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HISTOLOGY OF THE RETINAS OF THE PACIFIC SHARKS
CARCHARHINUS MELANOPTERUS
AND
TRIAENODON OBESUS

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF THE
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INTRODUCTION

This thesis is concerned with the retinal histology of two species of sharks which have wide Indo-Pacific distribution, the blacktip shark *Carcharhinus melanopterus* Quoy and Gaimard of the family Carcharhinidae, and the whitetip shark *Triaenodon obesus* Ruppell of the family Triakidae. The common names, blacktip and whitetip, will be used throughout this report.

The investigation was conducted at the Eniwetok Marine Biological Laboratory on Eniwetok Atoll, Marshall Islands, and at the Hawaii Marine Laboratory located in Kaneohe, Oahu, from January, 1960 to August, 1961.

The primary aims of this study were: (1) to describe the retinal structures and organization of the two species, (2) to determine whether their retinas bear cone visual cells, and (3) to study changes that occur in their retinas during development. An additional goal of this study was to investigate various fixatives, stains, and procedures, to determine which combination was best suited for the microscopic examination of the shark retina.

Although all parts of the vertebrate visual system are involved in visual physiology, the structural organization of the retina has long been known to be a good indicator of the visual capabilities of vertebrates (Detwiler, 1943). A close relationship exists between the cellular structure and organization of the retina and the visual capability
Acuity, sensitivity, and wave-length discrimination, for example, are visual functions associated with certain structural elements and retinal organization. According to Walls (1942), visual acuity is dependent on the diameter of the visual cells, the proximity in their spacing, and the number associated with an optic nerve fiber. Thus the more visual cells contained within a unit area, and the more bipolar and ganglion cells, the better the acuity. Sensitivity, on the other hand, is dependent on the size of the outer segments of rods, the amount of rhodopsin contained within them, and the extent of summation within the layers of retinal cells. Hence there is a mutual exclusion of these functions. This problem has been resolved in many vertebrates by having in the retina one or two specialized areas containing slender visual cells and an increased number of bipolar and ganglion cells (Walls, 1942). These specialized areas are then equipped for good acuity, but are less sensitive than other parts of the retina which typically contain many visual cells and few bipolar and ganglion cells, and hence have high sensitivity (Detwiler, 1943 and Walls, 1942). Polyak (1941) states that all vertebrate classes, though not all species, have such a specialized region in the retina, called the area centralis, that is "... distinguished by the accumulation of nerve cells and photoreceptors and the general refinement of structure."
In regard to wave-length discrimination, Brett (1957) in a review of the literature, reported that cone visual cells were present in the retinas of all vertebrates known to have the ability to discriminate between different wave lengths of light. There is some controversy in connection with the implications (although they may not have been intended as such) of Brett's statement: that cones, and not rods, are the mediators of wave-length discrimination, and that animals without cones do not have the ability to discriminate between different wave lengths of light. This will be discussed later.

The literature on the histology of the shark retina will be reviewed in a later section when the results of this investigation are compared with those of others. It might be noted here that many retinologists (Frans, 1913, Rochon-Duvigneaud, 1943, Verrier, 1929, and Kolmer, 1936, etc.) agree that sharks have relatively simple retinas which are organized for efficient use of low intensities of light, but which have only minor if any, adaptations for visual acuity.
MATERIALS AND METHODS

Sharks were caught on hook and line, either by casting or by set lines. All whitetips and most blacktips were collected at Eniwetok Atoll in the summer of 1960. Additional blacktip adults and embryos were collected at Palmyra Atoll in January, 1960. Several species of sharks were also collected at Oahu between March, 1959 and November, 1960. Their eyes were used in the study of microtechnical methods.

Preparation of the eyes prior to fixation

All shark eyes were excised while the animals were still alive. In some cases an anesthetic (tricaine methanolsulfate) was used before dissection. Both light and dark-adapted eyes were preserved. In the latter case, the sharks were kept in a dark tank for at least three hours, and all work was done under a deep-red heat lamp.

Prior to fixation, each eye was operated upon in one of three different methods used by retinologists (Polyak, 1941, Greff, 1899, etc.): (1) "dissection", in which the eye was excised, the cornea, iris, and lens removed, and the eye immersed in the fixative; (2) "piercing", in which the eye was excised, the cornea and iris pierced with a scalpel, and the eye immersed in the fixative; (3) "injection", in which the eye was excised, the cornea and iris pierced with a scalpel (or a small "window" made in the cornea with a pair of scissors), and the fixative injected
into the vitreal cavity through the optic nerve and papilla. Alternately, an 18 gauge hyperdermic needle was inserted into the vitreal cavity through the ventral part of the cornea, and the fixative was injected slowly into the vitreal cavity with a 22 gauge needle through the dorsal part of the cornea; the eye was then excised and immersed in the fixative.

A total of 43 eyes from blacktips, whitetips, and two species of Carcharhinids from Oahu, were examined in the investigation of microtechnical methods. Of 35 adult and juvenile eyes examined, 13 were dissected, 8 pierced, and 14 injected. In addition, 3 embryonic eyes were dissected, 3 pierced, and 7 injected.

Piercing the cornea with a scalpel before fixation produced the best results. The chief advantages of this method were: the operation was quick and simple; the intact vitreous humor gelled firmly and kept the retina in place, and also protected the retina; intraocular pressure was avoided. The dissection method offered only the advantage of a faster diffusion rate of the fixative which was already adequate with piercing. The injection method introduced the problem of intraocular pressure which sometimes damaged the delicate retina. This danger existed even after a careful and successful operation because the needle-holes tended to close when the eye was immersed in the fixative, and diffusion of water into the vitreal cavity caused swelling.
(as evidenced by the turgidity of the embryonic eyes). Another disadvantage was the reduction in diffusion rate of the fixing, washing, and drying agents.

**Fixatives**

Of the large number of fixing fluids used by retinologists (Polyak, 1941, McEwan, 1938, Lee, 1950, etc.), the following were selected for this study: Kolmer, Susa, Zenker, Helly, Zenker-formal, Bouin, and FWA (Flemming's fixative without acetic acid). The formulas for these fixatives are given in the reference texts dealing with micro-technique.

In general, none of the fixatives yielded completely satisfactory results. Retinal structures, especially the visual cells, were often distorted or damaged. However, the poor results were not necessarily the fault of the fixatives. Handling of the retina during dissection, and perhaps some factors involved in the paraffin process, probably also contributed to the poor results.

Kolmer and Susa's fixatives consistently yielded the best results of all the fluids used. Both produced smooth, non-brittle retinas, with little or no shrinkage (which was roughly measured by the distance the retina had retracted from the choroid). Cell structures, including the rods in some cases, were preserved well, and most stains were compatible with these fixatives. One drawback with Kolmer and Susa's fixatives however, was the formation of many large
globular inclusions in all layers of the retina. These artifacts were not found with other fixatives, and were also absent in teleost fish eyes which were fixed in Kolmer and Susa’s fluids. Thus they were specific to shark eyes and with only these two particular fixatives.

Bouin, Zenker, and Zenker-formal fixatives were less satisfactory. All produced heavy wrinkling, shrinkage, and/or brittleness. However, the condition of the cells was only a little poorer than with Kolmer and Susa’s fixatives.

Results with FWA were completely unsatisfactory. The retinal layers were poorly preserved and failed to stain properly. Inasmuch as Bayliss (1936) found this fixative especially suitable for shark retinas, the results of the present study must be attributed to unknown differences in technique.

**Procedure following fixation**

All eyes were kept in the fixing fluids for 24 hours and then washed in running water for an equal length of time. They were then dehydrated and hardened by passing them through a graduated series of ethanol baths which were made increasingly stronger in concentration (20, 35, 50, 70, 80, 90, 95, and 100 per cent ethanol). Bouin-fixed eyes were transferred directly from the fixative into 70 per cent ethanol. Eyes fixed in solutions containing mercuric chloride were treated with a 70 per cent ethanol-iodine solution
in order to remove precipitates of mercuric salts. Following complete dehydration in 100 per cent ethanol, the eyes were cleared and preserved in cedarwood oil.

The eyes were cut open about midway between the front and back with a razor blade. The vitreous humor was removed and the retina was inspected for wrinkling, shrinkage, and brittleness. The sclera, or sclera and choroid, were then removed from the back half of the eye and the retina was divided into dorsal and ventral or nasal and temporal halves. In some cases, only small pieces of retinal tissue from particular regions of the eye were kept.

The tissues were imbedded in paraffin at 60° C. In all cases, left and right eyes had been marked during fixation. Orientation of the retinal tissues in paraffin was facilitated by making distinctive cuts in the corners of the tissues.

Serial sections were cut in two planes, "vertically" and "flat", with respect to the retinal layers. Vertical sections are defined as those that cut through all layers of the retina, while flat sections are those that are cut parallel with the retinal layers. All flat sections were cut at 4 µ, while vertical sections were cut at 4 µ and 7 µ. All flat sections were affixed on 25 x 75 mm. glass slides, but only alternate rows of about ten sections each, of the vertical serial sections were kept.
Stains and staining

As in the case of the fixatives, the stains employed were those recommended by various workers (Wolff, 1940, McEwan, 1938, Lee, 1950, etc.). The primary purpose for using a variety of stains was to attempt to insure the detection of cone visual cells if they were present in the retinas of blacktip and whitetip sharks. The so-called "differential stains" used here have been successfully employed with teleost or other vertebrate retinas, coloring the rods and the cones and/or their nuclei differentially (Wolff, 1940 and McEwan, 1938). In order to test the utility of the differential stains, and to facilitate interpretation of the results, cone-bearing retinas of teleost fishes were stained along with the shark retinas. The teleost fishes used were: Chaetodon auriga, a diurnal fish, both light and dark-adapted, Apogon sp., a nocturnal fish, and Conger marginatus, a nocturnal eel with a cone-free (?) retina.

In addition to five differential stains, three non-differential stains were used in an attempt to find a fixative-stain combination that afforded good detail of retinal structures. The differential stains were also tried with different fixatives with this aim in view.

The use of combinations of stains produced variable results due to slight differences in timing, condition of the stain, etc. In order to make meaningful comparisons,
the slides of tissues to be compared were carried together in a single stain rack. Thus, to test fixative-stain combinations, for example, slides of retinas fixed in Kolmer, Susa, and Bouin's fluids, etc., were all stained together in Mallory's triple or other stain.

Slight differences in staining ability were found between blacktip and whitetip retinas. The whitetip retinas tended to stain more feebly, especially with Mallory's triple stain, and appeared to have greater affinity for Orange G stain.

The results obtained with the various differential stains are given below. In each case, the name of the stain is followed by the name of the author whose particular modification was used. Although general procedures on the application of the stains were followed according to the authors' suggestions, the concentration of the stains and the staining times were usually altered. A higher concentration of dye and/or a longer staining time was usually necessary for adequate staining of shark retinas.

(1) Mallory's triple stain. Pantin (1946) and Heidenhain's modification (in Cowdry, 1952). This stain, following Kolmer's fixative, produced the best results of all combinations of fixatives and stains used, both with shark retinas and teleost retinas. (See Figures 8, 9, and 11). Although individual cellular elements were not always defined precisely, the various retinal layers and most nuclei
were clearly indicated by different colors.

Wolff (1940), using Mallory's triple stain after Zenker fixation found that in human retinas the inner segments of cones were stained red while the rod inner segments were stained blue. These results were not duplicated with the teleost fish and shark eyes used in this study, although a differential staining was achieved. Instead, teleost cones were stained orange to orange-red while the rods were stained light purple. Individual rods were not seen but dark-adapted Chaetodon retinas had a purplish coloration just above the external limiting membrane, presumably because the rods were retracted, while the cones which had migrated toward the choroid were recognizable individually. Although the outer segments of shark rods were stained blue, the inner segments were usually stained purple.

Kolmer (in Wolff, 1940) used this stain to color cone nuclei red and rod nuclei orange. Since the fixative was not specified, a number were tried. Shark rod nuclei were sometimes stained orange, sometimes red, and even purple with Mallory's triple stain following Kolmer fixation. (Figures 8 and 9.) Teleost rod nuclei were stained dark orange to red, in contrast with Kolmer's red cone nuclei. (Figure 11.) Individual cone nuclei were not seen, but all nuclei appeared to be stained uniformly.

Bouin's fixative followed by Mallory's stain yielded similar results with shark eyes as Kolmer's fixative.
After Susa's fixative, all nuclei of both sharks and teleosts were stained orange and the cytoplasm a vague pink, so that this combination was of little value.

After Helly, Zenker, and Zenker-formal fixatives, teleost rod nuclei were stained orange to red but shark rod nuclei were stained purple to blue. Definition of detail ranged from fair to good.

Similar results were obtained with both Pantin (1946) and Heidenhain's (in Cowdry, 1952) modifications of Mallory's triple stain. The latter was more convenient to use because of its simpler technique, only one step being required in the process, and was better in that the results were more consistent. Teleost retinas stained well in the recommended time of 5 minutes, but shark retinas had to be immersed in the stain for 45 to 60 minutes in order to achieve sufficient coloration.

(2) Heidenhain's azan stain. Cowdry (1952) and Gurr (1956). McEwan (1938) working with teleost eyes found that azan following Susa's fixative stained the inner segments of rods blue, the cone lentiform body red and myoid blue, and all outer segments gold. An attempt to reproduce these results proved unsuccessful, as all inner segments of both teleost and shark retinas were stained a uniform purple. The failure can probably be attributed to differences in techniques or composition of the different dyes.

Kolmer (in Wolff, 1940) reported that this stain
produced results similar to Mallory's triple stain, cone nuclei being stained red while rod nuclei were stained orange. This distinction was not observed in any of the teleosts, the rod nuclei staining red after Susa and Kolmer's fixatives (again cone nuclei could not be distinguished) and brown to blue after Helly and Zenker's fluids. With sharks, only Susa and Zenker-fixed material were treated with azan. After Susa's fixative, the rod nuclei were stained orange, as Kolmer reported, but after Zenker they were stained blue.

(3) Unna's orcein method (orcein followed by polychrome methylene blue). Lee (1950). Kolmer (in Wolff, 1940) using "Unna's orcein-polychrome methylene blue-tannin stain" colored the outer segments of cones deep blue while the outer segments of rods were unstained, in humans and other animals. This combination of stains, employing a solution of polychrome methylene blue and tannic acid, was not found in the literature. However, Lee (1950) described "Unna's method", which was essentially the same except for the omission of tannic acid. This method was used.

Teleost retinas fixed in Kolmer or Susa's fluids and stained by Unna's orcein method had traces of blue in the region of the outer segments. With sharks, the outer segments remained unstained or were stained only slightly with orcein. Thus, although the blue coloration in the teleosts could not be definitely attributed to cone outer segments,
the results may have been comparable to Kolmer's. In addi-
tion to the possible differential staining, this stain
coupled with Kolmer's fixative yielded good definition of
cell structures. However, great difficulty was experienced
in balancing the two dye components. Orcein was quickly
washed out during dehydration and polychrome methylene blue
tended to overstain. Best results were obtained after
fixation in Kolmer with the following modification of Unna's
method: stain in 1 per cent orcein in 100 per cent ethanol
at 55-60° C for 45 minutes, rinse in 50 per cent ethanol,
stain in polychrome methylene blue for 3 minutes, dehydrate
quickly.

(4) Unna's safranin and waterblue, Lee (1950). Wolff
(1940) quotes Kolmer who found, with man and the primates:
"After fixation in chrome-containing fluids and treatment
with nascent chlorine, Unna's epithelial stain colored the
inner and outer portions of cones a deep blue (with Wasser-
blau), while the inner and outer portions of the rods were
colored red (with Safranin)." There was some inconsistency
in the description as Carlton (1938), Lee (1937), and Conn
(1940) all gave "Unna's epithelial stain" as a combination
of waterblue and orcein, not safranin as stated by Kolmer.
Unna did in fact use such a combination, but this was called
simply "Unna's safranin and waterblue" by Lee (1950). This
stain is discussed here, while Unna's epithelial stain is
discussed below.
The "chrome-containing fluids" used in this study were Kolmer, Zenker, Helly, and Zenker-formal fixatives. The rods of sharks were stained blue, with waterblue, after all these fixatives, in contrast with Kolmer's results. In a few cases, the outer segments were stained a light red, but the inner segments were always colored blue. Teleost retinas produced further inconsistencies. After Kolmer's fixative, the cones were stained blue to purple and traces of red were seen in the region of the outer segments, as reported by Kolmer. However, after Zenker and Helly's fluids, cones were stained red to purple, while the rod outer segments were stained red. Thus, after fixation in Zenker and Helly's fluids, the rods of sharks apparently had a greater affinity for waterblue than teleost rods, and the differential-staining property of this stain-combination was not applicable to sharks. However, Unna's safranin and waterblue following Susa or Kolmer's fixatives produced good definition of cells, and was useful in this respect. (Figures 10 and 12.) The best times were safranin—24 hours, and waterblue—30 minutes, or safranin at 55° C.—1 hour, and waterblue—15 minutes.

(5) Unna's epithelial stain. As mentioned above, this stain was probably not the one used by Kolmer. However, it was tried because its components were readily available, being used also in other stain combinations. The results were similar no matter which fixative was used: all visual
cells of both teleost and shark retinas were stained blue, and structural detail was poorly defined.

In general, the reliability of the differential stains in distinguishing the rods from the cones was not conclusively established. Even with teleost retinas, in which cones were easily recognizable by shape, the results were inconsistent. However, the uniformity of staining in the visual cell layer of blacktip and whitetip sharks indicated that only a single type of visual cell was present in these retinas. The conger eel, *Conger marginatus*, which appeared to have an all-rod retina, also displayed this uniformity of staining of visual cells. (Adrian and Matthews, 1927, reported that a related species, *Conger vulgaris*, had both rods and cones. However, Bayliss, et al., 1936, did not find cones in the same species.) The failure to achieve consistent differential staining, at least in the teleost retinas, may have been due to differences in technique, as differential staining has been reported by workers (Wolff, 1940, and McEwan, 1938) in a variety of vertebrate retinas. It was not evident in the literature whether or not the retinas of cone-bearing sharks, e. g., *Mustelus* (Franz, 1913) have been stained with differential stains. Such a study would be invaluable as an aid to further investigations of shark retinas.

The non-differential stains failed to produce the
desired fineness of detail of shark retinal structures. All stained the nuclei well, but failed to color other structures sufficiently. Results were similar no matter which fixative preceded the stains. The non-differential stains are given below, with the staining times found best for shark retinas:

(1) Chlorazol black E. Gurr (1956). Stain in 1 per cent solution in 70 per cent ethanol for 1-1½ hours.

(2) Heidenhain's iron haematoxylin followed by Orange G or acid fuchsin. Pantin (1946) and Gurr (1956). Stain with haematoxylin—24 hours and 1 per cent aqueous Orange G—1 hour, or 0.1 per cent aqueous acid fuchsin—10 seconds.

RESULTS

Although serial sections were made of tissue from all areas of the retina, only the fundus was thoroughly studied and the discussion is limited to this region. Measurements of retinal structures from the dorsal-nasal, dorsal-temporal, ventral-nasal, and ventral-temporal quarters of the fundus were made. In addition, the retinal structures from the region judged to be on the optic axis, 5-10 mm. dorsal to the optic papilla, were measured separately. This region will be called the "central region". The measurements are presented in Tables 1, 2, 4, and 5.

Retinal layers

Good descriptions of shark retinas are given by Rochon-Duvigneaud (1943) and Franz (1913). The following description, which is applicable to both blacktip and whitetip adults, differs only slightly from Franz's, and generally follows his nomenclature. The retinal layers are labelled in Figure 1.

1. Retinal epithelium. This was a single layer of cells in loose contact with the distal tips of the visual cells and attached to the choroid at the ora serrata and optic papilla. The "pigment cells" were devoid of pigment, a condition which appears to be universal in shark retinas (Franz, 1913, Rochon-Duvigneaud, 1943, Kolmer, 1936, etc.). The cells appeared hexagonal in flat section, and were without processes.
Fig. 1. Retina of adult whitetip shark *Triasenodon obesus* (121 cm.), 1200X. Kolmer, Mallory's triple stain.
2. Visual cells. Ever since Max Schultze introduced the theory of the "duplex retina" in 1866, visual cells have been classified as being either of two types, rods or cones. However, most sharks appear to have all-rod retinas (Walls, 1942, Brett, 1957).

Rods. The term "rod" as used in this section refers to that part of the rod visual cell that extends from the external limiting membrane to the retinal epithelium (Polyak, 1941). In other sections, "rod" may also refer to the entire rod visual cell, for example, the "all-rod" retina mentioned in the preceding paragraph.

The outer and inner segments of the rods appeared long and slender in vertical section. In flat section, they appeared angular and not cylindrical as are most vertebrate rods (Walls, 1942). The outer segments were difficult to stain and were easily damaged, usually being compressed and bent or broken off. The inner segments were stained readily with nuclear stains, especially at the distal end, which corresponds to the lentiform body in cones.

Measurements of the rods, and all other structures of the retina, were complicated by the factor of shrinkage which is known to occur in slide preparations. Tamura (1957), working with fish retinas, used a correction factor based on Patten and Philpott's (1921) work in which shrinkage was estimated to be 25 per cent with the paraffin method. This correction was adopted in this paper. Further, shrinkage was
assumed to be consistent throughout, affecting all structures similarly in both the vertical and horizontal planes.

The average measurements of the rods, corrected for 25 per cent shrinkage, are given below in microns. The uncorrected figures are in parentheses.

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<th>Whitetips</th>
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<td>24 (18)</td>
<td>25 (19)</td>
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<tr>
<td>Length of inner segment</td>
<td>19 (14)</td>
<td>19 (14)</td>
</tr>
<tr>
<td>Total length of rods</td>
<td>43 (32)</td>
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<tr>
<td>Width of outer segment</td>
<td>2 (1.5)</td>
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<td>Width of inner segment</td>
<td>3 (2.2)</td>
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</tbody>
</table>

These figures are similar to those of Detwiler's (1943) who found rods 42 μ long at the fundus and 52 μ midway between the fundus and periphery in dogfish sharks.

Cones. Differentiation of visual cell types is based mainly on morphological and chemical characteristics. Of the many such criteria, the following were selected for this study:

a. Size and shape of the visual cells. The inner segments of cones are typically stouter than those of rods (Detwiler, 1943).

b. Shape and position of the visual cell nuclei. The cone nuclei are oval and are usually found in a single row just below the external limiting membrane; rod nuclei are round and situated below the cone nuclei (Wolff, 1940). Schaper (in Franz, 1913) depicted this nuclear arrangement in a diagram of the retina of the dogfish shark Mustelus,
but the rod nuclei were oval and the cone nuclei spindle-shaped, with their points often protruding past the external limiting membrane.

c. Chemical affinity. Rods and cones and their nuclei are stained differentially with certain combinations of dyes (Wolff, 1940).

Using the above criteria, cones were judged to be absent in the fundus of both the blacktip and whitetip retinas. All visual cells were similar in shape, which was the long and slender shape typical of vertebrate rods, and were stained alike. Further, although the shape, position and stain-uptake of the visual cell nuclei were not completely uniform, exceptions from the general condition were rare and inconsistent, and did not warrant a conclusion that two types of nuclei were present.

3. External limiting membrane. This "layer" appeared as a dark line separating the rods from their nuclei.

4. External nuclear layer (rod nuclei). The rod nuclei were arranged in two to four, usually three rows. Most were oval to spindle-shaped, with the tips of the nuclei in the top row situated slightly below the external limiting membrane. A few round nuclei were found, usually in the lower rows. The nuclei were easily stained with basic dyes. Most of the nuclei appeared granular, but a few clear, spindle-shaped nuclei were also found in the whitetip retina.

According to Schaper (in Franz, 1913) a few "displaced"
bipolar cells are occasionally found in the external nuclear layer. Due to the similarity in stain uptake and the density of cell bodies, the displaced bipolars, if they were indeed present, were not distinguished from the rod nuclei in whitetip and blacktip retinas.

5. External plexiform layer. The fibers of visual cells and bipolars that make up this layer were not made visible with the histological methods used. This layer was very thin, its relative width being less than half that of the teleost fishes studied.

6. Internal nuclear layer. This layer contained the cell bodies of horizontal, amacrine, and bipolar cells. In addition, stout radial fibers of Müller and their nuclei were also found here.

The horizontal cells were found in the area immediately below the external plexiform layer. In vertical section they appeared as huge blocks of cells and occupied most of the area which, in other vertebrates, is included in the external plexiform layer. There appeared to be at least two distinct layers of cells with large oval nuclei whose longer plane lay horizontally. In flat section, the horizontal cells presented a completely different appearance. (See Figure 2.) The cell processes of the horizontal cells were enmeshed in a cytoplasmic network, with large oval nuclei imbedded in the matrix, and smaller structures, often kidney-shaped, lining the holes in the network. The latter
Fig. 2. Region of the horizontal cells in the internal nuclear layer of blacktip sharks, flat section, 770X. Kolmer, Mallory’s triple stain.
were probably Müller fibers in cross-section, as no other structures were found in this area in vertical sections. Additional nuclei were found in the holes. These were probably bipolar cell nuclei, according to Schaper's diagram of the retina of Mustelus (in Franz, 1913). Schaper also believed that there were two different layers of horizontal cells, and that both formed networks in which the cells were joined together through stout branches. The presence of two distinct networks was not seen in flat sections, but the two layers of horizontal cells found in vertical sections indicate this possibility. Unlike the retinas of higher vertebrates in which horizontal cells have a nervous capacity, the horizontal cells of sharks appear to function only as supporting structures (Walls, 1942).

At least three different types of nuclei were found in the basal portion of the internal nuclear layer. One was that of Müller fibers, which were easily recognizable as they were definitely oval and were usually found within the stout fibers. Of the other two, one was large (5-8 \( \mu \)), round, and appeared granular, while the other was smaller (2-3 \( \mu \)), round, usually stained darker, and was not always granular in appearance. The former were probably the bipolar cell nuclei, as they appeared similar to the bipolars "displaced" in the region of the horizontal cells, as depicted in Schaper's diagram (in Franz, 1913). The latter would then be the amacrines, though positive identification was
impossible without the use of special neuro-histological techniques which stain the fibers as well as the entire cell body. Both Rochon-Duvigneaud (1943) and Schaper's (in Franz, 1913) diagrams of shark retinas, Lamma Cornubica and Mustelus, respectively, depict a single distinct row of amacrines at the base of the internal nuclear layer, with the bipolars lying above them in 3-4 rows, as well as being scattered in the region of the horizontal cells. In black-tips and whitetips however, the two types of cells appeared to be intermingled. Further, in the adult sharks, only one or two rows of nuclei were found, the central region of the internal nuclear layer usually being devoid of nuclei.

Müller fibers are radial supporting structures with processes extending from the internal limiting membrane to the external limiting membrane (Walls, 1942). The fibers were thin in the nerve fiber and internal plexiform layers, very stout in the internal nuclear layer, and gave rise to smaller branches which, according to Walls (1942), terminate as the fenestrated external limiting membrane. It is thought that the fibers may serve not only as supporting structures, but also as insulators, covering all nerve cells with a tiny neurological envelope (Arey, 1934).

7. Internal plexiform layer. Many fibers were visible in this layer, but their origin or termination were not apparent. They may have been the fibers of bipolar, amacrine, and ganglion cells, and the more prominent ones were
probably Müller fibers. A few nuclei were also found scattered in this layer. Their large size suggested that they were nuclei of displaced ganglion cells. Walls (1942) noted that such displacement of ganglion cells as well as other cell types was not unusual in Selachians.

8. Ganglion cell layer. Most of the ganglion cell nuclei were located at the border of the internal nuclear and nerve fiber layers, but a few were also found in both adjoining layers. The nuclei were large and very few in number.

9. Nerve fiber layer. This layer is composed of the axons of ganglion cells. Its appearance in vertical sections was not consistent, its width depending on the state of preservation and/or plane of sectioning. Any section other than a perfectly vertical one made the nerve fiber layer broader than its true width. Further, the nerve fibers were cut in cross-section or longitudinally, depending on the area of the retina sectioned. In flat section, some fibers appeared to be beaded with small round bodies. Large ganglion cell nuclei were also found in this layer.

10. Internal limiting membrane. This thin membrane forms the border between the retina and the vitreous cavity. It was often missing or distorted in sections.

**Developmental changes in the retina**

**Blacktips**

Of the shark eyes used in this comparative study 7 were
from embryos 33 to 37 cm. in length, 6 from juveniles 43 to 56 cm., and 15 from adults 94 to 122 cm. Measurements of layers and counts of rods and nuclei from different areas of the fundus of the retina were made from 7 μ vertical sections and are given in Tables 1 and 2. All good slides were used in this survey, regardless of the fixative or stain.

Apart from the optic papilla, no regional differences were found in the structure of the retina within the fundic regions of each size group. Although some differences were found in the width of particular layers and in the numbers of nuclei, they were never consistent. For example, if there were more ganglion cells in the central region, an increased number of bipolar cells was not found above them. The figures along each row in the tables indicate the essential similarity among the different fundic regions.

Most of the figures in the body of Tables 1 and 2 are averages of actual measurements and counts, uncorrected for shrinkage. Graphs of the frequency distributions showed that these figures were near-normal in distribution. The uncorrected grand means were calculated from all measurements within each size group. The grand means and their 95 per cent confidence intervals (Snedecor, 1957) were corrected for 25 per cent shrinkage.

Differences in the retinal structure of the three size groups are demonstrated by the means and confidence intervals. They are further illustrated in Figures 3-5 and 8-10.
Table 1. Mean width of retinal layers (in μ) of blacktip sharks.

<table>
<thead>
<tr>
<th>Retinal layers</th>
<th>Sharks</th>
<th>Regions of the retina</th>
<th>Uncorrected grand mean</th>
<th>Corrected grand mean</th>
<th>95 per cent confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rod layer</td>
<td>embryo</td>
<td>A 27 B 29 C 26 D 20 E 21</td>
<td>22.9</td>
<td>30.6</td>
<td>28.6--32.5</td>
</tr>
<tr>
<td></td>
<td>juvenile</td>
<td>A 31 B 31 C 32 D 30 E 31</td>
<td>31.0</td>
<td>41.3</td>
<td>40.3--42.2</td>
</tr>
<tr>
<td></td>
<td>adult</td>
<td>A 26 B 36 C 35 D 31 E 32</td>
<td>31.9</td>
<td>42.5</td>
<td>40.0--45.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>External</td>
<td>embryo</td>
<td>A 18 B 20 C 20 D 18 E 18</td>
<td>18.8</td>
<td>25.0</td>
<td>24.1--26.0</td>
</tr>
<tr>
<td>nuclear layer</td>
<td>juvenile</td>
<td>A 20 B 18 C 17 D 19 E 20</td>
<td>19.2</td>
<td>25.5</td>
<td>24.7--26.3</td>
</tr>
<tr>
<td></td>
<td>adult</td>
<td>A 16 B 18 C 18 D 18 E 17</td>
<td>17.8</td>
<td>23.7</td>
<td>22.7--24.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>External</td>
<td>embryo</td>
<td>A 50 B 46 C 43 D 50 E 51</td>
<td>47.1</td>
<td>62.7</td>
<td>59.9--65.5</td>
</tr>
<tr>
<td>plexiform layer</td>
<td>juvenile</td>
<td>A 45 B 40 C 40 D 34 E 38</td>
<td>41.3</td>
<td>55.0</td>
<td>53.2--56.8</td>
</tr>
<tr>
<td>internal nuclear layer</td>
<td>adult</td>
<td>A 32 B 31 C 33 D 32 E 33</td>
<td>31.7</td>
<td>42.2</td>
<td>40.8--43.8</td>
</tr>
<tr>
<td>Retinal epithelium to end of internal plexiform layer</td>
<td>embryo</td>
<td>A 135 B 138 C 144 D 130 E 124</td>
<td>132.8</td>
<td>177.0</td>
<td>169.5--184.4</td>
</tr>
<tr>
<td></td>
<td>juvenile</td>
<td>A 129 B 125 C 125 D 128 E 135</td>
<td>129.5</td>
<td>172.7</td>
<td>168.5--176.4</td>
</tr>
<tr>
<td></td>
<td>adult</td>
<td>A 118 B 120 C 138 D 120 E 124</td>
<td>127.0</td>
<td>169.2</td>
<td>163.2--175.5</td>
</tr>
</tbody>
</table>

Table 2. Mean number of rods and nuclei in the retina of blacktip sharks.

<table>
<thead>
<tr>
<th>Retinal structures</th>
<th>Sharks</th>
<th>Areas of the retina/</th>
<th>Uncorrected grand mean</th>
<th>Corrected grand mean</th>
<th>95 per cent confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rods per 0.1 mm.</td>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>embryo</td>
<td>40</td>
<td>42</td>
<td>38</td>
<td>40</td>
<td>36</td>
</tr>
<tr>
<td>juvenile</td>
<td>40</td>
<td>42</td>
<td>46</td>
<td>44</td>
<td>42</td>
</tr>
<tr>
<td>adult</td>
<td>44</td>
<td>44</td>
<td>42</td>
<td>40</td>
<td>42</td>
</tr>
</tbody>
</table>

| No. of rod nuclei per 0.1 mm. (top row only) |        | A       | B | C | D | E |                  |                        |                                  |
| embryo                      | 22     | 23      | 23 | 23 | 20 |    | 22.7             | 17.0                   | 16.4—17.7                    |
| juvenile                    | 25     | 22      | 23 | 24 | 23 |    | 23.7             | 17.8                   | 17.2—18.3                    |
| adult                       | 25     | 23      | 23 | 26 | 23 |    | 23.3             | 17.5                   | 17.1—17.8                    |

| No. of nuclei in the internal nuclear layer per 0.1 mm. 2/ |        | A       | B | C | D | E |                  |                        |                                  |
| embryo                      | 21     | 23      | 24 | 23 | 20 |    | 22.1             | 16.6                   | 14.3—18.8                    |
| juvenile                    | 18     | 12      | 14 | 18 | 18 |    | 17.1             | 12.8                   | 11.9—13.8                    |
| adult                       | 10     | 10      | 11 | 10 | 10 |    | 10.4             | 7.8                    | 7.5—8.1                      |

| No. of ganglion cells per 1.0 mm. |        | A       | B | C | D | E |                  |                        |                                  |
| embryo                      | 16     | 10      | 16 | 21 | 19 |    | 20.3             | 15.2                   | 13.0—17.5                    |
| juvenile                    | 19     | 12      | 16 | 19 | 19 |    | 16.2             | 12.1                   | 10.5—13.7                    |
| adult                       | 8      | 9       | 13 | 14 | 15 |    | 12.0             | 9.0                    | 8.3—9.3                      |


2/Includes all nuclei in the internal nuclear layer except those obviously of Müller fibers and horizontal cells.
Fig. 3. Retina of embryonic blacktip shark (37 cm.), 770X. Kolmer, Mallory's triple stain.

Fig. 4. Retina of juvenile blacktip shark (56 cm.), 770X. Kolmer, Mallory's triple stain.

Fig. 5. Retina of adult blacktip shark (119 cm.), 770X. Kolmer, Safranin and Waterblue.
The major changes that occur during development are: (1) growth in length and width of the rods, (2) decrease in width of the internal nuclear layer and decrease in number of nuclei per unit area therein, and (3) decrease in number of ganglion cells per unit area. In addition, the width of the entire retina appears to decrease with overall retinal growth, the increase in length of the embryonic rods notwithstanding. Statistical proof for the last observation was not established, but indications were seen in the means for the three size groups, the embryonic retina being wider than that of the juvenile, which in turn was wider than that of the adult. In this connection, it should be noted that the nerve fiber layer was excluded from the measurements because of its inconsistent width. Of the few good sections of this layer available, the average width was about 40 μ. Thus, addition of 40 μ to the figures in the last column of Table 1 gives an approximation of the width of the entire retina, 219 μ for the embryonic retina, 211 μ for the juvenile, and 205 μ for the adult. These figures are somewhat lower than Detwiler's (1943) who found that the dogfish shark retina was 227-234 μ wide. Such differences in measurements are common, as Detwiler points out, and are caused mainly by the differences in techniques used in preparing the retina for microscopic examination.

Judging from the data, the growth of the shark retina is comparable to the expansion of a small balloon. The
retinal layers are stretched and become thinner resulting in a decrease in the number of nervous elements per unit area. This explanation of retinal growth is compatible with observations by other workers. Arey (1934), for example, states that all nervous components are already present at birth and no addition or replacement of retinal cells occurs thereafter. Wolff (1940) maintains that in the human foetus no new retinal elements are added after the sixth month of development.

In addition to changes in the nervous elements, a further developmental change was evident in the disappearance of the numerous globular structures found in the internal nuclear layer of the embryonic retina. These structures were definitely associated with the developmental stages, their number decreasing with growth of the shark. They are best illustrated in Figure 9 in which they are colored bright orange. Table 3 provides a measure of the comparative abundance of the structures which will be called for convenience "embryonic bodies".

In the case of the embryos, the Palmyra sharks were in an earlier stage of development than the Eniwetok specimens. The embryonic bodies filled the entire internal nuclear layer in the former, making it difficult to see the other structures, but were restricted mainly to the central portion in the latter, and appeared to be clumped around or near Müller fibers. With juveniles, clumping of the bodies
Table 3. Number of rows of "embryonic bodies" and the width (in μ, corrected for 25 per cent shrinkage) occupied by them in the internal nuclear layer.

<table>
<thead>
<tr>
<th>Sharks</th>
<th>Mean number of rows</th>
<th>95 per cent confidence interval</th>
<th>Mean width occupied in the internal nuclear layer</th>
<th>95 per cent confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryos from Palmyra (33 cm.)</td>
<td>13.2</td>
<td>(8.9--17.5)</td>
<td>49.8</td>
<td>(28.6--71.0)</td>
</tr>
<tr>
<td>Embryos from Eniwetok (37 cm.)</td>
<td>7.8</td>
<td>(6.5--9.1)</td>
<td>31.1</td>
<td>(29.9--32.3)</td>
</tr>
<tr>
<td>Juveniles (43-56 cm.)</td>
<td>3.9</td>
<td>(3.0--4.8)</td>
<td>19.0</td>
<td>(17.4--20.5)</td>
</tr>
<tr>
<td>Adults (84-122 cm.)</td>
<td>Few only, scattered.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
was also evident, as was a definite decrease in number compared with the embryos. Further, the number of bodies decreased with increase in size of the juvenile sharks. In the adults, of twelve specimens studied, four had a few scattered embryonic bodies, four had an appreciable number, but always less than juveniles, and four had none. Again, the decrease in number of embryonic bodies was associated with increase in length of the shark.

Identity of the bodies was not ascertained, but they are suspected to be related to structures found in the "transient non-nucleated layer of Chievitz" (Detwiler, 1943) found in human foetal retinas. According to Detwiler, this layer is formed during the sixth week of development when nuclei in the primitive neuro-epithelium divide and some migrate to form the ganglion cell layer. This results in the formation of a non-nucleated layer between the primitive and new nuclear layers. The structures found in the non-nucleated layer disappear by the third month of foetal development. Their function is unknown. Although Detwiler fails to give a detailed description of the elements comprising the non-nucleated layer of Chievitz, his figure (page 50) shows structures very similar to the embryonic bodies found in their internal nuclear layer of shark retinas.

The embryonic bodies were usually angular in shape, although some appeared to be without sharp edges. They were 3 to 5 μ in diameter, and appeared to be slightly larger in
embryos than in juveniles. The bodies were usually stained heavily with nuclear stains, taking on a clear, bright coloration. They sometimes appeared granular as did the nuclei in the embryonic retina. However, with Unna's epithelial stain, the embryonic bodies failed to stain with waterblue, while all nuclei were stained blue. With Mallory's triple stain following Zenker fixation, the bodies were stained orange and blue, with both types existing side by side. More commonly, however, bodies in some parts of the retinal section were stained orange while those in other parts were stained blue. Unstained preparations studied under a phase contrast microscope showed that the bodies were highly refractive, appearing very bright compared to the other retinal elements.

Until the function of these embryonic bodies is known, their effect on the visual capabilities of the blacktip cannot be ascertained. They may serve as trophic cells that are resorbed during growth and may not affect vision. On the other hand, they may be pigment granules that are selective filters, absorbing and/or reflecting particular wave lengths of light, in which case they would be quite important in vision.

Whitetips

Five adult and seven embryonic whitetip eyes were available for this study. A single juvenile whitetip eye was also preserved, but slides of its retina turned out very
poorly. The embryos, taken from a single female, were all 43 cm. long. The adults ranged from 119 to 147 cm., while the juvenile was 99 cm. long.

Measurements of retinal structures and layers are given in Tables 4 and 5. The retinas are illustrated in Figures 6, 7, 12, and 13. As with blacktips, no regional differentiation was found in the fundus of the whitetip retina. Further, the differences in the widths of the layers between the whitetip embryos and adults (Table 4) were similar in direction and magnitude to those found in the blacktips, and were all statistically significant, i.e., there was no overlapping in the confidence intervals of the adult and embryo. The counts of rods and nuclei (Table 5) were also similar to those of blacktips, and lead to the same explanation of retinal growth.

All retinal structures found in blacktips were also present in whitetips, including the "transient non-nucleated layer of Chievits". This layer was not examined as thoroughly as with blacktips, but in this respect no overt differences were found between the two species. The single juvenile whitetip retina showed the intermediate stage in which the numbers of embryonic bodies were reduced but still abundant.

Comparison of blacktip and whitetip retinas

Very little structural difference was found between the retinas of blacktip and whitetip sharks. Neither had
Table 4. Mean width of retinal layers (in μ) of whitetip sharks.

<table>
<thead>
<tr>
<th>Retinal layers</th>
<th>Regions of the retina</th>
<th>Uncorrected grand mean</th>
<th>Corrected grand mean</th>
<th>95 per cent confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Rod layer</td>
<td>embryo</td>
<td>25</td>
<td>22</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>adult</td>
<td>38</td>
<td>34</td>
<td>33</td>
</tr>
<tr>
<td>External nuclear layer</td>
<td>embryo</td>
<td>25</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>adult</td>
<td>20</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>External plexiform layer and internal nuclear layer</td>
<td>embryo</td>
<td>51</td>
<td>51</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>adult</td>
<td>24</td>
<td>29</td>
<td>31</td>
</tr>
<tr>
<td>Retinal epithelium to end of internal plexiform layer</td>
<td>embryo</td>
<td>140</td>
<td>142</td>
<td>144</td>
</tr>
<tr>
<td></td>
<td>adult</td>
<td>124</td>
<td>125</td>
<td>126</td>
</tr>
</tbody>
</table>

Table 5. Mean number of rods and nuclei in the retina of whitetip sharks.

<table>
<thead>
<tr>
<th>Retinal structures</th>
<th>Sharks</th>
<th>Regions of the retinal/uncorrected grand mean</th>
<th>Corrected grand mean</th>
<th>95% per cent confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of rods per 0.1 mm.</td>
<td>Embryo</td>
<td>34 44 42 38 40</td>
<td>39.8</td>
<td>29.8</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>41 50 44 38 46</td>
<td>42.6</td>
<td>32.0</td>
</tr>
<tr>
<td>No. of rod nuclei per 0.1 mm. (top row only)</td>
<td>Embryo</td>
<td>23 23 22 19 22</td>
<td>22.4</td>
<td>16.8</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>26 26 25 23 25</td>
<td>24.9</td>
<td>18.7</td>
</tr>
<tr>
<td>No. of nuclei in the internal nuclear layer per 0.1 mm. 2/</td>
<td>Embryo</td>
<td>23 25 28 22 23</td>
<td>23.8</td>
<td>17.9</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>11 15 13 10 11</td>
<td>12.6</td>
<td>9.4</td>
</tr>
<tr>
<td>No. of ganglion cells per 1.0 mm.</td>
<td>Embryo</td>
<td>12 13 19 15 15</td>
<td>14.6</td>
<td>11.0</td>
</tr>
<tr>
<td></td>
<td>Adult</td>
<td>20 13 13 14 10</td>
<td>11.6</td>
<td>8.7</td>
</tr>
</tbody>
</table>


2/ Includes all nuclei in the internal nuclear layer except those obviously of Müller fibers and horizontal cells.
Fig. 6. Retina of embryonic whitetip shark (44 cm.), 770X. Kolmer, Safranin and Waterblue.

Fig. 7. Retina of adult whitetip shark (119 cm.), 770X. Kolmer, Mallory's triple stain.
cones nor regional specialization in the form of a central area. Further, the development of the retina in the two species appeared to be similar.

Comparison of Tables 1 and 4 shows that the width of the various retinal layers in adult blacktips and whitetips were essentially the same. The only dissimilarity was in the width of the internal nuclear layer which appeared to be slightly thinner in the whitetips. In the case of the embryos, differences were found in the external nuclear layer and the entire retina (minus the nerve fiber layer), both of which were wider in the whitetip than in the blacktip embryo. It should be pointed out, however, that the differences may have been due simply to the fact that the blacktip and whitetip embryos were in different stages of development.

The data of Tables 2 and 5 serve to emphasize the similarity of the retinas of the two species. In the adults, the only noticeable difference was in the slightly greater number of nuclei in the internal nuclear layer of the white-tip. However, since the number of rods and ganglion cells were about the same for the two species, it is doubtful that the above difference results in a difference in visual capability.

The extent of summation of visual elements was about the same for the two species. Vertical sections were used for counting the number of rods, bipolars, amacrines, and
ganglion cells. Counts of rods made from a few flat sections were the same as the counts made from vertical sections. However, since the nuclei of the bipolar, amacrine, and ganglion cells were not situated in an orderly plane, flat sections could not be utilized for counting these nuclei. The use of vertical sections for cell counts made it necessary to count all bipolar, amacrine, and ganglion cells, since the depth of focus could not be restricted to a single plane as with the rods.

The linear summation of visual elements in adult blacktip and whitetip sharks, calculated from the corrected figures in Tables 2 and 5, are given below:

<table>
<thead>
<tr>
<th></th>
<th>Rods</th>
<th>Bipolars</th>
<th>Ganglion cells</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blacktips</td>
<td>315</td>
<td>78.2</td>
<td>8.98</td>
<td>35:9:1</td>
</tr>
<tr>
<td>Whitetips</td>
<td>320</td>
<td>94.3</td>
<td>8.73</td>
<td>37:11:1</td>
</tr>
</tbody>
</table>

It should be noted that all nuclei found in the internal nuclear layer were classed as bipolars, although some were undoubtedly amacrine cell nuclei.

The above ratios are probably too small, inasmuch as only a single row of rods was considered while the bipolars and ganglion cells were counted from the entire thickness of the 7 μ vertical section. If summation were calculated from a square unit of rods and the bipolars and ganglion cells situated under this unit, the ratios would probably be more accurate. For example, since 78.2 bipolar cells were found in an "area" 1 mm. X 0.007 mm. (the latter being the
thickness of the retinal section), then by expanding proportionately there would be 78.2/0.007 or 11,171 bipolar cells per square millimeter, as compared to 315² or 99,325 rods/mm². The width of the internal nuclear layer was disregarded because it remains constant and cancels out in the calculations, and all the bipolar cells were imagined as being in a single horizontal plane under the rods, instead of in a volume.

Using the above relations, the ratios were recalculated using a square millimeter as the unit area:

<table>
<thead>
<tr>
<th></th>
<th>Rods</th>
<th>Bipolars</th>
<th>Ganglion cells</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blacktips</td>
<td>315²</td>
<td>78.2/0.007</td>
<td>8.98/0.007</td>
<td>77:9:1</td>
</tr>
<tr>
<td>Whitetips</td>
<td>320²</td>
<td>94.3/0.007</td>
<td>8.73/0.007</td>
<td>82:11:1</td>
</tr>
</tbody>
</table>

The ratios between rods and ganglion cells thus become twice as great as those given previously, and are believed to be more accurate.
Fig. 8. Retina of embryonic black-tip shark (37 cm.), 650X. Kolmer, Mallory's triple stain.

Fig. 9. Retina of juvenile black-tip shark (56 cm.), 650X. Kolmer, Mallory's triple stain.
Fig. 10. Retina of adult blacktip shark (101 cm.), 650X. Kolmer, Safranin and Waterblue.

Fig. 11. Retina of Apogon sp., 650X. Kolmer, Mallory's triple stain.
Fig. 12. Retina of embryonic white-tip shark (44 cm.), 650X. Kolmer, Safranin and Waterblue.

Fig. 13. Retina of adult white-tip shark (119 cm.), 650X. Kolmer, Mallory's triple stain.
DISCUSSION

Retinal organization and life habits

The close association found between retinal structures and life habits of animals is so consistent that Detwiler (1943) was prompted to state: "So closely correlated is the mode of life of the animal with the structure of the retina that, from a histological section, one can predict something of the habits of the animal, as well as its visual ability."

Vertebrate animals are commonly classified as being one of two types according to their period of greatest activity, nocturnal or diurnal, and the retinas reflect these differences in mode of life (Detwiler, 1943). Functional morphology of the retina and the correlation with life habits have been discussed at great length by Detwiler (1943) and Walls (1942). According to their views, the retinas of vertebrates are constructed principally for either of two mutually exclusive visual functions, sensitivity or acuity. In most nocturnal vertebrates, the retina is constructed for sensitivity, there being a preponderance of rods over cones, or complete absence of cones, and extensive summation of visual cells upon ganglion cells (Detwiler, 1943). Further, Wunder (1926) found that the rods of nocturnal or "twilight" teleosts were much smaller and far more numerous than those of diurnal teleosts. In diurnal vertebrates, there are a greater number of cone, bipolar, and ganglion cells, so that summation is not as extensive, and acuity is enhanced (Walls, 1942). However, Wunder (1926) found that in cone-bearing
teleosts, the size and number of cones were not markedly different in nocturnal and diurnal fishes.

Most vertebrates have in the retina a region specialized for greater visual acuity, namely the area centralis (Detwiler, 1943) in which visual cells (usually cones) are concentrated, and there is an increased number of secondary and tertiary neurones (Walls, 1942). This area is thus especially adapted for mediating high visual acuity, even if the rest of the retina may be organized for sensitivity (Detwiler, 1943 and Walls, 1942). Thus, the problem of mutual exclusion of the two functions of acuity and sensitivity can be resolved by the presence of an area centralis.

Another visual function that is probably mediated by the cones, and not by rods, is that of color vision, "... the capacity to respond to lights which differ only in frequency" (Walls, 1942). The terms "color" and "wave length" will be used synonymously, although the subjective experience of lower animals may not necessarily be the same as that of humans. There is some controversy in the relegation of all color vision solely to the cones (Walls, 1942). The cone-free retina of the guinea pig has been investigated by Granit (in Morgan and Stellar, 1950). Using electrophysiological methods, Granit found that these animals have receptors in the retina that react differently to different wave lengths of monochromatic light, although the actual presence of color vision was not demonstrated by the animal's
behavior. Siven (in Arey, 1934) maintains that rods are instrumental in the discrimination of colors of the shorter wave lengths. Protasov (1960) holds that both rods and cones must be present in order for an animal to have color vision. His methods of investigation could not be determined from the paper (in Russian), but electrophysiological techniques were presumably used. Among the list of fishes he worked with, the all-rod sting ray Trygon pastinaca, and all-cone sturgeon Acipenser stellatus were found lacking in color vision. Most fishes with color vision had large numbers of both rods and cones in their retinas. However, the eel Anguilla anguilla was found to have only a few cones, yet, according to Protasov, it also had color vision. On the other hand, the dogfish shark Scyliorhinus, which allegedly also had a few cones, could not discriminate between wave lengths. In the dogfish, Protasov did not find a "Purkinje shift"—a change in spectral sensitivity with change in intensity, which presumably results from the presence of both rods and cones and their differential sensitivity to intensity (Walls, 1942). Thus, the cones, if they were indeed present, were not numerous enough to be of any consequence in regard to color vision in the dogfish.

Walton (1939) in behavioral experiments with rats, which have all-rod or rod-rich retinas, found that these animals could discriminate between certain colors. Walls (1942) questions Walton's results inasmuch as the latter was the
only one to find color vision in rats. Several workers, whose techniques were as good as Walton's—according to Walls—failed to show that rats could discriminate between different wave lengths. However, Walls notes that no one has yet proven that all-rod animals do not have color vision. On the other hand, neither has anyone proven, to Walls' satisfaction, that animals with all-rod retinas can discriminate between wave lengths. Despite the controversy, the bulk of evidence points to a close association between color vision and the presence of cones (Brett, 1957, and Walls, 1942).

According to the above relationships, the blacktip and whitetip sharks, with all-rod retinas and fairly extensive summation, are adapted for sensitivity rather than acuity of vision. Thus, their vision might be classed as "diffuse" (Walls, 1942). Apparently this is also true for most of the sharks which have been examined (Franz, 1913, Kolmer, 1936, Rochon-Duvigneaud, 1943, Verrier, 1929, and Walls, 1942) as shown by the following list with all-rod retinas (the scientific names are as given by the authors, with no attempt to resolve possible synonymies):

Verrier, 1929: Scyllium (presumably S. catulus and S. canicula) and Acanthias (presumably A. vulgaris).

Kolmer, 1936: Acanthias vulgaris, Carcharias sp., Centrophorus granulosus, Ethmopterus spinax, Galeus canis, Pristurus sp., Scyllium canicula, S. canicula stellare,
S. catulus, Scyliorhinus lichia, and Somniosus microcephalus. Rochon-Duvigneaud, 1943: Centrina Salviani, Centrophorus calceus, Galeus canis, Scyliorhinus sp. In addition, Rochon-Duvigneaud described parts of the ocular anatomy of some 30 sharks, but he did not specifically state whether or not their retinas were cone-free.

Thus, omitting duplications, at least 14 sharks are known to have all-rod retinas. In addition, we can add the blacktip and whitetip in which no cones were found despite an intensive search. A thorough search of the literature would undoubtedly uncover many more sharks with all-rod retinas (e.g., in Franz's works), and lend further support to the general statement that most sharks have all-rod retinas, which appears in many publications (Brett, 1957, Walls, 1942, etc.).

In contrast to the large number of all-rod shark retinas, only 3 or 4 genera and about 6 or 7 species of sharks are known to have cones. Greef (in Detwiler, 1943) and Franz (1913) found a few cones in several species of the shark genus Mustelus. The latter also found cones in the ray-like shark Squatina sp. Rochon-Duvigneaud (1943) reported the presence of cones in Lamna cornubica as did Protasov (1960) in Scyliorhinus canicula. Nicol (1961) did not mention having found cones in S. canicula, but in a personal communication he stated that he may have overlooked them as he was not particularly interested in the visual
cells. However, Rochon-Duvigneaud (1943) also reported the absence of cones in Scylliorhinus (sic) sp..

Most sharks are considered to be nocturnal (Walls, 1942) and their all-rod retinas generally reflect this life habit. However, the number of rods, the width of the external nuclear layer, and extent of summation of rods upon ganglion cells appear to be quite different from those of most other nocturnal vertebrate eyes, which typically contain numerous slender rods, a wide external nuclear layer, and a great degree of summation (Walls, 1942). Blacktips and whitetips have relatively few and robust rods. Other sharks appear to have similar rods (Franz, 1913, Rochon-Duvigneaud, 1943, and Verrier, 1929). The number of rods in blacktip and whitetip retinas was calculated to be about 99,000/mm.$^2$ and 102,000/mm.$^2$ respectively. These figures fall in between Franz’s (in Walls, 1942) who found 24–75,000 rods/mm.$^2$ in “various small sharks” and 132,000/mm.$^2$ in Etmopterus sp..

Wunder (1926) gives comparative figures for 24 species of teleost fishes. He separates these teleosts into four classes, and gives the number of rods found “auf einer Strecke von 80 μ.” The range and average diameter of the rods are also given:

1. “Hellfische” or fish active during the day in well-lit waters—8 species. 18–61 rods, average 37. Diameter 2–4 μ, average 2.7 μ.
2. "Hell und Dämmerungsfische" or fish active both during the day and at night—2 species. 62-105 rods, average 84. Diameter 2 μ.

3. "Dämmerungsfische" or fish active at night or found in dimly-lit habitats—7 species. 74-260 rods, average 139. Diameter 1-1.5 μ, average 1.2 μ.

4. "Dämmerungsfische, Augen schlecht entwickelt" or fish active at night or found in dimly-lit habitats, with eyes that are poorly developed—7 species. 11-38 rods, average 27. Diameter 2-4.5 μ, average 2.9 μ.

Walls (1942) expanded a few of Wunder's figures into rods per square millimeter. Two of these are given below, with Wunder's figures in parentheses: Lota, a nocturnal teleost, 180,000 rods/mm.² (260); Ameiurus, a nocturnal teleost with poorly developed eyes, 18,400 rods/mm.² (11). How Walls' figures were derived was not explained and could not be determined from Wunder's (1926) paper. If Walls' figures are correct, blacktip and whitetip sharks have far fewer rods per unit area than nocturnal animals. However, one must take into account probable differences in technique between Wunder's and the writer's methods. Wunder (1926) also used the paraffin method of imbedding, and noticed shrinkage of the retinal tissue. He did not appear to make any corrections for shrinkage in his counts of retinal structures. Thus, if the uncorrected counts of blacktip and whitetip rods are used, the number of rods becomes similar...
to that of Lota—177,000/mm.² for blacktips and 181,000/mm.² for whitetips. Although Lota has a few cones, they are so few as to be insignificant in the counts; Lota has 3 cones as compared to 260 rods in an 80 µ section (Wunder, 1926). This similarity is highly unlikely, considering the diameter of the rods. Lota's rods are only 1 µ thick (Wunder, 1926) while the blacktips and whitetips's rod inner segments are 1.8-2.2 µ (uncorrected for shrinkage) in diameter.

Under the circumstances, the only comparison that can be made then must be a rough one based on the diameter of the rods. The shark rods (uncorrected) are most similar in diameter with those of the so-called "Hell und Dämmerungsfische". The number of rods per unit area should then be in the same order of magnitude, which is far less than the number found in truly nocturnal fishes with well-developed eyes. The "Hell und Dämmerungsfische" have an average of 4 more cones than the "Dämmerungsfische" for the same unit space. If these cones are "converted" into rods at the rate of 1 cone equals about 3 rod diameters, the "rod" count of the "Hell und Dämmerungsfische" (and thus that of the sharks also) would rise to 96, which is still considerably less than the 139 of the "Dämmerungsfische".

Further, differences between the number of rods in blacktips and whitetips and those in nocturnal vertebrates can be easily seen by comparing Detwiler's (1943) photographs with those included herein. Most nocturnal
vertebrates have very wide outer nuclear layers, with numerous tiny rod nuclei. This can be seen also in Figure 11, which shows the retina of the generally nocturnal teleost, *Apogon sp.*

The extent of summation in shark retinas is likewise atypical of nocturnal vertebrates, in which it is not unusual for thousands of rods to be summed in a single optic nerve fiber (Walls, 1942). However, Wunder (in Brett, 1957) found a ratio of rods to ganglion cells of only 263:1 in *Lota*, a nocturnal species with extremely fine and numerous rods. The ratio of rods to ganglion cells was 77:1 in blacktips and 82:1 in whitetips. Verrier (1929) found a ratio of only 8:1 in *Scyllium*, and 5:1 in *Acanthias*. Franz (in Walls, 1942) found a ratio of about 20:1 for "various small sharks" and 147:1 in *Etmopterus*. The difference in ratios among the sharks may be due in part to differences in microtechnique or in the calculation of the ratios. Further, Rochon-Duvigneaud (1943) pointed out the difficulties involved in making accurate counts, especially in regard to the ganglion cells, which are often found in the internal plexiform layer. Moreover, he found that not all nuclei in this layer were ganglion cell nuclei, so that one might either over-enumerate the number of ganglion cells by counting all nuclei in the internal plexiform layer, or conversely, underestimate the number by ignoring the nuclei in this layer.

Despite the relatively few rods and moderate summation,
shark retinas may still be highly sensitive to light of low intensity. According to Walls (1942) the total mass of the rods, and the rhodopsin contained within them are as important as numbers of rods, so that summation of a few robust rods upon a ganglion cell may be equal in effect to that of many slender rods summated upon a ganglion cell.

The lack of an area centralis in the retinas of blacktips and whitetips further precludes a high degree of visual acuity. Only a few sharks are known to have an area centralis. In these, it appears only as a slight modification of the retinas, and is less complex in organization than that of most other vertebrate classes. Scyllium, Mustelus, and Acanthias have an area centralis, according to Hesse, Franz, and Slonaker (in Rochon-Duvigneaud, 1943). Nicol (1961) also found a region with very long rods in the retina of Scyliorhinus canicula. In the case of Mustelus, Franz (in Walls, 1942) found cones in the area centralis, as well as a slight increase in number of bipolar and ganglion cells.

The absence of cones in the retinas of blacktips and whitetips probably precludes color vision in these sharks, but because of conflicting evidence one cannot be as certain of this conclusion as that of the association between diffuse vision and an all-rod retina (Walls, 1942).

It should be noted that the preceding discussion has been limited to the retinal basis of the visual functions of acuity, sensitivity, and color vision. A discussion of
other aspects of the visual apparatus is beyond the scope of this study.

**Life habits and vision in blacktips and whitetips**

Although sharks are generally thought to be nocturnal animals (Walls, 1942), the blacktip is probably more diurnal than nocturnal, or at least is equally active in both light and dark conditions. These sharks were frequently seen in the shallow waters of both the lagoon and seaward shore flats at Palmyra and Eniwetok Atolls apparently foraging at all hours of the day. Smaller individuals were more commonly seen during the daylight hours, but it was not unusual to find sharks up to four feet in length in waters barely deep enough to cover their dorsal fins. In fact, large blacktips were only rarely seen in the deeper waters of Eniwetok lagoon by Hobson (1961) who spent a considerable amount of time observing this habitat. Although adult blacktips were captured on hook and line at night as well as during the day, no juveniles were caught at night. Further, on several night excursions, no blacktips were seen on the seaward reef flat at Eniwetok by the writer. Thus, it is possible that the juvenile blacktips may move to a different habitat at night, but whether or not they are less active at night is highly conjectural.

The role of vision in the blacktips' feeding behavior has been investigated by Hobson (1961). He found that vision played a major role in the final phase of the search
and attack pattern during feeding activity. The main visual cues utilized by blacktips appeared to be brightness - contrast and movement.

Whitetips are sluggish sharks that occurred in both shallow and in fairly deep waters in Eniwetok lagoon. Unlike blacktips, they were usually found near coral heads and ledges (Hobson, 1961). In deeper water, whitetips usually swam slowly close to the bottom during daylight hours and were sometimes found resting on the bottom in holes and under coral ledges. However, Hobson found that they were responsive to stimuli during the daytime, and were quite capable of swimming quickly after their prey, and unusual for sharks, they even entered small holes in the coral in pursuit of prey. Further, he found that whitetips were very adept in finding food placed deep in holes, but were very awkward when feeding at the surface or mid-depths, where the food was in plain view and was undoubtedly seen by the sharks.

Whitetips were less commonly seen in the shallower waters of Eniwetok lagoon. Only a few whitetips were seen by the writer at two shallow water (10-15 feet in depth) piers during the daytime, and only one took a baited hook. (Blacktips, which were commonly seen at the piers, took bait readily.) However, fishing at night at these piers yielded as many whitetips as blacktips.

Both the whitetip and the blacktip then were active
during the day as well as at night. Their habits differed somewhat; whitetips were found more commonly in deeper water while blacktips occurred mainly in shallow water, although both species were found in the two habitats. Further, in shallow water, whitetips were either more abundant or more active at night than during the day, while no such difference was found with blacktip adults. Juvenile blacktips, however, apparently moved to deeper waters at night.

The retinas of both species are very similar and are adapted mainly for high sensitivity. Both blacktips and whitetips have a vertical-slit pupil, which is very efficient in protecting the sensitive rods from excessive illumination (Walls, 1942), and is probably the chief adaptation that allows the sharks to have a 24-hour visual activity in spite of the nocturnal retina. The unique occlusible tapetum lucidum found in blacktips and whitetips is also an adaptation useful in both bright and dim-light. In bright light, the tapetum is occluded by pigments which absorb the incident light that falls on the retina. In low illumination, the pigments are retracted, exposing the shiny tapetal plates, which reflect incident light back through the retina, making for efficient use of dim light (Walls, 1942).

Thus, although the retinas of the blacktips and whitetips are adapted mainly for sensitivity, both species can use their eyesight under both bright and dim-light conditions.
The differences in their life habits are probably not the result of visual limitations, but are more likely connected with other factors.

It should be pointed out that juvenile and adult black-tips and whitetips differ slightly in retinal structure. Both the greater number of cells per unit area and the presence of embryonic bodies in the juvenile retinas of both species suggests that visual ability may be associated with the stage of development of the retina.
SUMMARY AND CONCLUSIONS

The retinas of two species of Pacific sharks, *Carcharhinus melanopterus* and *Trianodon obesus*, were subjected to histological examination. The structure and organization of the retinas were described in detail. The standard paraffin method was used for making slide preparations of the retinas. A study was made of the various procedures, fixatives, and stains recommended by retinal histologists. The method that yielded the most consistently good results with shark eyes was the following: excise the eye from a living or freshly killed shark, pierce the cornea with a scalpel, use Kolmer's fixative, stain with Mallory's triple stain (Heidenhain's rapid one-step method).

The retinas of the two species were essentially similar; the following conclusions are applicable to both:

1. No regional differentiation in the structure of the retina was found in either species; the number of cells and width of the retinal layers were uniform throughout the fundus.

2. Cone visual cells were absent in both species.

3. Rod visual cells were thicker and fewer than those found in nocturnal teleost fishes. Blacktip sharks had about 99,000 rods/mm\(^2\) while whitetips had about 102,000 rods/mm\(^2\).

4. The ratio of rods to ganglion cells was low compared with nocturnal fishes: blacktips had a ratio of 77:1
and whitetips had a ratio of 82:1.

5. No photomechanical changes of visual cells occurred with changes in illumination; light and dark-adapted retinas appeared identical in all respects.

6. Developmental changes occur in the retinas of both species until the sharks have nearly attained their maximum length. Both species showed a decrease with development in the relative and absolute widths of most retinal layers, a decrease in numbers of cells per unit area, and a disappearance of the "embryonic bodies" (presumably comprising the "transient non-nucleated layer of Chievitz") found in the internal nuclear layer of embryonic and juvenile sharks.

7. The life habits of the sharks and their visual abilities were discussed in association with their retinal organization. The evidence points to a good discrimination of brightness but poor visual acuity in C. melanopterus and probably also in T. obesus. The absence of cones probably precludes color vision in the two species.
REFERENCES: General


REFERENCES: Microscopical Technique


Polyak, S. L. 1941. See REFERENCES: General.