

THE EYE OF THE HARBOUR SEAL, PHOCA VITULINA

by

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October, 1970

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Abstract

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The structural and functional organization of the pinniped eye, as represented by the harbour seal, Phoca vitulina, is much more complex than previously realized. The retina of the harbour seal is similar to that of the harp seal, in that it shows little zonation of the inner nuclear layer, possesses large horizontal cells, and has similar nuclear densities within the different retinal layers throughout the retina. The outer nuclear layer is the thickest of all the retinal layers. Photoreceptor cells of types B and C were observed with a ratio of roughly 1:23 respectively. This ratio and photoreceptor density along with a well-developed and extensive tapetum, indicates a highly light-sensitive retina. The existence of retinal folding in marine mammals is also questioned and discussed.

Refractive observations obtained through retinoscopy show that the harbour seal eye is very myopic and astigmatic in air, and hypermetropic with no discernable astigmatism in water. The axis of least corneal curvature is horizontal; current theories predict that the stenopeic pupil would thus correct for astigmatism in air. The functional significance of the astigmatism, which is in the order of 7 to 13 diopters, has not been adequately

determined to date, however. These observations are in agreement with those recently reported for the harp seal.

Behavioural studies have also confirmed the high sensitivity of the harbour seal eye to light, as suggested by the histological observations. Two captive harbour seals were trained to select a two-bar pattern over a one-bar pattern in both air and water, discriminating for the presence of a gap. Their abilities to carry out this task were measured as the gap in the two-bar pattern was gradually reduced in size. The light intensity of the white parts of the stimulus cards was $284-369 \text{ candela/m}^2$. Under these conditions, the harbour seal can detect a gap as small as 1 mm at a distance of 1.7 m in both air and water. These studies fail to provide values of the visual acuity of the harbour seal in the strict sense, however, owing to the physics of the pattern utilized. Rather, they indicate a measure of the absolute sensitivity of the eye to light.

Acknowledgments:

Appreciation is expressed to Dr. A. Q. McCormick, Dept. of Ophthalmology, U.B.C.; Drs. D. Tennant and B. Jervis, Vancouver optometrists; and Dr. G.G.E. Scudder, Dept. of Zoology, U.B.C., for many helpful discussions. Allan Mitchell, of the Pathology Laboratory of the Department of Ophthalmology, U.B.C., and Miss Joan Meredith, of the Department of Zoology, U.B.C., are thanked for their technical assistance. The staff of the Vancouver Public Aquarium is also thanked for their assistance, as are Jane Crocker and Peter Chappell who helped in testing the seals.

Particular thanks are expressed to Dr. H. D. Fisher, my supervisor, and to my wife, Dorothy, for their unrelenting support.

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Introduction

Present evolutionary theory dictates that the vertebrates evolved in water and hence the eye was initially an aquatic structure: its basic plan being established in the fishes. The physical properties of water which influence the eye and vision - properties such as the absorption of light, scattering effects and friction - are essentially exaggerations of the same properties that occur in air. Thus, when the vertebrates finally took to land, they were faced primarily with a quantitative disparity for most of the physical properties which influence vision. A few qualitative changes were required for aerial vision, however, mostly relating to the difference in refractive index of the medium. New features in the adnexa were also required to protect and moisten the now important refractive surface in the eye, the cornea. All the above modifications involved in this change of media are quite well known (Wall, 1942).

The secondary adaptation to water with an aeri-ally-adapted eye is not so well documented and understood. This secondary adaptation is obviously present in the cetaceans, which in general may be considered completely aquatic. It is in those species which are amphibious, though, that an adaptation problem might arise since somehow they must see well in both air and water. Such forms are of interest from an evolutionary point of view since such a way of life may represent stages in the

land to water transition.

There is little information on vision in amphibious mammals, such as pinnipeds, which breed on land but spend most of their lives in water. Being only secondarily adapted to an aquatic habitat, one would assume that the visual difficulties of amphibious mammals, if any, lie primarily underwater. The problem of aquatic vision for the terrestrial eye is one of developing enough accommodation to neutralize the hypermetropia which results when the cornea is in contact with water. However, rather than using intra-ocular muscles to increase the curvature of the lens, as does the otter (Walls, 1942), pinnipeds have achieved a moderate degree of hypermetropia underwater (Piggins, 1970) by means of a spherical lens. The combination of a stenopeic pupil arranged along the axis of greatest corneal curvature and a sensitive retina is thought to make extensive accommodation in air unnecessary (Walls, 1942), although in air, the eyes are very myopic and quite astigmatic (Johnson, 1893; Piggins, 1970). In water, this myopia and astigmatism disappear owing to the similarity of the refractive indices of the cornea and water.

To date, most of the emphasis on the sensory capabilities of pinnipeds has been directed towards acoustics and whether pinnipeds employ active sonar (eg. Poulter, 1966; Schusterman et al., 1967; Shaver and Poulter, 1967), with relatively little emphasis being devoted to the study of the other senses, particularly vision. Previous research on the harbour seal, P. vitulina

(Feinstein and Rice, 1966), and the California sea lion, Zalophus californianus (Schusterman et al., 1965), has been concerned with the abilities of these animals to discriminate between targets of various sizes in water. Their size-discrimination tests have shown that these species can make fine discriminations and that both species perform almost equally well. No tests have been made to date, however, on the relative ability of phocids to see above and below water, although Schusterman and Balliet have reported the visual acuity of the harbour seal and Steller sea lion underwater (1970a) and the visual acuity of the California sea lion in air (1970b).

Accounts on the structural organization of the pinniped eye are also few and fragmentary. Initial studies by Wilson (1865) and Johnson (1893) on the harbour seal were primarily devoted to the description of the dioptric mechanism and contributed little to the understanding of the retina. It remained for Putter (1903) to describe the gross morphology of the retina, which he did for several pinniped species. However, owing to the histological techniques available to him at that time, he was unable to describe in detail the retinal organization at the cellular level. He did describe the tapetum and the gross characteristics of the different retinal layers. He also confirmed Johnson's (1901) earlier statement on the absence of an area centralis, which disclaimed Chievitz's (1889) report of the existence of such a feature.

The most complete account to date on the structure of the pinniped retina is by Nagy and Ronald (1970) on the harp seal, Pagophilus groenlandicus. These authors also reported the absence of an area centralis and were able to observe only rod photoreceptors, although terminal pedicles characteristic of cones were noted. However, as they were unable to discern any further morphological differences, they concluded that cones are not present in this species. The huge horizontal cells and apparent lack of zonation in the inner nuclear layer were also particularly emphasized.

To date, there has been no concerted attempt in pinnipeds to relate visual ability in both air and water with eye structure. Among the local pinnipeds, the harbour seal (Phoca vitulina) is perhaps the most convenient for study. It has large eyes and is hardy. Thus, this species was chosen for a study of the visual adaptations required for an amphibious habit. The objectives were:

1. a histological and morphological study of the harbour seal eye with particular emphasis on the structural organization of the retina.
2. a refractive study of the harbour seal eye in both air and water to see if the eyes are as astigmatic and myopic as claimed by Johnson (1893).
3. a behavioural investigation of the harbour seal's discriminatory abilities in both air and water with the intent of measuring visual acuity.

With this information, the degree and nature of the harbour seal's visual adaptations to an amphibious habit will be evaluated and discussed.

Materials and Methods

1. Morphology and Histology:

The structural aspects of the harbour seal eye were studied at the level of the light microscope. Eyes obtained from seals caught in the Strait of Georgia were fixed whole in 10% formalin. Eyes were fixed within a few minutes of the animal's death, embedded whole in Bioloid, 56-58° C. mp (Will Scientific Incorp., Ann Arbor, Mich.), and sectioned at 5-8 μ in the vertical plane, ie. parallel to the orientation of the photoreceptors. Staining was with Harris haematoxylin and eosin, periodic acid-Schiff (PAS), or thionin.

In addition to the above method, some eyes were removed from animals a few minutes after all signs of body movement had ceased following injection of a lethal dose of Barb-Euthol (Haver-Lockhart Lab., Kansas City) into the extradural vein. These eyes immediately after removal were slit open around the ora terminalis and the posterior hemisphere was cut into squares of approximately one centimeter. Large sections were found necessary as with smaller sections the retina detached itself from the choroid in postfixation and became lost in the solution. Owing to the thinness of the retinal layer, which was directly exposed to the fixation, no cases of poor penetration of the fixative arose. Fixation was with glutaraldehyde (Sabatini et al., 1963) of pH = 7.4 in Sorensen's phosphate buffer (Culling, 1962), followed by postfixation in osmium

tetraoxide, dehydration and embedding in Epon (Luft, 1961). Sectioning was done with glass knives on the Sorvall ultra-microtome, MT-1, at 0.5-1 μ in both the vertical and horizontal planes. Staining was with alkaline toluidine blue or alkaline Azur II.

Cell types in the retina were identified in accordance with Polyak (1941) and cell numbers were determined with the equation $P = A \frac{M}{L + M}$ as described by Abercrombie (1946), where P = the average number of nuclear points per section, A = the crude count of nuclear numbers seen in section, M = the thickness of the section in μ , and L = the average length of the nuclei in μ . Five cell counts (A) for each cell type were made from photographs of the central region of the retina.

Eyes were also observed in living individuals throughout the study and pupil shapes with different light intensities were noted.

2. Refraction:

The four seals considered in this study ranged in age from one to four years and were all captive animals that had been held at either the University of B.C. or the Vancouver Public Aquarium for at least one year. Retinoscopy measurements were taken by two optometrists (Drs. D. Tennant and B. Jarvis) and an ophthalmologist (Dr. A. McCormick), as I had neither the experience nor the equipment to undertake these studies myself. The eyes were refracted under cycloplegia, with 3 drops of 1% atropine sulphate solution (Alcon of Canada

Ltd., Toronto) instilled in each eye. Cycloplegia typically took at least forty minutes to complete and was judged by the absence of a direct pupil reflex to light. After our first attempt at refraction, I felt that more accurate results could be obtained if the seal was first anaesthetized, as a few were quite active and difficult to handle. Anaesthesia was accomplished with 2.5% sodium thiopental solution (Abbott Lab., Montreal) which was injected intravenously into the extradural vein. The seal was maintained just on the verge of complete anaesthesia so that breathing, which is a voluntary action in seals, could be maintained. Complete anaesthesia runs the risk of killing the animal if maintained for more than 5-10 minutes.

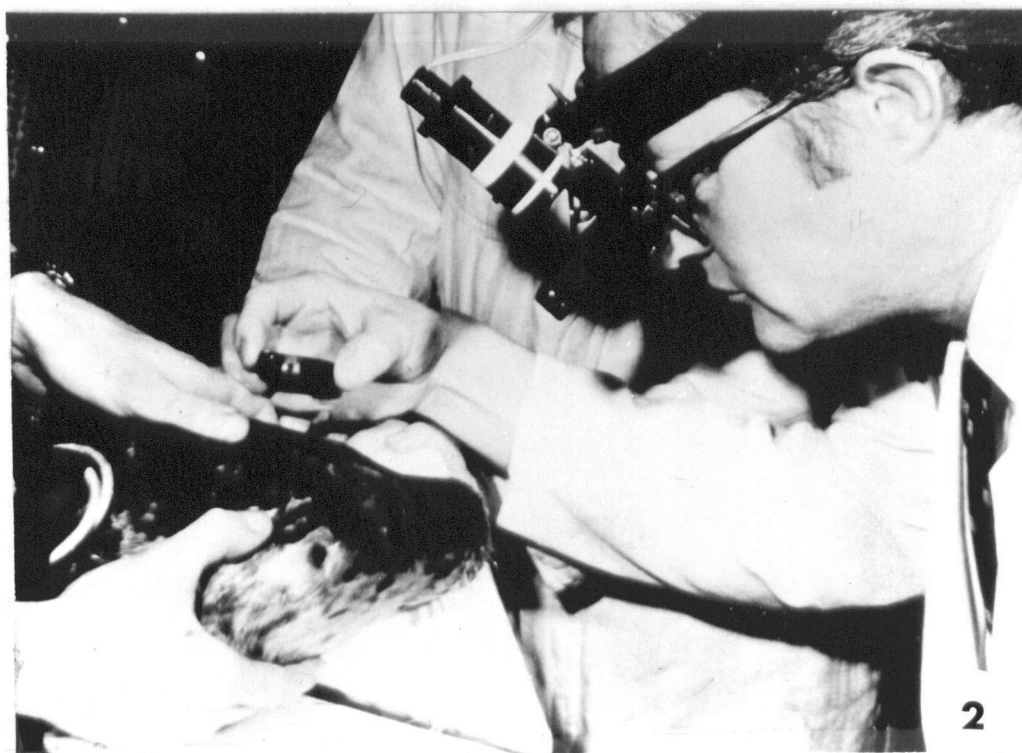
Refraction was carried out with both a slit retinoscope and a round, or point, retinoscope, the only difference between the two instruments being the shape of the light beam produced.

An attempt was also made to refract the eyes underwater, with the animal's head immersed in a plexiglass (methyl methacrylate) container filled with water (Fig. 1). This apparatus, made of 0.6 cm plexiglass, consisted of a rectangular container, 15 cm by 18.5 cm by 60 cm, mounted at an angle of 40° . The seal's head could be placed in this container and positioned so that it was possible to observe the eye through the plexiglass wall.

In conjunction with these retinoscopy measurements, a keratometer was used to measure the curvature of the anterior

Figure 1. Apparatus used in the underwater refraction of the harbour seal.

Figure 2. Measuring the refraction of the harbour seal in air.



surface of the cornea. Its principle is the reflection of an image off the anterior corneal surface, which is then viewed against a standard measurement built into the instrument. I found I was unable to obtain even an approximate measure with this technique, however, as due to the extreme flatness of the cornea, its measure of curvature was well outside the calibration of the keratometer which was calibrated for human use. By use of a Placido's disc, though, corneal astigmatism was observed. This disc consists of alternating black and white concentric rings and when held in front of the eye and viewed through a hole in its center, elliptical images on the surface of the cornea result if corneal irregularity exists.

Corneal moulds of the eye were then made to measure this refractive error. The plan was to make a plaster mould and from this, a plastic contact lens, which could be measured through ultrasonic techniques. An initial attempt to make a plaster mould on a live animal failed, for when the eyelids were forced apart to the extent required for a mould of the complete corneal surface, the pressure exerted on the globe distorted the corneal shape. Two attempts were then made on animals which had just been killed with Barb-Euthol. After removal of the eyelids, a preliminary mould of the eye was made with "Ophthalmic Moldite" (Obrig Lab., Inc.), a soft, fast-hardening mixture which hardens to the consistency of a hard-boiled egg white. A positive mould of coecal dental stone, a very fine plaster, was then made from the "Moldite"

mould as soon as it was removed from the cornea. This was necessary as the "Moldite" mould soon undergoes shrinkage and distortion as the water in it evaporates. From the plaster mould, a crude contact lens was later made and measured by the Plastic Contact Lens Co. (Canada) Ltd.

Funduscopy examinations were also made on two of the seals examined as shown in Fig. 2 to determine if retinal folds were visible in the living eye.

3. Visual Discriminations:

Behavioural experiments were carried out in the research compound of the Vancouver Public Aquarium in Stanley Park. Salt water was found essential to maintain the eyes in perfect condition as many of the animals maintained in freshwater sooner or later showed a whitening of the cornea. This condition was found to be usually due to an invasion of leucocytes into the cornea as a result of corneal ulcers. Why seals maintained in freshwater seem to be more susceptible to these ulcers is not presently clear.

A total of seven seals were initially selected, with Scar, a two-year old male, and Blondie, a four-year old female, being chosen as the most manageable. Each seal was required to discriminate between a two-bar pattern and a one-bar pattern as the gap in the two-bar pattern was gradually reduced in size, with the presence of a gap being the critical parameter.

All tests were undertaken in a plywood tank 1.22 m by 2.44 m filled with seawater to a depth of 51 cm. The stimulus

cards were presented in a smaller tank 0.86 m by 0.90 m adjacent to the testing tank (Fig. 3 and 4). Plexiglass windows, 1.27 cm by 25.4 cm by 55.9 cm, were built into both tanks at the same level providing a clear view between the two tanks. Two tanks were used, along with continuous flushing of the seawater, to ensure that suspended debris in the larger tank would not interfere with the view; this debris consisted primarily of fish scales shed from the rewards. The tanks were enclosed in a hut to remove any external stimuli and to allow control of lighting of the stimulus cards.

The walls of the testing tank were a light gloss blue. The light intensity of the white parts of the stimulus cards was 284-369 candela/m². The walls of the smaller tank were a flat black to remove the possibility of glare. Both patterns were illuminated independently with 75 w floodlamps and the interior of the hut was illuminated with three, four-foot, 40 w, cool white fluorescent tubes. Measurements were made with a "Photovolt Universal Photometer, Mod. 200", and the intensity of light directed onto the stimulus cards was controlled by means of a dimmer switch. This range of illumination was not considered to affect the results, since in all cases, the animals were adapted to an illumination of this magnitude before commencement of the day's testing.

The experimenter stood behind the smaller tank and presented both stimulus cards simultaneously (Fig. 5). The stimulus cards were white plexiglass, 0.32 cm by 30.5 cm by



Figure 3. Photo of the tanks at the Vancouver Public Aquarium where the behavioural aspects of this study were done.

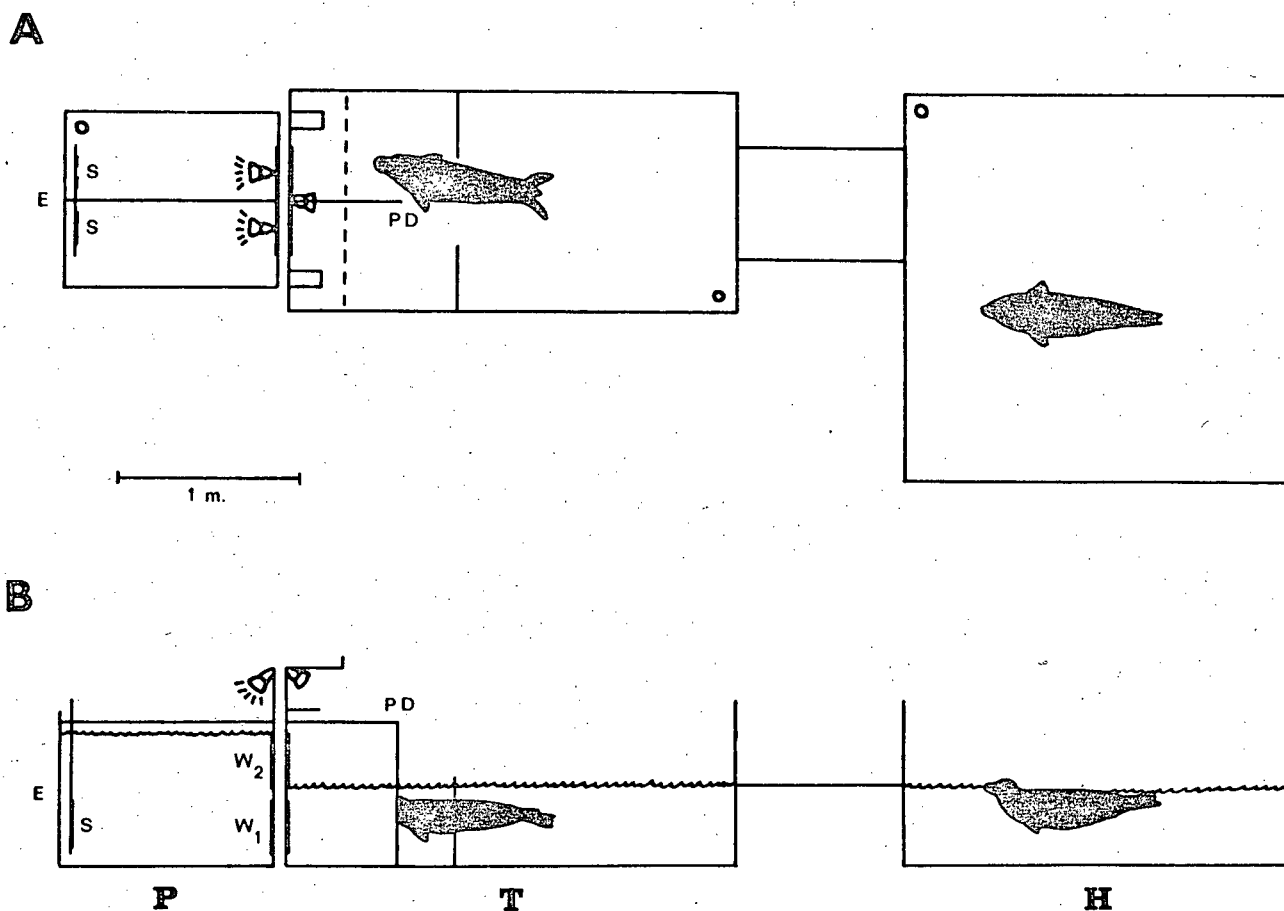
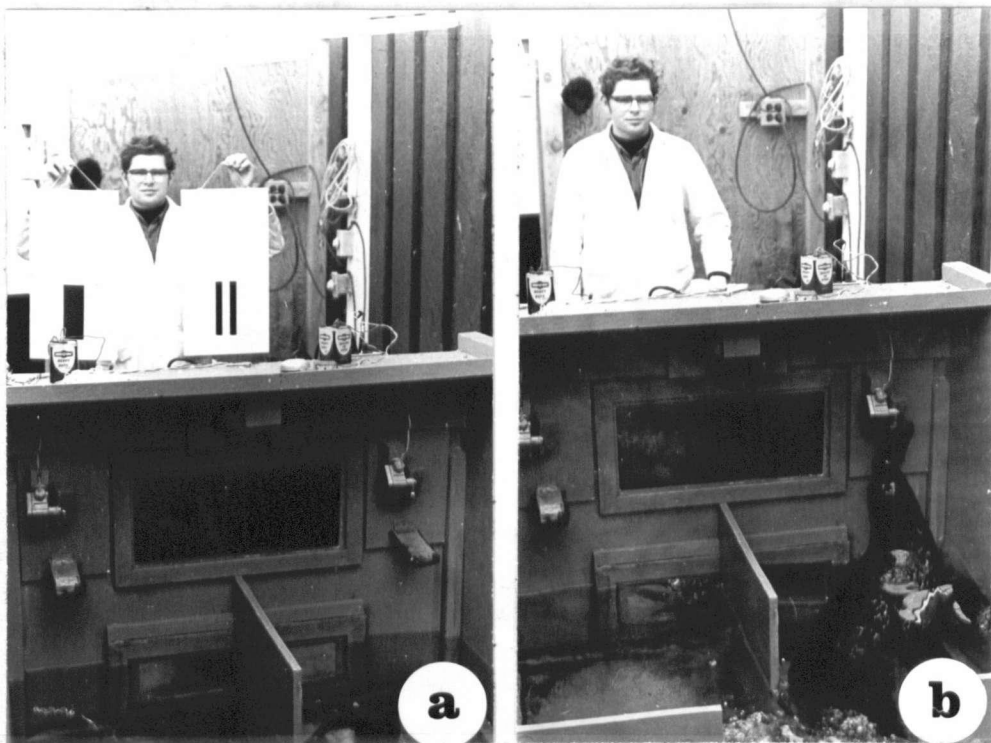


Fig. 4. Apparatus used in testing the harbour seal's underwater discrimination of one- and two-bar patterns presented simultaneously. The relationships of the tanks are shown in both dorsal (A) and lateral (B) views. For testing in air, the water was drained from the stimulus presentation tank (P) and the stimulus cards were presented at the level of the upper set of windows (W_2); the water level in the testing tank (T) remained the same but the seals were trained to swim at the surface. One seal at a time was let from the holding tank (H) into the testing tank (T) by means of sliding partitions.

E, experimenter; S, stimulus cards; W_1 , lower set of windows (for testing underwater); PD, point of discrimination for the seal.

Figures 5.a-5.b-5.c. Photos showing details of the behavioural discrimination tanks and the presentation of a typical problem.

- A. Photo of the stimulus cards, with the positive card at the reader's right. In a normal trial, the cards would not be held as high, so that the seal would be unable to see the cards.
- B. A correct response by the seal, as it chose the right-hand lever. The seal's approach was made underwater, with the cards viewed through the lower window.
- C. Photo showing details of the stimulus presentation tank. The stimulus cards were presented just to the fore of the bucket, which contained the herring rewards.



61.0 cm, with either one or two black bars. The bars were 20.3 cm long and were of 2.54 cm ACS tape. In the case of the one-bar (negative) pattern, the width of the bar was 5.08 cm plus the width of the gap on the corresponding two-bar (positive) pattern. The distance between the centers of any pair of patterns was 21 cm. By the use of vertical partitions separating the two targets in both tanks, the seals were forced to make their choice of targets at a distance of 1.7 m (Fig. 4). Any change of alleyways after entrance into one was considered a wrong response. A trial consisted of the seal waiting at the far end of the testing tank from the experimenter while the cards, a two-bar card with its corresponding one-bar card, were placed in position in the smaller tank. On the ringing of an electric bell, the seal was trained to swim forward and after making a choice of alleyways, raise a lever with its nose. A correct response was announced by a 5 second flash of light into the testing tank and was rewarded with 1/3 of a herring (Clupea pallasii). An incorrect response was followed by a two minute "time-out" period during which the seal was required to wait two minutes before the next trial was presented.

Both seals were first trained to push the levers after making an underwater approach. Sixty trials were presented daily and the amount of food fed to each seal daily was 2.7 kg of herring (included in this are the rewards for correct responses). This amount of food was such that both seals were

able to maintain their appetite and remain in a generally healthy condition.

The positive card was randomly alternated between the right and left sides in order to prevent lever preference. The following initial series of gap sizes were used (in cm): 2.54, 1.27, 0.63, 0.32, 0.25, 0.21, 0.13, 0.09 and 0.05. Each day's trials consisted of from two to three gap sizes randomly presented with the ratio biased in favour of the larger gap sizes. This ensured that a majority of the trials were such that the seals had no difficulty in making a correct response. A smaller gap size was only introduced when the data (Table 1) showed evidence that the seal was able to see the smallest gap size being presently used. Final measurements of their discriminatory abilities in both air and water were taken in the first half of 1969 and were based on approximately 600 trials with gap sizes between 0.05 - 0.3 cm (Table 1).

For testing in air, the water was drained from the smaller tank and the stimulus cards were presented at the level of the upper set of windows (W_2 in Fig. 4). The water level in the testing tank remained the same and the seals were trained to swim on the water surface.

It will be noticed that in Table 1, some of the gap sizes mentioned are not included in the initial series mentioned above. This is a result of shrinkage of the ACS tape due to the frequent immersions of the stimulus cards in water. It was therefore found necessary to remeasure the gaps after every

30 trials and note any changes in their sizes which may have occurred. High periods of humidity were also found to affect the tape, making daily measurements during aerial testing also essential. All measurements were made by eye with the use of a ruler calibrated in $1/50^{\text{ths}}$ of an inch. Each of these measurements was further divided on the basis of where the border of the gap fell and although it is realized that a few errors were no doubt made, it is felt that over 600 trials they would cancel out. Furthermore, a definite value below which the seals could not see and above which they could is not claimed, but rather simply that their lower threshold level is within a certain range of values.

Results

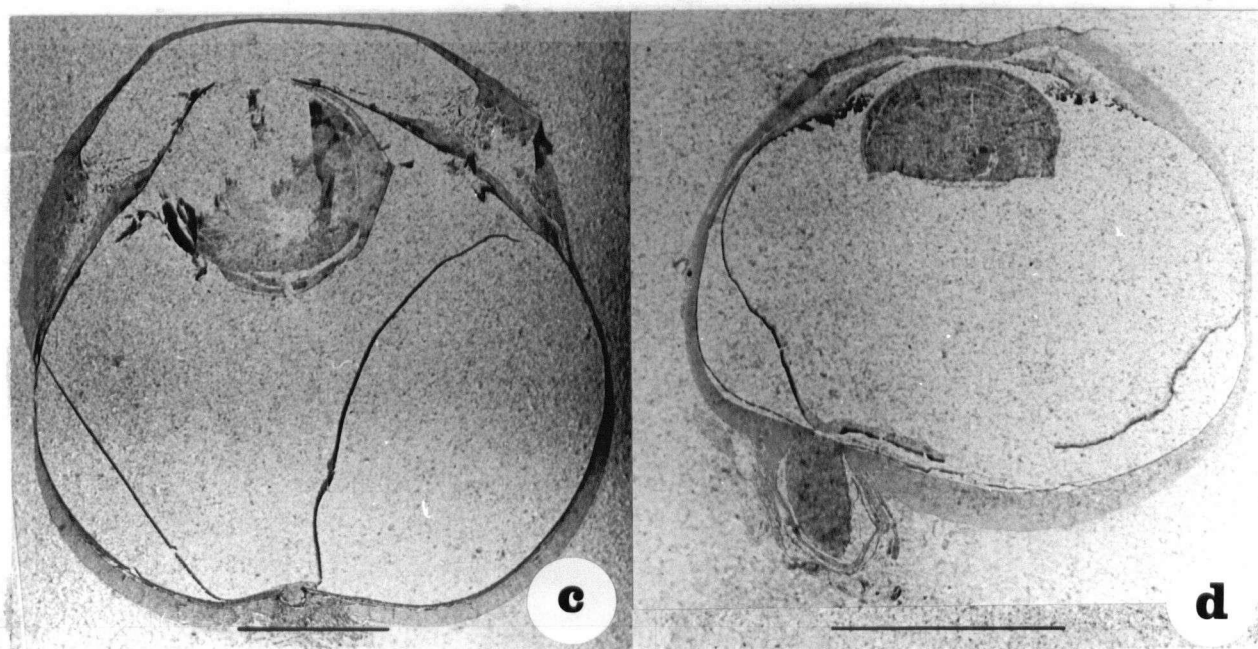
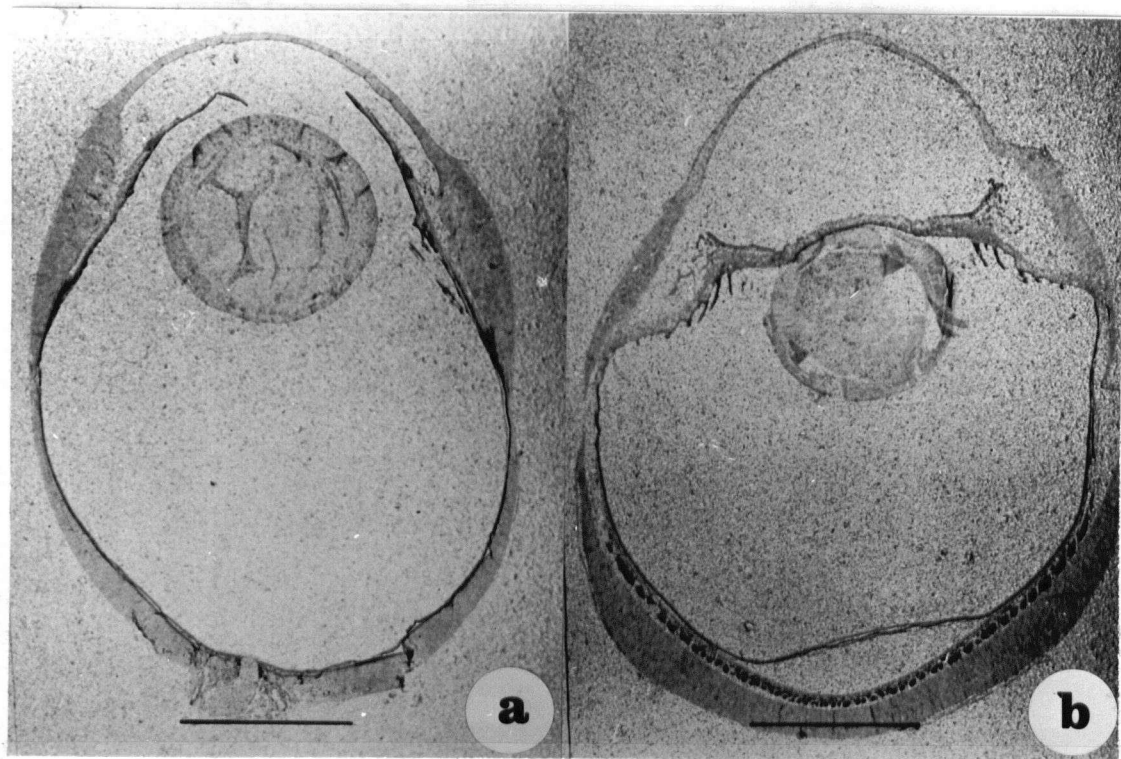
1. Morphology and Histology

The basic morphological features of the harbour seal eye are shown in Fig. 6. Briefly, the eyes are large, both in absolute size and in relative size to the body, with a globe diameter of 39-40 mm in the adult. The cornea is roughly circular and flattened, being only slightly arched over the anterior chamber which has a depth of 2-2.5 mm. The lens is spherical and although large, is not out of proportion to the rest of the eye; it has an equatorial diameter 37% that of the vertical diameter of the eye. The iris is very muscular and heavily vascularized, and is anchored to the inner aspect of the cornea by means of a very profuse fiber network (Fig. 7). The sclera is thickened slightly in the posterior portion of the eye but is also not out of proportion to the other component layers when compared to a typical terrestrial mammalian eye. The choroid is heavily vascularized and pigmented and in addition, contains an extensive tapetum which lines virtually the whole of the vitreous cavity. No fovea nor area centralis could be distinguished.

Pupil size and shape in air appear to be primarily influenced by the amount of light present, closing down to a pinhole at the uppermost end of a vertically constricted slit under high light intensities and opening up to an oval or elliptical shape under low light intensities (Fig. 8). No

Figures 6.a-6.b-6.c-6.d. Photos of histological sections of pinniped eyes. Scale = 1 cm.

- A. Harbour seal. (Phoca vitulina)
- B. Northern fur seal. (Callorhinus ursinus)
- C. Harp seal. (Pagophilus groenlandicus)
- D. Killer whale. (Grampus orca)



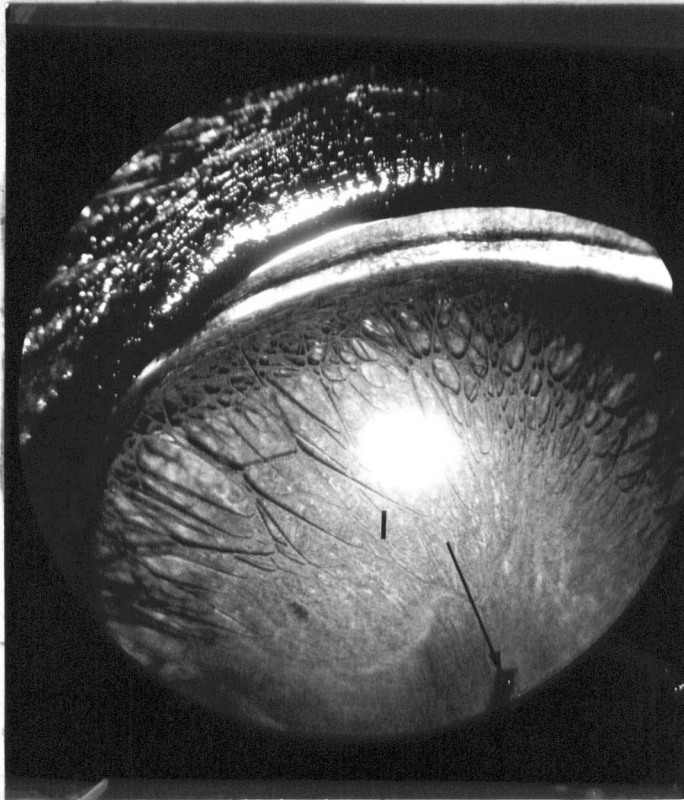
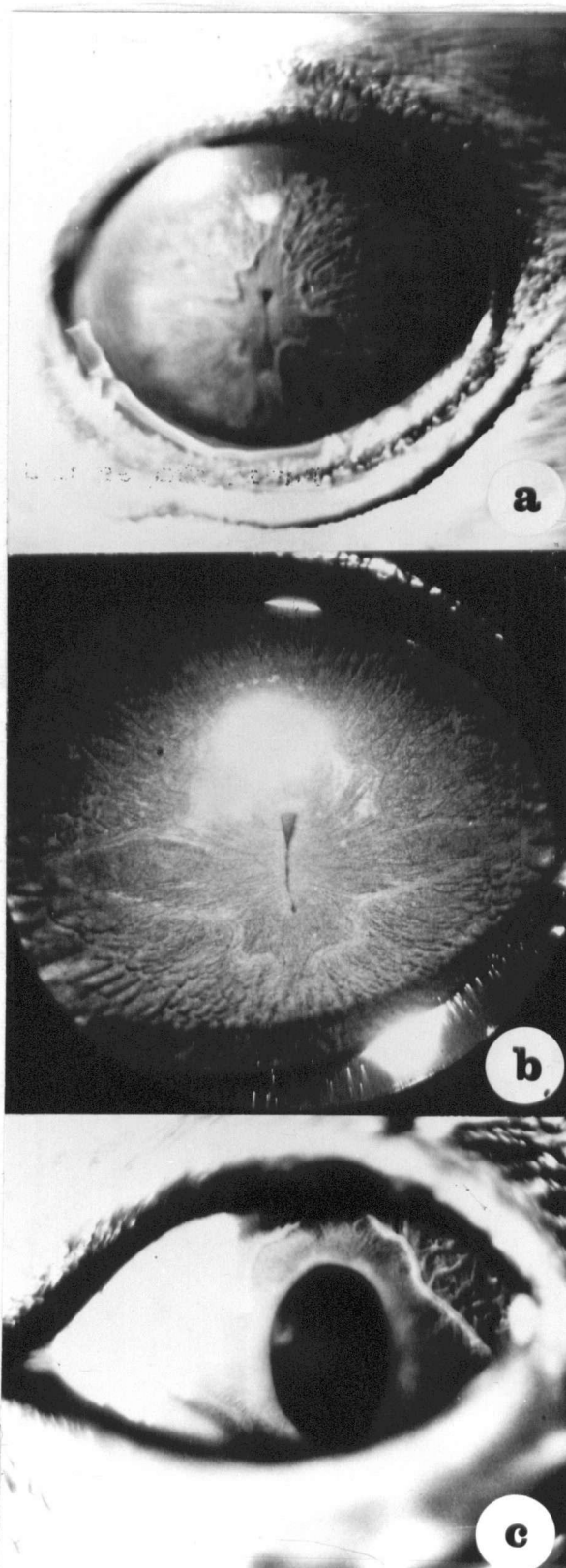


Figure 7. Close-up of the extensive fiber network characteristic around the periphery of the harbour seal iris (I). Arrow points to pupil.

Figure 8.a-8.b-8.c. Pupil shapes under different light intensities.

- A. Very high light intensity. This is as small as the pupil may be made.
- B. High light intensity. The pupil is more tear-shaped than in A.
- C. Effect of atropine. This shows the maximum size obtainable, and represents the pupil size found under very low light intensities.



pupil slit that is functional along its entire length is formed in bright light, such as would exist on a sunny day. In water, the pupil is the same size as in air, with pupil size and hence shape in water appearing to be also primarily influenced by light intensity.

The gross histological features of the harbour seal retina are seen in Fig. 9. Deep to the nerve fiber layer is a single layer of ganglion cells (Fig. 10) interspersed around the vertically transversing radial fibers of Muller. The ganglion cells vary little in size except for the occasional giant ganglion cell. These latter cells may be easily identified by their huge bodies (Fig. 11) and extensive dendritic branching.

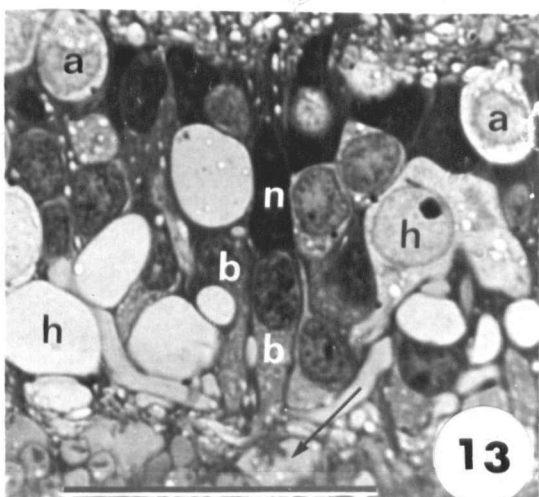
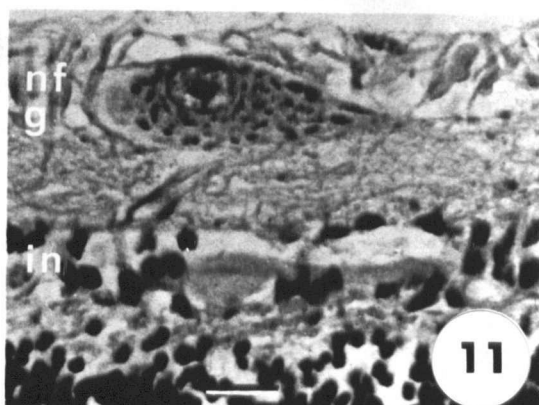
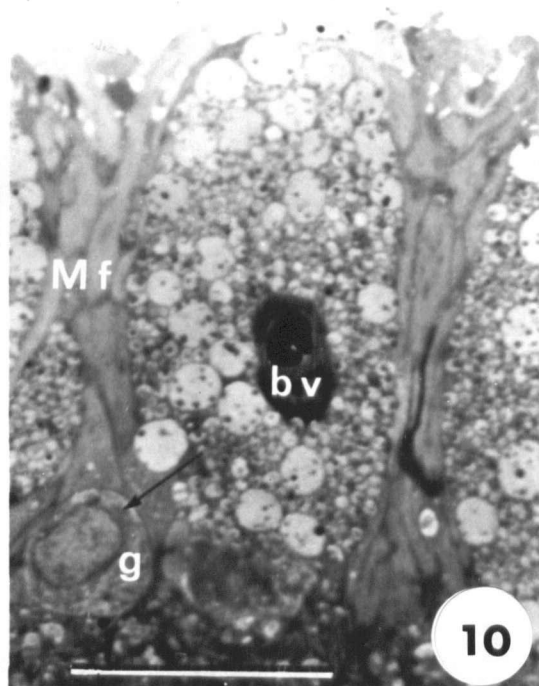
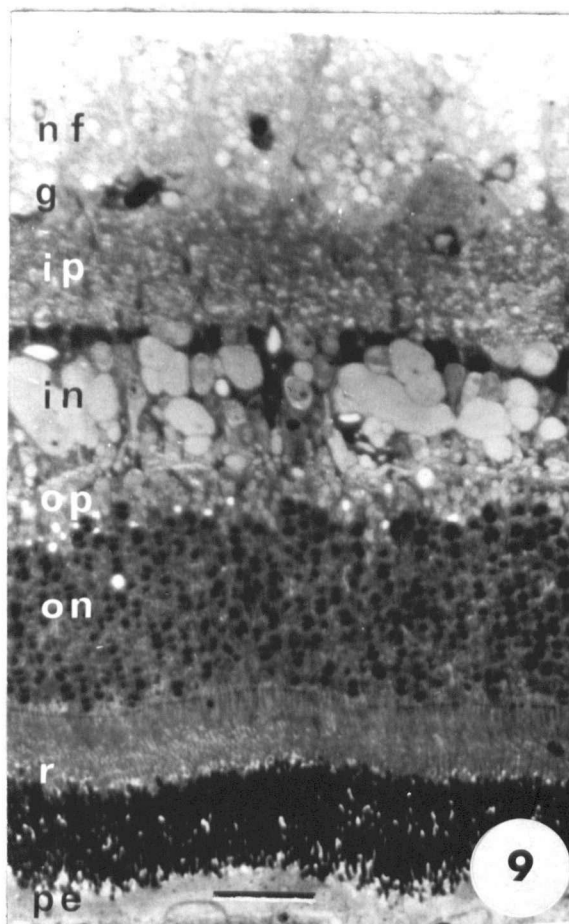
The inner nuclear layer is distinguished by the absence of any distinctive zonation and by the very large horizontal cells which extend throughout this layer (Fig. 12). Interspersed around these cells and their extensive axis cylinders are the bipolar, amacrine and neuroglial cells. The bipolar cells are characterized by processes which extend outward into the outer plexiform layer, where they synapse with the terminal pedicles of the photoreceptor cells, and inward into the inner plexiform layer, where they synapse with the ganglion cells. The bipolars are located predominately in the outer regions of the inner nuclear layer and may be identified in Fig. 12 by their outward extending processes and oval nuclei. Other occasional cells here identified as amacrine cells, with only broad, single processes extending into the inner plexiform

Explanation of Figures 9-21

Abbreviations used in Figures:

nf	optic nerve fiber layer
g	ganglion cell layer
ip	inner plexiform layer
in	inner nuclear layer
op	outer plexiform layer
on	outer nuclear layer
r	layer of photoreceptors
ris	receptor inner segments
ros	receptor outer segments
pe	pigment epithelium
t	tapetum
b	bipolar cell
h	horizontal cell
Mf	radial fibers of Muller
a	amacrine cell
n	neuroglial cell
bv	blood vessel
p	polysynaptic pedicle
o	oligosynaptic pedicle

- Fig. 9 Vertical cross section of the central area of the retina. Toluidine blue.
- Fig. 10 Vertical section through the optic nerve fiber and ganglion layers. Large radial fibers of Muller can be seen vertically transversing these layers. Arrows point to ganglion cells and a small blood vessel. Toluidine blue.
- Fig. 11 Vertical section through a giant ganglion cell. Haemotoxylin and eosin.
- Fig. 12 Vertical section through the inner nuclear layer showing large horizontal cells (h), amacrine cells (a), bipolar cells (b) and neuroglial cells (n). Horizontal cell axis cylinders are marked by arrows. Toluidine blue.
- Fig. 13 Vertical section through the inner nuclear layer showing the synapses between bipolar cells (b) and a polysynaptic pedicle (arrow). Toluidine blue.

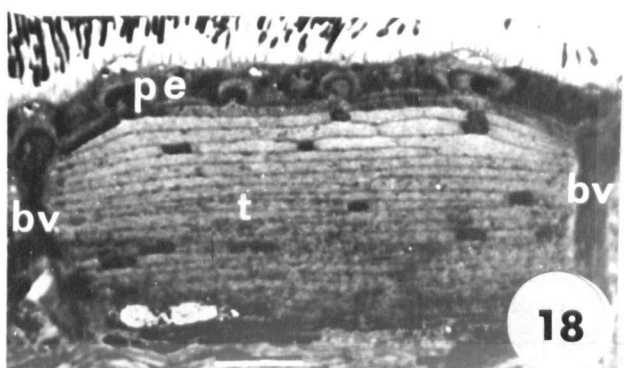
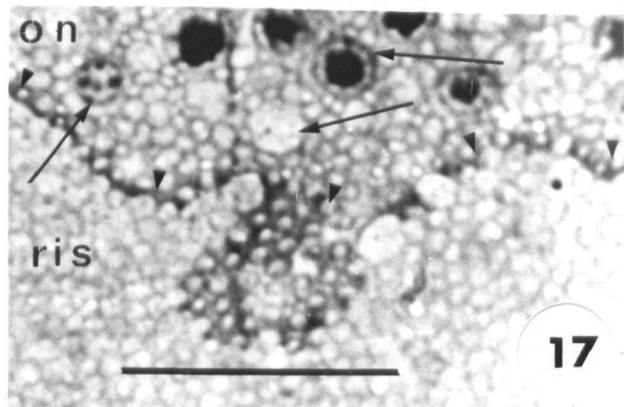
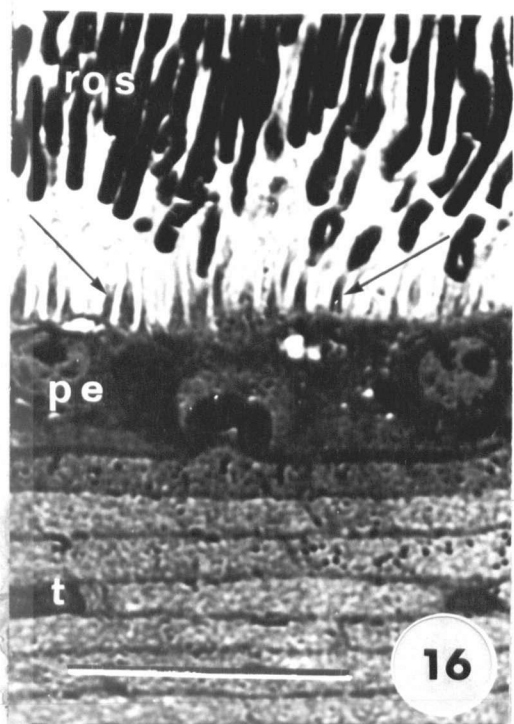
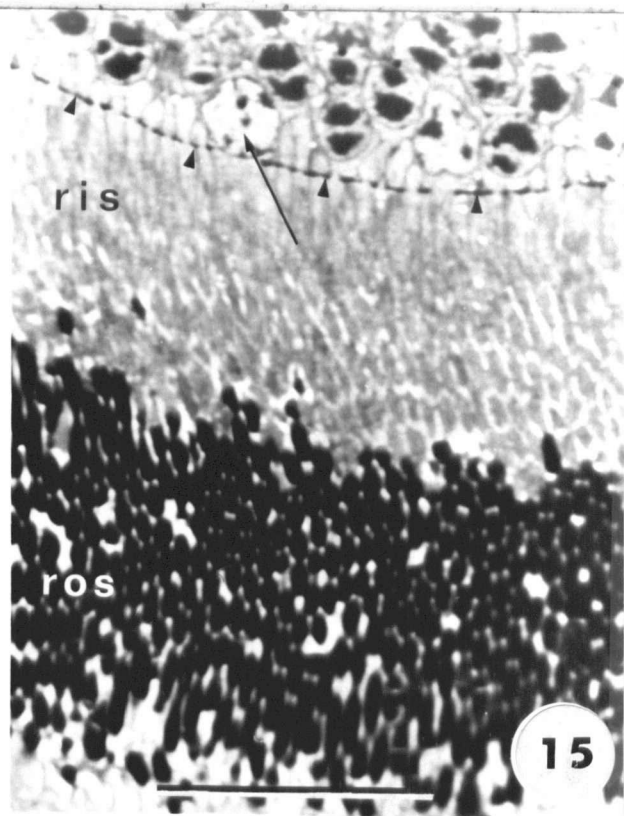
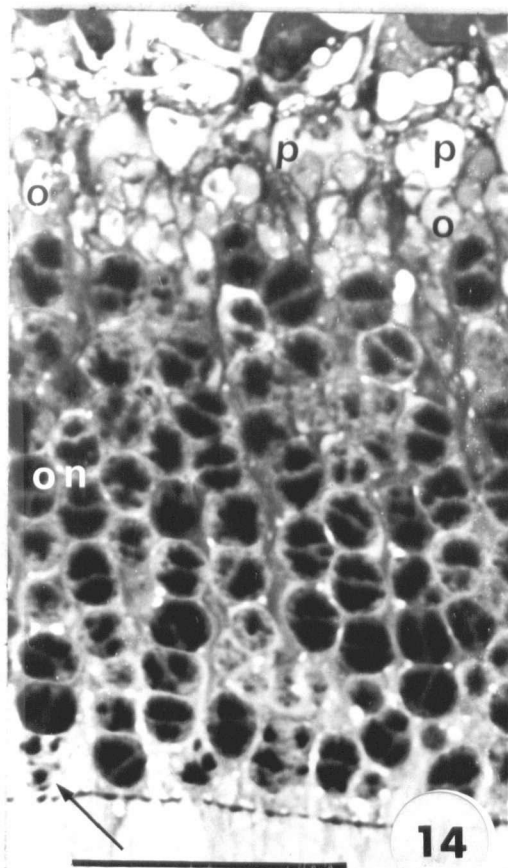


layer, were observed on the inner aspects of the inner nuclear layer. The small, darkly-stained nuclei surrounded by little cytoplasm in the inner regions of this layer (Fig. 13) are the nuclei of neuroglial cells.

Two varieties of photoreceptor pedicle can be distinguished in the outer plexiform layer, rod-like oligosynaptic pedicles and cone-like polysynaptic pedicles (Fig. 14). The oligosynaptic pedicles are most numerous, with a ratio of approximately 23:1. They are also much smaller than the polysynaptic pedicles and contain only one basal filament in contrast to the many short, converging filaments found in polysynaptic pedicles. The polysynaptic pedicles are also situated slightly closer to the inner nuclear layer than are the oligosynaptic pedicles. There is no outer fibre layer of Henle.

The outer nuclear layer is the thickest of all the retinal layers, being 10-12 cells deep. Regional variation in the thickness of this layer was only observed in the outer peripheral areas where it gradually thinned out. The photoreceptor nuclei appear to be of two types, with most of the nuclei containing two relatively large clumps of chromatic material and located in from the outer limiting membrane. A few, however, contain many smaller clumps of chromatin and are directly adjacent to this membrane (Fig. 14 and 15). These latter cone-type nuclei are also often associated with a large gap in the outer limiting membrane.

- Fig. 14 Vertical section through the outer plexiform and outer nuclear layers showing oligosynaptic (o) and polysynaptic (p) terminal pedicles. Arrow points to cone-type photoreceptor nucleus. Toluidine blue.
- Fig. 15 Vertical section through the outer limiting membrane and layer of rods and cones, showing photoreceptor inner (ris) and outer (ros) segments. Arrow points to cone-type photoreceptor nucleus. Toluidine blue.
- Fig. 16 Vertical section through pigment epithelium. Arrows point to fine cytoplasmic processes extending towards the photoreceptor outer segments. Toluidine blue.
- Fig. 17 Horizontal section (slightly oblique) through region of outer limiting membrane (points). Arrows point to photoreceptor nuclei. Azur II.
- Fig. 18 Vertical section through pigment epithelium and tapetum. Arrows point to chorio-capillaries transversing the tapetum. Toluidine blue.



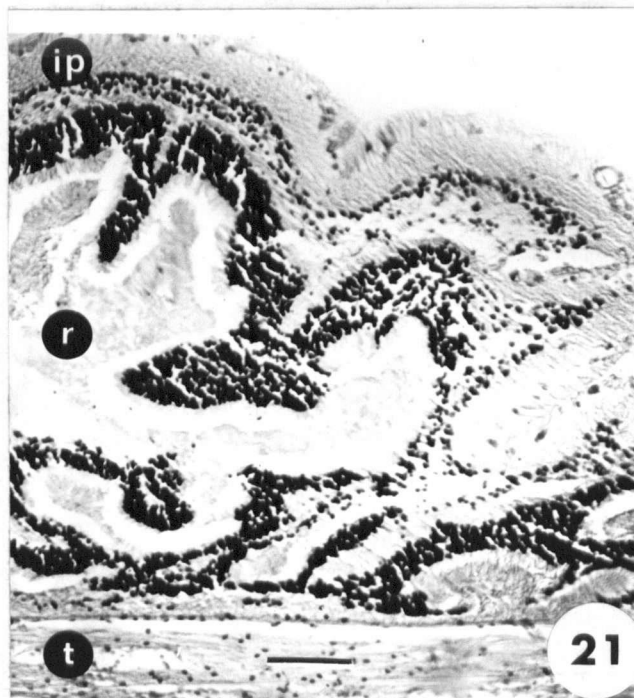
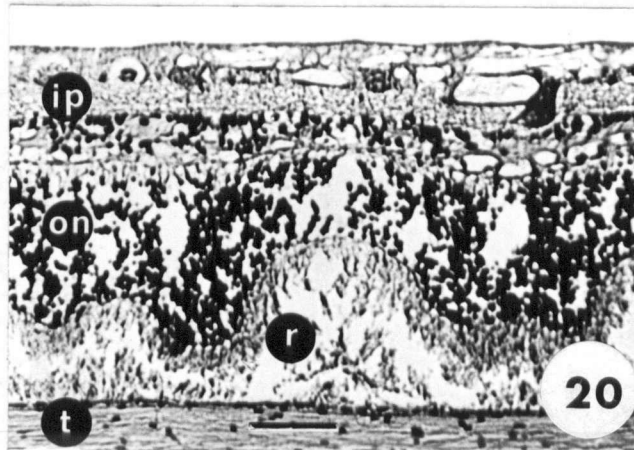
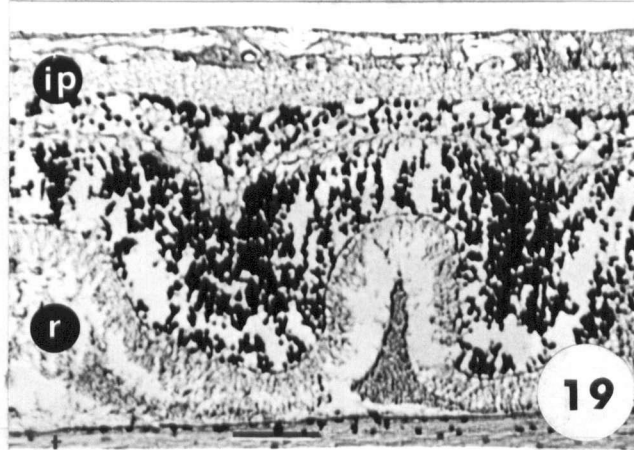
The photoreceptor layer shows two distinct zones when stained (Fig. 15), a lightly staining inner segment layer and a darker staining outer segment layer. Only one type of photoreceptor can be distinguished on the basis of inner segment morphology (Figs. 15 and 17). Closely associated with the photoreceptor cell layer is the pigment epithelium (Fig. 16), a unicellular layer, from the cells of which fine cytoplasmic processes extend down between the outer segments of the photoreceptor cells. No pigment granules could be observed.

Although not part of the retina, there also exists a very well-developed and extensive tapetum cellulosum (Fig. 18). It is composed of 20-22 layers of flattened cells lying in the superficial choroid deep to the pigment epithelium, thinning gradually towards the periphery. This layer is regularly penetrated by many small capillaries arising from the choroid which transverse laterally in the region between the pigment epithelium and tapetum.

Two types of "retinal folding" were observed in the whole sections embedded in paraffin. The most common type of folding was a slight one (Fig. 19 and 20) involving the outer nuclear and photoreceptor but not the inner layers of the retina. This folding was observed in four of the seven harbour seal eyes examined and in these eyes, was only absent in the peripheral regions of the retina. The second type of folding (Fig. 21), much more intricate and complex and involving the whole retinal layer, was observed in three of the seven harbour seals studied

Fig. 19 and 20 Vertical sections through the retina
showing the slight form of retinal folding. Only
the outer nuclear layer and layer of rods and cones
are folded. Haemotoxylin and eosin.

Fig. 21 Vertical section through the retina showing the
complex form of retinal folding. All the retinal
layers are folded. Haemotoxylin and eosin.



(these three also showed the first type of retinal folding). This folding is much more localized than the first type of folding mentioned, and was only observed to occur on a small proportion (10% at the most) of the retina shown through sectioning, usually in the central or middle retinal regions. Owing to its magnitude, this folding should be clearly visible through funduscopic examination. Since none was observed, it was not possible to confirm that this type of folding occurs as a natural phenomenon.

No folding of either type was observed in those sections embedded in Epon. It is not possible therefore to describe in detail the retinal organization about these folds.

The peripheral termination of the sensory retina, the ora terminalis, was observed to be at the base of the ciliary muscles. There was thus no pars plana as occurs in the human eye.

2. Refraction

Refractive measurements of the four harbour seals examined by retinoscopy are given in Table 1. Initial difficulties in controlling the seals were overcome by means of anaesthetics, but problems were still encountered in measuring the optical axes correctly. All three people that took these measurements are professional doctors and well-skilled in the measurement of refraction in humans, but none of them could state with certainty the values for the harbour seal. The effect of the tapetum, a layer not present in man, in reflecting back

<u>Seal</u>	<u>Refraction</u> <u>Axis</u> <u>Vertical</u> (Diopters)	<u>Refraction</u> <u>Axis</u> <u>Horizontal</u> (Diopters)	<u>Astigmatism</u> (Diopters)	<u>Instrument</u>	<u>Operator</u>
Susi.	-7.0 (B)	-7.0 (B)	0.0	Slit	1
Orphie	-5.0 (B)	-5.0 (B)	0.0	Slit	1
Scar	1.5 (B)	2.0 (B)	0.5	Slit	1
	-3.5 (R)	3.0 (R)	6.5	Round	2
	-2.5 (L)	4.5 (L)	7.0		
	3.0 (L)	5.5 (L)	2.5	Slit	2
	8.0 (B)	1.0 (B)	7.0	Slit	3
Blondie	-11.5 (B)	1.5 (B)	13.0	Round	2
	-11.5 (B) *	1.5 (B) *	13.0*	Round	2
Orphie (Water)	4.0 (B)	4.0 (B)	0.0	Slit	1
<hr/>					
-	-13.0	-4.0	9.0	-	Johnson
Mean of 12 eyes (harp seal)	-12.5 (-16.5 to -11.5)	-8.0 (11.5 to -3.5)	6.0 (12 to 5)	-	Piggins
Mean of 12 eyes (harp seal) (Water)	5.25 (9 to 3.75)	4.70 (9 to 3.75)	0.0 (2 to 0)	-	Piggins

Table 1 : Refractive measurements of the harbour seal as obtained in this study and by Johnson (1893). Harp seal measurements were taken by Piggins (1970). These values were obtained in air by means of retinoscopy, with those values obtained in water noted.

B = in both eyes, R = right eye, L = left eye, * = without anaesthesia.

the retinoscopy light, combined with the large eye and its upward tilt, made all the measurements questionable; it was not possible to definitely state that measurements obtained were along the optic axis.

Both attempts at obtaining accurate measurements by the use of contact lenses failed, for when the lenses were later studied, it was noted that dimpling of the cornea had occurred. This was presumably as a result of a drop in blood pressure within the eye owing to death. Furthermore, the lenses were also found to be too flat to be measured by conventional commercial ultrasonic equipment, which is calibrated for human eye measurement. Owing to a shortage of seals, efforts in measuring corneal curvature by this technique were not pursued.

Corneal astigmatism was observed to occur, however, by means of a Placido's disc. This astigmatism had its distortion effect in the vertical, which indicates the radius of least corneal curvature in the horizontal. No quantitative values are obtainable with this technique however.

3. Visual discriminations:

The greatest problem encountered in this phase of the study was the long period of time required to train the seals to respond to the correct stimulus card (Fig. 22). As mentioned earlier, Blondie and Scar were the two seals chosen as being the most manageable from seven initially selected seals. However, even these two were very slow learners.

Figures 22.A-22.B. Performances of the two harbour seals during conditioning. Each day's trials were broken down into blocks of twenty trials each to better describe performance behaviour.

- A. The performance of the four-year old female, Blondie.
- B. The performance of the two-year old male, Scar.

Dotted line = stage at introduction of the baffles into the tank; Star = simplification of the problem from a two-bar (positive) : one-bar (negative) one, to a one card one (only the two-bar (positive) card was presented).

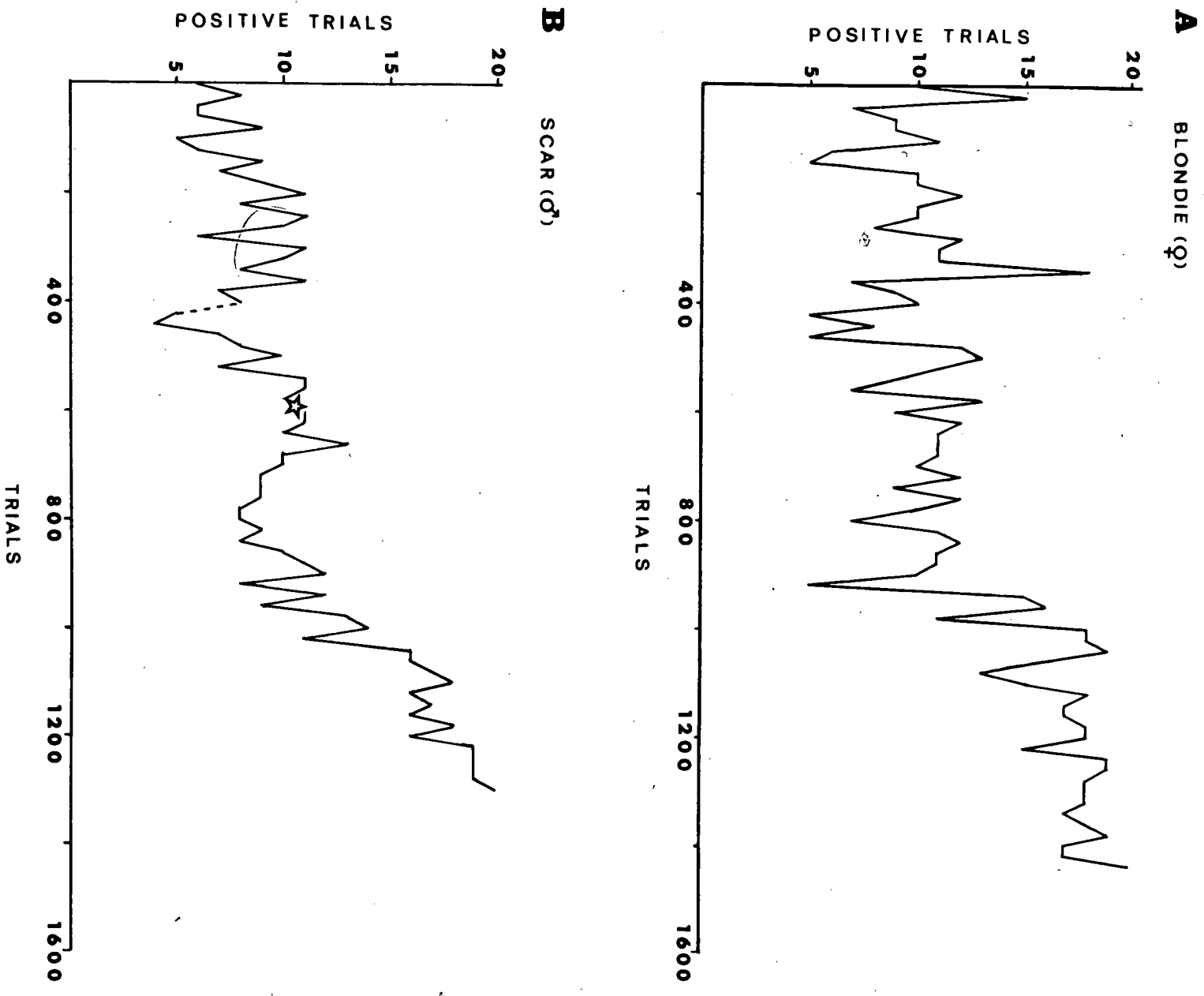


Figure 22.

Blondie, the female, was trained first and required 1540 trials before she was able to respond correctly to all of one block's trials (the day's 60 trials were subdivided into three blocks of 20 trials each), the criterion for solving the problem. The problem which she was required to solve was a three card one, consisting of one positive stimulus card, a two-bar pattern, and two negative stimulus cards, a blank white card and a blank black card. This combination was used as some generalization experiments were initially planned, but owing to the long period of time required in training the seals, this aspect was later dropped. The sudden improvement in Blondie's responses after 940 trials occurred after increasing the "time-out" period following an incorrect response from 30 seconds to 3 minutes. This suggests that perhaps one of the reasons for the large number of trials required to solve the problem was inexperience in training on my part. However, Scar was trained after Blondie and yet still required a comparable number of trials. Schusterman (1968) also encountered similar difficulties in his training of the harbour seal.

Scar, the male, required approximately the same number of trials (Fig. 22) to learn the two-bar (positive): one-bar (negative) problem as Blondie required to learn the three card one, even when the problem was simplified after 600 trials to a one card one (only the two-bar (positive) card was presented, with the only requirement being to push the lever on the same side as the card). Once the problem was learnt, however, both seals showed considerable retention of

the problem and would perform correctly even after a two month lapse in testing.

The responses of Blondie and Scar in both air and water for the final set of measurements, ie. for gap sizes between 0.05--3.0 cm, is given in Table 2 and Figs. 23 and 24 respectively. Weighted regressions were performed on the data for both air and water, and when the standard psychophysical criterion is applied to the data (the difference threshold being defined as the gap size corresponding to the 75% performance level), it is found that both seals could resolve a vertical gap between two black bars as small as 1 mm when at a distance of 1.7 m. There is no significant difference ($p = 0.05$) between the values in air and water for either seal and between seals in air. There is a significant difference between the two seals in water.

Table 2: Responses of both seals in both air and water for final testings. Values are given in % correct for each gap size.

Gap Size		Blondie				Scar			
(mm)	(min. of arc)	Air	n	Water	n	Air	n	Water	n
2.16	4.35	100	30	-	-	100	20	-	-
2.03	4.10	85	108	-	-	98	90	-	-
1.79	3.57	90	100	96	24	100	100	-	-
1.66	3.34	84	98	-	-	96	100	-	-
1.54	3.07	-	-	100	58	-	-	-	-
1.42	2.83	-	-	99	101	-	-	83	59
1.29	2.57	-	-	99	144	-	-	95	141
1.22	2.43	-	-	-	-	-	-	92	22
1.16	2.31	85	60	97	105	95	60	91	130
1.08	2.15	-	-	94	17	-	-	69	48
1.02	2.05	83	119	90	51	88	120	83	24
0.97	1.95	80	20	-	-	75	20	-	-
0.90	1.80	-	-	81	47	-	-	62	21
0.76	1.54	64	24	75	44	58	30	57	99
0.71	1.41	-	-	74	19	-	-	-	-
0.64	1.28	59	55	70	33	63	70	62	26
0.57	1.13	-	-	71	17	-	-	63	30
0.51	1.02	46	40	-	-	58	50	-	-
		654				660			

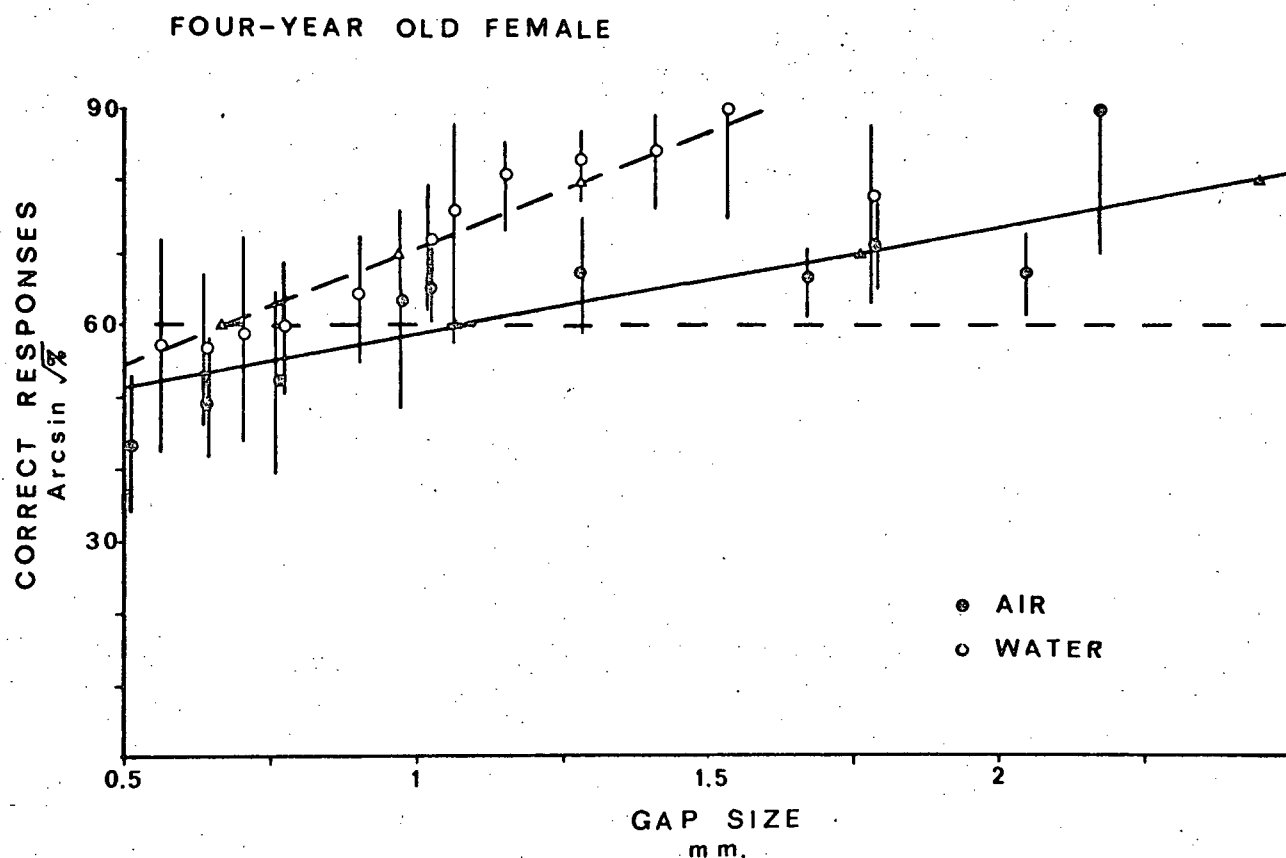


Fig. 23. A comparison of the ability of the four-year old female harbour seal to discriminate small differences in the gap size of the two-bar pattern in both air and water. A weighted linear regression was performed to determine the difference threshold which is defined as the gap size corresponding to the 75% (60 arcsin $\sqrt{\%}$) performance level.

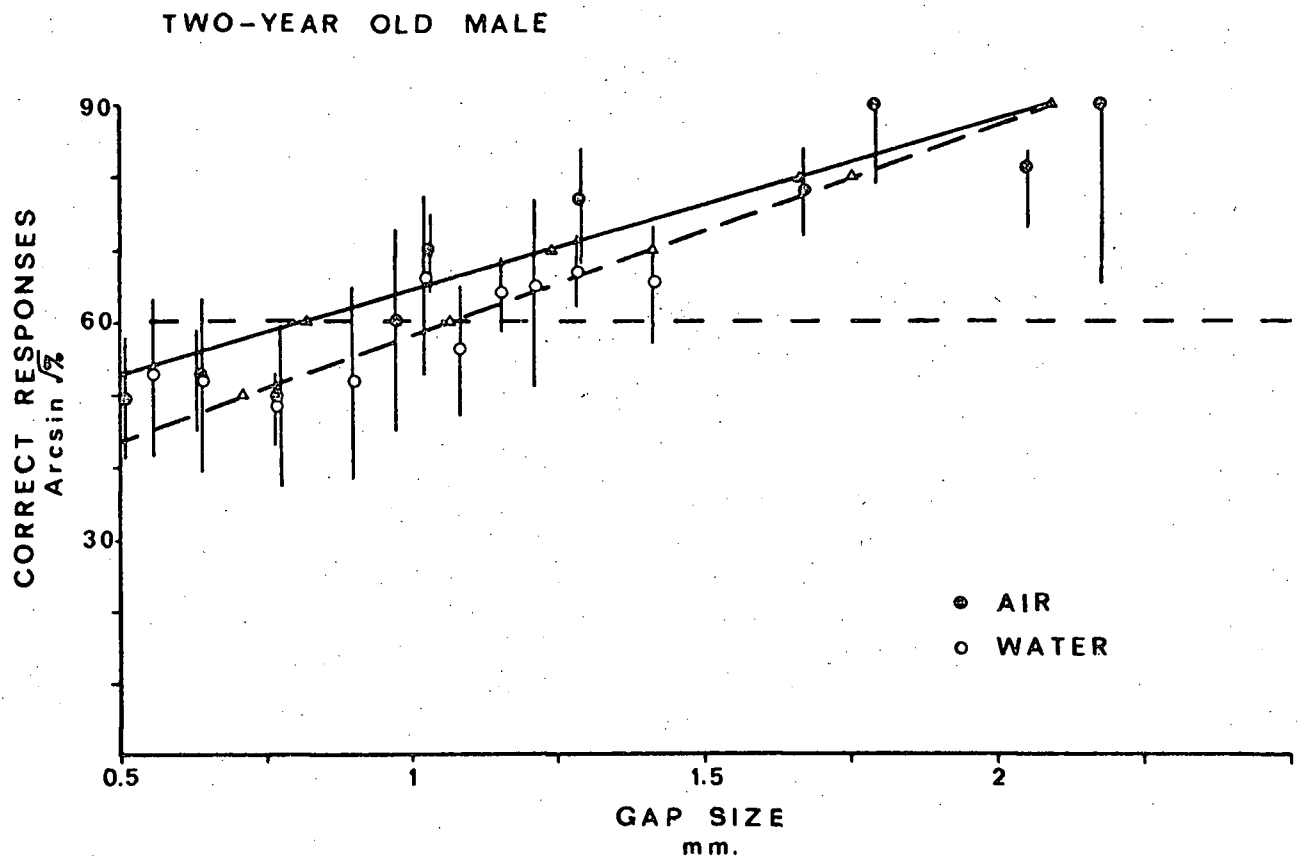


Fig.24. A comparison of the ability of the two-year old male harbour seal to discriminate small differences in the gap size of the two-bar pattern in both air and water. A weighted linear regression was performed to determine the difference threshold which is defined as the gap size corresponding to the 75%(60 arcsin $\sqrt{\%}$) performance level.

Discussion

The general aim of this study is to determine the nature and degree of the harbour seal's visual adaptations to an amphibious habit. I will first discuss the gross morphological and histological features, as an adequate understanding of the structure of the eye is essential before its functional capabilities may be assessed.

This study shows that the eye of the harbour seal is morphologically similar to that of other pinnipeds and confirms Walls' (1942) description of the pinniped eye. Briefly, the eyeball is large, both in absolute size and in relative size to the body, and is roughly spherical in shape. The cornea is flattened in its center regions, where it is much flatter than in humans. It is not more convex as stated by Johnson (1893). The anterior chamber is relatively deep, at least when compared to the aquatic teleost eye, which typically has the lens in direct contact with the cornea; it is not "very deep" as again stated by Johnson. The lens is essentially spherical in shape and although large, is not out of proportion to the rest of the eye and is not "nocturnal" in size. By this I mean that its equatorial diameter relative to the vertical diameter of the eye is only 0.37, a value much closer to that of the twenty-four-hour ungulates (0.40) than that of the nocturnal carnivores (0.50) (Walls, 1942). This increase in curvature, as Walls points out, seems thus to be an adaptation to the 'loss' of

the cornea under water and not to nocturnality, an hypothesis further supported by the diurnal habit of pinnipeds.

The sclera in the harbour seal is not out of proportion in thickness to the rest of the eye, as is the case in cetaceans; it is thickened slightly in the region surrounding the optic nerve. This would seem to question Walls' (1942) claim that the thickened sclera in cetaceans functions primarily as a supporting structure owing to the large absolute size of the eye, as the Pacific white-sided dolphin, Lagenorhynchus obliquidens, which has a smaller absolute eyeball size than the harbour seal, has a thicker sclera. However, perhaps the greater swimming speeds attained by cetaceans, with the resulting increase in corneal stress, necessitates this difference.

With regard to fine structure, this study shows that the retina of the harbour seal is histologically similar to that of other pinnipeds, especially that of the harp seal as described by Nagy and Ronald (1970). Both species show little zonation in the inner nuclear layer, possess large horizontal cells, have the outer nuclear layer as the thickest of all the retinal layers, and have similar nuclear densities within the different retinal layers throughout the retina. The degree of summation of receptor cells to bipolar cells to ganglion cells is also quite high and similar between the two species, roughly 100:10:1. The extensive horizontal cell network also suggests that a well-developed laterally conducting network exists, although the functional importance of such a network is still speculative.

In the inner nuclear layer, the bipolar, amacrine and neuroglial cells were initially identified and classified by the methods of Golgi and Ehrlich (Polyak, 1941). Since these methods were not used in this study, identification of these elements is uncertain, owing to their similar morphological appearances. The shape, size and position of the nuclei, and the appearances of the cellular processes, are not sure criteria.

In contrast to Nagy and Ronald (1970), however, it is felt that cone-type receptors are present, although not perhaps in the classical context as described by Polyak (1941). Pedler (1969) has recently proposed a new morphological classification of photoreceptors involving three basic varieties: Types A, B and C. The presence of type A, receptors relatively insensitive, polysynaptic cells representative of Polyak's (1941) "cones", was not conclusively demonstrated, as it was impossible to discern any definite morphological differences between the inner segments of the photoreceptors in vertical (Fig. 15) or horizontal (Fig. 17) section. However, there is some evidence indicating that this cell type may in fact be present on the basis of the large gaps in the outer limiting membrane which are characteristically associated with cone-type nuclei (Fig. 15).

In lieu of the inability to confirm the presence of type A receptors, type B receptors, relatively sensitive, polysynaptic cells, are suggested as the cone-like type observed in the retina

owing to the presence of polysynaptic pedicles. Those cells possessing oligosynaptic pedicles are type C receptors, sensitive cells of the type termed "rods" by Polyak (1941). These latter receptors comprise the majority of the photoreceptors found in the harbour seal retina. Type B and C photoreceptors would also appear to be present in the harp seal on the basis of the presence of both rod-like and cone-like receptor terminals (Nagy and Ronald, 1970). They also reported only one morphologically distinct inner segment variety.

Lacking neurophysiological data, the implications of such a photoreceptor composition in the phocid retina are not clear. The presence of type B receptors does not necessarily give them better visual acuity than an all rod eye, owing to the sparse numbers that are present. The extensive summation undergone by the sensitive receptors through the bipolar and ganglion cells likely creates highly sensitive receptor groups to deal with the relatively low light intensities such as would exist at great depths, beneath snow-covered ice, or at night. Those receptors possessing polysynaptic pedicles may in turn be connected to horizontal cells, connections which might impart a high information processing capability to the retina (Pedler, 1969) as these cells conduct impulses in a lateral direction. Such a system would certainly be advantageous to a predator required to hunt a fast-moving prey in a three-dimensional environment under low light intensities.

Retinal folding has been reported in the cetacean eye by

Mann (1946) on the basis of his observations on the sperm whale (Physter macrocephalus) and the fin whale (Balaenoptera physalus), and most recently, by Pilleri (1967) in the beluga (Delphinapterus leucas), the hippopotamus (H. amphibus), and the otter (Lutra lutra). The retinal folding reported by Mann (1946) involved only the outer nuclear layer and layer of rods and cones and was speculated to function in movement-detection. He theorized that since movements are better seen in the peripheral regions of the retina than centrally, the less the retina is adapted for acute vision, the better will be its ability to detect movement. Thus, the increased summation resulting from undulations of the photoreceptor layer, which increases the number of receptors per unit area of retinal surface without a corresponding increase in the number of nerve fibers in the nerve fiber layer, would decrease visual acuity and so aid in movement-detection. This situation with regard to physiological factors is not borne out, however, although according to Walls (1942), psychic factors support better movement-detection in the periphery, where movements have a saliency and attention-value quite out of proportion to the clarity with which they are actually discriminated. It seems unlikely, therefore, that retinal folding functions in movement-detection as Mann speculates.

The foldings reported by Pilleri (1967) involved all the layers of the retina with the exception of the pigment epithelium.

As three mammalian orders were involved, he concluded that folding was an adaptation to aquatic life, and suggested that it functioned somehow in diving, this perhaps being an adaptation to changes in water pressure to allow for fluctuating eye volumes. This is unlikely also - the eye contains no air pockets whose compression would cause distortion.

This raises the question of the existence of retinal folding as a "real" phenomenon in the mammalian eye, be it adapted to an aquatic, amphibious or terrestrial mode of life as it is hard to see how such a feature might function. If it is "real", it must have some functional significance (Cain, 1964). Is there, therefore, a possibility that it might be an artifact?

The appearance of the folds on histological examination supports their "real" status, as little shearing of the retina appears to have occurred. Often, the photoreceptor cells between folds are still connected to the pigment epithelium. This is especially evident in the first type of folding, which just involves the outer nuclear and photoreceptor layers. Nevertheless, this observation should be qualified. The second type of folding, involving all the retinal layers, is almost certainly an artifact, owing to our inability to observe any folding through funduscopic examination of the living seal eye. This second type, because of its magnitude, should be clearly visible if present.

That folding of any nature was prevented in the living animal and was an artifact in the prepared sections seems likely for the following reasons. The primary function of the outer limiting membrane is to orient the photoreceptor cells in the same meridian as the light passing through the retina. This is necessary in order to provide a reasonable degree of visual acuity, for if they were perpendicular or even obliquely oriented to the light passing through the retina, then the cells could be stimulated over a relatively large retinal area as opposed to just the diameter of the segment portion of the photoreceptor cell. Also the effect of having only parts of the retina with receptors in the same meridian as the light path is the formation of alternating regions of acute and fuzzy vision, a situation which would not seem advantageous in a predator. It is even more difficult to suggest a function for the second type of folding (gross folding), assuming that it is not an artifact.

An equally important consideration is the problem of retinal nutrition created by the folding. The region of the retina superficial to and including the outer plexiform layer is transversed by blood vessels originating from the optic nerve region of the retina and receives its nutrition from these vessels. The outer nuclear and photoreceptor layers however, receive their nutrition through diffusion from the choroidal blood vessels transversing between the pigment epithelium and tapetum (Fig. 18). Distance from these

vessels is therefore a critical factor. The distance created by folding, especially of the second type, would seem just too great to ensure adequate nutrition (McCormick, 1970).

However, these objections to folding as a "real" phenomenon are based primarily on an inability to associate any specific function to it, which as Cain (1964) points out, is not sufficient criterion. Too often in the past such conclusions have proven false. Further study is therefore required before this question may be fully resolved. Confirmation of its presence could be obtained if folds are observed in sections embedded in Epon, for the exact cellular relationships could then be observed to see if localized shearing has occurred. Unfortunately, my Epon-embedded sections showed no folds. Their absence here does not necessarily rule out their existence, however, as the distance between folds was often greater than the width of the retinal sections cut with this technique.

The tapetum cellulosum in the harbour seal is as extensive as indicated by Walls (1942) and lines the whole vitreous cavity, terminating just before the retina does. According to Walls (1942), this gives pinnipeds the most extensive tapetum among mammals. The mechanism of tapetal reflection is still uncertain, although several have been suggested. Walls has suggested interference effects but Pedlar (1963), although showing that this is likely, shows that interference as a sole mechanism is unlikely. There may in addition be a pigment,

although none has been isolated to date. Weitzel et al. (1956) have isolated zinc cysteine from the seal tapetum, but no connections have been shown to exist between the colour of this metal and the colour of the tapetum. Heller (1967) has shown that this compound possesses photoelectric properties, however, and states that this is related to the increased visual power of carnivores in the dark. Nagy and Ronald (1970) point out, however, that this would seem unlikely, when one considers the extensive knowledge relating to photoreceptor excitation as accomplished by the photochemical conversion of light energy into neural impulses.

If a pigment is indeed present, it may subserve the same function as suggested for riboflavin in the lemur (Galago crassicaudatus) retina (Pirie, 1959). The fluorescence maximum of riboflavin is 520 mμ at neutrality, and is maximally activated by light of 370 and 445 mμ. These wavelengths will penetrate the eye, be absorbed by the tapetum and then be re-emitted as light of 520 mμ, owing to the fluorescence of the riboflavin. Thus, if a pigment is present, it would be advantageous for it to reflect particular wavelengths of light which are more suitable to the optimum sensitivity of the visual cells, for it could therefore increase the stimulus received by the photoreceptor cells.

Refraction:

My refractive results (Table 1) agree basically with those reported by Johnson (1893) and Piggins (1970) for the

harbour seal and harp seal respectively; by means of retinoscopy, both noted considerable myopia and astigmatism in air, and in water, Piggins found moderate hypermetropia and negligible astigmatism. Hypermetropia, or far-sightedness, results if the eyeball is too short, so that the accommodation process is inadequate to pull the image forward onto the retina and the sharp image lies behind the eye. Myopia, or near-sightedness, is the opposite, where the eyeball is too elongated and the image lies too far forward, ie. in the vitreous cavity. The third and last refractive error, astigmatism, is when the retinal image of a point is not a point but a line, owing to one of the refracting surfaces being partly cylindrical as well as spherical in its curvature. This is almost always in the cornea in humans, but it is possible to have lenticular astigmatism or astigmatism resulting from the back of the eye itself. The location of the astigmatism in seals is especially critical, as only if it is corneal will it be removed under water, owing to the similarity in refractive indices between the cornea and water. This is why considerable effort was made to quantify the corneal astigmatism independently of any which might be inherent in the other eye components. Retinoscopy measures the refraction of the eye as a whole, and cannot be used to determine the refraction of any one refracting surface.

With respect to Johnson's (1893) and Piggins' (1970) results, owing to the difficulties encountered by very competent

doctors in the taking of my measurements, the accuracy of their results seems questionable, as neither has had his results confirmed. I found considerable variation in values taken by different doctors on the same seal on the same day and even by the same doctor with different instruments (a round retinoscope versus a slit retinoscope) on the same seal on the same day (see Table 1, Scar). The reasons for these difficulties are not certain, but I feel the major problems were the presence of a tapetum, the large eye, and the upward and horizontally (15°) directed optic axis, all features not characteristic of the human eye with which they were most experienced. These features combined made it very difficult to be sure that correct measurements along the optical axis were taken. Anaesthesia appeared to have no effect on eye refraction, as we were able to take the refraction of one of the seals (Blondie) without it; these values were similar to later ones taken while she was anaesthetized.

One further speculation as to why we had difficulty in determining the harbour seal's refraction is the influence of the animal's nervous state. It has been conclusively demonstrated that among humans, especially children, nervous tension can and does change the refractive state of the eye significantly, often up to 3 diopters (Radler, 1955). This change is typically one of increased myopia, and appears to be related to the difficulty of the task presented. There is no reason to suppose that a similar reaction to stress

could not occur in other animals, and if such changes took place while the seals were being tested, this could perhaps account for a part of the variation observed. Refraction involved tying the animal down and shining a light into its eyes and was definitely bothersome to at least some of the seals.

With a Placido's disc, I was, however, able to confirm Johnson's (1893) and Piggins' (1970) observation of the axis of least corneal curvature in the horizontal. This observation, therefore, questions some of the retinoscopy measurements, as some showed no astigmatism (Table 1, Operator 1, Susie and Orphie) and astigmatism with this axis in the vertical (Table 1, Operator 3, Scar). Only those measurements showing the axis of least corneal curvature in the horizontal are therefore consistent with my observations, as all other evidence to date suggests that the lens and posterior curvature of the eye are not significantly astigmatic. It is felt that these values are meaningful, for when combined with Johnson's measurements for the harbour seal, they should provide at least an indicative refractive value for this species. If this is indeed the case, and only further measurements will confirm it, then Walls' (1942) theorizing seems partly confirmed in that the astigmatism will be eliminated by the vertically constricted pupil. The power of a cylinder is directed at 90° to its axis of least curvature, ie. the horizontal. However, the function of astigmatism

in the dioptric mechanism is still not explained. A refractive study of the bearded seal, Erignathus barbatus, would now be interesting, as its pupil slit is almost horizontal in contrast to that of other pinnipeds (Walls, 1942). Its axis of least corneal curvature would thus be expected to be vertical, if indeed astigmatism is present at all.

This removal of the astigmatism, however, requires a stenopeic aperture, so that an approach to the performance of a pinhole aperture results. Only under very bright conditions though, such as occur on a sunny day, does the pupil ever fully approach this condition and often, as is the case on dull days and under the conditions of my behavioural experiments, the pupil adopts a rather more oval or elliptical shape, depending on the actual light intensity. Vision would thus likely be poorer under low light intensities than under high light intensities, as the effects of the corneal astigmatism would not be entirely eliminated by the now widened pupil. If the mean value of the degree of corneal astigmatism for the harbour seal is correct (0.6 diopter of hypermetropia in the vertical meridian and 8.5 diopters of myopia in the horizontal, resulting in 9 diopters of astigmatism) then this cannot be considered lightly, and any deviation from a stenopeic aperture would likely have a pronounced effect.

In water, the refractive results are questionable as the operator taking them was unable to detect astigmatism

when testing in air, astigmatism definitely shown to exist by use of a Placido's disc. However, they imply moderate hypermetropia with no astigmatism, results which seem reasonable when compared to those of the harp seal (Piggins, 1970) in Table 1. Both methods of measurement are comparable in technique, although again, individual variation should be expected. As these are the only underwater refractions carried out to date on pinnipeds, it would appear that these two species of phocids at least are similar in refraction underwater. These findings also agree with the inferences of Johnson (1893) and Walls (1942) that the astigmatism in phocids is resident in the cornea, for it is largely removed in water; underwater, corneal astigmatism should be lost with the reduction in effectiveness of the cornea (assuming that its refractive index is the same as other mammals in being similar to that of water). Vision under low light intensities would thus likely be poorer in air than water for the same reasons mentioned earlier, as the astigmatism would only be effective and detrimental in air.

The question is now raised as to why seals possess astigmatism at all and how they put up with such an extreme degree as 9 diopters. Among the carnivores and higher mammals, no significant astigmatism is the rule (Johnson, 1901), although it is found to a small degree, ie. between 0.5 - 1.0 D., throughout the orders. Only among the pinnipeds and cetaceans is it present to such an extreme degree. Matthiesen

(Johnson, 1901), who has refracted several Atlantic cetaceans' eyes reports a corneal astigmatism of between 4 - 4.5 diopters among all the species he observed. Presumably, however, it would be oriented differently from that of the pinnipeds, as their pupil is of a considerably different shape. Johnson's observations (1901) also show that among mammals, excluding the domestic species, in which refraction varies considerably in all directions of error, vision is characteristically hypermetropic. A slight degree of hypermetropia (under one diopter) is typical of the higher mammals, while higher hypermetropia (2-5 diopters) is found in wild species of rodents, the edentates and the marsupials. Pinnipeds are therefore unique, along with the primates, in possessing myopia as a common and persistent character.

Unfortunately, no acceptable explanation for these irregularities is presently available, for although this thesis confirms their presence, it is impossible to draw conclusions on the functional significance of these characters without further study. A stenopaic pupil will functionally correct the detrimental effects imposed by astigmatism and myopia, but these errors are still not required. In fact, the primary function of the slit pupil is presumably simply to cut down the intense light encountered by the rod-rich retina under photopic conditions. It is under low light intensities, when the pupil enlarges and ceases to be functionally stenopaic, that these errors become serious.

Visual Discriminations:

When the standard psychophysical criterion is applied to the data (the difference threshold being defined as the visual angle corresponding to the 75% performance level), it is found that both seals could resolve a vertical gap between two black lines as small as 1 mm at a distance of 1.7 m. Further, for each seal the visual discrimination was the same in air and in water (Fig. 23 and 24) although there was a significant difference between the two seals discriminations in water. This deviation is felt to represent individual behaviour differences, however, rather than morphological differences, owing to the seals personality differences.

Owing to the wide variety of stimulus patterns used in visual discrimination studies, and their different characteristics, it is always necessary to ask what physiological capacity has been measured with the pattern chosen. Lit (1968) has summarized the types of experiments possible for measuring visual acuity and states that "when the subject is required to discriminate only the presence or absence of the test stimulus..., the task of detecting an illuminated target against a black background becomes equivalent to that of measuring the absolute sensitivity of the eye to light." If both seals in the experiment reported on herein were therefore oriented to the detection of an illuminated gap against a black background rather than to the discrimination of a separation between the bars, target size would not be

critical, as the resolution of target detail was not involved. The smallness of an object that can be detected is not an adequate measure of visual acuity. Therefore, if visual acuity is defined as the capacity of visual systems to discriminate fine details of objects located in the field of view, the absolute sensitivity of the eye to light rather than visual acuity in the strict sense would seem to have been measured.

In the evaluation of the harbour seal eye's absolute sensitivity to light in both air and water, the effects of the different physical properties between the two media cannot be ignored. For example, the absorption and scattering of light are greater in water. As a result, the further the animal is away from the test stimulus, the greater will be the loss of object light in water relative to air. Since the seals in this study were discriminating at a presumably threshold level at a standard distance in both air and water, the absolute sensitivity of the eye would therefore be expected to be less in water; in this medium the light intensity per unit area would decrease at a greater rate than the square of the distance owing to the exaggerated properties of water. However, this was not the case - the results indicate similar values in both media. There are two possible explanations: either the range of illumination, 85 candela/m^2 , was great enough to hide any difference which might exist, with 1.2 m being too short a distance to significantly

influence the results, or there was an increase in pupil size, thereby counter-acting any scattering effects. Johnson (1893) has suggested that the latter occurs, stating that the pupil opens to the full, i.e., a complete circle, in water, as a correction for astigmatism is not needed in this medium. However, no significant change in pupil size of the seal eye in water has been observed. The first possibility, that this technique was not designed to detect this variation, would thus seem to be the case. It should be noted, however, that with increased testing distances this property difference between the two media could become significant.

A wide variety of test objects are available for measuring visual acuity, but both Riggs (1965) and Lit (1968) have discussed visual acuity in terms of a classification system which identifies four different tasks: detection, recognition, resolution, and localization. In each case, visual acuity is specified in terms of the reciprocal value of the visual angle formed by some detail of the test object which is discriminated. As discussed above, the task in this study appears to be one of detection, where only the presence or absence of the object is asked but owing to the broad definition of visual acuity, it is possible to consider this task as one of resolution, where discrimination of a separation between elements of the pattern is required. Wilcox and Purdy (1933) have discussed the ambiguity associated with

this double line target in detail, and distinguish two aspects relating to the separation threshold, the "minimum visible threshold" is the least perceptible distance between two stimulus lines of infinitesimal width, whereas the "minimum ^{separable} ~~visible~~ threshold" is that resolution threshold where the breadth of the lines is so great that a further increase in their width would not improve the discrimination of a gap between them. With these definitions, my measure was of the latter, but it is still possible to consider these values as a measure of visual acuity as this difference is not distinguished in the broad definition of visual acuity.

Therefore, if one defines the resolution threshold as the "minimum separable", i.e. the minimal angular distance between two objects in order for them to be seen as separate, it is possible to calculate values for this property for the harbour seal in both media (see Table 3).

In considering the visual acuity of the harbour seal in air, it has been shown that the eye is astigmatic with the axis of most curvature being vertical, like its slit pupil (Johnson, 1893); an approach to the characteristics of a pinhole aperture is thus thought to result. However, it has been observed that only under high light intensities, such as occur on a bright day, does the pupil ever fully approach this narrow slit condition and that under low light intensities, such as in the experiments, the pupil adopts a rather more oval or elliptical shape. If Johnson's (1893) measurements

Table III: Estimated resolution thresholds for both seals in air and water. Values in parentheses refer to the 95% confidence limits.

	Resolution Thresholds (min. of arc)		$t_{\infty df}$
	Air	Water	
Two-year old male	1.64 (3.35-0.06)	2.13 (2.96-1.30)	0.65
Seals			
Four-year old female	2.13 (4.11-0.15)	1.32 (2.48-0.16)	0.69
$t_{\infty df}$	0.44	2.20	

$$t_p = .05 \infty df \quad 1.960$$

of the degree of corneal astigmatism (9 diopters) are correct for the harbour seal then this cannot be considered lightly, and any widening of the pupil would have a pronounced effect in air, even at the short distance of 1.7 m. Therefore, on the basis of current theories, visual acuity under the test conditions would likely be poorer in air than in water, as the effects of the corneal astigmatism would not be entirely eliminated by the now widened pupil.

Since my results showed no difference in air and water discriminations, and the pupil in air was rather oval (and the eye hence astigmatic), it seems most likely that the absolute sensitivity of the eye to light, not visual acuity, was measured. Thus, my experiments do not necessarily imply that the visual acuity of the harbour seal is the same in air as in water. Instead, I suggest the opposite, that under low light intensities and moderate distances, visual acuity would be better in water.

Further support that visual acuity per se was not measured in this study is provided by Schusterman and Balliet (1970a). Their patterns were gratings consisting of lines varying in width, thereby correcting the problems inherent in my patterns. Threshold values of 8.5' and 8.1' were obtained for Phoca vitulina in water, values considerably different from those obtained in this thesis (2'). It should be noted, however, that since all these measurements were made on only a few individuals, it is not possible to decide the degree to which

these values are characteristic of the species.

These values, therefore, are not as suitable as a measure of visual acuity might have been in the estimation of the effectiveness of the dioptric mechanism in both media. However, they do support my histological observations and indicate that the harbour seal possesses a very sensitive visual system; it is comparable to that of many nocturnal species (Table 4). It must be borne in mind, however, that these values are not easily comparable with other values published in the literature. Different workers may use different definitions, as pointed out by Lit (1968), and often different experimental methods (Table 4). For example, Walls (1942) presents a table of the visual acuities of a few animals for parallel lines (from various sources), but as he fails to specify the patterns, distances and lighting used in each case, the values are meaningless for accurate comparative purposes. Indeed, visual acuity in the strict sense may not have been measured, as in this thesis.

The amphibious pinniped eye as represented by the harbour seal is significantly different from the terrestrial mammalian eye. It possesses a spherical lens for vision in water, with a complex dioptric mechanism for air. The most unique features are high myopia and astigmatism, characters not commonly found in terrestrial mammals. It should be noted that although the eyes of many small nocturnal mammals also possess spherical lenses, this is an adaptation to nocturnality.

Table 4. A comparison of visual acuity thresholds (V.A.T.) and retinal structure for a number of animals from various sources.

(¹=Walls, 1942; ²=McCormick, 1970; ³=Ordy & Samorajski, 1968; ⁴=Schusterman & Balliet, 1970a; ⁵=Nagy & Ronald, 1970; ⁶=Pilleri, 1961; ⁷=Spong, 1968 (behavioural aspects only)). D = diurnal; N = nocturnal; D/N = 24 hr. habit; P.L. = parallel lines

Species	# tested	Pattern	V.A.T. (min.)	Outer nuclei	Rows of Inner nuclei	Gang- lion cells	Habit
Domestic pigeon ¹ (Columba livia)	-	P.L.	2.7	3	15	2	D
Human adult ^{1,2}	-	P.L.	0.44- 0.83	4.5	3.4	1	D
S.A. owl monkey ³ (Aotus trivirgatus)	4	Striae	4.0	10	4	1	N
Tree shrew ³ (Tupaia glis)	4	Striae	1.0	2	5	2.5	D
Bush baby ³ (Gagalo crassicaudatus)	4	Striae	4.0	13	5	1	N
Lemur ³ (Lemur catta)	4	Striae	0.5	8	4.5	2.5	D
Harbour seal in water ⁴ (Phoca vitulina)	1	Striae	8.3	10	5	1	D/N
Harbour seal in air and water (Phoca vitulina)	2	2 P.L.	2.0	10	5	1	D/N
Harp seal ⁵ (Pagophilus groenladicus)	-	-	-	10	5	1	D/N
Beaver ⁶ (Castor canadensis)	-	-	-	7.5	5	1	N
Killer whale ⁷ (Grampus orca)	1	2 P.L.	6.0	9	4.5	1	D/N
Pacific white-sided dolphin ⁷ (Lagenorhynchus obliquidens)	1	2 P.L.	6.0	9	3	1	D/N

In these animals, the lens is always very large in proportion to eye size, thereby placing the optical center of the eye deep within the eye. This is not the case in pinnipeds. The net result in nocturnal mammals is a small but bright image equally good in the periphery of the retina as in the center. This feature in small mammals is also typically associated with a broad cornea in order to maintain a wide-angled visual field (Walls, 1942).

It is difficult to compare the amphibious pinniped eye to the aquatic mammalian eye, for as in amphibious mammals, relatively little is known about their visual systems and capacities. Gross eye morphology is well-documented (Putter, 1903; Walls, 1942) but little is known about the structural organization of the eye. The cetacean eye, however, is reported to contain many rods which are bigger than those of terrestrial mammals (Slipper, 1962), as well as cones, a tapetum lucidum, and a well-developed horizontally conducting network within the retina (Mann, 1946). Morphologically, the ciliary muscles are well-developed in odontocetes but are almost non-existent in mysticetes. The lens is spherical and has a greater refractive index than that of terrestrial mammals, while the cornea, aqueous humor, and probably the vitreous humor have the same refractive index as sea water (Slipper, 1962). According to Mann (1946), however, the main feature of the cetacean eye is that the distance between the lens and retina is greater in the upper portion of the eye and smaller in the lower portion of the eye. The eye is thus

divided into two parts, with air images received at the bottom of the eye and water images received on the upper half of the eye, the long-axis. This special method of seeing in air and water he terms "amphyophthalmia". This structural organization has not been confirmed, however, and owing to the visual habits of cetaceans, it is hard to see how such a visual apparatus would be required.

Nevertheless, it would seem from these fragmentary observations that the cetacean eye is indeed aquatically adapted and that the pinniped eye has many basic similarities. It would thus seem that the pinniped eye has passed the "halfway point" in the transition between a terrestrial and an aquatic eye, in that its visual system is most "efficient" in water. However, such a state is difficult to assess, as exactly what stages are involved in the transition are uncertain. The presence of a spherical lens, as opposed to wringing the lens (the method employed by the turtle, the cormorant, and the otter), would seem a major achievement, though. Further studies on the extent of this adaptation are now required, utilizing electron microscopy and physiological techniques. The exact nature of the visual pigments and whether or not a pigment is present in the tapetum are suggested subjects for further study.

SUMMARY

This study shows that the harbour seal eye is morphologically different from that of terrestrial mammals and aquatic mammals, being adapted for vision in both air and water. It possesses a spherical lens to compensate for the loss in effectiveness of the cornea under water. In air, a unique dioptric mechanism incorporating the retina and pupil prevent the need for excessive accommodation. This mechanism also corrects the refractive errors under high light intensities, but loses effectiveness as the pupil enlarges with decreasing light intensity.

A detailed study of retinal organization was also completed, with type B photoreceptor cells being positively identified for the first time in a pinniped. A very extensive network of laterally conducting elements was also observed, implying perhaps a high information processing ability within the retina. Owing to the very large number of type C photoreceptor cells also present, the retina may be assumed to be also very light sensitive, a condition which meets the requirements of the dioptric mechanism; a sensitive retina is required for photopic vision owing to the effect of the stenopeic aperture, as well as for nocturnal vision. Retinal folds were also observed, but their existence in the normal living animal is considered unlikely on the basis of present knowledge. The organization of the harbour seal retina is thus basically

similar to that reported for the harp seal (Nagy and Ronald, 1970).

Refractive observations have shown that the harbour seal eye is very myopic and astigmatic in air, and hypermetropic with no discernable astigmatism in water. The axis of least corneal curvature is horizontal, confirming Johnson's (1893) observations and his and Walls' (1942) theorizing as to its function in air. This latter observation is also in agreement with that reported by Piggins (1970) for the harp seal.

Behavioural experiments also confirmed the relatively high sensitivity of the harbour eye to light, as suggested by the histological observations. Both seals tested were capable of discriminating a 1 mm gap at a distance of 1.7 m in both air and water. The similarity of these results in both media supports the contention that visual sensitivity and not visual acuity in the strict sense was measured.

Glossary of Terms

cycloplegia - paralysis of the ciliary muscle; paralysis of accommodation.

diopter - the refractive power of a lens with a focal distance of one meter (assumed as a unit of measurement for refractive power).

dioptric mechanism - pertaining to the eye structures involved in refraction

fundusoscopic examination - examination of the fundus of the eye

keratometer - an instrument for measuring the curves of the cornea

Placido's disc - a disc with concentric circles marked on it used in examining the cornea.

stenopeic - having a narrow slit or opening.

retinoscopy - an objective method for investigating, diagnosing, and evaluating refractive errors of the eye, by projection of a beam of light into the eye and observation of the movement of the illuminated area on the retinal surface and of the refraction by the eye of the emergent rays.

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