THE NUMERICAL RELATIONS OF THE HISTOLOGICAL ELEMENTS IN THE RETINA OF NECTURUS MACULOSUS (RAF.)

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TWELVE FIGURES

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I. INTRODUCTION

Although the vertebrate eye has long attracted the attention of investigators and has been the subject of numerous researches, few attempts to enumerate the histological elements of the retina and of the optic nerve have been made. The exceedingly delicate nature of the retina has always been a serious difficulty in the way of its histological study, but with the introduction

of Golgi's bichromate method and of Ehrlich's methylene-blue method for staining nervous tissues, this difficulty has been, at least partially, overcome, and as a result great advance has been made in our knowledge of the visual apparatus. We owe much, also, to the use of osmic acid, which has been so successfully employed, not only in the study of the finer structure of nerve cells and their processes, but also in the enumeration of nervous elements. In the optic nerve this has not always been satisfactorily carried out, because the fibers of this nerve are very small and difficult to stain. The usual treatment has been to blacken the medullary sheaths with osmic acid, which, however, leaves the axis-cylinders unstained.

Many theories have been elaborated to explain the structure and functions of the retinal elements, but where the numerical relations of these units have been concerned investigators have, for the most part, confined their enumerations to regions of special interest, leaving untouched the broader aspect of the entire retina and optic nerve. It has seemed to me, therefore, that a complete enumeration of the histological elements of the retina and of the optic nerve, which have to do directly with the transmission of visual impulses, would be a desirable addition to our knowledge of the eye; and I have, therefore, undertaken to complete such an investigation on one of the lower vertebrates.

There is great similarity in the structure of the retina in all classes of vertebrates. From the standpoint of the neurone theory, the visual apparatus in these animals consists of three distinct layers of neurones. These neurones bear so constant a relation to one another that a definite terminology has become associated with them. The first neurones to receive the visual impulses are the visual cells, which include the rod-and-cone layer, a majority of the nuclei in the outer nuclear layer, and processes terminating in the outer reticular layer. The second set of neurones is represented by the nuclei of the inner nuclear layer exclusive of Müller's fibers and are of two distinct forms, viz., amacrine cells and bipolar cells. Processes from the latter form synapses with similar processes arising from the visual and from the ganglion cells. The third set of neurones consists of

the ganglion cells, which are described by Ramón y Cajal ('94) as sending free branching processes into the inner reticular layer, and axis-cylinders to the central organ through the optic stalk.

My problem is concerned with enumerations of the rods, cones, and double-cones, and the nuclei associated with them, of the nuclei of the inner nuclear layer, and the ganglion layer, of the Müller's fibers, and the fibers of the optic nerve.

All my investigations have been made in the Zoölogical Laboratory of Harvard University under the personal supervision of Prof. G. H. Parker, to whom I am greatly indebted for advice and valuable criticism.

II. HISTORICAL REVIEW

A. Retina

Although the literature on the retina and optic nerve in vertebrates is extensive, it is surprising that so little has been done on the numerical relations of the retinal elements. For the most part, those who have investigated the retina in this respect have limited their statements to the fovea and to comparisons between the number of visual and ganglion cells in central and peripheral regions. An epitome of the morphology and physiology of the retina has been published by Greeff ('00), but it is not satisfactory for numerical relations of the elements because of the lack of sufficient data.

Much of what has been written concerning the number of retinal elements in mammals relates to the human retina. One of the earliest to consider the question was Krause ('76), who estimated the number of rods in the human retina to be 130,000,000 and of cones to be 7,000,000. Salzer ('80) also worked on the human retina and estimated the number of cones to lie between 3,000,000 and 3,600,000, but gave no estimate of the number of rods or of other retinal elements. Foster ('91) and many other physiologists have apparently accepted the estimates of Salzer. In regard to the distribution of the rods and cones Foster writes "Over the retina (including the ora) the rods are much more numerous than the cones, there being two or three rods

in the line joining two cones," and "Toward the extreme periphery of the retina the cones become more numerous and close to the ora are alone present." Hesse ('00) gives the total number of visual cells in the human retina as 50,000,000. Stöhr ('06) believed that the rods were more numerous than the cones and that the latter occurred at regular intervals, so that three or four rods lay between adjacent cones. Pütter ('02) has given a great deal of attention to enumerations of the histological elements in the retina and in the optic nerve of marine mammals. The total number of elements in the different layers of the retina was found to be enormous (table 1). Estimates of the number of rods ranged from 227,000,000 in the adult *Phoca vitulina* to 800,000,000 in *Macrorhinus leoninus* and *Balaenoptera physalus*. As no mention was made of cones, except in the case of *Hyperoodon rostratus*, where they were said to be wanting, the retinas were presumably pure rod retinas. The nuclei in the outer nuclear layer were said to be from six to thirteen times as numerous

TABLE 1

Estimated numbers of retinal elements and optic nerve fibers in certain species of marine mammals (after Pütter, '02)

SPECIES	NUMBER OF RODS PER SQUARE MILLIMETER	MAXIMUM NUM- BER OF RODS IN RETINA	NUMBER OF NUCLEI PER SQUARE MILLIMETER IN		NUMBER OF OPTIC NERVE FIBERS		RATIO OF OPTIC NERVE FIBERS TO RODS
			Outer nuclear layer	Inner nuclear layer	Per square milli- meter	Total	
<i>Macrorhinus leoninus</i>	90,000	800,000,000	1,250,000	110,000	103	767,000	1:1050
<i>Phoca barbata</i> ..	120,000	363,000,000	1,367,000	119,000	68	174,000	1:2086
<i>Phoca vitulina</i> ..	110,000	227,000,000	1,512,500	78,000	74	147,000	1:1544
<i>Odobaeus rosmarus</i>	110,000	256,000,000	722,000	82,000	62	111,000	1:2300
<i>Otaria jubata</i>	250,000	475,000,000	2,000,000	181,000	74	140,000	1:2000
<i>Balaenoptera physalus</i>	62,000	800,000,000	550,000	62,000	13	157,000	1:5095
<i>Phocaena communis</i>	200,000	175,000,000	1,350,000	184,000	29	36,100	1:4850
<i>Delphinapterus leucas</i>	150,000	735,000,000	794,000	98,000	28	137,000	1:5560
<i>Hyperoodon rostratus</i>	111,000	557,000,000	918,000	90,000	15	77,000	1:7200

as the rods, which would make the enormous total for the outer nuclear layer in *Phoca barbata* more than 4,000,000,000 nuclei. In all the species mentioned in table 1, the number of nuclei in the inner nuclear layer approached more closely that of the rods, being slightly less in most cases. Equally important is the comparatively small number of optic nerve fibers associated with this enormous number of retinal cells. Pütter estimated that in *Balaenoptera physalus* there were only 13 optic nerve fibers per square millimeter, giving a total of 157,000 for the entire nerve. In this case there would be about 5000 visual cells to a single optic nerve fiber. Considerable difference was found between the number of elements in embryonic and adult retinas. As a rule the nuclei were more abundant in the former.

Investigation into the numerical relations of the retinal cells in other mammals, though meagre and fragmentary, indicate a condition similar to that in man. Chiarini ('06) expressed in a general statement concerning the visual cells of the dog, the unsatisfactory condition in which we find this problem in mammals, when he said, "The cones are less numerous than the rods."

Franz ('09) has given a definite enumeration of the numerical relations of the retinal elements for the central and peripheral regions of the retina in birds. The nuclei were found in eight species to be more numerous at the center than at the periphery. In the fundus of the retina of *Motacilla alba* there were on a line 0.1 mm. in length 266 nuclei in the inner nuclear layer, 60 in the outer nuclear layer, and 50 in the ganglionic layer; but over the same distance at the periphery there were only 40 nuclei in the inner, 20 in the outer, and 4 in the ganglionic layer. To find the total number in one square millimeter, Franz multiplied these numbers by 10 and squared the product. On this basis the fundus was stated to have in *Motacilla* 250,000 ganglion cells and 360,000 rods and cones, in *Bubo* 36,000 ganglion cells and 78,400 rods and cones to a square millimeter.

I have been unable to find any enumerations of the retinal elements of reptiles. It is important to note, however, that the so-called rods are said to be wanting in some species. Thus Ramón y Cajal ('94) says, "In die Retina der Eidechse die Stäb-

chen fehlen." The number of elements involved is probably greater in eyes of equal size in reptiles than in amphibians, because of the larger size of the elements in the latter.

Detailed enumerations of retinal elements in amphibians have not been made, so far as I can learn, but Howard ('08) estimated that the rods, cones, and double-cones in the retinas of *Necturus* based on estimates in the fundus, were in the relation 4 : 1 : 1 respectively; but he made no mention of his method of obtaining this result. The outer nuclear layer is said by him to consist of a single layer of nuclei in the fundus and a double layer at the periphery, each rod and each cone is described as having a nucleus and each double-cone, two nuclei. Schultze ('67) believed that there was only one nucleus to each double-cone.

Franz ('05) has found the number of nuclei per square millimeter in the outer nuclear and ganglionic layers in the number of selachians. The nuclei, in most cases, were found to be more numerous about the center than at the periphery. In *Acanthias blainvilli* the number of nuclei in the outer nuclear layer near the center of the retina was 24,000 per square millimeter and in the ganglionic layer in the same region 1200; in *Galeus galeus* near the center there were 75,000 nuclei to a square millimeter in the outer nuclear layer and 1500 in the ganglionic layer. In the deep-sea selachians there was a great increase in the number of nuclei in the outer nuclear layer and a decrease in the ganglionic layer. Thus, in the fundus over an area of one square millimeter there were in the retina of *Chimaera monstrosa* 100,000 nuclei in the outer nuclear layer and only 600 in the same area in the ganglionic layer.

Enumerations of the visual elements in the eyes of invertebrates have been made in some cases. Parker ('90) placed the number of facets in an adult lobster's eye at 13,500 and ('95) the number of ommatidia in the eye of an adult crayfish (*Astacus*) at 2500. Hesse ('00) estimated that there were between 2100 and 2400 rods in the eye of *Pecten jacobaeus*. Among cephalopods, the retina of *Loligo* was said to contain 162,000 rods per square millimeter and of *Scaevargus* only 26,000, with other species ranging between the two.

B. Optic nerve

Estimates of the number of optic nerve fibers, have been confined chiefly to the optic nerve of man. Kuhnt ('75) found 200 fibers in the diameter of the optic nerve of a new-born child, and 220 to 240 in that of a man forty years old, giving totals of approximately 31,400 and 40,000 respectively for the entire cross-sections. Krause ('76) put the number at 1,000,000 at least, but later ('80) reduced it to 400,000 including both large and small fibers, and an equal number of very small fibers. Salzer ('80) also worked on the optic nerve of man and from three nerves obtained an average of 437,745 fibers.

The only important contribution, to which I have had access, relating to the number of optic nerve fibers in amphibians is a short paper by Lauber ('02), who counted 450 fibers in a cross-section of the optic nerve of *Cryptobranchus japonicus*.

Among invertebrates Parker ('95) determined that seven fibers were connected with each ommatidium in the crayfish eye. This gave a total of 8085 retinal fibers in the case of a young crayfish and 16,625 in an old one. Proximal to the fourth optic ganglion these totals were reduced to 2021 fibers in the former and 4156 in the latter.

It is a generally credited theory that the majority of the optic nerve fibers originate in the retina and pass centripetally along the optic stalk to the brain, and that a smaller number arise in the brain and pass centrifugally to the retina. This view has the support of such investigators as His ('90), Ramón y Cajal ('91) and Robinson ('96). On the other hand Balfour ('81) did not hold this view, believing rather that the fibers of the optic nerve were derived from a differentiation of the epithelial cells of which the nerve was at first formed.

The axis-cylinders of the optic nerve in a majority of vertebrates are small and medullated. In some amphibians, viz., urodeles, the optic nerve was described by Osborn ('88) as 'greatly reduced,' and in some examples of *Necturus* there was stated to exist, in the adult condition a persistent lumen which opened into the *T*-like expansion of the third ventricle of the brain. Kings-

bury ('95), likewise, describes the optic nerve of *Necturus* as hollow for a portion of its length and states that its fibers are entirely amyelinic. This latter condition is said by Edinger ('92) to be the case in the young of certain other amphibians.

After a careful survey of all the literature at hand bearing on the numerical relations of the histological elements of the optic nerve and retina, I have failed to find for any vertebrate a consistent enumeration of these elements which has been carried out in such a way as to give reasonably safe grounds for comparisons. I have, therefore, undertaken this task in reference to *Necturus*.

III. MATERIAL AND TECHNIQUE

A. Material

It has long been known that the retinal elements in amphibians are very large as compared with those in other vertebrates. Howard ('08) gives the dimensions of the outer and inner segments of the rods in several species of amphibians as follows: In the outer segment the length varied from 24μ in Triton to 76μ in Bufo, and the width from 6μ in the frog to 12μ in both Triton and Salamandra. The outer segments of the rods in *Necturus* are said to be 36 to 40μ long and 12μ wide. Measurements made by myself on the diameter of the cones of *Necturus* just distal to the ellipsoids averaged 4.8μ , and through the ellipsoids, where visual cells first come in contact with neighboring visual cells, they averaged 10μ . The diameters of the rods through the ellipsoids was slightly larger than that of the cones. Slonaker ('97) states that "Amphibia have not only long rods, but the thickest found in vertebrates." In speaking of the cones, he says, they have the "greatest diameter in mammals." Table 2 shows the relative sizes in micra of the rods and cones in representatives from all classes of vertebrates. The length of the elements is non-essential for my purpose, but the diameter is of especial significance because it is one of the most important factors in determining the number of elements which can occupy a given space, when they are crowded together as are the visual cells in the vertebrate retina. From the measurements quoted

it will be seen at once that, because of the large diameter of the visual cells, the number of these elements in a given area will be fewer in the amphibia than in any other class of vertebrates. The two conditions, viz., large diameter and fewer elements, unite to make the amphibian retina especially favorable for a study of the numerical relations of the constituent parts. With this point in mind I have selected for my investigation the adult form of *Necturus maculosus* (Raf.), the common 'mud-puppy' of the fresh water streams and lakes of eastern Canada and of middle and southern United States (Cope, '89). The specimens were secured by the Zoölogical Department from Venice, Ohio.

TABLE 2

Comparative sizes of the outer segments of rods and cones in representatives of the different classes of vertebrates (after Müller '56)

SPECIES EXAMINED	DIMENSIONS IN MICRA OF THE OUTER SEGMENTS OF THE			
	Rods		Cones	
	Diameter	Length	Diameter	Length
Man.....	1.5 - 1.8	40.0 - 60.0	4.0 - 6.0	32.0 - 36.0
Pigeon.....	2.6 - 3.3	20.0 - 28.0	1.0 - 5.0	25.0 - 30.0
Chameleon.....			1.0 - 1.3	60.0 - 80.0
Frog.....	6.0 - 7.0	40.0 - 60.0	5.0	20.0 - 28.0
Perch.....	2.6	40.0 - 50.0		8.0 - 12.0

Upon their arrival at the laboratory they were transferred at once to a fresh-water cement aquarium in a dimly lighted part of the basement of the Museum of Comparative Zoölogy, where they were easily kept in a healthy condition.

B. Technique

I have found that the technique usually employed for the retina does not give satisfactory results when applied to the optic nerve; for this reason I have been obliged to make separate preparations for the two structures.

In order to secure material for a reliable enumeration of the retinal elements, a fixation fluid was necessary which would not wrinkle the retina, and at the same time could be followed by

successful double staining, to which I shall refer later, whereby small fragments of rods could be distinguished from bits of cones. The large size of all the retinal cells in *Necturus* has been a great aid in the identification, and in the accuracy of the counts, of the separate elements of the retina. The most successful results were obtained by fixation in Kleinenberg's picro-sulphuric mixture. My method was as follows: Live animals were placed in a bowl of tapwater in which a few small crystals of chloretone had been dissolved as a means of preventing the discharge of slime (Cole, '02). Chloroform was then added gradually until the animals were thoroughly anesthetized. The eyes were quickly removed and placed in picro-sulphuric acid, care being taken to free them from as much superfluous tissue as could be done quickly. To keep the orientation, a piece of skin was left attached to the dorsal side of the eyeball, differences in shape of the pieces serving to distinguish right and left eyes. I found the pieces of skin useful also in orientating the eyes in paraffin. The best results were obtained by immersing the unopened eyeballs in the picro-sulphuric mixture for four or five hours. They were then rinsed in distilled water a few minutes and dehydrated by passing them gradually through 35 per cent, 50 per cent, 90 per cent, and 100 per cent alcohol over a period of two days. When the eye was sufficiently hardened (90 per cent alcohol), the front face was cut away with a sharp razor, and the lens removed. Early in my work I found that the heat of a paraffin bath, extending over a time sufficient to insure saturation with paraffin, caused considerable shrinkage of the sclera and wrinkling of the retina. I, therefore, followed Bütschli's chloroform method of de-alcoholization. Transfer from the chloroform-paraffin mixture of this method to hard paraffin was completed by the evaporation of the chloroform over a water bath at about 60°C. and a five minute immersion in hard paraffin melting at about 56°C.

Sections 8μ thick were made in one set of eyes parallel to the antero-posterior¹ plane of the eye and passing through the optic

¹ The terms 'anterior,' 'posterior,' 'dorsal' and 'ventral' as applied to the eyeball in this account, are used in the sense of comparative anatomy; i.e., 'anterior'

nerve, and in another set parallel to the dorso-ventral plane, and passing through the optic nerve. Series of sections 6μ thick were made through the entire thickness of the retina tangential to the surface of the eyeball in the anterior, posterior, dorsal, and ventral regions and in the fundus. The sections were stained in Heidenhain's iron haematoxylin as a base, and in a 70 per cent alcoholic solution of eosin as a counter stain. Thirty minutes in the mordant (2 per cent ferric alum) and one hour in the haematoxylin gave very satisfactory results. The excess of stain was washed out in ferric alum of the same strength as the mordant, and the washing was continued until all traces of the haematoxylin had disappeared from the outer segments of the rods and from the reticular layers. At this stage the nuclei of the outer and inner nuclear layers and of the ganglionic layer were clearly defined and light blue in color. The nuclei of Müller's fibers were stained a deep blue to blue-black and contrasted sharply with the lighter blue of the surrounding nuclei. Control was kept over the process of destaining by examining the slides every few seconds under the microscope. Two minutes in the counter-stain were sufficient to color the outer segments of the rods bright red. The outer and inner reticular layers appeared as broad red fibrous bands separating the inner nuclear layer from the outer nuclear and the ganglionic layers, respectively. The strands of Müller's fibers, which stretched radially outward from the internal limiting membrane, were stained an intense red. It is clear that the selective qualities of the stains employed rested primarily with the haematoxylin, for in the process of reducing the over-stain of the base the outer segments of the cones, the nuclei of Müller's fibers, and the ellipsoids, retained their color longer than the other elements, thus indicating their greater chemical affinity for the basic stain. Although the success which attended fixation in picro-sulphuric acid and double staining with haematoxylin and eosin made other methods superfluous, nevertheless some entirely successful preparations were

refers to that part of the eyeball which is nearest the anterior end of the animal ('internal' in human anatomy), 'dorsal' to that part which is nearest the dorsal midline ('superior' in human anatomy), etc. The deep part of the eyeball, often called the posterior part, is here referred to as the 'fundus.'

made with the use of other reagents. Both the vapor of 2 per cent osmic acid and vom Rath's picro-osmo-platinic-chloride-acetic mixture gave excellent preservation of the retinal elements, though the outer segments of the rods and cones were over-blackened. Good preservation and successful double-staining were obtained by fixation in either Perenyi's fluid, Fol's mixture, or 7 per cent nitric acid, followed as before with haematoxylin and eosin stains.

I have already called attention to the non-medullated character of the optic nerve fibers in *Necturus*, hence the usual fixation fluids in which osmic acid is used to stain the medullary sheaths are ineffective for the optic nerve fibers, in this animal. Vom Rath's fluid gave excellent preservation of the supporting and vascular tissues, but was unsatisfactory for the nerve fibers. Ranson's ('09) modification of Ramón y Cajal's silver-nitrate method for non-medullated nerve fibers was tried without success. With another modification of this method, that of Mullenix ('09), I succeeded in staining the fibers, but the definition was poor. The only method tried which brought out the fibers distinctly was a modified form of Bielschowsky's ('03) method. In order to secure the necessarily rapid fixation of the proximal portion of the optic nerve, I cut away the tissues surrounding the skull, which was then split at both anterior and posterior ends. This permitted the formalin to enter the brain cavity quickly. The non-medullated fibers when impregnated by this method appeared in longitudinal section as sharply defined somewhat undulatory, brownish-black to black lines. In cross-section (figs. 10, 11, 12) they were irregular black spots or streaks in a yellowish-brown matrix. Dehydration and de-alcoholization were carried out as with the retina. Cross-sections of the optic nerve 5μ thick were made close to the chiasma and as near as possible to the eyeball.

The results obtained with Bielschowsky's fluid have justified its use in this case. There is no doubt in my mind that the irregular black spots and streaks referred to above are nerve fibers. I was fortunate in having a longitudinal section of an optic nerve turn up slightly so that the fibers could be seen to end as small

black spots of different sizes. In a longitudinal section individual fibers could be traced only a short distance. At the distal end they spread out in bundles along the inner margin of the retina and disappeared radiating outward between the nuclei of the ganglionic layer. I was wholly unable to detect a union between the ganglion cells and the optic nerve fibers, and I am unable therefore to state the exact relation of the optic nerve fibers to the retinal elements.

IV. OBSERVATIONS

A. *Measurements*

a. *Retinal layers.* The eyeballs of *Necturus* lie well forward on the dorso-lateral aspect of the head, where they appear as slightly arched whitish bodies. They are unusually small for the size of the animal, and are approximately spherical in shape. The retina, which is closely applied to the sclera, has, therefore, the shape of a *zone of one base* whose area is only slightly less than that of a hemisphere. Since the area of a zone of one base is equal to the area of a circle whose radius is the chord of the generating arc, the area of the retina in *Necturus* is equal to the area of a circle whose radius is the shortest distance from the periphery of the retina to the center of its fundus. To obtain this center I made a camera drawing ($\times 101$) of a median section of the eye, and erected a perpendicular at the middle point of the chord joining the ends of the retina at the ora. The point of intersection of the perpendicular and the retina marks the center required. The chord joining this center to the periphery is equal to the radius of a circle whose area is that of the zone of one base, which marks the limits of the retina. In many cases the center lay directly in the optic nerve, while in nearly all the remaining eyes, it lay very close to that nerve. By this method I have calculated the minimum, maximum, and average areas of zones of one base (which hereafter I shall refer to as *zones*) for 14 retinas, the three zones coinciding with the external limiting membrane, the middle part of the inner nuclear layer and the middle of the ganglionic layer (fig. 1, *cd*, *xz*, *vw*). The results

of my measurements are given in table 3. Eight retinas were measured in the antero-posterior and six in the dorso-ventral plane. Variations in the thickness of the retinas (figs. 2 to 4), measured from visual cells to internal limiting membrane, and irregularities of the retinas, especially at the periphery, are the causes of the differences of area in retinas nos. 3 and 8, 5 and 6, and 10 and 14.

In order to enumerate the retinal elements, it was necessary to adopt unit areas small enough to avoid the distortion at the margin of the microscopic field and large enough to include a great number of elements. Convenient sizes were found in a circular area of 0.013 sq. mm. for the visual cells and one of 0.0078 sq. mm. for the other elements. The areas of the retina in the zones at the different levels were duly considered, so that the number of visual cells and nuclei of the outer nuclear layer was

TABLE 3

Calculated areas of zones corresponding to the area of the retina in Necturus and passing through (1) the external limiting membrane, (2) the middle of the inner nuclear layer, and (3) the ganglionic layer

DESIGNATION OF EYE	PLANES OF SECTIONS	AREAS OF ZONES IN SQUARE MILLIMETERS AT THE		
		External limiting membrane	Middle of inner nuclear layer	Middle of the ganglionic layer
1	Antero-posterior..	12.5664	11.7094	10.9073
2	Antero-posterior..	13.4484	11.8309	10.3138
3	Antero-posterior..	15.1777*	13.5815	12.0693
4	Antero-posterior..	13.8940	12.2255	10.6535
5	Antero-posterior..	15.0399	13.6455	12.3176
6	Antero-posterior..	15.0399	13.8940	12.8165
7	Antero-posterior..	12.0693	10.9133	9.8120
8	Antero-posterior..	15.1777*	14.0721*	13.0690*
9	Dorso-ventral....	14.6303	12.1947	9.9743
10	Dorso-ventral....	10.8184†	9.7028†	8.6916†
11	Dorso-ventral....	12.5664	11.4616	10.4257
12	Dorso-ventral....	13.2541	11.8005	10.4257
13	Dorso-ventral....	13.9602	12.3796	10.9073
14	Dorso-ventral....	10.9351	9.7569	8.6916†
Average areas of zones in square millimeters.....		13.4698	12.0834	10.7910

* Maximum areas

† Minimum areas

based on an area obtained for the zones at the external limiting membrane; Müller's fibers and ganglion cells for the zone through the ganglion layer; and nuclei for the inner nuclear layer from the area of the zone passing through the middle of this layer. The number of unit areas in each zone was found by dividing the number of square millimeters in the zone by the number of square millimeters in the unit area. The total number of elements in a layer obviously equaled the product of the number of areas into the number of elements per unit area. The methods employed for the other layers were evidently unsuited for enumerations of the inner nuclear layer, for in this layer we have to do with solid content rather than surfaces. For this layer I have resorted to counting the nuclei in a cylinder with a head of 0.0078 sq. mm. and with its long axis placed radially. The length of the cylinders was determined by the number of microtome sections necessary to pass completely through the layer, the number of sections being eight in each of the two cases counted. The number of such cylinders was found by dividing the area of the zone of the inner nuclear layer by the area of the cylinder head. In all cases where elements touch the boundaries of the unit areas (figs. 5 to 9) they were rated at one-half their value within the field.

b. Cross-sections of the optic nerve. The areas of the cross-sections of the optic nerve varied according to their location (figs. 10 and 11). In all cases examined the nerve was very much smaller proximally than distally, in one instance the former amounting to little more than one-third the latter (table 15). My method of calculating the cross-sectional areas of the nerves was modelled after that employed by Parker ('95) on the optic nerve of *Astacus*. Camera drawings of all the sections to be measured were made on paper of uniform thickness and weight; these were then cut out and weighed. A standard unit 0.0015 sq. mm. of the same magnification was also cut out and weighed. I could then easily obtain the required areas from the arithmetical formula:

$$X \propto Y \text{ when } T \text{ is constant}$$

where X equals the area of the cross-section, Y equals the weight

of the drawing, and T equals the thickness of the paper. In sections near the chiasma allowance was necessarily made for the lumen of the stalk, which becomes obliterated distally.

B. Enumerations

a. Visual cells. The sections used in enumerating the visual cells were cut tangentially to the surface of the retina in the five regions already referred to, passing through the outer segments of the rods and cones, and through the paraboloids of the double-cones (fig. 1, *ab*). The visual cells, therefore, were seen in cross-section, and because of their structure, large diameter, and differential staining qualities they were easily distinguished from one another. I was unable to detect any plan in the arrangement of the rods and cones, such as Schwalbe ('87) has depicted, but noted a marked tendency for five or six elements of a kind to run in lines, a feature which I think is without real significance. The association of rods or cones in groups of six or eight. (figs. 6 and 8) was of frequent occurrence. Their usual distribution may be described as irregularly scattered, and free from contact along their outer segments with neighboring elements (figs. 5 to 9). With a magnification of 615 diameters the cross-sections of the rods were bright red, circular to oval in outline, and with the characteristic vacuolated structure described by Howard ('08). They were larger than the cones and somewhat more numerous. The cones appeared as small round, blue-black, homogeneous bodies scattered irregularly over the field; the double-cones were far less numerous and much larger than either the rods or the cones. As one should expect, the paraboloids were fused along one side (figs. 5 to 9), giving the appearance of double-elements. Their structure was reticular and took the stain only slightly. In addition to the elements already mentioned, there were a few whose identity could not be established with certainty, due, I think, to disintegration having set in before they were reached by the fixative fluids. They constituted only a very small percentage of the total number of visual cells.

Studies of the cross-sections of the cells in the five regions described, showing the close relation between the number of rods, cones, and double-cones, in the different positions, are

clearly set forth in table 4. I have taken the averages from five retinas in each case, right and left eyes being used indiscriminately. Twenty eyes were used, and in five cases counts from two different regions of the same eye were made, and in two cases right and left eyes from the same animal were considered. Averages per unit field for the right and left eyes respectively show a slightly greater number of rods and cones, and a smaller number of double-cones and doubtful elements in the left eye. These differences are shown in table 5. The maximum and minimum counts are, however, so close in every case as to lead me to believe that the number of elements involved in right and left sides is approximately the same, and I shall therefore make no discrimination between the two.

TABLE 4

Number of rods, cones, double-cones and uncertain elements in unit areas of 0.013 sq. mm.

DESIGNATION OF EYEBALLS	REGIONS OF THE EYEBALL IN WHICH THE UNIT AREAS WERE LOCATED																			
	Anterior				Posterior				Dorsal				Ventral				Fundus			
	R*	C	D	X	R	C	D	X	R	C	D	X	R	C	D	X	R	C	D	X
7 r																	46.0	39.5	12	1
8 l	70.5	49.5	14	0													56.5	47.5	14	1
16 r					35.0	44.5	21	0												
21 l									42.5	41.0	14	2	52.5	46.5	14	4.0				
22 l	49.2	41.0	14	2																
23 r																	41.0	37	17	0
23 l													65.5	28.5	10	6.0	37.5	31	16	0
24 r																	50.0	26	20	2
24 l	66.0	42.5	25	0																
25 l													64.0	42.0	8	9.5				
26 r	47.5	47.0	14	0	59.0	37.5	19	1												
28 r									50.5	37.0	12	1								
31 r									43.0	42.5	15	4	70.5	43.0	5	4.0				
33 l													45.0	49.5	18	2.5				
38 r	56.0	29.5	12	0																
40 l					48.0	54.0	10	1												
56 r					43.5	38.0	14	0												
57 r					55.5	41.5	25	0												
59 r									42.5	36.5	10	0.5								
60 l									40.5	28.0	8	3								
Average of 5 retinas	57.9	41.9	15.8	0.4	48.2	43.1	17.8	0.4	43.6	41	11.8	1.9	59.5	41.9	11	5.2	46.2	36.2	15.8	0.8

*R, rods; C, cones; D, double-cones; X, uncertain elements; r, right eye; l, left eye.

From tables 4 to 7 it will be seen that the rods are generally more numerous per unit field than the cones and double-cones. Exceptional cases exist, however, where the cones are found to be more numerous than the rods (table 4, nos. 16 *r*, 33 *l*, 40 *l*), and I am convinced after examination of a great number of retinas that such areas are not infrequent in every one of the five regions. The actual relative values of rods, cones, and double-cones and the total average number per unit fields, as well as the total average number per five unit field, are given in table 6, where the rods consistently outnumber the cones. In table 7 I have brought together many data for the visual cells. Between the maximum and minimum retinas there is a variation of 25 per cent, at least, in the total number of visual cells.

TABLE 5
Average number of retinal elements per unit area in right and left eyes

SIDE EXAMINED	UNIT AREA IN SQUARE MILLIMETERS	AVERAGE NUMBER OF			MAXIMUM NUMBER OF			MINIMUM NUMBER OF		
		Rods per unit field	Cones per unit field	Double-cones per unit field	Rods per unit field	Cones per unit field	Double-cones per unit field	Rods per unit field	Cones per unit field	Double-cones per unit field
Right	0.013	49.2	38.2	15	70.5	47	25	35	26	5
Left	0.013	53	41.7	12.7	70.5	49.5	25	37.5	28	8

TABLE 6
Average numbers of visual cells in the five selected unit areas (see table 4)

POSITIONS OF UNIT FIELDS	AVERAGE NUMBERS OF THE SEVERAL KINDS OF RETINAL ELEMENTS PER UNIT AREA IN THE FIVE REGIONS OF THE EYE				AVERAGE NUMBER OF ELEMENTS PER UNIT AREA
	Rods	Cones	Double-cones	Uncertain elements	
Anterior.....	57.9	41.9	15.8	0.4	116.0
Posterior.....	48.2	43.1	17.8	0.4	109.5
Dorsal.....	43.6	41.0	11.8	1.9	98.3
Ventral.....	59.5	41.9	11.0	5.2	117.6
Fundus.....	46.2	36.2	15.8	0.8	99.0
Total number of the several kinds of elements in five combined unit areas.....	255.4	204.1	72.2	8.7	540.4

To correlate the number of visual cells with the number of nuclei in the outer nuclear layer, it was necessary to count each double-cone as two units, on the assumption that each double-cone has two nuclei. This view is held by Howard ('08), who found two nuclei associated with each double-cone in macerated material. My own observations on sectioned material confirm this relation. The proportions of rods to cones and to double-cones approximates 3.5 : 2.8 : 1, respectively, which is a decided increase in the relative number of cones over that given by Howard.

b. Outer nuclear layer. The outer nuclear layer, when examined in radial sections of the eye (figs. 1 to 4) was seen to consist of a complete single external row of nuclei with here and there additional nuclei lying directly against its inner side. In calculating the number of nuclei in the 'external' sheet of which the 'single row' is the section, I have used the method already employed for the visual cells. The numbers of nuclei per unit area in the external sheet are given in table 8. In the fundus the numbers are slightly less than in the other regions. In order to include the partial 'internal' sheet in my enumerations, I counted the nuclei in the external and internal rows in median sections from periphery to periphery in eight retinas. In the

TABLE 7

Total numbers of visual cells in retinas of minimum, maximum, and average sizes. The enumerations are based on the following average numbers of elements in a unit field of 0.065 sq. mm. area: rods, 255.4; cones 204.1; double-cones, 72.2; uncertain elements 8.7; total visual cells, 540.4 (table 6)

RELATIVE SIZE OF EYEBALLS	CALCULATED AREAS OF RETINAS IN SQUARE MILLIMETERS AT EX- TERNAL LIMITING MEMBRANE (TABLE 3)	NUMBER OF UNIT FIELDS IN RETINAS	TOTAL NUMBER OF				
			Rods	Cones	Double- cones	Uncertain elements	Visual cells
Minimum...	10.8184	166.437	42,508	33,970	12,017	1,448	89,943
Maximum....	15.1777	233.503	59,637	47,658	16,859	2,032	126,185
Average of 14 retinas.....	13.4698	207.228	52,926	42,295	14,962	1,803	111,986

external row there was an average of 422 nuclei, as compared with 121.1 in the internal row. Since the nuclei of the two layers have the same average diameters in the plane tangential to the surface of the eyeball, the ratio of the number of nuclei in the external layer to the number in the internal layer is 422 : 121. The total number of nuclei in each of these layers as well as in the whole outer nuclear layer is given in table 9. By comparing tables 7 and 9, the number of nuclei in the outer nuclear layer is seen to exceed the number of visual cells by about 10 per cent; or, if directly compared for total numbers of elements, there are 121,000 nuclei in the outer nuclear layer to 111,000 visual cells.

c. Ganglionic layer. The nuclei of the ganglionic layer, which in radial sections of the eye (figs. 1 to 4), are seen to be a single

TABLE 8

Number of nuclei of the outer nuclear layer per unit area of 0.0078 sq. mm.

DESIGNATION OF THE EYEBALLS	REGIONS OF THE EYEBALL IN WHICH THE FIELDS WERE LOCATED				
	Anterior	Posterior	Dorsal	Ventral	Fundus
7 <i>r</i> *					55.5
8 <i>l</i>					54
16 <i>r</i>		53.5			
21 <i>r</i>	51.5				
21 <i>l</i>			59		
22 <i>l</i>	60	60			
23 <i>r</i>					49
23 <i>l</i>				51.5	
24 <i>r</i>					47
24 <i>l</i>					43
25 <i>r</i>	42	60			
25 <i>l</i>			61		
26 <i>r</i>	58.5	59			
28 <i>r</i>			53	42.5	
31 <i>r</i>				57	
32 <i>r</i>			60.5	51	
38 <i>r</i>	49				
39 <i>l</i>				71.5	
40 <i>r</i>			56		
40 <i>l</i>		62			
Averages of 5 retinas	52.2	58.9	57.9	54.7	49.7

**r*, right eye; *l*, left eye.

layer thick, are larger, more rounded and less closely grouped than are the nuclei of the other retinal layers. Sections from the five visual regions (table 10) in five retinas show as before a slight variation in the average number of nuclei per unit area, but this I believe is due to accidental conditions and is not at all consequential. Contrary to the condition found in the outer nuclear layer, the number of nuclei in the ganglionic layer is greater in the fundus than elsewhere. In table 11 the average number of nuclei per area of 0.39 sq. mm. is given as 111.1 and the total number of nuclei in a retina of average size is about 30,000.

Thus we see that in passing centripetally through the layers of the retina there is a gradual decrease in the number of elements composing each layer, except in the inner nuclear layer, so that according to the views usually expressed, visual impulses arising in a number of visual cells are transmitted to the central organ over a greatly reduced number of elements.

d. Müller's fibers. Müller's fibers were counted to best advantage in tangential sections through the inner reticular layer close to the ganglion-cell layer, where they appeared as intensely red spots in a net-work of small light-red fibers. They occurred in pairs and triplets as well as singly (figs. 1 to 4). I have assumed that each fiber represents a cell with a nucleus lying generally in the inner nuclear layer. Slight variations in the number of

TABLE 9

Total numbers of nuclei of the outer nuclear layer in retinas of minimum, maximum, and average sizes

RELATIVE SIZE OF EYEBALLS	CALCULATED AREAS OF RETINAS IN SQUARE MILLIMETERS AT EX- TERNAL LIMITING MEMBRANE (TABLE 3)	AREA IN SQUARE MILLIMETERS OF UNIT FIELD	NUMBER OF UNIT FIELDS IN RETINAS	AVERAGE NUMBER OF NUCLEI PER UNIT FIELD IN EXTERNAL SHEET*	TOTAL NUMBER OF NUCLEI IN EXTERNAL SHEET	TOTAL NUMBER OF NUCLEI IN INTERNAL SHEET	TOTAL NUMBER OF NUCLEI IN OUTER NUCLEAR LAYER
Minimum....	10.8184	0.039	277.394	273.4	75,840	21,764	97,604
Maximum....	15.1777	0.039	389.172	273.4	106,400	30,558	136,958
Average of 14 retinas.....	13.4698	0.039	345.379	273.4	94,427	27,097	121,524

TABLE 10

Numbers of the nuclei of the ganglionic layer per unit area of 0.0078 sq. mm.

DESIGNATION OF THE EYEBALLS	REGIONS OF THE EYEBALL IN WHICH THE FIELDS WERE LOCATED				
	Anterior	Posterior	Dorsal	Ventral	Fundus
7 r*					31.5
9 r					19.5
16 r		25.5			
21 r	14	16.5			
21 l			19		
22 l	23				
23 r		23			23
23 l				16	
24 r					27.5
24 l	16.5				33.5
26 r	18.5	26.5			
28 r			18.5		
30 l				13.5	
31 r				25	
31 l			20		
32 r			25	24	
33 l				20	
38 r	18				
40 r			25.5		
40 l		32.5			
Averages of 5 retinas	18.0	24.8	21.6	19.7	27.0

*r, right eye; l, left eye.

TABLE 11

Total numbers of nuclei of the ganglionic layer in retinas of minimum, maximum, and average sizes

RELATIVE SIZE OF EYEBALLS	CALCULATED AREAS OF RETINAS IN SQUARE MILLIMETERS AT MIDDLE OF GANGLIONIC LAYER (TABLE 3)	SIZE OF UNIT AREA IN SQUARE MILLIMETERS	NUMBER OF UNIT AREAS IN RETINA	AVERAGE NUMBER OF NUCLEI IN FIVE UNIT AREAS	TOTAL NUMBER OF NUCLEI OF GANGLIONIC LAYER
Minimum.....	8.6916	0.039	222.861	111.1	24,760
Maximum.....	13.0690	0.039	335.102	111.1	37,230
Averages of 14 retinas.....	10.7910	0.039	276.723	111.1	30,744

fibers per unit area occurred, but not sufficiently great to call for more than passing notice. In table 12 I have given the average numbers of fibers per unit area and the average numbers for five retinas in each of the selected regions. The fibers were counted in unit areas, in some cases in right and left eyes of the same animal, and in others in two regions in the same eye; but I could detect no characteristic differences. In table 13 the average number of fibers in five unit areas is given as 97.5 and the total number in the whole retina is between 21,729 and 32,672.

e. Inner nuclear layer. The inner nuclear layer presents in Necturus a variable condition, which makes it, of all the retinal layers, the most difficult in which to count the nuclei. It con-

TABLE 12
Number of Müller's fibers per unit area of 0.0078 sq. mm.

DESIGNATION OF EYEBALLS	REGIONS OF THE EYEBALL IN WHICH THE UNIT AREAS WERE LOCATED				
	Anterior	Posterior	Dorsal	Ventral	Fundus
7 <i>r</i>					21
8 <i>r</i>			21		
16 <i>r</i>		24			
17 <i>l</i>				24	
21 <i>r</i>	18	13			
21 <i>l</i>			17		16
23 <i>r</i>		21			13
24 <i>r</i>					21
24 <i>l</i>	22.5				20
25 <i>l</i>	13				
26 <i>r</i>	22				
28 <i>r</i>			15		
30 <i>l</i>				16	
31 <i>r</i>				20.5	
31 <i>l</i>			19		
32 <i>r</i>				24	
33 <i>l</i>				21	
38 <i>r</i>	20				
22 <i>l</i>		19.5			
40 <i>r</i>			18		
40 <i>l</i>		27			
Averages of 5 retinas	19.1	20.9	18.0	21.3	18.2

r, right eye; *l*, left eye.

TABLE 13

Total numbers of Müller's fibers in retinas of minimum, maximum, and average sizes

RELATIVE SIZE OF EYEBALLS	CALCULATED AREAS OF RETINAS IN SQUARE MILLIMETERS AT MIDDLE OF GANGLIONIC LAYER (TABLE 3)	SIZE OF UNIT AREA IN SQUARE MILLIMETER	NUMBER OF UNIT AREAS IN RETINA (TABLE 11)	AVERAGE NUMBER OF FIBERS IN FIVE UNIT AREAS	TOTAL NUMBER OF MÜLLER'S FIBERS
Minimum.....	8.6916	0.039	222.861	97.5	21,729
Maximum.....	13.0690	0.039	335.102	97.5	32,672
Averages of 14 retinas	10.7910	0.039	276.723	97.5	26,980

sists in its usual form of three layers of nuclei (fig. 1), of which the middle one has fewer nuclei than either of the other two. In addition a large part of the nuclei of Müller's fibers lie within it (figs. 1 to 3). The number of layers may be as numerous as four (fig. 3), or even five, or they may be as few as two (fig. 2); in one case I found only a single layer in a part of one side of the retina. Such variation makes any estimate of the number of cells unsatisfactory. Since the most frequent condition is the three layered one, I have determined the number of nuclei in a zone of this kind. Figs. 1 to 4 show that there is little difference in the 'tangential' diameters of the nuclei of the outer and inner nuclear layers. With any increase or decrease in the average tangential diameter of the nuclei of one of these layers over that in the other, the number of nuclei in a given belt, such as I have used in the inner nuclear layer, would be found to vary and I could not then make the proportion as stated, but if the average tangential diameters of the nuclei of the two layers is the same the relations between the two would be constant and my results would hold true. Since this is the case, the total number of nuclei in the two layers respectively should be nearly proportional to the number of rows as seen in radial section, and the area of the zone. If, then, the number of nuclei in the outer nuclear layer is 121,000 and this number varies as the number of rows and the area of the zone, it varies as their product, and the number of nuclei in the typical inner nuclear layer in the ret-

ina of average size should be about 167,000. This is a variation from the average estimates of two retinas of less than 5 per cent, which is negligible when we consider that the numbers in minimum and maximum retinas vary nearly 45 per cent of the former.

Since Müller's fibers are regarded as merely supporting structures and not a part of the nervous mechanism of the retina, they have been excluded from the final count of the nuclei properly belonging to the inner nuclear layer. The data for the inner nuclear layer of minimum, maximum, and average sized retinas are found in table 14. The extreme lower limit for the number of nuclei in this layer is approximately 97,000.

TABLE 14

Total numbers of nuclei of the inner nuclear layer in retinas of minimum, maximum, and average sizes

RELATIVE SIZE OF EYEBALLS	CALCULATED AREAS OF RETINAS IN SQ. MM. AT MIDDLE OF INNER NUCLEAR LAYER, (TABLE 3)	CALCULATED NUMBER OF CYLINDERS IN RETINA	NUMBER OF NUCLEI PER CYLINDER			NUMBER OF NUCLEI		TOTAL NUMBER OF NUCLEI IN INNER NUCLEAR LAYER
			Retina 1	Retina 2	Average	In inner nuclear layer exclusive of Müller's fibers	Of Müller's fibers, (table 13)	
Minimum.....	9.7028	1243.948	121	142	131	141,228	21,729	162,957
Maximum.....	14.0721	1804.115	121	142	131	203,667	32,672	236,339
Averages of 14 retinas	12.8034	1549.153	121	142	131	175,959	26,980	202,937

f. Optic nerve fibers. As I have already said, the optic nerve was transected in two planes, viz., near its two ends, and the difference in the cross-sectional areas in these two regions was clearly established. When the fibers in the two regions were studied, those in the proximal portion seemed smaller and slightly more numerous to a unit area than in the distal portion. This led me to suppose that the total number of fibers was less proximally than distally, and my opinion was borne out by actual counts in the two regions in two optic nerves. Examination of other optic nerves indicated that similar numerical relations of the fibers existed in the two regions and was a characteristic feature of the optic nerve of Necturus. All counts were made on cross-sections of the optic nerves. Outline camera drawings ($\times 755$) were made of cross-sections together with the nuclei

of the supporting tissue, all the largest fibers, and all the prominent markings of the optic nerve carefully sketched. The drawing was then marked off with coördinating lines and the rest of the optic nerve fibers were drawn in place and counted. By practice in counting I was able to make out and record the fibers in the sections with accuracy and speed.

I have counted the fibers in three of the nerves given in table 15 twice each. The results of the second counts were in each case so close to the first counts that I did not think it necessary to continue the process for every nerve; thus, in nerve no. 1 (fig. 12) I counted 2001 fibers on the first trial and 2003 on the second; in nerve no. 2, 1657 fibers on the first count and 1661 on the second;

TABLE 15
Areas of cross-sections of optic nerves and numbers of nerve fibers counted at different planes of transection

DESIGNATION OF NERVES	CALCULATED AREA IN SQUARE MILLIMETERS OF CROSS-SECTIONS OF THE OPTIC NERVE		TOTAL NUMBER OF FIBERS IN CROSS-SECTIONS	
	Close to eye	Close to chiasma	Close to eye	Close to chiasma
1	0.017		2002	
2	0.019		1659	
3	0.019	0.008	1780	853
4	0.026	0.009	1858	1071
5	0.027		2613	

and in nerve no. 5, 2616 fibers on the first count and 2611 on the second. The number of fibers appearing in table 15 for these optic nerves are averages obtained from the two counts. The complete results of my enumerations, together with the areas of the cross-sections of the optic nerve, are given in table 15. The average area near the eye was 0.0216 sq. mm. and contained by actual count an average of 1982.4 fibers or about 92,000 per square millimeter; the average of the cross-sections near the chiasma was 0.0085 sq. mm. with an average of 962 fibers, or approximately 113,000 per square millimeter. Although the proximal portion of the optic nerve is smaller in diameter than the distal part, it is, on the other hand, seen to be richer in fibers per unit area.

V. DISCUSSION

My intention in this investigation has been, as far as possible, to make a consistent enumeration of all the nervous elements in the retina and optic nerve in a single species of animal, and I have believed the logical choice of species to be some animal whose retinal cells are very large and few in number. Such an animal was naturally sought among the amphibians, and especially among those species whose eyes and optic nerves have undergone reduction in size, probably through diminished functioning powers, and whose histological elements were well known to be of unusual size. In *Necturus* I have found an animal in which both eyeballs and optic nerves are reduced in size; the retinal elements are extremely large, if not the largest known; and the optic nerve fibers are entirely non-medullated.

The nearly spherical shape of the eyeball in *Necturus* and the close application of the retina to the sclera have made the measurements of the retina comparatively easy and have led, I believe, to accurate results. The method employed has been described on page 417. The individual retinal cells are so large and free from contact with one another that I have been able to count and number the individual cells in each unit area. Following this the application of the unit area to the area of the retina has been a matter of simple mathematical calculation.

An attempt to estimate the total number of visual cells in the retina of *Necturus* by counting the rods, cones, and double-cones in a median section of the eye extending from periphery to periphery gave a total of 143,000 visual cells for an average sized retina. This number exceeds that found by the method finally adopted by about 31,000 cells or 28 per cent. Since in the latter method every element per unit area was counted and averages obtained as already stated, and the number of areas in a given retina is not subject to variation, I am convinced that the number of retinal cells in *Necturus* is close to the number obtained by this means, and that the method of counting elements in a line as a basis of enumeration of the visual cells should be rejected on the ground that it gives too large a number of cells.

Where the retinal cells are very small and numerous the difficulties of obtaining accurate counts are greatly increased. Hess ('05) has demonstrated in the retinas of *Eledone* and *Sepia* the great variation which exists in the number of visual cells in closely approximated regions in the same retina, and thereby implies the impossibility of obtaining reliable estimates of the total number of visual cells in a retina from the number in a given 'belt.'

It is commonly believed that the retinal cells in vertebrates are not evenly distributed over the retina. Franz ('05, '09) states that the visual cells are more numerous about the fundus than near the periphery; Howard ('08) calls attention to the double layer of nuclei near the periphery in the outer nuclear layer in *Necturus*; and my own observations on the same species show a slight variation in the numbers of the different elements in the different regions. Consequently I believe that estimates based on counts in a restricted region, as for example, the fundus, or the periphery, give no fair idea of the number of elements in the retina as a whole, and my observations show that estimates based on counts of elements in a line of definite length exaggerate the number of cells in a given retina. Nor can I accept Pütter's ('02) method, viz., that of computing from the diameter of a rod the number of visual cells that may be found in a square millimeter and in the retina as a whole. Figs. 4 to 9 show that in *Necturus* the interstices between the visual cells make up a large portion of the area of a zone passing through the outer segment of the visual cells, and these spaces must be considered in the retinas of all species of vertebrates. Without consideration of these spaces the number of elements obtained would greatly exceed the actual number present.

In order to avoid, then, what appear to me to be errors of method, I have taken great care to secure accurate counts per unit area in a number of regions of the retina in several animals. The retina of *Necturus* lends itself to a count of this kind, because the visual cells, the external sheet of the outer nuclear layer, Müller's fibers, and the ganglion cells are each represented by a single layer of nuclei.

The fovea, when present in the amphibian retina, is small. I have not been able to find it in *Necturus*, and Howard ('08) makes no mention of such a 'spot,' but Hulke ('67) and Chievitz ('91)² have seen it in *Triton* and *Salamandra*. The frequent grouping of 6 to 8 cone cells (figs. 5 to 9) in different parts of the retina of *Necturus* may, perhaps, compensate for the absence of a definite fovea.

Enumerations of nuclei in the outer nuclear layer (tables 8 and 9) bring out several important features. On the ground that each visual cell has its own nucleus there should be in the outer nuclear layer a number of nuclei equal to the number of rods and cones together, unless elements of another kind should be found in either or both the layers compared. The nuclei of the outer nuclear layer were found to outnumber the rods and cones by about 10,000. Undoubtedly a majority of the nuclei in the internal layer together with all those in the external layer should be associated with the visual cells; but there is a characteristic group in the internal layer, having their long axes at right angles to the long axes of the nuclei of the visual cells, which appear to me to have a different function. They may, perhaps, be horizontal cells, which Ramón y Cajal ('94) states are represented in amphibians by large and small nuclei at two different levels and constitute the outermost part of the inner nuclear layer. There are, too, in the internal layer a small number of Müller's-fiber nuclei (fig. 4) whose identity is clearly established by their heavy stain and fibers. A fourth kind of nucleus may occasionally wander into this layer. Fig. 2 shows how the outer limits of the inner nuclear layer may be thrown out of line and individual nuclei be protruded far into the outer reticular layer. This would seem to favor Bernard's ('00) contention that the nuclei of the retinal layers are constantly migrating outward to aid in the formation of new elements in the more outwardly lying layers. I have been able to observe an apparent migration of this kind only in the case cited.

Conditions in the inner nuclear layer show that it is the most variable of all the layers in respect to the number of its elements.

² See Slonaker, 1897.

I have called attention to the three-layer condition of its nuclei as the most characteristic condition, but two or four layers were not uncommon. Ramón y Cajal ('94) points out that the inner nuclear layer of amphibians consists of a number of 'types' of both amacrine and bi-polar cells, but I have made no attempt in this study to distinguish between them, and have considered the layer merely as a whole. Though the results obtained cannot be adapted to every retina because of the great individual variation, I am convinced that they are as accurate as possible for the three-layered average sized zone.

The ganglion-cell layer presents few features of special note in connection with the number of its nuclei. It consists uniformly of a single layer of loosely associated, large nuclei. A consideration of vital importance is the failure of Bielschowsky's impregnation method to demonstrate a union between the ganglion cells and the optic nerve fibers. Just what the real significance of this is, I am unable to say, and, when considered in relation to the number of optic nerve fibers in proximal and distal parts of the optic nerve, the interpretation becomes even more difficult.

Enumerations of the cross-sections of the strands of Müller's fibers per unit area in the inner reticular layer gave better results than enumeration of their nuclei per unit area in the inner nuclear layer, because at the plane of sectioning in the reticular layer there is no other structure with which they might be confused; secondly, the fibers stand out clearly in cross-section, and each fiber may be supposed with reasonable certainty to represent a complete unit; and, lastly, the chance of missing even a single fiber in the unit areas, was very small.

The objections to using the nuclei as a means of enumeration lie in the difficulty of distinguishing the nuclei of Müller's fibers from other nuclei in the layer; and secondly, in the fact that the nuclei of Müller's fibers are not always found within the limits of inner nuclear layer (fig. 4), in which case it would be impossible to count with certainty all the cells in a given field.

Enumerations of the optic nerve fibers were more tedious than difficult. The black ends of the fibers contrasted sharply with

the surrounding tissues. Over-blackened margins and the apparent fusion of occasional fibers did not prove to be insurmountable difficulties, for by focusing, the separate fibers could usually be distinguished, so that I believe the number of fibers counted in a cross section is very close to the actual number present.

In addition to variations in diameter in a given nerve, there are also individual variations which are considerable. Measurements near the chiasma are fairly uniform, but at the distal end of the nerve I found a range in area of 62.9 per cent. The number of fibers here correlates in some degree with the cross-sectional areas of the nerves. Thus in table 15, nerve no. 2, with a cross-sectional area of 0.019 sq. mm. has an average of 1659 fibers, and nerve no. 5, with an area of 0.027 sq. mm., has an average of 2613 fibers.

The number of fibers in other nerves than the optic nerve of *Necturus* have been counted or estimated in some instances. From Salzer's ('80) calculations there are between 60,000 and 70,000 fibers per square millimeter in the human optic nerve; Birge ('82) has counted 3550 fibers per square millimeter in the 7th spinal nerve of the frog, and 14,133 in the 10th; Hatai ('03) found a variation in the number of fibers in the spinal nerves of the white rat according to the position of the plane of transection, there being more in the proximal than in the distal planes, which is the reverse of the condition in the optic nerve of *Necturus*; Donaldson and Bolton ('91) record an average of 11,900 fibers to every square millimeter in the dorsal roots of the spinal nerves of man; Dunn ('00) has found that the total number of fibers innervating the hind foot of the frog is between 5000 and 6000; and on page 430 I have shown that in the optic nerve of *Necturus* there are about 100,000 fibers per square millimeter.

Thus we see that, although in general the visual apparatus of *Necturus* is described as degenerate, and the animal's habits have apparently brought about a reduced functional activity of the visual cells, the optic nerve appears to have more nerve fibers per unit area than any other nerve so far studied.

VI. THEORETICAL CONSIDERATIONS

Theoretical consideration of the optic functions is not the primary object of my investigation, and I shall enter into it only so far as my research indicates exceptional conditions in *Necturus*.

I have found in the visual cells that rods and cones alike develop at the extreme margin of the retina, the initial element being indifferently a rod or a cone. Since this is so, whatever differences there may be in the light perceiving potentialities between central and peripheral regions, they must depend largely on the degree of the functional development of the visual cells in these regions. The presence of rods and cones alike over the whole retina—if the theory that rods are organs for light and shadow perception and cones for color preception is true—indicates power to distinguish colored lights as well as light and shadow at the periphery as well as in the fundus. In this connection Reese ('06) found that *Necturus* responded to both red and blue lights, but since no analysis of the lights for purity of color or intensity was made, it is not evident to what the reactions were due. Pearse ('10) has shown that *Necturus* reacts readily to ordinary light stimuli through both the eye and the skin. From these investigations it appears that *Necturus* is capable of distinguishing colored as well as white light, but on this point there is need of further investigation.

I know of no positive evidence that horizontal cells exist in the retina of *Necturus*, but I know of no other explanation for the group of cells referred to on page 423 (figs. 2 and 3, *n*). The arrangement of the nuclei in the inner nuclear layer seems to me to preclude the existence of such cells in that layer. Since I have already shown that the nuclei of Müller's fibers may migrate or be pushed outward in some way from the inner nuclear layer into the outer nuclear layer, it is possible that a similar change in position has taken place with the horizontal cells, and that in time they have become permanent constituents of the outer nuclear layer. Investigation into the identity of these cells by Golgi's, or some other stain equally valuable for nerve processes, is very desirable.

Because of the great variability of the inner nuclear layer, I cannot help wondering what the influence of such variation may be on the sight of the animals. Can animals with five layers of cells in this region see better than those with two? Or does the number of cells involved have no effect whatever on the clearness of vision?

Enumerations of the optic nerve fibers in cross-sections of the optic nerve show that distally there are nearly double the number of fibers that there are in the proximal portion; a condition which suggests at once a dichotomous division of the axis-cylinders. Such an interpretation of the increase of the fibers distally would mean that a majority of the optic nerve fibers in *Necturus* have their origin in the brain, which is the reciprocal of the condition found by His ('90), Assheton ('92), and Robinson ('96) in other vertebrates. But Robinson also states that the optic nerve fibers arise in the retina and are more numerous near the retina even when the fibers have come to occupy the entire length of the optic stalk. The implication is that some of the fibers fail at this stage to reach the central organ.

In my treatment of the optic nerve of *Necturus* with Bielschowsky's fluid, I have been unable to demonstrate a morphological connection between the ganglion cells and the optic nerve fibers, but the fibers seem to pass between the nuclei of the ganglion cells and enter the inner reticular layer. If the fibers can in any way be shown to be joined to the ganglion cells, then we should have in the adult *Necturus* a condition similar to that found by Robinson in the embryos of higher vertebrates.

Since a direct union, then, between the optic nerve fibers and the ganglion cells is not an established fact, the origin of the fibers is a matter of speculation. Do the fibers originate wholly in the brain and pass centrifugally to the retina, branching within the optic nerve and ending freely in the retina? Or do they have their origin chiefly in the retina, in spite of the facts that staining with Bielschowsky's fluid fails to show positive connection between the ganglion cells and the fibers, and that only a half of them reach their destination in the brain? In consideration of these questions the influence of the degeneracy of the

eye, and the fallibility of the stain must be given due weight. I am unwilling to make any statement as to the origin of the optic nerve fibers in *Necturus*, and await with interest further investigation of this subject by other methods.

VII. SUMMARY

1. The proportion of rods to cones is about the same in all regions of the retina. Double-cones are wanting at the extreme periphery.

2. There is no particular plan of arrangement of the visual cells.

3. In the retina of average size there are about 110,000 visual cells, of which 53,000 are rods, 42,000 cones, and 15,000 double-cones.

4. The total number of visual cells varies with the size of the retina. In maximum sized retinas the number of visual cells is about 126,000; in minimum sized retinas, about 90,000.

5. The number of visual cells is less than the number of nuclei in the outer nuclear layer.

6. The total number of nuclei in the outer nuclear layer in an average-sized retina is 121,000. The number varies from 137,000 in maximum sized retinas to 97,000 in minimum sized retinas.

7. The outer nuclear layer consists of two sheets; an external complete, and an internal loosely scattered sheet, with 94,000 and 27,000 nuclei, respectively, in the average-sized retinas.

8. The nuclei of the internal sheet of the outer nuclear layer consists of nuclei of the visual cells, of Müller's fibers, and possibly of horizontal cells.

9. The inner nuclear layer is the most variable of all the layers in the number of its elements. In an average sized retina there are approximately 176,000 nuclei in a layer composed of three sub-layers. The range in the number of nuclei in maximum and minimum retinas of this structure is 204,000 to 45,000.

10. The inner nuclear layer may have as many as five sub-layers or as few as two.

11. Nuclei of Müller's fibers lie chiefly in the inner nuclear layer, but a small number may be found in the outer nuclear layer. In an average sized retina there are about 26,734 of these nuclei, the maximum and minimum numbers being 33,000 and 22,000, respectively.

12. The number of ganglion cells in the smallest retina measured, was 24,758; in the largest, 37,229; and in an average-sized retina 30,464.

13. The optic nerve of *Necturus* varies in diameter with the plane of transection. Near the chiasma its cross section has on the average an area of 0.0085 sq. mm., and an average of 962 nerve fibers; near the eyeball the average area of the cross section is 0.0216 sq. mm., with an average of 1982 nerve fibers.

14. The proportion of histological elements of the retina and optic nerve are approximately as follows: visual cells 111, nuclei in the outer nuclear layer 121, nuclei in the inner nuclear layer, exclusive of Müller's fibers, 175, ganglion-cell nuclei 30, Müller's fibers 26, optic nerve fibers distally 2, optic nerve fibers proximally 1.

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EXPLANATION OF FIGURES

The drawings are all from *Necturus maculosus* (Raf.) All the outlines in figs. 1 to 12 were made with the aid of the camera lucida. The sections of the retina were stained in Heidenhain's iron haematoxylin followed by a 70 per cent alcoholic solution of eosin. Radial sections of the visual cells (figs. 1 to 4) were cut 8μ thick, and cross-sections (figs. 5 to 9) 6μ thick.

The optic-nerve fibers were stained in Bielschowsky's fluid in every case, and all sections were 5μ thick.

Fig. 1 Semi-diagrammatic drawing of a radial section of a normal retina of *Necturus*; *cd*, *xz*, *vw* designate three zones in which the areas of the retinas were obtained; *ab*, *mn*, *xz* and *vw* designate the 'levels' in which average counts, per unit areas, were obtained for the different layers of the retina. $\times 331$.

Fig. 2 Semi-diagrammatic drawing of a radial section of a retina with two sublayers or 'sheets' in the inner nuclear layer. *n*, horizontal cell. $\times 331$.

Fig. 3 Semi-diagrammatic drawing of a radial section of a retina with four to five sublayers in the inner nuclear layer. *n*, horizontal cell. $\times 331$.

Fig. 4 Semi-diagrammatic drawing of a radial section of a retina with two nuclei, *m*, of Müller's fibers, in the outer nuclear layer. $\times 331$.

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Figs. 5 to 9 Semi-diagrammatic drawings of cross-sections of the visual cells in five regions of the retina. Fig. 5, anterior; fig 6, posterior; fig. 7, fundus; fig. 8, dorsal; fig. 9, ventral; *r*, rod; *c*, cone; *cd*, double-cone. $\times 232$.

Fig. 10 Semi-diagrammatic drawing of a cross-section of the optic nerve (no. 4, table 15) near the eyeball. $\times 431$.

Fig. 11 Semi-diagrammatic drawing of the same optic nerve near the chiasma. *o*, lumen of the nerve. $\times 431$.

Fig. 12 Semi-diagrammatic drawing of a cross-section of nerve (no. 1, table 15) near the eyeball. $\times 485$.

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