nucleus. Therefore we infer that the larger cell-body is associated with larger nucleus, and vice versa. We can also

find from the correlation table the diameter of the nucleus corresponding to the any given diameter of the cell-body. The value of the nucleus thus obtained is however affected by a variable probable error owing to insufficient number of observations combined with a random sampling. We therefore need to find the most probable values from the observed data, or the characteristic equation which can best represent the data with minimum error. We have two kinds of characteristic equations, linear and non-linear. Whether or not a given expression can be best represented by the linear or non-linear characteristic equation is of the utmost importance, and it is necessary to determine which equation applies to our present data. PEARSON ('04) has introduced a new constant, η, called the correlation ratio and this is used to test the linearity of the regression. The correlation ratio according to PEARSON is the ratio of the variability of the means of the arrays of one correlated character to the total variability of that character and is shown in the following formula:

$$\eta = \frac{\sigma_{my}}{\sigma_y}.$$

The constant η has the same value as the coefficient of correlation when the regression is perfectly linear. If the regression is not linear η will be greater than r. Then evidently $\eta - r$ is a measure of the approach of the regression to linearity. I have calculated the value of η by the formula given above and found that when this value is compared with the coefficient of correlation the former is significantly greater than the latter as is shown in the following:

$$\eta - r = .9267 - .8616 = .0651$$

However, the difference between the value of these two constants will in practice deviate more or less from zero. It is therefore necessary to find whether or not the difference found between the two constants is significant. Recently BLAKEMAN ('05) has given methods of obtaining the probable error of various functions of $\eta - r$. If we let

$$\zeta = \eta^2 - r^2$$

an approximate formula for the probable error of ζ, *i.e.*, E_ζ, is

$$\frac{\zeta}{E_\zeta} = \frac{\sqrt{n}}{0.6745} \cdot \frac{1}{3} \sqrt{\zeta} \frac{1}{\sqrt{1+(1-\eta^2)^2-(1-r^2)^2}}$$

Applying this formula it was found that

$$\zeta = .1164 \pm .0135$$

Thus the difference is certainly significant and data demand a non-linear characteristic equation. I have applied PEARSON's method of parabola ('04, '05) to the present data and obtained very satisfactory results as will be seen later. The general formula of parabolas of any order is as follows:

$$y = y_0 \left\{ \varepsilon_0 + \varepsilon_1 \left(\frac{x}{l}\right) + \varepsilon_2 \left(\frac{x}{l}\right)^2 + \varepsilon_3 \left(\frac{x}{l}\right)^3 + \ldots\ldots\ldots \right\}$$

where l is a half range of variates and ε_s are the constants to be determined from the observed data. I found that for the present

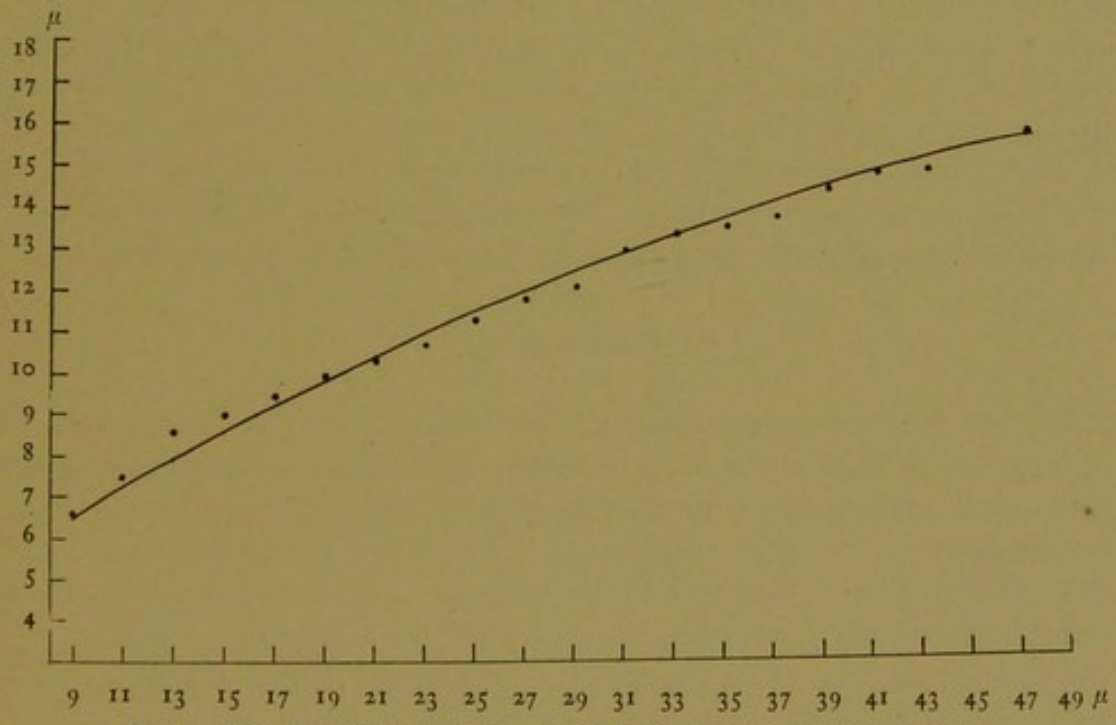

FIG. 4. Probable diameter of the nucleus for given diameter of the cell-body.
..... observed; ————, calculated.

data the parabola of the second order makes a very close fit to the observed means of the arrays. The smooth curve in Fig. 4, where the observed and calculated results are graphically represented, was plotted from the following equation:

$$y = 12.2939 \left\{ 1.0252 + .3564 \left(\frac{x}{l}\right) - .0758 \left(\frac{x}{l}\right)^2 \right\}$$

As will be seen from Fig. 4, the two curves agree very satisfactorily. It is therefore concluded that there certainly exists some definite mass relation between the cell-body and nucleus and its relation is mathematically expressed by the parabolic formula of the second order, as given. Since the regression is not linear but is best represented by parabola we may say that gain in the diameter of the nucleus following increase in the diameter of the cell-body varies in every stage and although the curvature is not pronounced from the nature of the parabola, the diameter of the nucleus is relatively greater in the small cells than in the large cells. For example, when the volume of the cell-body is compared with that of the corresponding nucleus, the following relation is found: In the cell-body whose diameter is 9 micra the volume of the same is 2.64 times that of the nucleus, while in the cell-body whose diameter is 47 micra its volume is 25.34 times that of the corresponding nucleus. This fact indicates, as was mentioned already, a predominant growth of the cell-body over that of the nucleus.

This gives us a method for comparing at some future time the relations in the small cells in the adult ganglion with that of the small cells having the same size in the immature ganglion.

CONCLUSIONS.

We see from the preceding observations that: 1. The method of section modifies the true frequency distributions of the cells and nuclei when their diameters are considered. 2. Under the circumstances the skew curves of Type 1 for the cell-bodies and that of the Type 4 for the nuclei may be considered the best and most reasonable representation of the frequency distribution of the diameters. 3. The theory that the entire group of small cells with the structural characters of the large cells represents unchanged small cells is probably erroneous in view of the unavoidable modification of the large cells by the method of section. 4. The diameters of the nucleus and that of the cell-body are highly and positively correlated ($r = 0.8616$). 5. There exists a definite mass relation between cell-body and nucleus, and the diameter of the nucleus corresponding to any given diameter of the cell-body is best represented by a parabola of the second order. 6. The spinal ganglion cells in a given ganglion may be considered as a homogeneous group, so far as the size is concerned. 7. Spinal

ganglion cells are classified into two groups according to their structural characters: (*a*) *Pycnomorphic cells*, those cells which appear dark owing to a stronger affinity to the staining reagents; and the cell-outline of the same is usually irregular. Such cells are usually small in size. (*b*) *Apycnomorphic cells*, those spherical or oblong cells which stain lightly and have cell-outlines which are regular. Such cells range from small to large in size. These two groups however grade into one another

APPENDIX. TABLE II.

Correlation between cell-bodies and nuclei.

Cell-bodies \ Nuclei	4.65–5.30μ	5.30–5.95	5.95–6.60	6.60–7.25	7.25–7.90	7.90–8.55	8.55–9.20	9.20–9.85	9.85–10.50	10.50–11.15	11.15–11.80	11.80–12.45	12.45–13.10	13.10–13.75	13.75–14.40	14.40–15.05	15.05–15.70	15.70–16.35	16.35–17.00	17.00–17.65	
8–10μ	2	1	1	2	2		1														9
10–12		3	2	6	6	4	3	2													26
12–14			2		4	5	8	6	1												26
14–16				1	3	7	14	16	3	1											45
16–18			2			8	12	44	22	2		1									91
18–20						4	3	55	50	20	4	2									138
20–22						6	1	37	76	36	25	11	2		1						195
22–24						1	1	14	58	47	35	17	6	1							180
24–26								7	20	22	25	14	14	4	1	1					108
26–28									8	7	14	26	14	3	2						74
28–30									1		11	10	7	3		1					33
30–32											8	8	10	13	8	5				1	53
32–34										1		5	5	5	11	6					33
34–36											1	2	9	4	9	4	1	2			32
36–38									1			1	3	1	6	6	3				21
38–40											1			1	10	3	2	6			23
40–42														1	2	4	4		2		13
42–44															1	1				1	3
44–46															1	2					3
46–48																	1		1		2
Totals.....	2	4	7	9	15	35	43	181	240	136	124	97	70	36	52	33	11	8	3	2	1108

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