THE STRUCTURE AND CONCENTRATION OF SOLIDS IN PHOTORECEPTOR CELLS STUDIED BY REFRACTOMETRY AND INTERFERENCE MICROSCOPY

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PLATES 9 AND 10

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Vertebrate photoreceptor cells resemble one another in general structure, each comprising an outer segment, an inner segment, a nuclear region, and an efferent process which synapses with a bipolar cell. Only the outer and inner segments will be considered in the present study. The outer segment lies furthest from the light entering the eye and contains most or all of the photosensitive pigment. The inner segment contains the mitochondria, glycogen, and ribonucleoprotein of the cell and may be subdivided, beginning distally, into oil droplet, ellipsoid, paraboloid, and myoid. Not all of these subdivisions are to be found in all species (2).

Several uncertainties persist concerning the cytology of rods and cones. A variety of fibrillar structures on the surface or within the substance of the outer and inner segments has been reported (2), but few of these have been closely studied or their actual existence verified. The nature of the junction between the outer and inner segments is uncertain, as are also the relations of the several subdivisions of the inner segment to one another. Finally, the distribution of sizes in a population of outer segments has not been measured accurately in living rod cells, and the concentrations of solids and water in the various cell regions are unknown.

In the present investigation these problems are studied by the use of refractometry, a method involving the examination of living cells by phase contrast microscopy in non-toxic media of various refractive indices (7–9). The observations on cell structure are confirmed by variable amplitude phase contrast microscopy and some of the quantitative data have been checked by interference microscopy. A critique of these new methods of microscopy has been presented by Barer (4, 5).

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Material and Methods

Retinas were studied from 30 frogs (*Rana temporaria*), 4 salamanders (*Salamandra maculosa*), 11 turtles, 2 chicks, 2 pigeons, 5 white rats, 2 mice, and 3 monkeys (*Macaca macaca*).

All animals were decapitated except the monkeys which were anesthetized with nembutal. The eyes were quickly enucleated and bisected. The retinas were lifted out with forceps and portions were cut into fragments about 1 mm. square. Each fragment was suspended on a microscope slide in a large drop of mounting fluid with or without further teasing, and gently covered with a thin coverslip. Tissues were prepared in this way for all the methods of microscopy.

The mounting fluids were prepared from powdered bovine plasma albumin (Armour Fraction V) which had been dialyzed against distilled water, frozen-dried, and measured for salt concentration as described in detail by Barer and Joseph (8). Albumin solutions at concentrations up to 53 per cent in NaCl provided a series of non-toxic mounting media ranging in refractive index from 1.334 to 1.428, which covers the range found in living cells (10). The concentration of NaCl was varied to provide hypotonic, isotonic, and hypertonic solutions. Some retinal fragments were suspended in saline alone, for comparison with those mounted in albumin solutions.

The refractometric method involves comparison of the brightness of the living cell and its albumin medium by the use of phase contrast microscopy. When viewed with positive phase contrast a cell having a higher refractive index than the medium appears dark, one having a lower refractive index than the medium appears bright, and at the "match point" when cell and medium have the same refractive index, the cell virtually disappears from view. Refractometry offers a useful method for detailed examination of the structure of living cells because small differences in contrast between the cytoplasm and its inclusions are enhanced when the medium and the cytoplasm are near the match point. Also scatter of light at the surface of refractile cells decreases as the match point is approached.

Many of the observations on the structure of the photoreceptor cells were checked and further extended by examining the cells with a Cooke, Troughton, and Simms variable amplitude phase contrast microscope. The contrast could be adjusted according to the delicacy of the details being examined.

In addition to providing a tool for examination of cell structure, refractometry can yield certain quantitative information (4, 5). The match point establishes the refractive index of the portion of a cell which is in contact with the medium. The concentration of solids in that portion of the cell is related to the refractive index according to the formula

$$C_s = \frac{\mu_c - \mu_m}{\alpha}$$

in which C_s = the concentration of solids in grams per 100 ml., μ_c = the refractive index of cell, μ_m = the refractive index of medium, and α = the specific refractive increment defined as the increase in refractive index for every 1 per cent increase in C. The specific refractive increment may be taken as 0.0018 for protoplasmic solids in general (7). Thus measurement of refractive index by the match method allows calculation of the concentration of solids. The concentration of water in grams per 100 ml. (C_w) is obtained by the formula (5)

$$C_w = 100 - 0.75\ C_s.$$

Some of the quantitative data were confirmed by the use of a Baker shearing interference microscope. Interferometry measures the retardation of light passing through the cell. This retardation is the product of cell thickness and the difference between the refractive indices of the cell and its medium. Since rod outer segments are cylindrical, one can measure thickness

directly and obtain the refractive index alone. For rod outer segments of measured size the methods of refractometry and interferometry are thus interchangeable and yield data on wet weight, dry weight, and concentrations of solids and water. Refractometry is a more accurate and less tedious method because it requires only observation of the brightness of all the cells in the field of the microscope, whereas interferometry involves measurement of both the phase retardation and the size of each individual cell. Refractometry has the further advantage of measuring the concentration of solids independent of cell size or shape and is applicable to segments of photoreceptor cells other than the cylindrical rod outer segment.

Photographic records were obtained on Kodak Microfile 35 mm. film with a Zeiss-Winkel phase contrast microscope. Details in many of the photomicrographs unavoidably are somewhat blurred because of movement of the teased cells during the required 5 to 10 seconds of exposure.

Measurements of length and width were made directly on the living cells with an ocular micrometer and were confirmed on the photomicrographs.

OBSERVATIONS

1. The Structure of the Photoreceptor Cells

(a) The Rod Cell:

The Outer Segment.—The most detailed observations were made on rod outer segments of the frog and salamander. The outer segment is cylindrical and slightly rounded at its distal end. There are longitudinal serrations on the surface of the rod but no surface fibers. Rods examined within a few minutes after removal of the eye show no internal structure at magnifications up to 1600 (Fig. 1). No sheath was noted.

A series of degenerative changes begins within minutes, allowing some inferences to be made concerning the finer structure of the rod. Usually the first change is the appearance of transverse striations. These may be few (Figs. 7, 15) or they may cross the entire rod at intervals of less than 1 μ, as demonstrated clearly with the variable amplitude phase contrast microscope (Figs. 13, 14). In very hypotonic fluids rods elongate rapidly through increase in the interval between transverse striations (Fig. 17) and soon fragment to unrecognizable debris. The lower density (bright appearance) and exaggerated development in hypotonic media suggest that the transverse striations develop because of imbibition of water, as proposed long ago by Schultze (26).

In many rod outer segments a bright longitudinal streak will appear, sometimes within 3 minutes of the time the eye is removed from the animal (Fig. 6). The streak is eccentrically located near the surface of the rod and runs the length of the rod outer segment or any portion of it. Since the degree of brightness of a cell constituent is determined both by its thickness and its refractive index relative to the medium (5), one might interpret the bright streak either as a linear groove thinner than other parts of the rod outer segment or as a streak of lower refractive index. The bright line could not represent a groove for the following reason: as a cell and its medium approach the match point, contrast decreases and thickness of the cell becomes progressively less important a factor in determining brightness until at the match point a cell of any thickness

is invisible. Yet the streak appears brightest precisely at the match point (Figs. 6, 8, 11). It must, therefore, signify a zone of lower refractive index.

The longitudinal streak might be continuous in the form of a fiber or discontinuous as a local area of lower refractive index located uniformly in each of the submicroscopic lamellae which are stacked to comprise the rod outer segment (28). At the base of the rod outer segment the streak clearly represents a fiber, for a fiber often may be seen projecting freely into the medium from the proximal end of an outer segment which has broken away from its ellipsoid (Figs. 10, 11). Tentatively the streak will be considered to represent a fiber throughout the length of the outer segment and hereafter will be referred to as such. The thickness of the fiber is somewhat variable (Figs. 6, 15) and at some sites it may show gross swelling (Fig. 12). One gains the impression that *in vivo* the fiber is submicroscopic in diameter and becomes visible as a bright streak by rapidly swelling to microscopic dimensions in the teased preparations.

An irregular "core" differing in its staining properties from the periphery of the rod outer segment has been described in retinas fixed in alcohol (31, and others). This core appears to be an artifact resulting from shrinkage during dehydration of the rod and has been reproduced in unstained outer segments (Fig. 16).

Supplementary observations were made on rod outer segments of the pigeon, rat, mouse, and monkey. As in the amphibia, rods show no internal structure in the first few minutes after the retina is teased (Figs. 2 to 5). Thereafter a single longitudinal fiber of variable thickness may appear. It seems to lie directly on the surface of the rod (Figs. 8, 18), rather than slightly beneath the surface as in the frog and salamander.

The Inner Segment.—The myoid is considered to be the equivalent of ordinary cell cytoplasm (27). In the amphibia most teased photoreceptor cells break across the proximal portion of the myoid, just distal to the nucleus. Sometimes the broken end of the myoid appears as a tapered filament but more commonly the myoid rounds up proximally (Figs. 6, 9). In contrast no such rounding was noted in injured outer segments, which seem to have a more rigid structure. The myoid also differs from the outer segment in being considerably less refractile. The myoid may appear homogeneous, have scattered granules along its lateral margins, or contain irregularly shaped large aggregates of somewhat greater density within its substance (Figs. 1, 3, 6, 9). The distal border of the myoid is contiguous with the proximal border of the outer segment (Figs. 1, 6, 8, 9) and in teased rod cells of all species that junction is a second point at which breaking often occurs.

The myoids of amphibian photoreceptor cells may elongate or shorten *in vivo* in response to light (17). In teased preparations also one occasionally observes marked elongation, and such myoids show remarkable elasticity. They may bend 90° or more when light pressure is applied to the coverslip

and spring back to their previous shapes immediately when the pressure is released (Figs. 29 to 32).

The ellipsoid lies within the distal portion of the myoid and is completely surrounded by myoid substance. Its distal and lateral borders appear smooth and slightly convex, while the proximal border often bulges eccentrically toward the nuclear region of the cell (Figs. 1, 6, 9). The ellipsoid is closely packed with mitochondria, which are seen particularly well when the cell swells in a hypotonic medium (Fig. 17). In favorable preparations a fiber may be seen crossing diagonally through the ellipsoid (Fig. 9). The same fiber torn free from the ellipsoid and still attached to the proximal part of the outer segment is seen in Figs. 10 and 11. The ellipsoid is difficult to delineate in teased rod cells of the avian and mammalian species (Figs. 2, 4, 8, 18).

The "chromophobic disc" (2), sometimes considered to be a structure interpolated between inner and outer segments of the cell, is an artifact caused by shrinkage of the myoid and its separation from the outer segment during dehydration. Its formation has been observed in teased drying cells (compare Figs. 6 and 16). The failure of the "chromophobic disc" to accept any stains (31) is thus accounted for.

(*b*) *The Cone Cell:*

The Outer Segment.—The structure of the outer segment of the cone cell is more difficult to observe because of its fragility. Only a rare cone cell is seen with outer segment attached and intact, even in the freshest preparations. Moreover in some species, such as the pigeon, the outer segment is obscured by the pigment granules adhering to its surface. Three shapes of outer segments are observed in teased preparations: a truncated cone tapering distally at an angle of about 5 to 9° (Figs. 19, 28, 33), a cone with a wide base proximally and a tapered point distally (Fig. 20), and a needle-like outer segment (Figs. 21, 23). One can only guess which of these shapes, if any, occurs *in vivo*. The very thin form (Figs. 21, 23) seems hardiest and possibly represents a mere residual portion of the outer segment rather than a variety of intact outer segment. A bright longitudinal fiber thicker than the rod fiber may appear along one edge of the cone outer segment (Figs. 28, 33), and as in the rod, this fiber may become irregularly thickened.

The Inner Segment.—The myoid is comparable to that of the rod cell in having a bright appearance and a variable assortment of granules and dense aggregates within it (Fig. 25).

The ellipsoid of the cone likewise resembles that of the rod except that, as is well known, it contains an oil droplet within its distal portion in many species (Figs. 19 to 23, 25 to 27, 33). The lateral surfaces of the ellipsoid are slightly curved and taper at an angle of 14 to 22° (Figs. 19, 25, 33). The small zone distal to the oil droplet has a rigid form, with a slightly narrowed neck and rounded tip (Figs. 23, 25). This zone commonly retains its normal shape even

after the outer segment has disappeared and the more proximal portions of the cell have become swollen and distorted.

A paraboloid is embedded within the substance of the myoid, proximal in position to the ellipsoid, in cones of some species (Fig. 25). The paraboloid is homogeneous and has a refractive index different from those of the myoid, ellipsoid, and outer segment. The paraboloid may remain intact for some time even in distilled water, after the other segments have fragmented to unrecognizable debris. The paraboloid appears specialized also on histochemical study and stains only for glycogen (27).

In the frog and turtle double cones were observed. Typically one member of the pair contains an oil droplet and the other a paraboloid (Fig. 26). Corresponding segments of single and double cones have similar structure.

Among the photoreceptor cells teased from retinas of the frog, salamander, turtle, and monkey one sees occasionally cells having a low refractive index in all segments (Fig. 24). These cells require further characterization.

2. *Quantitative Measurements of the Photoreceptor Cells*

Cell Size.—Refractometry provides a reliable method for determining the size of rod outer segments. In a medium near the match point the outlines of the cell are seen without distortion and dimensions can be measured accurately. Observation of the brightness of the cell during the period of measurement provides a critical check on the constancy of size; a change in volume of only 1 per cent is easily noted by the altered brightness when the cell is mounted near its match point, although the changes in linear dimensions producing such a change in volume would be too small to observe. Thus errors from even minor shrinkage or swelling are detected. As a further check, cells were measured in slightly hypertonic and hypotonic, as well as in isotonic, albumin solutions.

A summary of measurements on the diameters of 60 rod outer segments of the frog is presented in Table I. The rods were suspended in albumin at tonicities of 0.65, 0.70, and 0.75 per cent NaCl. The mean diameter was 6.83 $\mu \pm 0.55\ \mu$.

The variation of length of the frog rods is more difficult to determine statistically because many teased rods break transversely and appear unduly short. The most reliable measurements were considered to be the largest values obtained for rods near their match points and constant in brightness. The value of 55 $\mu \pm 8\ \mu$ (Table II) is thus biased by selection and no attempt was made to determine the true variability of rod lengths in the frog. In the mammalian species the outer segments commonly remain intact. The dimensions of rod outer segments in several species are recorded in Table II and the volumes in Table IV. It should be pointed out how remarkably different in size are the rods of amphibia and mammals.

Concentrations of Solids.—The refractive indices and concentrations of solids for various segments of rod and cone cells in the several species are presented in

Table III. The values of 40 to 43 per cent obtained for the concentrations of solids in rod outer segments are far higher than those found for ordinary cytoplasm and are among the highest measured for any biological material (10). Cone outer segments are considerably less dense than rods and contain about 29 to 34 per cent solids. The myoids resemble ordinary cell cytoplasm in their

TABLE I

Distribution of Diameters of 60 Frog Rod Outer Segments

Diameter	Rods suspended in albumin in			Total No. of rods
	0.65 per cent NaCl	0.70 per cent NaCl	0.75 per cent NaCl	
μ				
5.5	0	1	2	3
6.0	3	3	2	8
6.5	5	12	8	25
7.0	6	3	5	14
7.5	6	1	3	10
Total No. of rods	20	20	20	60

Mean diameter = 6.83 μ.
Standard deviation = 0.55 μ.

TABLE II

Dimensions of Rod Outer Segments

Species	Diameter	Length
	μ	μ
Frog	6.83 ± 0.55	55 ± 8
Salamander	9.2 ± 1.1	46 ± 7
Rat	1.4 ± 0.2	13 ± 1.0
Mouse	1.3 ± 0.2	11.2 ± 0.6
Monkey		
Peripheral retina	1.8 ± 0.3	21 ± 1.0
Central retina	1.3 ± 0.2	17 ± 1.8

concentrations of solids, while the other divisions have values intermediate between those of rod outer segments and myoids.

The way light will traverse photoreceptor cells depends upon the shapes and relations of the cell parts and their refractive indices. Idealized rod and cone cells and average refractive indices for their various parts are shown diagrammatically in Text-fig. 1. In rods the light passes through zones of increasing refractive index save for the thin rim of myoid cytoplasm of low refractive index interposed between ellipsoid and outer segment. In cones the path of light is even more complex because of the inclusions within the inner segment. The ellipsoids of cones have slightly higher refractive indices than the outer segments (Text-fig. 1, Table III).

TABLE III

Average Refractive Index and Concentration of Solids in Photoreceptor Cell Segments‡*

	Frog	Salamander	Turtle	Chick	Pigeon	Rat	Mouse	Monkey
Rod outer segment								
R.I.	1.4106	1.4056			1.4076	1.4076	1.4056	1.4076
Solid conc.	43.0	40.3			41.4	41.4	40.3	41.4
Rod ellipsoid								
R.I.		1.3978						
Solid conc.		36.0						
Rod myoid								
R.I.	<1.3638							1.3605
Solid conc.	<17.1							15.3
Cone outer segment								
R.I.	1.3883	<1.3865	1.3865	<1.3958				<1.3985
Solid conc.	30.7	<29.7	29.7	<34.9				<34.9
Cone ellipsoid								
R.I.	1.3978	1.3939		1.3902				
Solid conc.	36.0	33.8		31.8				
Cone paraboloid§								
R.I.			1.3775					
Solid conc.			24.7					
Cone myoid								
R.I.	<1.3638	1.3638	1.3605					1.3605
Solid conc.	<17.1	17.1	15.3					15.3

* The relationship of refractive index (μ) to solid concentration (C) is $C = \frac{\mu_c - \mu_m}{\alpha}$.

‡ Each value represents measurement of a substantial series of cells in many retinas. The values recorded as "greater than" or "less than" represent smaller series, in which exact matching points were not obtained.

§ The paraboloid is composed almost entirely of glycogen and α for glycogen is unknown; as explained in the text (Discussion), a value of 0.0014 was used for calculating concentration of solids in the paraboloid. For all other segments $\alpha = 0.0018$ was used.

TABLE IV

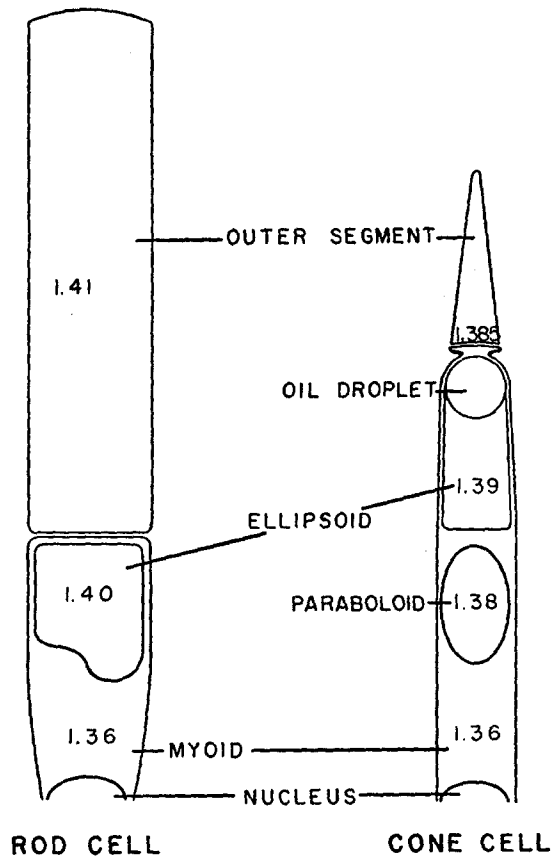
Volume, Solid Concentration, and Wet and Dry Weight in Rod Outer Segments of Various Species*

Species	Volume	Solid concentration	Wet weight	Dry weight
	cc.	*gm./100 cc.*	*gm*	*gm*
Frog	2.021×10^{-9}	43	2.22×10^{-9}	8.69×10^{-10}
Salamander	2.962×10^{-9}	40.3	3.26×10^{-9}	11.9×10^{-10}
Rat	2.000×10^{-11}	41.4	2.20×10^{-11}	8.28×10^{-12}
Mouse	1.539×10^{-11}	40.3	1.69×10^{-11}	6.18×10^{-12}
Monkey (central retina)	2.255×10^{-11}	41.4	2.48×10^{-11}	9.35×10^{-12}

* Wet weight = density × volume (density of rod outer segments was calculated to be 1.1 according to equations derived by Barer and Joseph (9)).

Dry weight = solid concentration × volume.

Corresponding cell segments in the various species are remarkably uniform in concentration of solids even though the size, and therefore the total amount of solids, may vary enormously from species to species. A comparison of volumes, solid concentrations, and wet and dry weights for rod outer segments is presented in Table IV.



Text-Fig. 1. Schematic outlines of rod and cone cells, indicating average refractive indices in the various segments.

A number of photoreceptor cells were studied by interference microscopy as an independent check on the data obtained by refractometry. The phase retardation by rod outer segments suspended in isotonic saline solutions or in albumin solutions was determined in three ways: by measuring the displacement of interference fringes along the rod; by transferring the minimum illumination condition from the background to the central axis of the rod using green light; and by transferring various colors from the background to the central axis of

the rod using white light. The last method proved most precise with objects of cylindrical shape.

Each frog rod outer segment suspended in saline produced a phase retardation of very nearly 1 wavelength and measured about 6.5 μ, or 13 wavelengths, in thickness. Using the equation

$$\Phi = (\mu_c - \mu_m)t$$

in which Φ = phase retardation, μ_c and μ_m = refractive indices of cell and medium respectively, and t = thickness of cell, one may substitute:

$$1 = (\mu_c - 1.334)13,$$

or

$$\mu_c = 1.411.$$

This agrees well with the value of 1.406 obtained by refractometry (Table III). A few measurements on the refractive indices of rod and cone myoids were made and these also fell within the range obtained by refractometry. However, the data obtained by interference microscopy were somewhat more variable, as well as far more tedious to obtain because of the errors inherent in measuring the dimensions of each cell in addition to determining the phase retardation. Refractometry was far more suitable for statistical measurements.

DISCUSSION

Cell Structure.—A number of fibrillar structures on the surface or within the substance of photoreceptor cells have been described but not confirmed (2). Most serious consideration should be accorded the single, longitudinal, eccentrically-placed rod fiber described in 1904 independently by Fürst (18), Held (19), and Kolmer (21) and confirmed a year later by Retzius (24). The fiber was demonstrated with iron hematoxylin or silver in fixed cells. More recently a fiber in a similar position and less than 0.3 μ in diameter was demonstrated in electron photomicrographs of rod cells fixed in osmium tetroxide (29). De Robertis (16) has named the fiber a "connecting cilium" on the basis of its internal structure and connections. The high refractility of living rod cells and the relatively slight difference in refractive index between the fiber and the surrounding substance of the outer segment have precluded the demonstration of the fiber by ordinary methods of microscopy. However, it is easily seen by refractometric methods in teased unfixed rods, though possibly only after it swells to microscopic size (Figs. 6, 8, 18). This structure may have been observed even earlier and designated a "canal" by Kühne (22). Its function is unknown.

None of the other fibers previously described on the surface or within the substance of rod outer segments (2, 30, and others) have been seen in living cells studied by refractometry. However, in amphibia the rod surface is serrated longitudinally and the diffraction of light by the uneven surface of this very

refractile object may account for earlier descriptions. A central "core" differing in its staining properties from the periphery of the rod outer segment, and a "chromophobic disc" separating the outer from the inner segment (2, 31) are interpreted in the present study as artifacts caused by shrinkage and separation of the segments during dehydration (Fig. 16). Finally no sheath was seen surrounding teased rods.

The structure of the cone outer segment is less certain. Its normal shape and size are elusive. The structure sometimes seen along its surface (Figs. 28, 33) may be a swollen fiber analogous to the rod fiber, or perhaps a portion of a damaged sheath.

Finally the elasticity of the myoid (Figs. 29 to 32) merits special attention since no clues are available regarding the molecular pattern which underlies it.

Quantitative Considerations.—Collins, Love, and Morton (13) isolated rod outer segments of cattle by differential centrifugation in sucrose, drained off excess water, and then weighed the outer segments before and after complete drying. The dry weight was 27 gm. per 100 gm. of wet tissue. Davies, Engström, and Lindström (14) briefly recorded the concentration of solids in the rod outer segment of the frog as 40 gm. per 100 ml. using the fringe method of interference microscopy, and our results obtained by refractometry and interference microscopy are similar (Table IV). The apparent difference in concentrations obtained by the chemical and optical methods may be reduced somewhat for purposes of comparison if the value of 40 gm. per 100 ml. is converted according to the formula derived by Barer (5) to its equivalent of 36 gm. per 100 gm. of wet tissue.

Refractometry has limitations as a quantitative method for deriving the concentrations of solids in cells. Refractive indices may be measured accurately but their translation into concentrations of solids requires knowledge of the value of α, the specific refractive increment of the solid. Most chemical constituents of ordinary cytoplasm have similar refractive increments and α equal to 0.0018 generally may be used (7). On the other hand, the photoreceptor cell is specialized. Its paraboloid, for example, is composed almost entirely of glycogen (27), a complex carbohydrate for which the value of α is unknown. Since α for simple carbohydrates and for the complex carbohydrate, dextran, is nearer 0.0014, that value was used arbitrarily to calculate the concentration of solids in the paraboloid. Table III records the measured refractive indices of the various segments as well as the calculated concentrations of solids. The calculations can be modified should the values for α become known more accurately.

Another possible error stems from the observation that the ellipsoid and paraboloid are surrounded by a rim of myoid substance and are not directly in contact with the medium for determination of the match point. Since the rim of myoid substance is thin relative to the diameter of the ellipsoid or paraboloid, this source of error is unimportant. The true refractive index for these internal

bodies might be slightly higher by a factor in the third decimal place according to a formula given by Barer and Joseph (9).

Another difficulty in measuring heterogeneous cells is that one portion, such as an outer segment or an oil droplet, may superimpose a bright halo on an adjacent segment of lower density (Figs. 19, 22, 25). The halo may obscure the match point with an albumin medium of low refractive index. This difficulty accounts for some of the gaps in Table III.

Finally, the dichroism and birefringence of rod outer segments (15) may influence the measurements. In the present study polarized light was not used and the values given are average values for the refractive index and the concentration of solids in each cell.

Having sounded these notes of caution, one may point to certain advantages of refractometry over conventional biochemical methods. Changes are easily detected in the volumes of individual cells regardless of cell shape, so that the influence of the medium upon the concentration of solids may be observed directly in each cell. Alterations in the distribution of solids and water may develop quickly after a tissue is removed from the organism, and other degenerative changes may follow. These changes may be too subtle for detection even if the state of the tissue is checked by ordinary methods of microscopy. Such considerations limit the accuracy of many chemical studies on living tissues, for the biochemist rarely assesses and corrects for the agonal and postmortem alterations of volume, composition, and metabolism in the tissues under study.

Refractometry has the added merit of providing data directly on the concentration of solids, rather than on mass. Concentration would seem to be the critical quantitative measure for photoreceptor cells when one contrasts the variability in volume and mass of rod outer segments, differing more than 100-fold between amphibian and mammalian species, with the relative constancy of the concentration of solids (Table IV). Also the similarity between species in the concentration of solids for any given segment of the photoreceptor cells should be noted (Table III), though the various segments differ considerably from one another with regard to this measurement.

Refractive Index and the Absorption of Light by Photoreceptor Cells.—O'Brien (23) has presented some stimulating ideas on the relation of visual acuity in the central retina to the structure of cone cells, with particular reference to the Stiles-Crawford effect, namely that cone cells but not rods have a higher threshold for light entering them obliquely than for light arriving along their main axes. O'Brien developed a postulate, first proposed in 1843 by Brücke (12) (see also 32), that if the refractive index of the receptor cell is suitably higher than that of the surrounding medium, multiple reflection within the cell will confine the light and increase the efficiency of the absorption process leading to a visual response. The pathway of light cannot be analyzed ade-

quately by ordinary geometrical optics because the diameter of the outer segment is small relative to the wavelength of light. Therefore O'Brien tested the postulate by applying the theory of wave guides and dielectric antennae to a model system in which radar waves were directed at a large polystyrene "cone cell" scaled to the size and shape of the foveal cones in the monkey. The Stiles-Crawford effect could be reproduced quantitatively for monkey cones when the ratio of refractive indices of "cells" and "medium" was 1.022.

Data on the actual refractive indices of retinal elements would provide a test of this postulate. Zenker (33), Schultze (26), Helmholtz (20), and W. J. Schmidt (25) recorded the refractive index of rod outer segments as approximately 1.5. This value is too high to have validity for hydrated living cells and actually is the value expected for dried protein.

Refractometry provides a suitable technique for obtaining the refractive indices of living cells (Table III). Rod outer segments average 1.41 and cone outer segments 1.385. The other necessary datum, not yet available, is the refractive index of the substance surrouding the outer segments. Presumably a thin layer of fluid, perhaps with metachromatic staining properties (31), intervenes between the outer segments and the processes of the pigmented epithelial cells. Its refractive index probably is between 1.334 (saline) and 1.347 (serum) and would yield a ratio of refractive indices compatible with O'Brien's explanation of the Stiles-Crawford effect. A ratio of precisely 1.022 would not be expected since that value applies only to the particular geometry of the average foveal cone in the monkey. The absence of a Stiles-Crawford effect for rod vision can be explained by the high refractive index and the non-tapering shape of rod outer segments.

The path of light through the photoreceptor cells actually is less simple than these rough calculations would imply. The cone cell, and the rod to a lesser extent, is a complex structure with multiple surfaces curving in different ways, and with internal segments of varying shapes and refractive indices (Text-fig. 1). Some light will pass through the paraboloid and ellipsoid, while some will by-pass these internal bodies through the rim of myoid substance. Calculation of how much scattering and dispersion would occur before light gained access to the outer segment may be possible theoretically. However, the only practical approach to such an analysis would be with models, albeit more complex ones than O'Brien used in his pioneering studies.

Barer (6) has suggested the interesting theoretical possibility that dispersion and refraction in the outer segment might provide a mechanism accounting for variations in spectral sensitivity among cones which would require but a single cone pigment with a broad spectral absorption.

Spectrophotometric Analysis of Intact Rod Outer Segments.—The data on the refractive index of rod outer segments serve yet another purpose. If cells are suspended in an albumin solution matching their refractive index, light scatter-

ing is greatly reduced and the suspension is sufficiently clarified for spectrophotometric analysis (3). In a preliminary study (11) the absorption maximum for unbleached suspensions of intact rods was recorded between 500 and 510 mμ and the difference spectrum obtained after bleaching gave a maximum at about 515 mμ. Arden (1) prepared turbid suspensions of frog rods in saline and likewise recorded difference spectra of rhodopsin with a maximum at 515 mμ, shifted about 15 mμ toward the long wavelengths from the maximum of rhodopsin in solution.

SUMMARY

Fragments of freshly obtained retinas of several vertebrate species were studied by refractometry, with reference to the structure of the rods and cones. The findings allowed a reassessment of previous descriptions based mainly on fixed material. The refractometric method was used also to measure the refractice indices and to calculate the concentrations of solids and water in the various cell segments. The main quantitative data were confirmed by interference microscopy.

When examined by the method of refractometry the outer segments of freshly prepared retinal rods appear homogeneous. Within a few minutes a single eccentric longitudinal fiber appears, and transverse striations may develop. These changes are attributed to imbibition of water and swelling in structures normally too small for detection by light microscopy. The central "core" of outer segments and the chromophobic disc between outer and inner segments appear to be artifacts resulting from shrinkage during dehydration. The fresh outer segments of cones, and the inner segments of rods and cones also are described and illustrated.

The volumes, refractive indices, concentrations of solids, and wet and dry weights of various segments of the photoreceptor cells were tabulated. Rod outer segments of the different species vary more than 100-fold in volume and mass but all have concentrations of solids of 40 to 43 per cent. Cone outer segments contain only about 30 per cent solids. The myoids, paraboloids, and ellipsoids of the inner segments likewise have characteristic refractive indices and concentrations of solids.

Some of the limitations and particular virtues of refractometry as a method for quantitative analysis of living cells are discussed in comparison with more conventional biochemical techniques. Also the shapes and refractive indices of the various segments of photoreceptor cells are considered in relation to the absorption and transmission of light. The Stiles-Crawford effect can be accounted for on the basis of the structure of cone cells.

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EXPLANATION OF PLATES

PLATE 9

FIG. 1. Frog's rod cell in 43 per cent albumin. The outer segment (between brackets) is at the match point, where contrast between the cell and medium is minimal. The ellipsoid (*E*) appears slightly brighter than the medium. The myoid (*M*) is brightest and contains denser coarse particles. The nucleus (*N*) is partially obscured by the deeper retinal layers. × 1,100.

FIG. 2. Monkey's rods in 43 per cent albumin. Bright rod outer segments lie freely suspended in the medium. Most retain their more highly refractile, damaged inner segments. Refractometry allows rapid assessment of the refractility of a statistically significant number of rods. × 225.

FIG. 3. Monkey's rod outer segment in 35 per cent albumin. One end of the rod has curled. No sheath, surface structures, or internal fibers are seen. The rod appears dark. × 1,700.

FIG. 4. Monkey's rod outer segment in 41 per cent albumin. The distal end of the rod is cleaving transversely at two sites. The rod and the medium almost match in refractive index. × 2,000.

FIG. 5. Monkey's rod outer segment from the periphery of the retina suspended in 43 per cent albumin for 55 hours. The rod is still brighter than the medium more than 2 days after removal from the retina and would seem to retain its impermeability to the molecules of albumin (compare with Fig. 16). × 1,500.

FIG. 6. Frog's rod cell in 40 per cent albumin. A thin bright fiber (*F*) is seen along the dark outer segment. The distal half of the outer segment has broken away. The myoid is contiguous with the base of the outer segment; no "chromophobic disc" is seen. × 1,100.

FIG. 7. Frog's rod outer segment in 44 per cent albumin. Although bent and showing several delicate transverse striations, the rod remains brighter than the medium. × 775.

FIG. 8. Monkey's rod cell in 41 per cent albumin. A bright fiber with a single swelling is seen along the edge of the outer segment. × 2,000.

FIG. 9. Frog's rod cell in 27 per cent albumin. A bright fiber is seen diagonally crossing the ellipsoid (*E*). The fiber appears broadest at the distal end of the ellipsoid. × 575.

FIG. 10. Frog's rod outer segment in 46 per cent albumin. A fiber projects into the medium from the proximal end of the outer segment. × 775.

FIG. 11. Frog's rod outer segment in 46 per cent albumin. A bright fiber is seen along the surface of the outer segment and projects proximally into the medium. × 775.

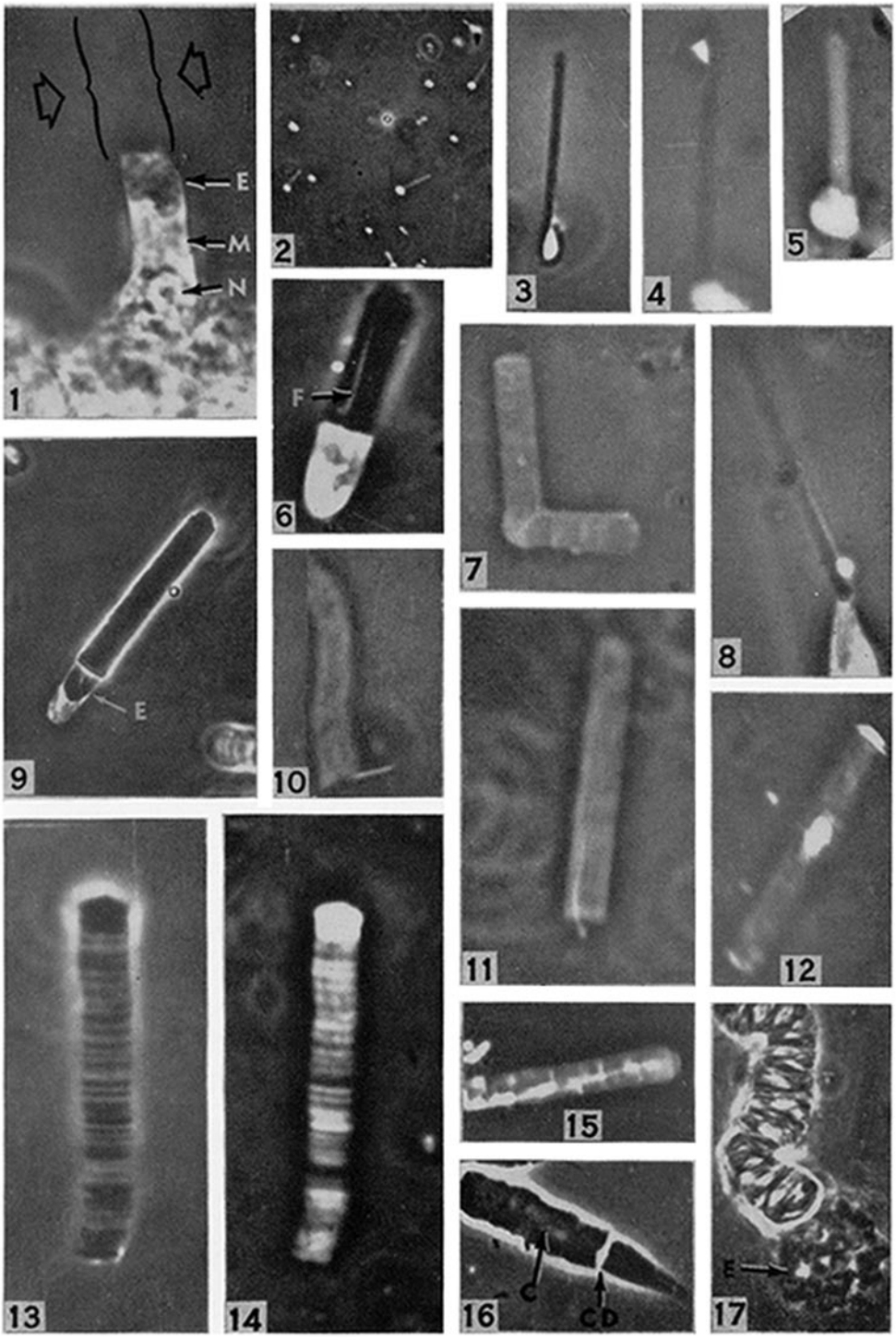
FIG. 12. Frog's rod outer segment in 44 per cent albumin. A bright irregular swelling is seen on a rod fiber. The longitudinal fiber itself is not in focus. × 650.

FIGS. 13 and 14. Frog's rod outer segment in 50 per cent albumin. Photomicrographs were taken through a variable amplitude phase contrast microscope, with negative phase (Fig. 13) and positive phase (Fig. 14) settings, using a mercury lamp as the source of light. Note the closely spaced transverse bands, an early degenerative change. × 1,000.

FIG. 15. Frog's rod outer segment in 50 per cent albumin. Transverse bands and an irregular longitudinal streak are seen. The outer segment remains bright. × 700.

FIG. 16. Frog's rod cell in 53 per cent albumin for 6 hours. The cell is darker than the albumin medium for it has become permeable to the molecules of albumin. The cell is shrunken and artifacts have appeared—the "chromophobic disc" (*CD*) between the inner and outer segments, and the irregular central "core" (*C*) within the outer segment. × 1,000.

FIG. 17. Frog's rod cell in 0.3 per cent NaCl. The outer segment has assumed a sinuous shape and is swelling rapidly, especially in the lengthwise direction as the transverse striations widen. The ellipsoid (*E*) also is swelling and the rounded mitochondria within it are seen. × 1,000.



(Sidman: Solids in photoreceptor cells)

PLATE 10

FIG. 18. Pigeon's rod cell in 42 per cent albumin. A moderately thick, slightly irregular fiber extends along the entire length of the outer segment and appears to enter or blend with the bright inner segment. × 750.

FIG. 19. Frog's cone cells in 27 per cent albumin. The outer segments present as truncated cones. × 750.

FIG. 20. Turtle's cone cell in 38 per cent albumin. The outer segment has a broad base and tapers to a point distally. Contrast on the one hand with Fig. 19 and on the other hand with Figs. 21 and 23, which show outer segments of other shapes. × 600.

FIG. 21. Frog's cone cell in 35 per cent albumin. The myoid (*M*) appears bright, the ellipsoid (*E*) within its distal portion dark, the oil droplet (*OD*) very bright, while the outer segment (*OS*) resembles a bright needle approximately constant in diameter from base to tip. × 750.

FIG. 22. Chick's cone cell in 29 per cent albumin. The cell is viewed obliquely and its outer segment has broken off. × 2,000.

FIG. 23. Turtle's cone cells in 0.9 per cent NaCl. Note the small zone of dark ellipsoid substance distal to the oil droplets and the thin outer segments (arrows), which resemble mounted bayonets. × 1,100.

FIG. 24. Monkey's cell in 41 per cent albumin. This cell resembles a foveal cone in shape, but has an unusually low and constant refractive index throughout its length, from the synaptic zone (*S*) which is above the plane of focus, through the nuclear region (*N*), to the finely tapered outer segment (*OS*), whose extremely delicate tip is below the plane of focus. × 1,600.

FIG. 25. Turtle's cone cell in 14 per cent albumin. Since the medium and the myoid have similar refractive indices, the contrast among internal structures of the inner segment is enhanced and one may observe the myoid (*M*) which contains granules and denser aggregates, the paraboloid (*P*), the ellipsoid (*E*), the very bright oil droplet (*OD*), and the distal tip of the ellipsoid (arrow). The outer segment (*OS*) appears to be at the match point but actually is out of focus. × 750.

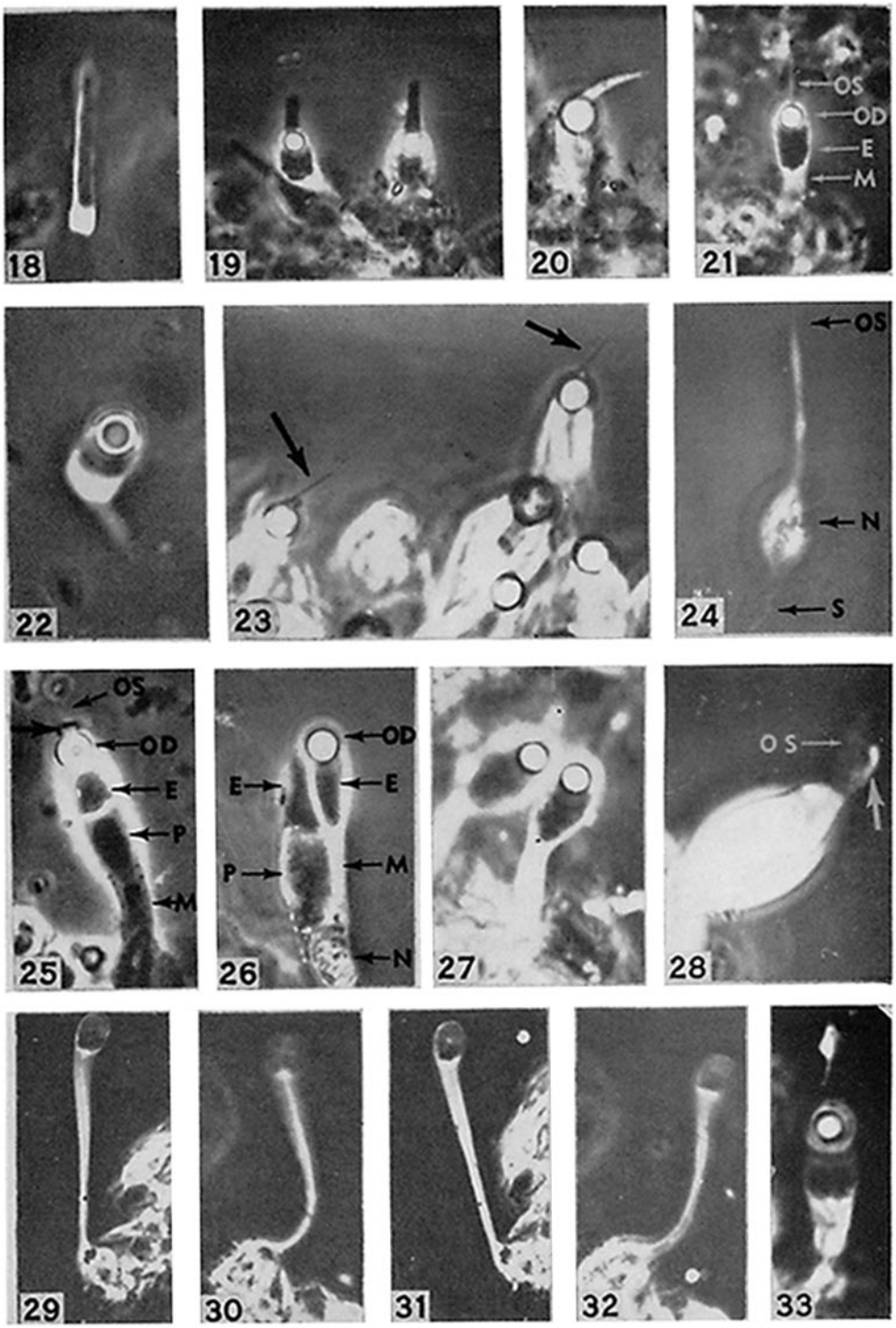
FIG. 26. Turtle's double cone cell in 26 per cent albumin. Only one nucleus (*N*) is visible. One member has a myoid (*M*), ellipsoid (*E*), and oil droplet (*OD*) while the other shows a paraboloid (*P*) and ellipsoid (*E*). The outer segments have broken off. × 700.

FIG. 27. Frog's cone cells in 36 per cent albumin. The myoids appear elongated. The ellipsoids, surrounded by a thin rim of myoid substance, are approximately at the match point with the medium. × 1,000.

FIG. 28. Salamander's cone cell in 30 per cent albumin. The outer segment (*OS*) is almost at the match point. Note the coarse fiber (arrow) along the margin of the outer segment. × 600.

FIGS. 29 to 32. Frog's inner segment in 53 per cent albumin. The myoid is elongated. When light pressure is applied to the coverslip the myoid promptly flexes (Fig. 30). When the pressure is released the myoid immediately snaps back to its former position (Fig. 31) and the maneuver can be repeated many times (Fig. 32). The molecular basis for this elasticity is unknown. × 650.

FIG. 33. Frog's cone cell in 30 per cent albumin. The slightly tapered outer segment is at the match point. It is outlined on one edge by a bright, irregularly thickened fiber or fragment of a sheath. × 1,000.



(Sidman: Solids in photoreceptor cells)