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Symposium



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Symposium

COLOUR VISION

Physiology and Experimental Psychology

Edited by A. V. S. DE REUCK, M.Sc., D.I.C., A.R.C.S.
and
JULIE KNIGHT, B.A.

With 98 illustrations



1965

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Preface

THIS symposium represents the first of a series of four or five small international and interdisciplinary conferences planned at the Ciba Foundation to extend over a corresponding period of four or five years, on the general theme of Sensory Function. At the British Association meeting in Manchester in 1962 the Deputy Director proposed such a series to Professor Otto Lowenstein, who responded generously and enthusiastically with offers of advice in planning the scope of the symposia and selecting the members. Professor Lowenstein was himself persuaded to take the Chair at this first meeting of the series, where he made a further contribution to its success by his skilful but unobtrusive guidance of the proceedings.

The inevitable pressure on places was particularly severe at this meeting and much care had to be exercised in identifying the growing points of the subject and in choosing active workers in the field. In this task and in the subsequent one of editing this book, valuable assistance was given by Dr. H. J. A. Dartnall, Director of the M.R.C. Vision Research Unit at the Institute of Ophthalmology, London, to whom the Editors and the Ciba Foundation are greatly indebted.

The Editors must also record the regret of both the participants and the Foundation that a senior member of the group, Professor H. Autrum, was prevented at the last moment from attending the symposium by his wife's illness. His paper which was mailed to London was delayed by a postal strike and so could not be read and discussed at the meeting, but it is now published here with the rest of the proceedings.

It is intended that the next symposium in the series on Sensory Function shall be entitled "Touch, Heat and Pain".

Editors' note

THE recommendation† of the British Standards Institute that the unit nanometre (nm., 10^{-9} metre) should be used to replace millimicron (m μ) has been followed in this book.

† British Standards Institute (1960). Amendment no. 3, to B.S. 1991, part I, p. 3; British Standards Institute (1954). B.S. 1991, part I, p. 33.

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Membership

Symposium on Colour Vision: Physiology and Experimental Psychology held 21st-23rd July, 1964.

- | | | |
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CHAIRMAN'S INTRODUCTION

O. E. LOWENSTEIN

WE are assembled under the hospitable and auspicious roof of the Ciba Foundation in what I think is the justifiable hope that in the next few days we shall accomplish a good piece of work together. We are all aware that our topic is, and will be for a long time to come, in need of clarification, and when I began to assist Mr. de Reuck in the planning of these symposia on sensory function it was a selfish wish of mine that colour vision should be put first. As a teacher of comparative physiology who has many discussions with students, it has invariably been my experience that I as the tutor was sometimes as lost as they, when it came to the clarification of fundamental points in this difficult topic.

I would like first to say something about my legitimation as the chairman of this meeting, since it is, I am sure, not a secret among the majority of you that I have never published a paper on vision. It may seem terrible to have a chairman who doesn't know anything about the subject, but I wouldn't go so far as this! First of all, when I was a student, I was present when most of the work on colour vision in bees and in fish was carried out in von Frisch's department. So I may say that I grew up with colour vision, and ever since I have been teaching comparative physiology and have been face to face with all its problems.

The title of our symposium is "Colour Vision: Physiology and Experimental Psychology", and the programme will show that quite a lot of our papers are going to be physiological in the true sense of the word. However there will be, as there should be, a good body of evidence provided by morphological facts. I have already mentioned my teacher Karl von Frisch. Whenever he

called somebody to work under his guidance he said to him: "Remember this, never do any physiological work without doing a parallel piece of morphology on the organ system you are studying", and I have kept that very much in the forefront of my mind ever since, and have kept my promise to him. And so I see it as quite natural that we should interest ourselves in morphological results. Then, turning to the other aspect of our programme, the psychology of colour vision, there are nominally very few truly experimental psychological papers in this programme but it is quite clear that our hopes run high; a lot of the evidence which will be discussed here will have been derived from work done in laboratories where experimental psychological methods are practised.

Finally, may I say one or two words about the planning stages of the symposium. The interesting thing about planning such a symposium is that there is a primary seeding by somebody like myself and from that moment onwards, the subterranean roots begin to grow and the growth of the whole thing is sometimes hidden from the spiritual father! It is true that I have been consulted, but I have had little share in the hard work of persuading you all to come. It is therefore my earnest desire to express my indebtedness to Dr. Dartnall, who gave so much help in the planning stages here in London, when I was in Birmingham at the end of long reins.

VISUAL PIGMENTS

THE CLUSTERING OF FISH VISUAL PIGMENTS AROUND DISCRETE SPECTRAL POSITIONS, AND ITS BEARING ON CHEMICAL STRUCTURE†

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ALL the known visual pigments are chromoproteins. They consist of a special class of proteins called opsins in association with prosthetic groups that are based either on retinene₁ or on retinene₂. In the last quarter century, and particularly in the last ten years, the systematic exploration of the animal kingdom has begun, and through the efforts of the several workers now active in this field a considerable number of different visual pigments have now been described.

Since the visual pigments each have a single absorption band in the visible spectrum it is convenient to characterize them by their λ_{\max} , that is, the wavelength at which absorption is greatest. In the retinene₁ series the known pigments have λ_{\max} ranging from about 430 nm. to 562 nm.; in the retinene₂ series the range is similarly broad, the lowest known λ_{\max} being 510 nm. and the highest 620 nm., this being the λ_{\max} of the synthetic pigment cyanopsin.

The absorption bands of these two series of visual pigments are shown in Fig. 1. This shows that the λ_{\max} ranges are roughly equal in the two kinds of pigments, being 132 nm. in the retinene₁ series and 110 nm. in the retinene₂ series. The retinene₂ pigments in

† This paper was presented to the symposium under the title "Relations between retinene₁ and retinene₂ visual pigments."

general have absorption bands situated at longer wavelengths than those of the retinene₂ pigments, but there is a considerable region of overlap. Another obvious fact is that the retinene₁ pigments are much more numerous than those based on retinene₂. Future dis-

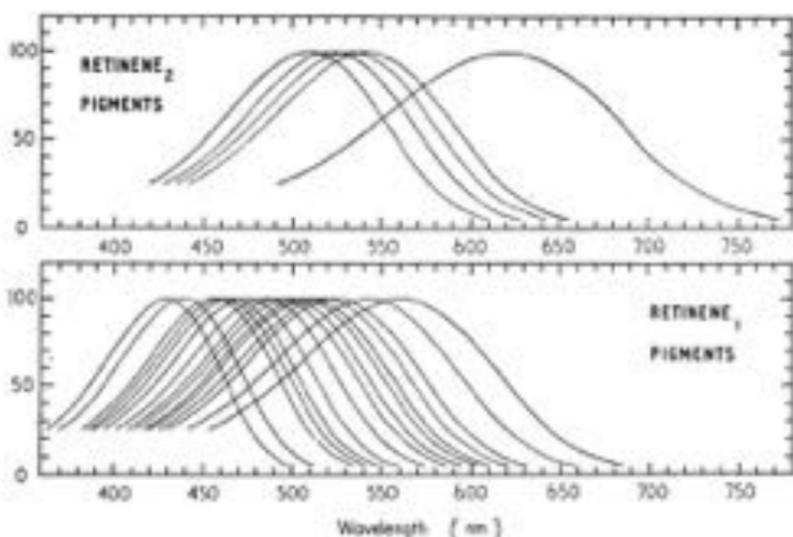


FIG. 1. The main absorption bands of the visual pigments in the two series. The λ_{max} of retinene₂ pigments range from 510 nm. in the wrasse (*Labrus merula*, Brown and Brown, cited by Wald, 1960) to 620 nm. (cyanopsin, Wald, Brown and Smith, 1953), and those of the retinene₁ pigments from 430 nm. in the green-rod pigment of the frog (Dartnall, 1957) to 562 nm. in the chicken cone pigment, iodopsin (Wald, Brown and Smith, 1955). Curves constructed from the visual pigment nomogram (Dartnall, 1953). (From Dartnall, 1964.)

coveries may narrow this difference in numbers, and in this connexion there certainly seems to be a gap to be filled between the synthetic cyanopsin, maximal at 620 nm., and the next highest retinene₂ pigment at 543 nm.

How are we to account for the remarkable variation in spectral properties of the visual pigments, a variation that enables species to evolve pigments suitable for their photic environment, and, in some species where more than one pigment is possessed, possibly to

enjoy colour vision? It is instructive to consider, point by point, possible ways in which this variability might be achieved.

In the first place, as already mentioned, the visual pigments have prosthetic groups based either on retinene₁ or on retinene₂. Now the retinenes and their alcohols, the vitamins A, can exist in a number of different shapes. Present indications are that eight isomeric forms of retinene₁ and of vitamin A₁ should be possible. The structural formulae of these are shown in Fig. 2. Since retinene₂ and vitamin A₂ differ from their "A₁" counterparts only in the possession of an additional double bond in the ring structure, it is probable that similar formulae apply to them also.

The upper formulae in Fig. 2 are the unhindered isomers, all of which have been prepared. The lower formulae are the four hindered isomers, that is, those in which there is some degree of steric hindrance between a hydrogen atom, shown by a small white circle, and an adjacent methyl group shown by a large black circle. These regions are ringed in the figure. Of the four hindered isomers only the first and third forms, that is 11-*cis* and 11-13 di-*cis*, have been prepared so far, but it is probable that the remaining two can also exist.

Can the existence of these various *cis-trans* isomers be invoked to account for the variability of the visual pigments? Apparently not. According to Wald (1958) "the retinene precursor of a visual pigment is always *neo-b* [11-*cis*], whether one is dealing with vitamin A₁ or A₂, or the corresponding retinenes; and whether vertebrate or invertebrate visual pigments". This generalization should be accepted with some reserve for the 11-*cis* shape of the prosthetic group has been established in only a few pigments, and these all based on retinene₁. Nevertheless no positive exception to this statement is known.

If, therefore, we may not invoke different shapes of the prosthetic groups to account for the variability, is the answer to be found, perhaps, in different numbers of prosthetic groups per opsin molecule? In 1954 Hubbard showed that in the 498 nm. rhodopsin of cattle

there is only one prosthetic group to each opsin molecule. Most investigators accept this conclusion as valid for all pigments, presumably because of the similarity between visual pigments in all

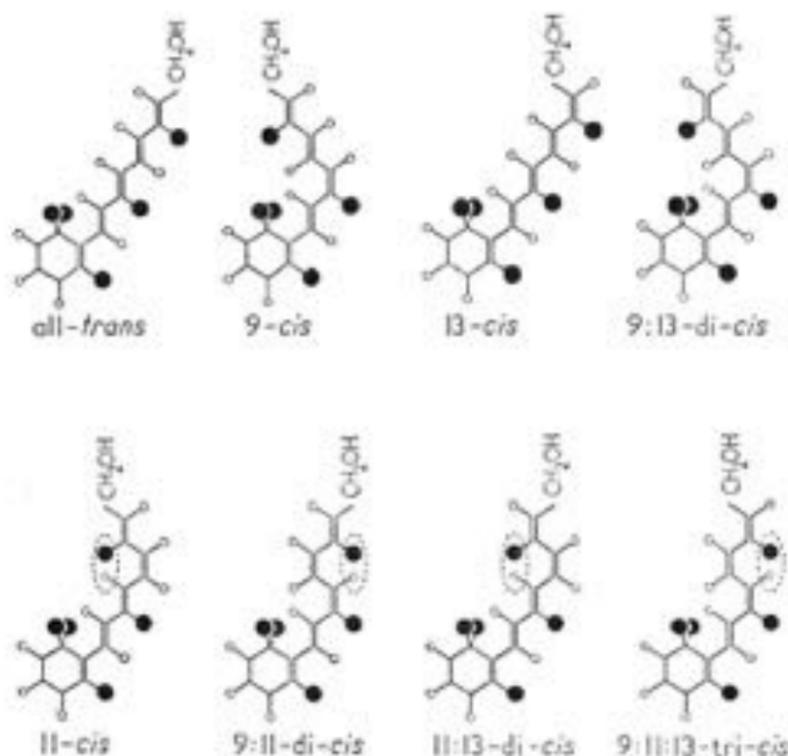


FIG. 2. Structural formulae for vitamin A₁ isomers showing regions of steric hindrance (dotted rings). The upper formulae are the unhindered isomers, all of which have been prepared. The lower formulae are the four hindered 11-*cis* isomers, only the first and third of which have been prepared to date. The small unfilled circles represent hydrogen atoms, the large filled circles methyl ($-\text{CH}_3$) groups. Similar formulae apply to the vitamin A₂ isomers (additional conjugated double bond at carbon 3 in the ring structure), and to the aldehydes (retinenes) of the vitamins. (From Dartnall, 1962b.)

respects save in the spectral location of their absorption bands. Thus, when plotted to a regular frequency scale the absorption bands of all the pigments have closely similar shapes (Dartnall, 1952,

1953, 1962a) and those pigments so far examined all have comparable photosensitivities (Dartnall, 1958). Moreover, one would, perhaps, expect that a replication of prosthetic groups on such a relatively large molecule as an opsin would cause an intensification of absorbance rather than an alteration to its spectral location.

It seems, therefore, that the cause of variability is to be sought in the opsins, the protein moieties of the pigment molecules, or in the nature of the attachments between prosthetic group and opsin. Wald, Brown and Smith (1952, 1953) have proposed that the opsins of cone pigments be called "photopsins" and those of rod pigments "scotopsins". This distinction was a reasonable one to make when it seemed that there was a large spectral separation between the classical rod pigment "rhodopsin" ($\lambda_{\max} = 500 \pm 2$ nm.) and the classical cone pigment iodopsin ($\lambda_{\max} = 562$ nm.) in the retinene₁ series and between the analogous "porphyropsin" ($\lambda_{\max} = 522 \pm 2$ nm.) and cyanopsin ($\lambda_{\max} = 620$ nm.) in the retinene₂ series. With the passage of time, however, the terms "rhodopsin" and "porphyropsin" have come to embrace ever-widening ranges of "rod" pigments, until there is now no clear indication in the spectrum where "rod" pigments cease and "cone" pigments begin (Fig. 1), while the recent spectrophotometric measurements on cones of the goldfish, macaque monkey, and man (Marks, 1963; Marks, Dobbelle and MacNichol, 1964; Brown and Wald, 1964; see also Marks, 1965) have disclosed cone pigments with absorption bands in positions that interdigitate with those of rod pigments.

Thus "cone" pigments can no longer be set aside as distinct from "rod" pigments by their spectral location, nor by any other criterion such as the shapes of their absorption bands or the nature of their prosthetic groups. In fact there is not a single chemical or physical property by which one can distinguish them as in different classes. The variability of opsin is now required to "explain" the variability of pigments in general, independently of their origin. In short, it seems preferable to regard all visual pigments, whether of rod, cone, or uncertain origin, as belonging to one variable family,

and to shelve, for the time being at least, the still-current terms "scotopsin" and "photopsin".

We have recently completed a survey of the visual pigments of teleost fishes. The results of this survey seem to provide a clue to pigment variability. But before describing this work it is necessary to demonstrate the relationship between the λ_{\max} of pigments in the retinene₁ and retinene₂ series.

RELATION BETWEEN λ_{\max} OF RETINENE₁ AND RETINENE₂
PIGMENTS IN IDENTICAL-OPsin PAIRS

Wald, Brown and Smith (cited by Wald, 1953) found that the opsin of a retinene₁ pigment could be induced to combine with a suitable isomer of retinene₂ to form a synthetic analogue. The opsin used was derived from the 498 nm. visual pigment of cattle. The synthetic retinene₂ pigment so formed had $\lambda_{\max} = 517$ nm., that is, 19 nm. higher than the natural retinene₁ pigment. Wald, Brown and Smith (1953) also performed a similar experiment using the opsin of chicken iodopsin ($\lambda_{\max} = 562$ nm.). The retinene₂ analogue ("cyanopsin") formed in this case had $\lambda_{\max} = 620$ nm., namely 58 nm. higher than the natural retinene₁ pigment.

These two examples are the only direct substitution experiments that have been carried out. There are several instances, however, where a retinene₁/retinene₂ pair of pigments occur naturally together in a retina, and where there are good reasons for believing that both pigments are based on a common opsin.

Thus Dartnall, Lander and Munz (1961) found that the rudd, a freshwater fish, possesses a retinene₁ pigment of $\lambda_{\max} = 510$ nm. and a retinene₂ pigment of $\lambda_{\max} = 543$ nm. Under natural conditions the proportions of these two pigments vary according to season, the 543₂ pigment preponderating in the winter (short days) and the other pigment in the summer (long days). Moreover the pigment composition of the retina can be varied in the laboratory by putting the fishes either into darkness, which causes an increase in the pro-

portion of the retinene₂ pigment, or into light, when the reverse change occurs. For these reasons it is considered that the two rudd pigments are based on the same opsin. Since this work, other fishes possessing a retinene₁/retinene₂ pair of pigments have been found to behave similarly (Dartnall, 1962*a*, p. 411; Bridges, 1964*a*).

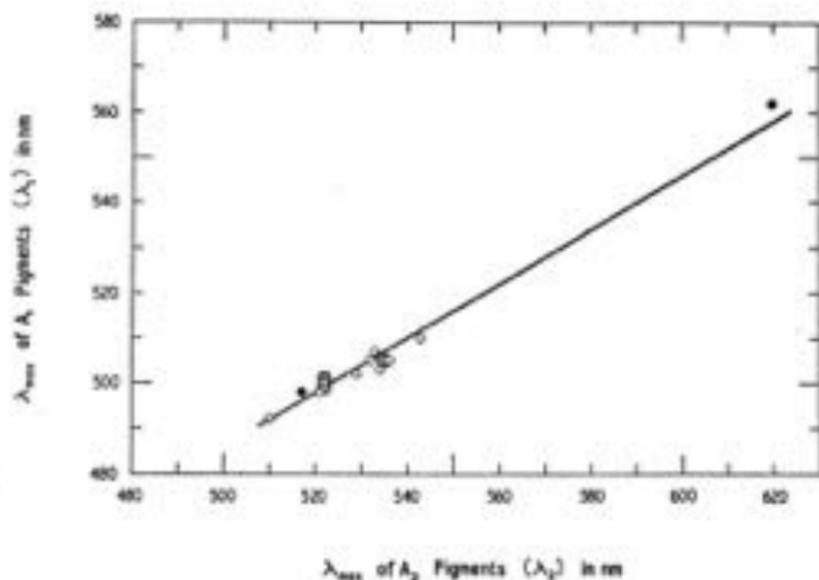


FIG. 3. The relationship between the λ_{\max} of retinene₁ and retinene₂ pigments in identical-opsin pairs. Empty circles represent the sixteen naturally occurring pairs and filled circles, the data obtained in Wald, Brown and Smith's direct substitution experiments (see text). Equation to the line is $\lambda_1 = 0.60 \lambda_2 + 186$.

In general whenever a species contains *only* a retinene₁/retinene₂ pair it may be argued that both pigments are necessarily based on the same opsin. For otherwise, since both retinenes could combine with the different opsins, one would expect not two but four pigments to be present.

In the pigment list of Table I there are eighteen teleost species shown as possessing *only* a retinene₁/retinene₂ pair. In Fig. 3 the λ_{\max} of the retinene₁ pigment is plotted against that of its retinene₂ counterpart for sixteen of these pairs (empty circles). The two

species omitted from this plot are the tench (467₁ and 533₂) and the scarid, *Sparisoma cretense* (486₁ and 520₂). The tench pair are omitted because it is not absolutely certain that the 467 pigment is based on retinene₁ (Dartnall, 1952), and the scarid pair because we could not exclude from the results of our partial bleaching experiments the possibility that a third pigment was present having λ_{\max} intermediate between those reported.

The results of Wald, Brown and Smith's two substitution experiments (mentioned above) are shown in Fig. 3 by the filled circles. The figure shows that these results are consistent with those of the natural pairs and that the relation between the λ_{\max} of pigments in the retinene₁ and retinene₂ series is approximately linear (cf. also Dartnall, 1962*a*, p. 420).

ARE THE λ_{\max} OF VISUAL PIGMENTS CONTINUOUSLY VARIABLE?

In 1963 we made expeditions to a number of localities including Marsilles, Plymouth, Aberdeen, The Bay of Biscay, Malta and the Isle of Man, with the object of collecting fishes for visual pigment analyses. About 40 different teleost species were satisfactorily analysed by the method of partial bleaching, and the results obtained are listed in Table I ("present work"). The great majority of these fishes had single or mixed pigments based on retinene₁, although retinene₂ pigments were also present in a few instances (see Table I). The λ_{\max} of the retinene₁ pigments were found to extend from 468 nm. to 528 nm., a result that significantly widens the known range of retinene₁ pigments in marine fishes (previous range 478 to 520 nm.).

The results also seem to provide an answer to one of the questions we had posed, namely whether the absorption bands of the visual pigments are continuously variable from species to species, or whether the λ_{\max} are clustered around discrete positions in the spectrum. When pigment λ_{\max} is plotted versus frequency (number of species) there is clear evidence of a clustering of pigments at

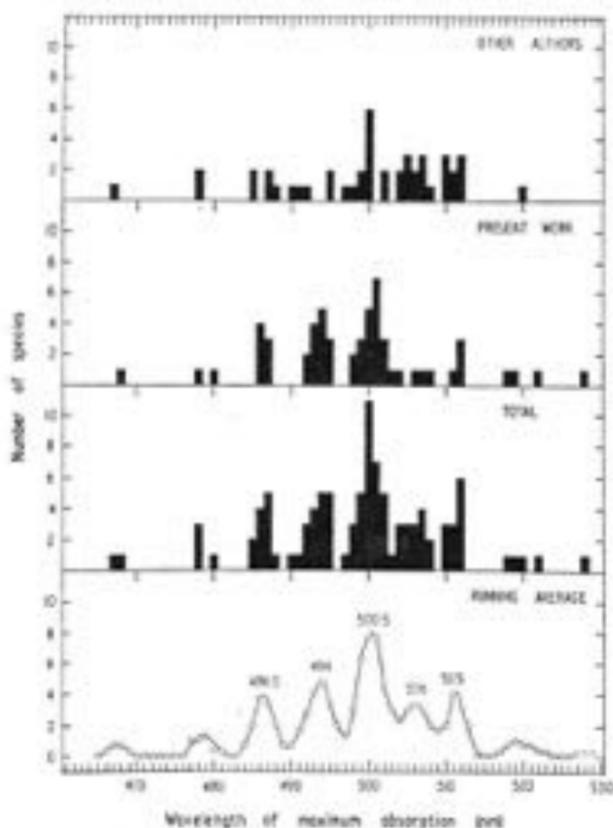


FIG. 4. The distribution of retinene₁ visual pigments in teleost fishes. The histograms give the plot of pigment λ_{max} against frequency of occurrence (number of species). Previously published data, present work, and the total data are separately shown. The "running average" curve was obtained from the total histogram by averaging the frequencies over three consecutive wavelengths, and gives the points about which clustering is centred.

positions near 487, 494 and 500 nm. (Fig. 4, histogram labelled "present work"). When these results are added to those already published in the literature (listed in Table I, and plotted in the Fig. 4 histogram labelled "other authors") the total histogram reveals clustering at two additional positions, namely 506 and 512 nm.

The present investigation provides 57 retinene₁ pigments from 41 species; the work of previous authors gives 45 retinene₁ pigments from an additional 42 species. Thus the total survey includes 102 retinene₁ pigments (all of which have been tested for homogeneity by the method of partial bleaching—a criterion for inclusion in Table I) from 83 different species of teleost fishes obtained from widely separated parts of the world, and from various depths in the oceans. Although this total is minute in comparison with the total number of teleost species, the representation of families (37) is a significant fraction of the whole.

A more precise estimate of the five spectral positions about which the retinene₁ pigments are centred can be obtained by plotting the running averages of the frequencies taken over three consecutive wavelengths. This has been done in the bottom quarter of Fig. 4, which shows them to be 486.5, 494, 500.5, 506 and 511.5 nm. There is also just a suggestion of two other positions at about 478 nm. and 519 nm., but the numbers of pigments involved are insufficient to be certain.

The broader question of whether pigments with λ_{\max} above and below those of the present sequence of five groups are also clustered can only be answered, of course, by the analysis of further material from sources providing pigments in ranges centred in regions outside that (500 nm.) of the present survey (cf. Dartnall, 1964).

From the relationship between the λ_{\max} of pigments in the retinene₁ and retinene₂ series (Fig. 3) it can be calculated that the retinene₂ analogues of our retinene₁ series (486.5, 494, 500.5, 506 and 511.5 nm.) would have their λ_{\max} at 501, 513, 524, 533 and 543 nm. (Dartnall and Lythgoe, 1964).

By a fortunate coincidence Bridges has been studying the distribution of the natural retinene₂ pigments. He has examined the visual pigments of 18 North American freshwater and freshwater/marine fishes and reports (Bridges, 1964*b, c*) that when his results are added to those for the twelve British freshwater species, and three marine labrids already in the literature, the λ_{\max} are found

to cluster around the positions 511.5 (this should read 512), 523.5, 534 and 543 nm.

These four positions are at, or very close to, four of the five positions for retinene₂ analogues of our retinene₁ series. This correspondence, in our view, gives *additional* substance to the hypothesis that λ_{max} occur at discrete spectral positions in both series. It could, of course, be objected that such a correspondence would be expected on account of the fact that the naturally paired retinene₁/retinene₂ pigments form a significant portion of both lists (particularly that of Bridges) and that these pairs provide most of the data used for establishing the relationship between retinene₁ and retinene₂ pigments (Fig. 3). To answer this objection we need to consider the distribution of pigment λ_{max} in fishes that possess *only* retinene₁ pigments in the one case, and *only* retinene₂ pigments in the other.

Twenty of the species listed in Table I possess retinene₂ as well as retinene₁ pigments. Removal of these still leaves 63 species having retinene₁ pigments only (81 pigments). These, when plotted in histogram form, show clustering at almost exactly the same positions as before (actually at 486.5, 494, 500.5, 506.5 and 512 nm.). In other words, removal of the "paired pigment" species from the list (Table I) has no material effect on the distribution pattern of retinene₁ pigments.

The retinene₂ list (33 species) is more seriously depleted by removal of "paired pigment" species. Thus Bridges' 18 new species (Bridges, 1964c) include 9 such members, while the 15 retinene₂-containing species in the literature harbour another 7, including the only two 543₂ examples (rudd and chub with 543₂ and 510₁). The 17 species possessing *only* retinene₂ pigments that remain after this operation are listed in Table II in ascending order of λ_{max} . They still show evidence of clustering, around the positions 513 nm. (two examples), 524 nm. (ten examples) and 534 nm. (five examples).

Thus we may conclude that the spectral positions about which clustering of λ_{max} is observed in fishes that contain *only* retinene₁

pigments on the one hand, and *only* retinene₂ on the other, are related in the same way as are the λ_{\max} of naturally paired pigments (Fig. 3), which are based on the same opsins.

STRUCTURAL IMPLICATIONS OF CLUSTERING

Although this paper deals exclusively with fishes, a study of the lists of visual pigments of other animals (Dartnall, 1960c) suggests that clustering of λ_{\max} is not confined to this class.

A conceivable explanation for the clustering phenomenon in fishes is that it is an example of convergent evolution towards certain spectral regions. However, we do not consider this a likely explanation for, even if it were accepted that the λ_{\max} in the retinene₁ series, viz. 486.5, 494, 500.5, 506 and 511.5 nm., are environmentally favoured positions, to suit, for example, the optical properties of certain (marine) waters, it is difficult to see why the *analogous* retinene₂ positions, viz. 501, 513, 524, 534 and 543 nm.—four of which, in fact, are found in *natural* retinene₂ pigments—should suit the optical properties of another set of (fresh) waters.

Since, as we have seen, the prosthetic groups of all visual pigments are presumed to be of identical shape (*11-cis*) the only way in which pigment variability can be achieved would seem to be by variations in opsin or in the linkages between opsin and prosthetic group that are *additional* to the main carbon-nitrogen bond. We incline to the view that clustering has a chemical basis, that is, that the λ_{\max} positions relate to discrete structural forms of the opsins or their linkages to the prosthetic group.

The prosthetic group is conjugated, that is, it consists of a string of carbon atoms linked by alternate single and double valency bonds. Now a conjugated structure such as



can exist in a number of different conditions in which the shared electrons constituting one of a pair of double bonds move closer to

one of the carbon atoms, thus creating a dipole. Moreover this dipole (through interchange of single and double bonds) can extend over less or greater lengths of the molecule. In this way several conditions, such as the following, are possible:



The conventional formula (1) represents the condition of a normal unexcited molecule: the formulae (2), a few of the possible resonance structures for an excited one.

This suggests to us a way in which variability of visual pigments might be achieved. For the *stabilization* of different ionic forms of the prosthetic group could be effected through electrostatic attraction to oppositely charged atoms in the opsin molecule. In Fig. 5 fifteen possible positions for the prosthetic group dipole are shown. There are another fifteen in which the charges are reversed. According to this suggestion the dipole condition into which the *11-cis* prosthetic group is "frozen" in a particular instance would be determined by the distance between the positive and negative charges on the opsin moiety. After partial detachment of the prosthetic group from opsin (for example, through isomerism following exposure to light) no trace of the "frozen" dipole would remain.

Not all of the structures shown in Fig. 5 are equally likely. It seems likely that those dipoles on the prosthetic group that are separated by only a single carbon-carbon distance (1, 6, 10, 13 and 15 in Fig. 5) are not likely to be "interested" in charges on the relatively remote opsin molecule. In other words, twenty (not thirty) forms seem more probable to us. In this connexion it may be noted that the spectral separation between the shortest (430 nm.) and longest (562 nm.) known λ_{\max} in the retinene₁ series is 132 nm.,

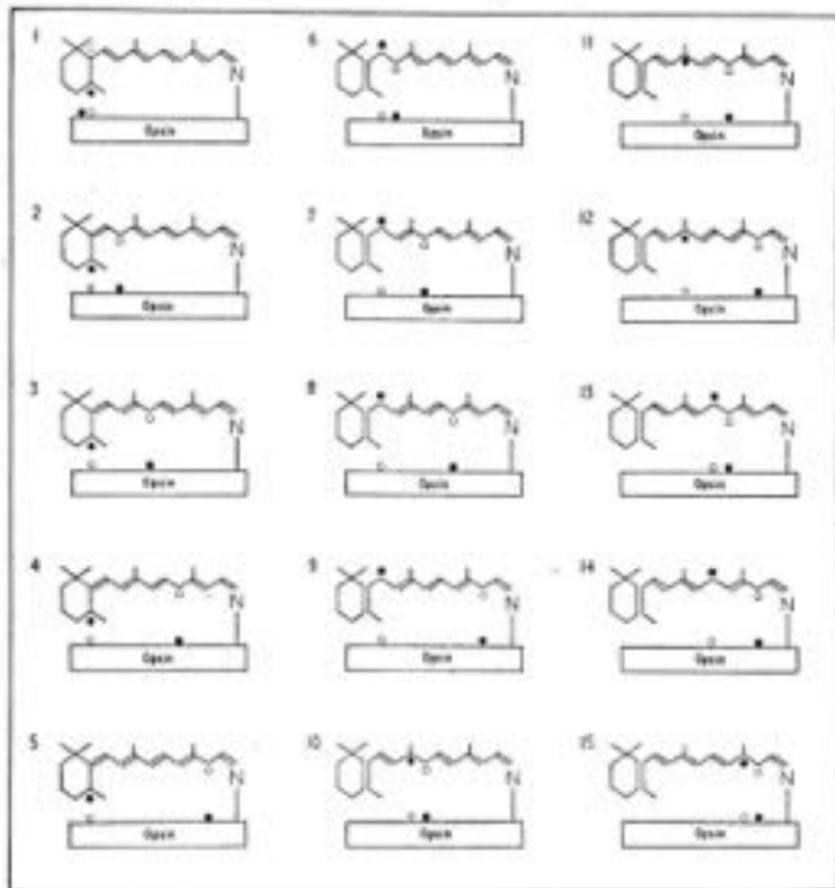


FIG. 5. Proposed schematic formulae for visual pigments based on retinene₁. The filled and unfilled circles on the prosthetic groups represent dipoles that are "frozen" in position by oppositely charged atoms on the protein moiety. Thirty such forms are possible, according to whether either the filled or unfilled circles are considered to be positive or negative. Formulae 1, 6, 10, 13 and 15 are considered less probable than the others, leaving twenty more-probable forms (see text). In these formulae the prosthetic groups are drawn without prejudice to their configuration, which is probably 11-*cis*.

and this figure, divided by nineteen (spaces between twenty positions) gives 7 nm., or about the interval between successive members in the retinene₁ series.

VISUAL PIGMENTS OF TELOSTY FISHES POSSESSING AT LEAST ONE RETINENE₁ PIGMENT

Family	Species	English name	Pigments	Reference
Alepocephalidae	<i>Xenodermichthys copei</i> Gill		480 ₁ †	present work
Clupeidae	<i>Clupea sprattus</i> L.	Sprat	501 ₁ ‡	present work
Sternopychiidae	<i>Argyroteleus affinis</i> Garman	Hatchet fish	478 ₁	Munz, 1958a
	<i>Sternopyx obscura</i> Garman	Hatchet fish	485 ₁	Munz, 1958a
Salmonidae§	<i>Oncorhynchus kisutch</i> (Walbaum)	Coho salmon	507 ₁	Munz, 1957
	<i>Oncorhynchus tshawytscha</i> (Walbaum)	King salmon	507 ₁	Munz, 1957
	<i>Salmo gairdneri</i> Richardson	Rainbow trout	507 ₁	Bridges, 1956
Argentidae	<i>Bathylagus microphalus</i> Norman		498 ₁ , 468 ₁	present work
	<i>Bathylagus weselii</i> Bolin		501 ₁ , 478 ₁	Munz, 1958a
	<i>Nausenia groenlandica</i> (Reinhardt)		492 ₁	present work
Myctophidae	<i>Searsia koefoedi</i> Parr		478 ₁	present work
	<i>Lamparyctus mexicanus</i> Gilbert		490 ₁	Munz, 1958a
	<i>Lamparyctus</i> sp.		486 ₁	present work
Cyprinidae	<i>Alburnus lucidus</i> Day	Bleak	510 ₁	533 ₂ , c. 550 ₂ Dartnall, 1955
	<i>Hybopsis biguttata</i> (Kirtland)	Hornhead chub	505 ₁	535 ₂ Bridges, 1964c
	<i>Leuciscus cephalus</i> Day	Chub	510 ₂	543 ₂ Dartnall, 1962a
	<i>Notemigonus crysoleucas auratus</i> (Rafinesque)		504 ₁	532 ₂ Bridges, 1964c
	<i>Notemigonus crysoleucas boeii</i> (Valenciennes)	South-east golden shiner	502 ₁	529 ₂ Bridges, 1964c
	<i>Notropis cornutus frontalis</i> (Agassiz)		505 ₁	536 ₂ Bridges, 1964c
	<i>Pimephales notatus</i> (Rafinesque)	Bluntnose minnow	505 ₂	535 ₂ Bridges, 1964c
	<i>Scardinus erythrophthalmus</i> (L.)	Rudd	510 ₁	543 ₂ Dartnall <i>et al.</i> , 1961
Poeciliidae	<i>Belonesox helizans</i> Kner		467 ₁	533 ₂ Dartnall, 1952
	<i>Anguilla anguilla</i> (L.)	Eel	498 ₁	521 ₂ Bridges, 1964c
Anguillidae	<i>Anguilla anguilla</i> (L.)		502 ₁ , 487 ₁	523 ₂ Brown and Brown, <i>ex Wald</i> , 1960
Congridae	<i>Conger conger</i> (L.)	Conger	487 ₁	Walker, 1956

† The extract also contained a minor amount of a retinene₁-based pigment of λ_{max} in the region of 472 nm.

‡ The extract also contained a minor amount of a pigment of undetermined category and of λ_{max} in the region of 506 nm.

§ Munz (private communication), however, will report that in five species of salmon (*Oncorhynchus gorbuscha* (Walbaum), *O. keta* (Walbaum), *O. kisutch* (Walbaum), *O. nerka* (Walbaum) and *O. tshawytscha* (Walbaum)) and in four species of trout (*Salmo clarki* Richardson, *S. gairdneri* Richardson, *S. salar* L., and *S. trutta* L.) the retinal pigments are mixtures of 503₁ and 527₂.

Table I—continued

Family	Species	English name	Pigments	Reference
Gadidae	<i>Gadus aeglefinus</i> L.	Haddock	495 ₁ ¶	present work
	<i>Gadus merlangus</i> L.	Whiting	501 ₁	present work
	<i>Gadus minutus</i> L.	Poole cod	494 ₁	present work
	<i>Gadus morhua</i> Day	Codling	500 ₁	present work
	<i>Astionora rostrata</i> Günther		486 ₁	present work
Melanphaidae	<i>Melanphaes bipinnatus</i> Gilbert		488 ₁	Munz, 1958a
Zeidae	<i>Zeus faber</i> L.	John Dory	493 ₁	present work
Caproidae	<i>Capros aper</i> (L.)	Boar fish	494 ₁	present work
Serranidae	<i>Epinephelus alexandrinus</i> (Cuvier and Valenciennes)	Grouper	500 ₁ , 495 ₁	present work
	<i>Epinephelus guaza</i> (L.)	Grouper	502 ₁ , 495 ₁	present work
	<i>Moreno labrax</i> (L.)	Sea bass	503 ₁	534 ₁ present work
	<i>Paracentropistis scriba</i> (L.)	Painted comber	499 ₁ , 403 ₁	present work
	<i>Serranus cabrilla</i> (L.)	Comber	494 ₁	present work
Carangidae	<i>Caranx trachurus</i> Day	Scad	499 ₂	present work
	<i>Trachurus symmetricus</i> (Ayres)		495 ₁	Munz, 1957
Sciaenidae	<i>Coreina nigra</i> = <i>Sciaena nigra</i> Bloch	Drumfish	501 ₁	present work
	<i>Scorpaenopsis polina</i> Ayres	Queenfish	504 ₁	Munz, 1958b
Sparidae	<i>Oblada</i> sp.		506 ₁	present work
	<i>Oblada melanura</i> (L.)	Saddle bream	501 ₁	present work
	<i>Pagellus erythrinus</i> (L.)	Pandora	500 ₁ , 494 ₁	present work
	<i>Sarpa salpa</i> (L.)		501 ₁	present work
	<i>Spondylionoma cantharus</i> (L.)	Black bream	519 ₁ , 499 ₁	present work
Centracanthidae	<i>Merolepis chryselis</i> (Cuvier and Valenciennes)	Blotched picarel	512 ₁ , 501 ₁	present work
	<i>Merolepis maena</i> (L.)		501 ₁	522 ₁ present work
Embiotocidae	<i>Embiotoca jacksoni</i> Agassiz	Bay black perch	506 ₁	Munz, 1958b
	<i>Hyperprosopon argenteum</i> Gibbons	Wall-eye surf perch	506 ₁	Munz, 1958b
Pomacentridae	<i>Chromis punctipinnis</i> (Cooper)	Blacksmith	495 ₁	Munz, 1957
Labridae	<i>Labrus merula</i>	Brown wrasse	492 ₁	510 ₁ Brown and Brown, ex Wald, 1960
	<i>Labrus mixtus</i> L.	Cuckoo wrasse	528 ₁ , 487 ₁	present work
	<i>Pomelometopon pulchrum</i> (Ayres)	Californian sheep head	520 ₁ , 497 ₁	Munz, 1958c

Scaridae	<i>Sparus cretense</i> (L.)	Parrot fish	486 ₁	520 ₂	present work
Tripterygiidae	<i>Axoclinus carminalis</i> (Jordan and Gilbert)		500 ₁		Munz, 1957
Scombridae	<i>Scomberomorus concolor</i> (Lockington)	Monterey Spanish mackerel	485 ₁		Munz, 1957
	<i>Scomber scombrus</i> L.	Mackerel	522 ₁ , 487 ₁		present work
	<i>Pseudotophorus japonicus diego</i> (Ayres)		491 ₁		Munz, 1957
Gobiidae	<i>Coryphopterus nicholai</i> (Bean)	Crested goby	500 ₁		Munz, 1958b
	<i>Clevelandia ios</i> (Jordan and Gilbert)	Arrow goby	512 ₁		Munz, 1958b
	<i>Eucyclogobius newberryi</i> (Girard)	Tidewater goby	512 ₁		Munz, 1958b
	<i>Gillichthys mirabilis</i> Cooper	Mudsucker	512 ₁		Munz, 1956
	<i>Callionymus lyra</i> L.	Dragonet	512 ₁ , 486 ₁		present work
Mugilidae	<i>Mugil cephalus</i> L.	Striped mullet	499 ₁	522 ₂	Bridges, 1964c
	<i>Mugil curema</i> Cuvier and Valenciennes	White mullet	499 ₁	522 ₂	Bridges, 1964c
Atherinidae	<i>Atherinops affinis</i> (Ayres)	Topsmelt	508 ₁		Munz, 1958b
Scorpaenidae	<i>Scorpaena guttata</i> Girard		500 ₁		Munz, 1957
Triglidae	<i>Trigla ocularis</i> L.	Red gurnard	493 ₁ †		present work
	<i>Trigla gurnardus</i> L.	Grey gurnard	512 ₁ , 492 ₁		present work
Cottidae	<i>Cottus bubalis</i> Euphrasen	Sea scorpion	504 ₁		present work
	<i>Cottus</i> sp.	Sculpin	511 ₁		Munz, 1958b
	<i>Leptocottus armatus</i> Girard	Staghorn sculpin	511 ₁		Munz, 1958b
Gasterosteidae	<i>Gasterosteus aculeatus</i> L.	Three-spined stickleback	501 ₁	522 ₂	Munz, 1957
Bothidae	<i>Amoglossus megastoma</i> Day	Megrin	494 ₁ , 487 ₁		present work
	<i>Rhombus laevis</i> Day	Brill	498 ₁		present work
	<i>Rhombus maximus</i> Day	Turbot	518 ₁ , 500 ₁		present work
Pleuronectidae	<i>Pleuronectes flexus</i> Day	Flounder	511 ₁ , 508 ₁		present work
	<i>Pleuronectes limanda</i> Day	Dab	507 ₁ , 500 ₁ ‡		present work
	<i>Pleuronectes microcephalus</i> Day	Lemon sole	502 ₁ , 493 ₁		present work
	<i>Solea vulgaris</i> Day	Dover sole	502 ₁		present work
Tetraodontidae	<i>Sphaeroides annulatus</i> (Jenyns)	Network puffer	500 ₁	522 ₁	Munz, 1958b

¶ The extract also contained a minor amount of a pigment of undetermined category and of λ_{max} in the region of 499 nm.

|| λ_{max} (with 13 nm. correction) reported as 496 nm. Mean λ_{max} of difference spectrum reported as 497 nm.

† Walker (1957) gives λ_{max} = 497 nm.

‡ Location of λ_{max} at 507 nm. only approximate.

Table II

VISUAL PIGMENTS OF FISHES POSSESSING ONLY RETINENE₂ PIGMENTS

Species	Pigment	Reference
<i>Crenilabrus festinus</i>	513 ₂	Brown and Brown, ex Wald, 1960
<i>Crenilabrus massa</i>	513 ₂	
<i>Carassius carassius</i> (L.)	523 ₂	Dartnall, 1962c
<i>Cyprinus carpio</i> L.	523 ₂	Crescitelli and Dartnall, 1954
<i>Lepisosteus platyrhincus</i> De Kay	523 ₂	
<i>Catostomus commersonnii</i> (Lacépède)	524 ₂	Bridges, 1964c
<i>Chaenobryttus coronarius</i> (Bartram)	524 ₂	
<i>Amia calva</i> L.	525 ₂	
<i>Lepomis macrochirus purpureus</i> Cope	525 ₂	
<i>Lepomis microlophus</i> (Günther)	525 ₂	
<i>Micropterus salmoides floridanus</i> (Le Sueur)	525 ₂	
<i>Umbra limi</i> (Kirtland)	526 ₂	Dartnall, 1952
<i>Esox lucius</i> L.	533 ₂	
<i>Tinca tinca</i> (L.) (var. <i>auratus</i>)	533 ₂	Dartnall, 1962c
<i>Ameiurus nebulosus marmoratus</i> (Holbrook)	534 ₂	Bridges, 1964c
<i>Gobio gobio</i> (L.)	535 ₂	Dartnall, 1962c
<i>Rutilus rutilus</i> (L.)	535 ₂	

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DISCUSSION

Rushton: Dr. Dartnall, when you put forward your nomogram about ten years ago, the important thing was to plot upon a basis not of wavelength but of frequency, and the curves you gave us so plotted were all the same shape. They were, in fact, constructed from the nomogram. Now, all the new work you have described here, the linear relation, is on a basis of wavelength and not frequency. The five peaks which are equidistant seem to me to be equally spaced on frequency also. I had an idea that frequency was the proper thing to plot.

Dartnall: There are really two points here. First, there are the five spectral positions about which the λ_{max} of retinene₂ pigments are clustered, namely 486.5, 494, 500.5, 506 and 511.5 nm. I spoke of these as separated by approximately equal wavelength intervals, but the range

of values is so small (486.5 to 511.5) that, as you suggest, one could just as well say that they were separated by equal frequency intervals. My reason for choosing the former of these equal alternatives is that in certain homologous series, for example, the diphenylpolyenes, of formula $C_6H_5-(CH=CH)_n-C_6H_5$, the absorption band moves from the ultraviolet for $n=1$ and 2 through the violet, blue and green to the red as n is increased to 15 and, I believe, the displacement for each increment of n is approximately the same on a *wavelength* basis.

Rushon: But the linear result that you gave at the beginning of your paper would be a hyperbolic result if it was a frequency and not a wavelength.

Dartnall: This is the other point. In Fig. 3 I have plotted on a wavelength basis the λ_{max} of retinene₁ and retinene₂ pigments in identical-opsin pairs, and you ask whether this should not be presented on a frequency basis. One might expect that this would make a great deal of difference, for the results extend over a wide spectral range. In this instance, however, it doesn't make much difference, for this is not a case of altering the plot of some *independent* function such as sensitivity from a wavelength to a frequency scale, but of altering a wavelength *versus* wavelength plot to a frequency *versus* frequency plot. The wavelength basis, in fact, gives a slightly more convincing linear plot than does a frequency basis.

Cresitelli: Dr. Dartnall, I would like to discuss one point that you brought up. I suspect nothing has been done here, but perhaps someone will be stimulated to do something about it. This is the change from a retinene₂ to a retinene₁ pigment in nature, as occurs in the bullfrog and in certain other amphibia during metamorphosis. This is a definite, measurable change and at certain stages during metamorphosis both pigments exist. You mentioned dehydrogenation, and there are two questions here. Is there any evidence that such a dehydrogenase or hydrogenase exists, and can you make a test-tube system in which this transformation can be achieved? Secondly, what is the rôle of the thyroid hormone here, because it seems to be implicated in metamorphosis?

Dartnall: We observed with the rudd that when fishes that have been kept in darkness are put into light, the retina goes over from a predominantly retinene₂ system to a predominantly retinene₁ system.

Now, of course, the effect of light on a visual pigment is to bleach it, whether it is a retinene₂ or a retinene₁ pigment. Hence we envisage that the mechanism was that the retinene₂ pigment was bleached by light, and that since retinene₁ was, perhaps, more plentiful in the bloodstream, a retinene₁ pigment was reconstituted. In the dark the opposite happens; a predominantly retinene₁ retina goes over to retinene₂. But there can be no turnover of visual pigment in the dark by photochemical bleaching, so how are we going to get it over there? This is why we have postulated an *in situ* dehydrogenation. No test-tube experiments have been done so far.

Crescitelli: There is also the morphological question of whether the outer segment of a visual cell has to break down and be reconstituted in the conversion of a retinene₁ system from a retinene₂ system or whether the visual cell is replaced by another visual cell containing the new visual pigment. In J. E. Dowling's work, when retinene was removed from the opsin, the opsin apparently became quite unstable and the outer segments broke down anatomically ([1960], *Proc. nat. Acad. Sci. [Wash.]*, **46**, 587). What is the critical point here? Does one retinene slip in as the other slips out, to prevent the opsin becoming unstable, although there seems to be a safety factor in the sense that opsin does not break down immediately after losing its chromophore? Someone should also attack the question of whether these two pigments are present in the same cell or in different cells. Modern spectrophotometric methods ought to be capable of showing this.

Wolbarsht: There is another point here. All the precursors of vitamin A₂ are rare in nature and you almost always find the A₁ forms in carotenoids and other substances found in plants and animal tissues. So if an animal uses a retinene₂ pigment it has almost to manufacture the vitamin A₂ itself.

Dartnall: I don't know the answers to the morphological questions. We believe that the same opsin is involved in the two pigments and, because of certain quantitative relations between the two pigments, that they occur mixed in the same photoreceptors. We were much impressed by the rapidity of this changeover to the retinene₂ system in the dark. It is noticeable within a few days. How does a retinene₁ system change over to a retinene₂ system when there is no turnover of pigment by bleaching? This is the problem.

Marks: Perhaps it is by the dark light of Stiles, H. B. Barlow and Rushton; the pigment is actually breaking down in the dark.

Dartnall: But in circumstances where there certainly is breakdown (that is, in the light) the change is in the *other* direction.

Rushton: Dr. Pirenne is the authority on dark light in this connexion, that is to say, the dark light that follows no bleaching. And if I remember rightly, in the paper of E. J. Denton and M. H. Pirenne ([1954]. *J. Physiol.* [Lond.], **123**, 417) each pigment molecule in the cell has enormous stability—a stability equal to that of a university lecturer; it only breaks down once in seven years!

Sjöstrand: Is it really known that there is no breakdown in the dark? Have any isotope labelling experiments been made?

Dartnall: Visual pigments in solution are very stable; I have kept a digitonin extract of frog rhodopsin at 0° c for fourteen months and have been unable to find any loss at all in that period. I cannot say that it would be the same *in vivo*, of course.

Pirenne: The argument for the greater stability *in vivo* was that the rate of breakdown observed *in vitro* some years ago for rhodopsin would, if it occurred in the living eye, result in our being night blind (assuming, of course, that thermal breakdown of the pigment produces visual excitation). On this assumption the breakdown of pigment by thermal action in the dark must be exceedingly small in the visual cells in order not to interfere with the extremely small light signals which the dark-adapted eye can detect. The molecules in the cells must therefore be under constraint so that they decompose thermally much more slowly than in solution.

This brings us to another problem; what happens when light acts on these pigments and produces indicator yellow and so on? What does cause the nervous excitation? Is it the splitting of the molecule, that is to say, the bleaching of the molecule, which causes excitation with regard to vision, or not? I feel it is not proved that it is. Nervous excitation of the cell might be caused by an excitation of the molecule by light, without requiring the breaking down of the molecule.

Dartnall: I agree with you that bleaching is not, philosophically speaking, necessary for the visual act. I envisage that the only thing necessary is that a quantum of light should be absorbed in the chromophore that is situated in the prosthetic group. This would raise the energy

level of the molecule, following which there might be electrical changes due to the "pulling away" of the prosthetic group from the protein. These could initiate the visual process. But, such changes having occurred, there might be a rapid reinstatement of the original condition.

Pedler: Dr. Dartnall, do you think it useful any longer to speak of a rod or a cone pigment?

Dartnall: No. The distinction frequently made between rod and cone pigments seems to me to be quite baseless. There is not a single known chemical or physical property by which they can be distinguished in solution. The cone pigments are sometimes stated to regenerate *in vivo* faster than do the rod pigments, but this may be a difference not so much between pigments as between their environments. Even this distinction is not always valid, for the 500 nm. "rod" pigment of the Mississippi alligator has a regeneration rate *in vivo* quite equal to that normally assigned to "cone" pigments.

Stiles: Dr. Dartnall, would you enlarge further on your model, and say what kind of theoretical basis there is for this concept? On old ideas one would expect to have the wavelength of maximum absorption dependent in a general way on the length of the conjugated chain. It is, of course, the case that the visual pigments have a much longer λ_{\max} than the isolated carotenoids (such as carotene) which have a conjugated chain double the length of that in the prosthetic group of the visual pigment. This problem apart, the new scheme seems to correspond to cutting the prosthetic conjugated chain into shorter lengths, and this would seem to favour still shorter λ_{\max} values.

Dartnall: The surprisingly long λ_{\max} of the visual pigments in comparison with those of isolated carotenoids must be due, one supposes, to participation of the opsin moiety. It would be tempting to suggest that in the visual pigment molecule there is extension of the prosthetic group conjugation into the protein part *via* the nitrogen-carbon bond linking the two: it would be tempting to suggest this, were it not for the fact that the visual pigment photoproducts, in which the prosthetic group and opsin are still linked, show a common absorption pattern; that is, they do not vary like the parent pigments.

I take your point about the cutting up of conjugation in the prosthetic group. It is difficult to meet it except, perhaps, by developing the ideas unjustifiably further. Thus the dipoles postulated to exist in prosthetic

group and protein when these two are in intimate contact might lead to an *effective* extension of conjugation into the latter, an extension that would have to disappear—together with the dipoles—once the close association of the two were lost.

Land: Are the longest wavelengths associated with the greatest uninterrupted chain of conjugation? They are in dyes, but are they here?

Dartnall: No. This is the difficulty we are labouring to solve. The visual pigments in the retinene₁ series, for example, have λ_{\max} ranging from 430 to 562 nm. and yet they are all supposed to have the same prosthetic group.

Stiles: Length of conjugation is related to λ_{\max} in the carotenoids.

Pirene: R. Kuhn in about 1930 described the synthesis of compounds with longer and longer conjugated chains, the compounds becoming first yellow and then red as the chain became longer.

Dartnall: Another example is the series of diphenylpolyenes which I mentioned earlier in discussion with Dr. Rushton.

Land: Dr. Stiles, is not your question here whether the structure proposed does indeed constitute an interruption and not a conjugation?

Stiles: That is my point, yes.

ADAPTATION OF VISUAL PIGMENTS TO THE PHOTIC ENVIRONMENT

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THERE is an approximate correspondence between the absorption bands of visual pigments and the spectral region in which the most quanta of sunlight are available. This is hardly surprising, but it carries an implication of some interest. Retinene, the prosthetic group of the visual pigment molecule, does not of itself absorb appreciably at these wavelengths (from 450 to 650 nm.), but in the ultraviolet. The shift in absorption to the spectral region that we know as "visible" is brought about by combination with the proteins called opsins. This is desirable, because there are fewer quanta of ultraviolet light in sunlight, because of the poor transmission of ultraviolet light by water and because these quanta can damage living tissue. Surely the processes of natural selection have operated in the evolution of visual pigments effective in the only spectral region well represented in sunlight, well transmitted by water and not physiologically deleterious.

Marine fishes

Within this general framework, however, the visual pigments of different species are not all identical. Wald (1960, p. 324) has stated that "the few aberrations that have appeared among them have taken on exaggerated importance as workers grow bored with the prevalent forms and concentrate their attention and energies on discovering exceptions." The investigations of Dartnall and Lythgoe (1965) must surely refute this view. Let us ask whether these "aberrations" have a recognizable biological significance.

For example, marine fishes are known to possess a series of "rhodopsins" ranging in λ_{\max} at least from 468 to 528 nm. By "rhodopsins," I mean any visual pigments based on retinene₁, whether contained within rod cells or not. "Porphyropsins" are the corresponding series of retinene₂ pigments. Thus, VP (visual pigment) 503₁ is a "rhodopsin" with $\lambda_{\max} = 503$ nm., and VP 527₂ is the corresponding "porphyropsin." Presumably the retinene₁ pigments of marine fishes come largely or entirely from retinal rods, but this has not been tested. Although the relative photosensitivities of these pigments have not been measured, it seems reasonable to assume that they are about equal in magnitude (see Dartnall, 1958) and are approximated by the density spectra.

Perhaps Clarke (1936) first suggested that visual sensitivity (which must depend partly upon the visual pigments) might conform to the spectral distribution of light available in a particular habitat. He was referring to the relatively blue photic environment of deep-sea fishes. This was later demonstrated by Denton and Warren (1956, 1957); Munz (1957, 1958a); and Wald, Brown and Brown (1957). These workers agree that the λ_{\max} of these visual pigments is in the range from 478 to 490 nm. and that they have probably become adapted to the light available in the deep-sea environment, possibly even to that emitted by these animals themselves, of which many are luminescent (Munz, 1958a).

Correlation between the spectral distribution of light and λ_{\max} of visual pigments has been extended to fishes inhabiting turbid marine environments (Munz, 1958b) and to rocky-shore and epipelagic fishes (Munz, 1964). Epipelagic animals swim or drift freely in the ocean, like bathypelagic animals, but live nearer the surface, at depths of 200 m. or less. Examples of these different visual pigments are shown in Fig. 1. The species in a particular habitat do not all have the same visual pigment; indeed, glaring exceptions to these trends may be revealed by future surveys. Two facts, however, make this correlation seem plausible. The occurrence of a particular visual pigment cuts across phylogenetic rela-

tionships, the "deep-sea rhodopsins" of bathypelagic animals occurring in unrelated groups of teleosts, and in deep-sea elasmobranchs and crustaceans as well (see below). Secondly, some epi-

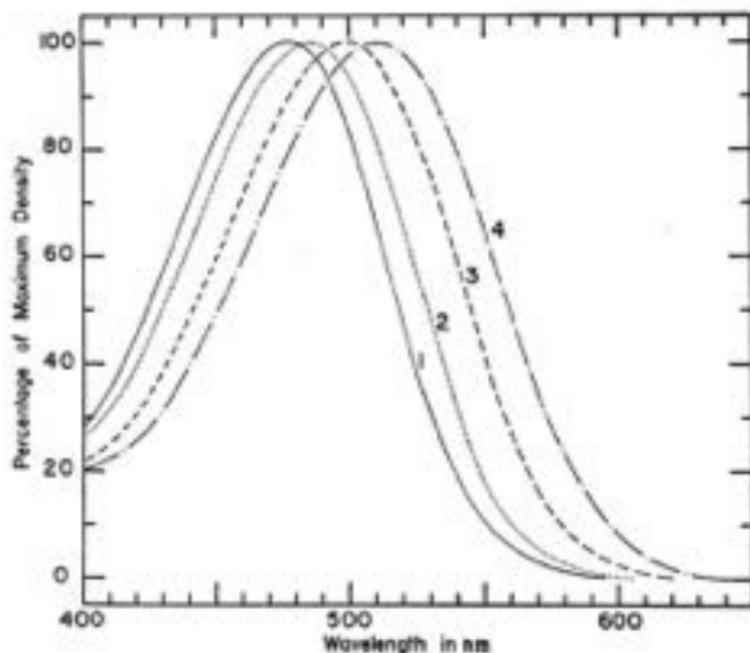


FIG. 1. Density spectra of representative "rhodopsins" of marine fishes, scaled to the same maximum. Curve 1, VP 478₂ of a bathypelagic hatchet-fish, *Axyropelecus affinis*; curve 2, VP 486₂ of the epipelagic Spanish mackerel, *Scorpaenopsis sierra*; curve 3, VP 500₂ of the rocky-shore kelp bass, *Paralabrax clathratus*; curve 4, VP 512₂ of the mudsucker, *Gillichthys mirabilis*, from turbid coastal habitats. Data for curve 1 taken from Munz (1958a), curves 2 and 3, from Munz (1964); and curve 4, from Munz (1956). All curves derived from density and difference spectra, except at shorter wavelengths, where Dartnall's (1953) nomogram was used.

pelagic fishes also have these so-called "deep-sea rhodopsins." The visual pigment of the epipelagic *Scorpaenomoropus* (Curve 2) is almost identical to that of the bathypelagic hatchet-fish, *Sternoptyx obscura* (not shown; $\lambda_{\max} = 485$ nm.; Munz, 1958a). The single connexion between these forms is that they live in a blue photic environment.

Other factors are all diverse: average light intensities, phylogenetic relationships, morphology (including that of the retinal receptors) and physiology. It would seem difficult to avoid concluding that the visual pigments of marine fishes have become adapted to the several photic environments that they inhabit. Nicol (1963) has reviewed this topic, together with several other aspects of photo-reception in fishes.

Denton and Shaw (1963) have recently shown that deep-sea sharks (family Squalidae) have blue-sensitive "rhodopsins" similar to those of bathypelagic teleosts. Denton and Nicol (1964) obtained evidence of a similar blue-sensitive visual pigment in *Hydrolagus affinis*, a deep-water holocephalan. Rather little information is available on the visual pigments of shallow-water elasmobranchs, but Wald (1939) reported a "rhodopsin" with $\lambda_{\max} = 500$ nm. in the spiny dogfish (Squalidae). This has been confirmed by Denton and Nicol (1964), who also obtained evidence of a similar visual pigment in *Scyliorhinus caniculus*, a cat shark. To these species may be added two shallow-water stingrays, *Myliobatis californicus* and *Rhinoptera steindachneri*, which have "rhodopsins" with $\lambda_{\max} = 500 \pm 2$ nm. (Munz, unpublished observations). Experiments with additional species are desirable, but it appears that elasmobranchs probably exhibit the same correlations between visual pigment and habitat that have been described for bony fishes.

Photic environments

It is apparent, particularly to anyone who has been a skin-diver, that aquatic habitats are vastly more variable than terrestrial habitats in their predominant colour. The diverse visual pigments of fishes reflect this fact, contrasting with the rather more "conservative" situation in terrestrial vertebrates. Geckos, however, will receive their undoubted due in another part of this symposium (Crescitelli, 1965).

The data of Jerlov (1951) have been used to calculate the relative numbers of quanta remaining at each wavelength after sunlight has

passed through 5 m. of several different seawater types (Fig. 2). All types transmit red light poorly, but they differ greatly in their transmission of shorter wavelengths. Clearest (Curve 1) and average (Curve 2) oceanic waters have maximum transmission

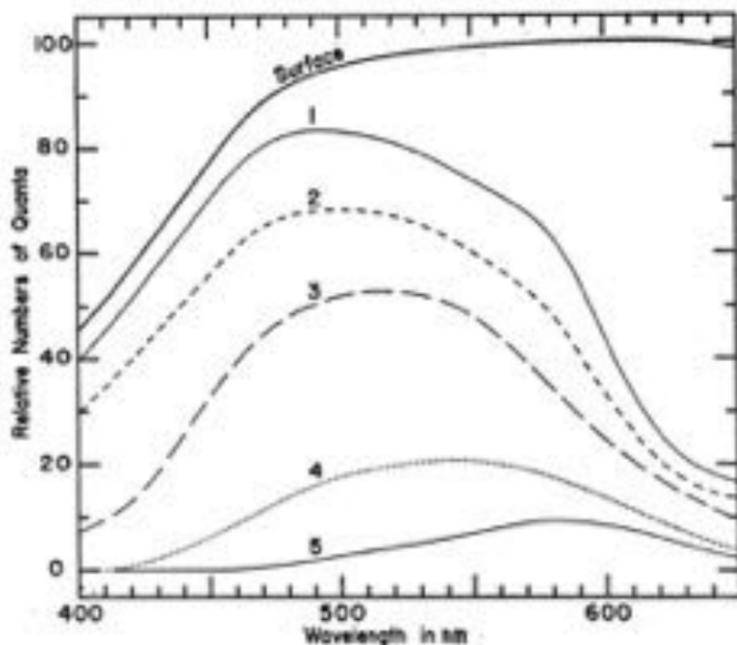


FIG. 2. Relative spectral distribution of sunlight at 5 m. depth, in five different seawater types. Data for solar radiation incident at surface are taken from Birge and James (*in Clarke, 1939*), data for water types from Jerlov (1951). Curve 1, clearest oceanic; curve 2, average oceanic; curve 3, clearest coastal; curve 4, average coastal; and curve 5, average inshore waters.

below 500 nm., clearest coastal (Curve 3) at about 500 nm., and average coastal (Curve 4) and average inshore (Curve 5) above 500 nm. Variable light absorption by chlorophyll and scattering by particulate matter are responsible for these differences (Yentsch, 1960). It must be admitted that these curves are only approximate, for several reasons. For instance, cloud cover and solar altitude will appreciably influence the spectral distribution of sunlight at the

earth's surface. But the curves presented should serve to represent the general range of variation among different water types.

This information is presented more dramatically as the spectral distribution of sunlight remaining after the number of quanta at the

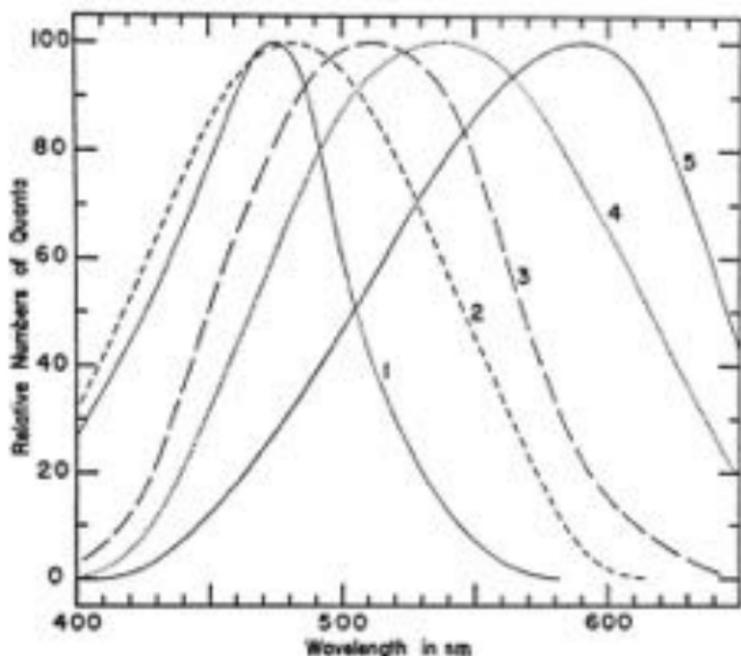


FIG. 3. Relative spectral distribution of sunlight remaining in different marine waters, after number of quanta at the respective maxima is reduced to 20-25 per cent of the maximum number incident at surface (λ 600 nm.). Curves re-scaled to 100 for comparison. Water types as in Fig. 2; curve 1, representing spectral distribution of quanta in clearest oceanic water at 70 m. depth; curve 2, average oceanic at 22 m.; curve 3, clearest coastal at 12 m.; curve 4, average coastal at 5 m.; and curve 5, average inshore at 3 m.

respective maxima has been reduced to about one-fourth of the maximum number incident at the surface (Fig. 3). The curve for each water type has been re-scaled to 100, to facilitate comparison. Differences in transmission make the depth necessary to reduce the intensity as described range from 70 m. (clearest oceanic, Curve 1) to 3 m. (average inshore, Curve 5). As the light intensity is reduced,

the transmission curves become progressively narrowed (accounting for most of the difference between Curves 1 and 2; see Tyler, 1959). There are significant differences between the spectral regions transmitted by the various water types, and these differences will be sharpened by any further increase in penetration and reduction in intensity. Thus, under water, at low light intensities where the spectral sensitivity of the visual pigments may begin to be important, there are great differences in light quality or colour. In general, it seems probable that the "rhodopsins" of marine fishes, including both teleosts and elasmobranchs, have been selected for maximum sensitivity to the light available. This has been accomplished by translation of the absorption band along the wavelength axis, rather than by an absolute increase in photosensitivity, which is already very great, even in frog "rhodopsin" (Dartnall, 1957, p. 77). The best example is afforded by the blue photic environment of the deep sea, in which the intensities are extremely low and the spectral range of illumination very narrow. These considerations apply not only to deep-sea fishes, however, but to the epipelagic species living in blue environments near the surface, and to coastal forms, for which the photic environment is predominantly greenish or yellowish, as well.

Invertebrates

The same trends might be expected in the visual pigments of those marine invertebrates which have well-developed eyes. This subject has been reviewed recently by Kennedy and Bruno (1961) and by Kampa, Abbott and Boden (1963). The visual pigments of too few cephalopods have been studied for any comparison with fishes, and a more extensive survey is desirable.

More information is available on the spectral sensitivity of representative crustaceans. Wald and Hubbard (1957) extracted the "rhodopsin" of the American lobster (*Homarus americanus*) and found that its $\lambda_{\text{max}} = 515$ nm. From electrical recordings, Kennedy and Bruno (1961) estimated that the maximum sensitivity of the

eye is at 525 nm. They attributed this shift to screening of the visual pigment by astaxanthin. Kampa, Abbott and Boden (1963) measured the spectral sensitivity of the European lobster (*H. vulgaris*) with electrical methods and also found a maximum at about 525 nm. They concluded that the lobster visual pigment is well adapted to the spectral distribution of light available in its environment. The eye of a hermit crab (*Eupagurus*) has maximum sensitivity near 500 nm. (Stieve, 1960), in keeping with its coastal habitat. The visual pigment and spectral sensitivity of several species of pelagic euphausiid crustaceans have been studied by Kampa (1955), Fisher and Goldie (1959), Dartnall (unpublished experiments, cited by Kampa, Abbott and Boden, 1963) and Boden, Kampa and Abbott (1961). A substantial shift toward shorter wavelengths (λ_{\max} of the euphausiid visual pigment = c. 460 nm.) parallels the trend in pelagic fishes; the visual pigment is based on retinene₁ (Fisher and Goldie, 1959). Further recent work by Kampa (cited by Kampa, Abbott and Boden, 1963) on a pelagic galatheid crab has yielded similar results. In freshwater crayfish, on the other hand, Kennedy and Bruno (1961) obtained evidence of a greater sensitivity at long wavelengths (λ_{\max} = 570 nm.). They related this to the predominance of longer wavelengths in the light available in freshwater habitats. Wald (1943) extracted vitamin A₁ and retinene₁ from the crayfish eye, which indicates that the red-sensitive visual pigment is probably based on retinene₁. In summary, crustaceans have retinene₁ pigments that cover a spectral range in λ_{\max} of at least 100 nm. and that are well suited for vision in the habitat occupied by each species.

Freshwater fishes

Let us then return to the fishes, to examine the situation in fresh water. The early work of Köttgen and Abelsdorff (1896) indicated that the visual pigments of freshwater fishes are more sensitive to red light than those of other vertebrates. Wald (1939) confirmed this and related it to the substitution of retinene₂ for retinene₁ as the

prosthetic group. He named the retinene₂ pigments "porphyropsins." Freshwater fishes, at least some species, do not have the retinene₂ visual system exclusively, however (summarized by Dartnall, 1962, pp. 529-530). Wald (1957) doubted that any visual significance could be attributed to the presence of "porphyropsin" instead of "rhodopsin" and thought that the distribution of the two visual pigments is secondary to some more fundamental process. Willmer (1956) had suggested earlier that the distribution of "rhodopsin" and "porphyropsin" may be determined by an unknown rôle of vitamins A₁ and A₂ in salt or water balance. In this connexion, however, it seems significant that the freshwater crayfish has a red-sensitive "rhodopsin", visually more or less equivalent to the "porphyropsins" of freshwater fishes. Dartnall (1962) has considered retinene₂ pigments to be adaptations for vision in environments of light of long wavelength.

As a photic environment, fresh water is extremely variable; but there is a general shift toward longer wavelengths. This was demonstrated by James and Birge (1938), who studied the spectral transmission of 50 different bodies of fresh water in Wisconsin. Their data for the relative distribution of sunlight in two of these lakes (taken from Clarke, 1939) are shown after reduction to about one-fourth of the maximum number of quanta incident at the surface (re-scaled; Fig. 4). Curve 1 represents the sunlight remaining in heavily stained Rudolph Lake at a depth of 1 m. and Curve 2 that in the clearer Crystal Lake at a depth of 5 m. Curve 3 is an average sea water type (Curve 3 of Fig. 3), for comparison. Although the shift to longer wavelengths is undoubted, the great variability among different lakes makes any general treatment difficult. Seasonal variations in plankton density or sediments must make these differences very great, even within the same lake or river.

The two outstanding features of the freshwater photic environment would seem to be paralleled in the visual pigments of freshwater fishes. The "porphyropsins" may be regarded as an adapta-

tion to the predominance of longer wavelengths in the transmitted sunlight. The presence in some freshwater species of both retinene₁ and retinene₂ visual pigments, in changeable proportions, may be advantageous in a variable photic environment. Thus, a seasonal change from a "porphyropsin" to the corresponding "rhodopsin"

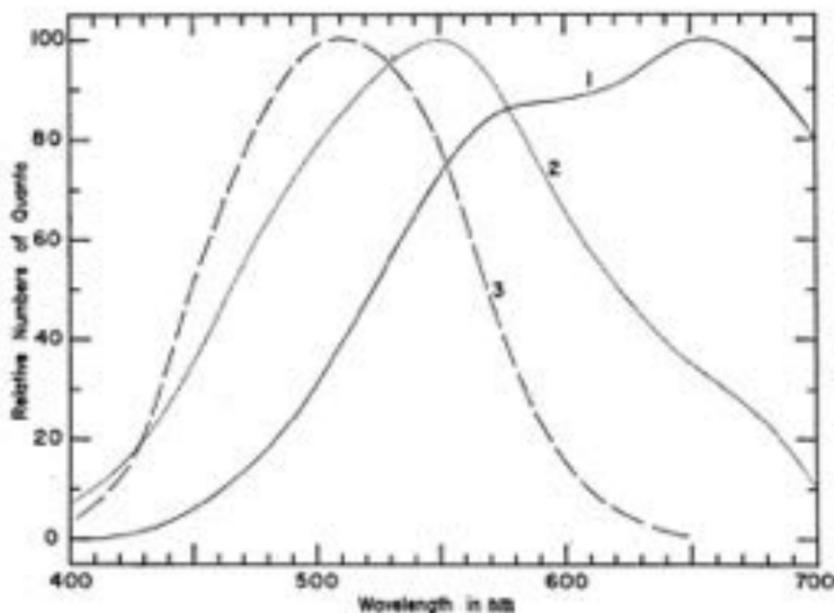


FIG. 4. Relative spectral distribution of sunlight remaining in representative freshwater types (calculated as in Fig. 3). Curve 1, a clear lake, Crystal Lake, Wisconsin, at 5 m. depth; curve 2, a heavily stained lake, Rudolph Lake, Wisconsin, at 1 m.; data taken from Birge and James (*in* Clarke, 1939). Curve 3, clearest coastal marine water (same as curve 3 of Fig. 3), for comparison.

would be visually comparable to undergoing a transition in opsins, which seems to be a rare occurrence. The migratory eel, *Anguilla*, is the only animal known whose opsin is converted from one type to another during the life cycle (Carlisle and Denton, 1959; Brown and Brown, *in* Wald, 1960). A succession of retinene₁ and retinene₂ visual pigments, on the other hand, has been studied in certain metamorphosing amphibians (Wald, 1947; Crescitelli, 1958; and

Wilt, 1959), in lampreys (Wald, 1957; see also Crescitelli, 1956), in *Anguilla* (Carlisle and Denton, 1959; Brown and Brown, in Wald, 1960), in the rudd (Dartnall, Lander and Munz, 1961) and suggested in certain other freshwater fishes (Dartnall, 1962, p. 411). Experiments conducted at the University of Oregon by Mr. D. D. Beatty (1964) and myself have established that a similar succession of visual pigments occurs in several species of salmonid fishes. This effect may be widespread, but we should remember that some freshwater fishes do have "porphyropsin" unmixed with retinene₁ pigment, throughout the seasons and the life cycle.

To assess the visual importance of a shift from retinene₁ to retinene₂ it is necessary to compare the photosensitivities rather than the density spectra of "rhodopsin" and "porphyropsin." Dartnall (1958) showed that the maximal photosensitivities of several visual pigments were not all identical, but ranged from 5 to 10×10^{-17} cm². Ideal for comparison of retinene₁ and retinene₂ pigments would be a pair based upon the same opsin. Trout and Pacific salmon have a "rhodopsin" with $\lambda_{\max} = 503$ nm. and a "porphyropsin" with $\lambda_{\max} = 527$ nm. (Munz and Beatty, 1965). A "rhodopsin"-rich extract of Pacific salmon was first exposed to deep red light (λ 660 nm.) for a period sufficient to bleach almost all of the VP 527₂ that it contained. Then its photosensitivity was measured at 500 and 540 nm., relative to that of frog "rhodopsin", by the method of photometric curves (Dartnall, 1958). Since the plot of photosensitivity against wavelength can be matched by the density spectrum of the visual pigment (Schneider, Goodeve and Lythgoe, 1939; Dartnall, 1958), the density spectrum of VP 503₁ was scaled to fit these experimental measurements (Fig. 5). Determination of the photosensitivity of the retinene₂ component is more complicated. The relative photosensitivity of a "porphyropsin"-rich salmon extract was measured at 540 nm., a wavelength at which its photosensitivity is about the same as that of VP 503₁. Under these conditions, Dartnall (1958) had shown that the method of photometric curves was applicable to a mixture of frog and carp

visual pigments. Establishment of the photosensitivity of VP 527₂ at 540 nm. permits the scaling of the entire density spectrum in photosensitivity units (Fig. 5). Also shown is the photosensitivity

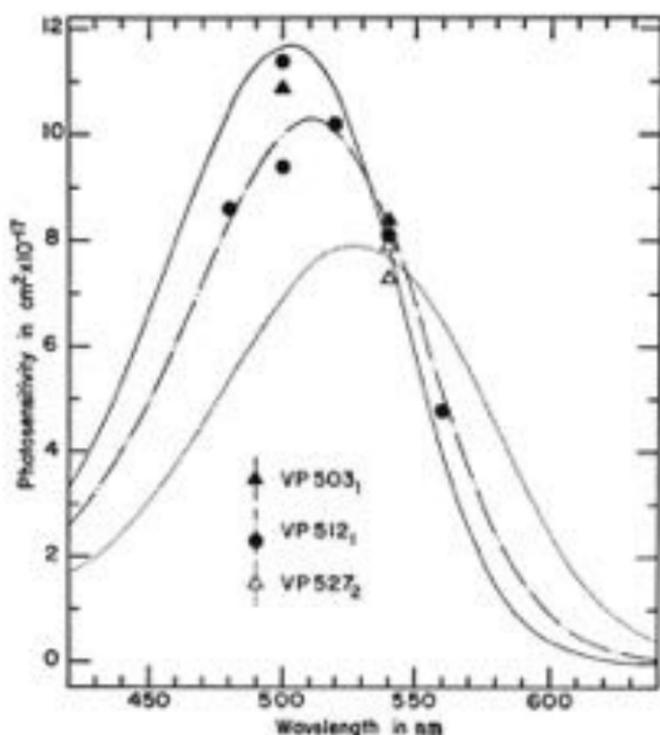


FIG. 5. The photosensitivities of three visual pigments of salmonid fishes. Symbols represent actual measurements of photosensitivity, relative to that of frog "rhodopsin", by the method of photometric curves. Curves are the density spectra, to fit the photosensitivity measurements. VP 503₁ and VP 527₂ are the "rhodopsin" and "porphyropsin" of Pacific salmon, and VP 512₁ is the "rhodopsin" of lake trout, *Salvelinus namaycush*.

of the "rhodopsin" of lake trout, *Salvelinus namaycush*. This species, which is related to Pacific salmon, often possesses the retinene₁ component without appreciable admixture of the corresponding "porphyropsin". The values for photosensitivity are all

approximately of the same order as those measured in other species by Dartnall (1958). They agree in another regard: the retinene₂ pigment has a lower photosensitivity than the retinene₁ pigments. In the present case this is particularly interesting, because the two salmon pigments are based upon the same opsin and differ only in the nature of their chromophore. Perhaps lower maximal photosensitivity is a general feature of retinene₂ pigments.

Table I

RATIO OF PHOTOSENSITIVITIES AT VARIOUS WAVELENGTHS

	Wavelength in nm.										
	420	440	460	480	500	520	540	560	580	600	620
VP 527 ₂ /VP 503 ₁	0.5	0.4	0.5	0.5	0.7	0.7	1.0	1.6	3.1	6.1	> 10
Mixture†/VP 503 ₁	0.8	0.7	0.7	0.8	0.8	0.9	1.0	1.3*	2.0	3.6	10
VP 512 ₁ /VP 503 ₁	0.8	0.7	0.8	0.8	0.9	0.9	1.1	1.3	1.7	2.3	> 5

† A 50:50 mixture of VP 527₂ and VP 503₁.

The visual consequences of a change from retinene₁ to retinene₂ or of the evolution of a new opsin may be estimated by comparison of the photosensitivities of the visual pigments of Pacific salmon and lake trout (Fig. 5, Table I). Complete conversion from "rhodopsin" (VP 503₁) to the corresponding "porphyropsin" (VP 527₂) results in an increased sensitivity to yellow light (λ 580 nm.) of threefold, to orange light (λ 600 nm.) of sixfold and to red light (λ 620 nm.) of more than tenfold. This is achieved at the expense of a halved sensitivity to blue light (λ 480 to 420 nm.). If the dominant spectral region in many freshwater photic environments is at wavelengths longer than 600 nm.—and measurements of light transmission indicate this—the retinene₂ pigment should offer a clear advantage in effective sensitivity. It is also interesting to compare the "rhodopsin" of lake trout with a mixture of the Pacific salmon visual pigments in equal proportions (these are presented in Table I, each in relation to pure VP 503₁). They are very similar, except in red light, to which the mixture of VP 527₂ and VP 503₁ is

more sensitive. The two processes, adaptation of the opsin or alteration of the prosthetic group, may lead to similar visual sensitivity, but only at one particular ratio of retinene₁ and retinene₂. Changing the ratio of retinenes should adapt the animal to the variable photic environments typical of streams, rivers and ponds, with a flexibility that is beyond most marine fishes, even those having "aberrant rhodopsins" (i.e. with λ_{\max} not 500 nm.).

If mixed retinene₁ and retinene₂ pigments are advantageous in variable freshwater photic environments, why have some freshwater fishes the "porphyropsin" system only? Normally vitamin A₂ must be synthesized from vitamin A₁ in an enzymically catalysed process (Naito and Wilt, 1962), although piscivorous species may obtain it in their diet. Carp (*Cyprinus carpio*), crucian carp (*Carassius carassius*), gudgeon (*Gobio gobio*), pike (*Esox lucius*) and roach (*Rutilus rutilus*) all have "porphyropsins" unmixed with the corresponding retinene₁ pigments (Crescitelli and Dartnall, 1954; Dartnall, 1962). To this list may be added the black and the white crappie (*Pomoxis nigromaculatus* and *P. annularis*), which have a retinene₂ pigment with $\lambda_{\max} = 525 \pm 2$ nm. (Munz, unpublished observations). One might speculate that these species so seldom inhabit a bluish photic environment that natural selection nearly always favours the red-sensitive retinene₂ pigment and that they have lost the capacity for conversion to the retinene₁ pigment.

Less easy to understand are the few known freshwater fishes that have little retinene₂ pigment. It has already been suggested that the lake trout, *Salvelinus namaycush*, often has little "porphyropsin". This lacustrine species frequently lives on the bottom at depths greater than 100 m.; it is rarely taken in the ocean. My samples came from the extraordinarily clear, blue Odell Lake, Oregon, and from Cayuga Lake, New York, which is clear also. Lake trout from Odell Lake had VP 512₁ alone, but some of the Cayuga Lake fish had a small proportion of the corresponding "porphyropsin", with λ_{\max} c. 545 nm. Tests of vitamin A in the liver with the Carr-Price reaction, however, showed a great preponderance of A₂, suggesting

that the eye tissues probably re-synthesize A_1 from A_2 . Thus, any special functions of vitamin A_2 that may be related to ionic regulation and water balance in fresh water would be served in the normal way. Only the eye of this fish is "unorthodox", indicating that the choice of retinenes is important in vision, whatever its possible relevance in other biochemical mechanisms. Now, VP 512₁ of the lake trout must have evolved from VP 503₁, the pigment commonly present in other species of *Salvelinus*, in trout (*Salmo*) and Pacific salmon (*Oncorhynchus*). Visually, it is roughly comparable to a 50:50 mixture of VP 503₁ and VP 527₂ (Table I). Even though this mixture of pigments is probably well suited to the relatively stable (both seasonally and over a long span of years) photic environments in which lake trout evolved (such as the Great Lakes of North America), natural selection would favour transition to a more red-sensitive "rhodopsin". When the retinene₁ pigment (VP 512₁) alone became well adapted to the photic environment, no longer would there be pressure to maintain the retinene₂ system. In the lake trout, the liver continues to have a great preponderance of vitamin A_2 , however; perhaps most of the dietary vitamin A of this fish-eating species is in the A_2 form. Kokanee, the landlocked sockeye salmon (*Oncorhynchus nerka*), also have relatively little "porphyropsin" at any time in their life cycle (Beatty, 1964). They have the same VP 503₁ as sea-going sockeye salmon, and in even higher proportion. In fresh water, both forms are pelagic in clear, glacial lakes. Investigations of the visual pigments of similar lacustrine species would be of great interest.

Let us consider the mechanisms that may control the conversion of retinene₁ and retinene₂. To save space, the biochemical and hormonal aspects of this problem are omitted (Wilt, 1959; Naito and Wilt, 1962). Changes in the photic environment stimulated a dramatic and relatively rapid conversion of retinene₁ and retinene₂ pigments in the rudd (Dartnall, Lander and Munz, 1961). Darkness favoured retinene₂ and light, retinene₁. It seemed possible that at least part of the rôle of light may result from the photoperiod, rather

than the amount of light; but this has not yet been fully tested. In Pacific salmon, Beatty (1964) has found that bright light also favours the retinene₁ pigment. It seems clear, however, that a long photoperiod will not, by itself, produce the same results (Beatty, experiments with coho salmon and rainbow trout). Nor does constant darkness favour the retinene₂ pigment. In rudd, juvenile salmon and trout, this response to the photic environment is expressed as a seasonal succession of the visual pigments. Why should they have more "porphyropsin" in winter than in summer? Measurements of possible seasonal changes in the spectral distribution of submarine illumination have not been made; but the lower the solar altitude, the greater is the predominance of red light at the earth's surface (Jerlov and Kullenberg, 1946). One need only look at the setting sun to verify this for oneself. Under water, where light intensities are quickly reduced to low values (to one per cent at 3 m. in heavily stained lakes; Clarke, 1939), this wintertime shift to more red-sensitive pigments may be of critical importance.

Salinity had no effect on the visual pigments of the salmonids tested (juvenile coho salmon: Beatty, 1964; juvenile steelhead, i.e., sea-going rainbow trout: Munz, unpublished experiments) at a time when bright light clearly favoured the retinene₁ pigment. It seems unlikely that salinity itself has any effect on the succession of visual pigments, at least in salmonid fishes. Experimental work with these factors is continuing.

SUMMARY

Several investigators have concluded that the visual pigments of deep-sea animals (teleosts, crustaceans and elasmobranchs) have become adapted to the predominantly blue light in that habitat by shifts in λ_{max} to wavelengths below 500 nm. This type of evolutionary response to the photic environment has occurred in other marine habitats. Some pelagic fishes living near the surface also have so-called "deep-sea rhodopsins", well suited to the pre-

dominantly blue light in this environment. In turbid, greenish or yellowish coastal waters, fishes have "rhodopsins" with λ_{\max} above 500 nm. and, in clearer coastal habitats, "rhodopsins" with λ_{\max} near 500 nm. These ecological groupings of fishes, with visual pigments correlated to the spectral distribution of sunlight in each habitat, also appear to be paralleled by the crustaceans.

In fresh water, both fishes and crustaceans have visual pigments adapted to the predominance of long wavelengths in the photic environment. In fishes, this is accomplished by the retinene₂ pigments, often mixed with the corresponding retinene₁ pigment. Mixtures of "rhodopsin" and "porphyropsin", in changeable proportions, may be adapted to the highly variable nature of the photic environment in many freshwater habitats. The rôle of light and environmental salinity in controlling the proportions of retinene₁ and retinene₂ is also discussed.

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DISCUSSION

Walbarsht: I wonder whether temperature has something to do with the ratio of retinene₁ to retinene₂? Many species of fish when moving from a warm to a cold environment tend to change their saturated storage fats to unsaturated fats, which are more fluid at lower temperatures. The only difference between retinene₁ and retinene₂ would be the addition or subtraction of the double bond, which would be the same thing as the unsaturation of fat.

Munz: This is a possibility, but our work with the rudd (Dartnall, H. J. A., Lander, M. R., and Munz, F. W. [1961]. In *Progress in Photobiology*, pp. 203-213, ed. Christensen, B. C., and Buchmann, B. Amsterdam: Elsevier), and also with the salmonids, both salmon and trout, shows that in these fishes, at least, this is not the case. We kept them at different temperatures and the ratio of the visual pigments did not change; then we kept them at the same temperature, and light changed the ratio.

Donner: Have you considered the change in ratio of the visual pigments from the point of view of cone vision? For instance, the shift from retinene₁ to retinene₂ pigments is likely to result in a change in the properties of the corresponding cone pigments, which determine the photopic sensitivity of the eye and also presumably subserve colour vision.

Munz: I have considered this, but I have not really been able to do anything about it. This may be a factor in many cases, but I am encouraged that the bathypelagic teleosts with pure rod eyes show this trend of a correlation of λ_{\max} with available light; that the crustaceans do, and they have no rods or cones at all; and that the elasmobranchs which are either rod rich or pure rod seem to show it also. But what you say may well be true.

Land: One should perhaps not project on to the fish the concept of adaptation in terms of maximum efficiency in using the wavelength that preponderates. The very "clever" fish might adapt itself to use the wavelength that has diminished in the number of quanta available. Thus any situation which cannot be explained by having the peak where you want it can be explained by saying that the fish has adapted to the shift in the peak by increasing its sensitivity to the different regions!

Stiles: To what extent does one know the photosensitivities, at peak, of the different rhodopsin types that one meets with in the different teleosts?

Muntz: Not many have been measured. Dr. Dartnall has measured several and they are roughly comparable, in the range $5-10 \times 10^{-17} \text{cm.}^2$

Stiles: When one is arguing about the advantage to the fish, working on the simple idea that the more light absorbed, the better, one integrates over the spectrum the product of spectral distribution of the light and spectral absorption of the visual pigment to obtain a single resultant quantity. The value of this quantity is used to gauge the adaptation of the visual pigment to the light environment. The value will depend critically on the position of λ_{max} for the pigment only if both the spectral distribution and the spectral absorption are narrow curves. But even if the spectral distribution is narrow (as at great depths in the sea), the rhodopsin type of absorption curve is an extended curve. Thus in imagining changes in the opsin that could produce a shift in λ_{max} of the pigment, possible concurrent changes in the absolute photosensitivity must be borne in mind. These might quite upset conclusions about the advantage of relatively small shifts in λ_{max} .

Muntz: There is only one bathypelagic fish pigment whose photosensitivity has been measured and it was a single measurement only. Its photosensitivity was slightly greater than that of frog rhodopsin, but there was little difference.

Stiles: I was wondering whether one has to think of a twenty per cent variation or a two to one variation in the absolute photosensitivity?

Dartnall: The range in photosensitivities at λ_{max} among the five visual pigments I have examined (Dartnall, H. J. A. [1958]. *In* Visual Problems of Colour, National Physical Laboratory Symposium, vol. 1, pp. 121-146. London: H.M.S.O.) was no more than two to one. Two of the pigments were based on retinene₂ and these had maximum photo-

sensitivities of about 6×10^{-17} cm.² compared with $7-9.5 \times 10^{-17}$ cm.² for the three retinene₁ pigments.

Stiles: This could be a factor, then, in the general argument as to the advantage of using retinene₂?

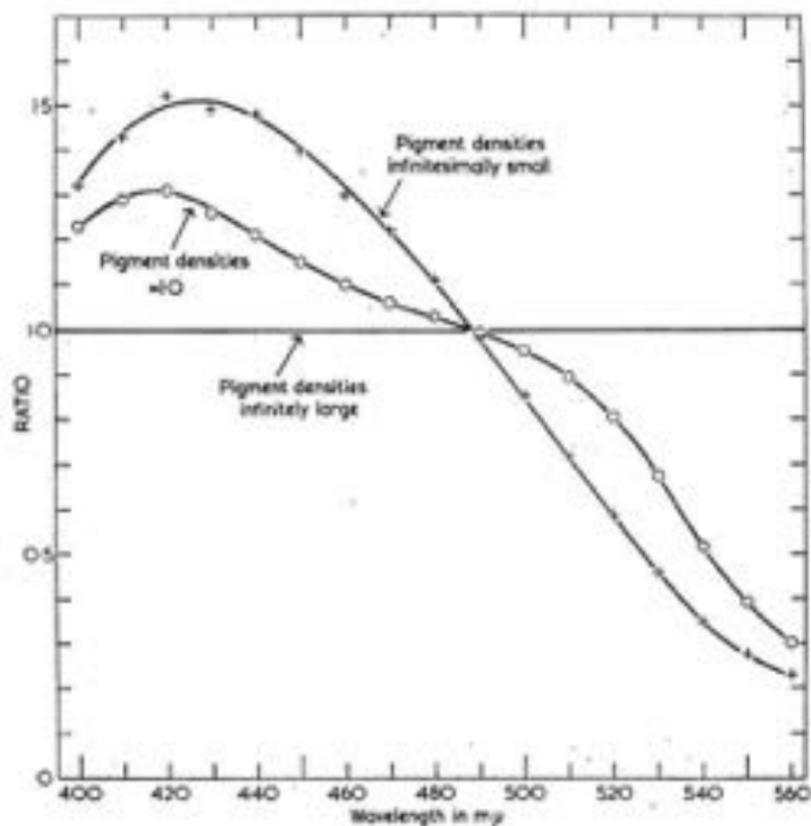


FIG. 1. (Dartnall). The ratio of light absorbed by pigment 480 to that absorbed by pigment 502 as a function of wavelength, for different pigment densities.

Dartnall: Yes. I would like to particularize and extend some of Dr. Stiles' remarks about the relationship between pigment λ_{\max} and environment. In the depths of the oceans the light is maximal at about 480 nm., and one does indeed find that some deep-sea fishes have pigments absorbing maximally in this region. One might say, what a

wonderful adaptation to environment! But how necessary is this adaptation? The point I want to make is illustrated in Fig. 1, which I first showed at the Symposium on Photoreception at New York in 1958. This figure compares the light-absorbing properties throughout the spectrum of two pigments; one a conventional "rhodopsin" with λ_{\max} at 502 nm., as is present in many surface marine fishes, and the other a "deep-sea rhodopsin" with λ_{\max} at 480 nm.

At depths in the oceans the light is not only maximal at about 480 nm.; it is approaching monochromaticity as well, a situation in which one might expect the λ_{\max} of the visual pigment to be more critical. This is not the case, however, because of the broadness of visual pigment absorption bands and because the short-wave arm of these bands, unlike the long-wave arm, does not descend to zero. As the figure shows, a 480 nm. pigment has only a 10 per cent greater extinction at 480 nm. than a 502 nm. pigment. Moreover it is not fair to compare extinctions; one should compare quantities of light absorbed, and this depends on the optical density of the pigments. At a retinal density of 1.0, which as Denton's work has shown, is not unusual in deep-sea fishes, the advantage of a 480 nm. over a 502 nm. pigment is seen in Fig. 1 to be less than 3 per cent.

If such a small advantage as 3 per cent is important it is a matter for some surprise that this is not achieved more easily in other ways, for example, by increasing the photosensitivity or the retinal density of the pigment.

From considerations such as this I sometimes wonder whether we have the boot on the wrong foot. Is it, perhaps, not so much that a 480 nm. pigment is a good adaptation to a near-monochromatic 480 nm. environment as that a 502 nm. pigment is better—some three to four times better—at absorbing the longer wavelengths present in the environment of a surface fish?

Menz: This is a valid point, and it means that if there is any selective advantage in having a "deep-sea" rhodopsin in the deep sea, then the forces of natural selection can operate over a long time-period with a small advantage. This is a concept that is accepted by most systematists, but it is rather hard for physiologists to allow. Another point is that in small or immature deep-sea fishes the densities of the pigments may well not be 1.0, but something less than that. Perhaps the critical time of life

is not the adult phase, when Denton measured the densities, but some earlier stage when the densities may be smaller. I do not know this, but I think it is a possibility.

Land: We have to be careful about the importance of the number of quanta compared to the importance of contrast. As far as the fish is concerned, *quantity* of light may not be important. What may count is the contrast between the object and the surroundings. The amount of scattering increases very rapidly with decreasing wavelength. You could argue that the real advantage would be to have a long-wave sensitivity and not a short-wave sensitivity.

Munz: Only if there is some long wavelength light present in the environment.

The importance of contrast in the deep sea is shown in another way, in the position of the photophores. Many of these fishes, of course, are bioluminescent, and their photophores typically point downwards. It is when a predator looks up against a lighted background that it can see a fish above it, unless confused by the downward-shining luminescence. This shows the importance of contrast as a selective factor in the evolution of these adaptations.

Ripps: Will you tell us again under what natural conditions the rudd shows this conversion from retinene₁ to retinene₂?

Munz: There is a conversion from retinene₁ to retinene₂ in darkness. In other words, in darkness sensitivity goes towards the longer wavelengths. But this is not true in salmonids.

Ripps: This seems to be a contradiction, a reversal of the usual trend of going to a longer wavelength sensitivity in a darker environment.

Dartnall: Why should that be a contradiction? Moonlight is "redder" than sunlight and consequently the Purkinje shift is in the wrong direction!

Ripps: A reversed Purkinje shift is what I was going to call it. But what are the natural conditions under which this change occurs?

Munz: During the winter there is more porphyropsin; in the summer there is more rhodopsin. The functional reason behind this is hard to see. It would be very valuable if there were seasonal measurements of the spectral distribution of submarine illumination in the fresh waters where these fishes live, but such measurements are not available. Just possibly the reduction in solar altitude in the winter may enter into this;

in northern latitudes, as the solar altitude is reduced, there is a very definite shift toward the red in the sunlight incident at the surface. But why should this not have an equal effect upon terrestrial animals? Perhaps because the light is reduced in intensity so rapidly under water, or for some other reason which I do not know.

Wolbarsht: The fish to look at is perhaps *Latimeria*, the Crossopterygian recently found as a living fossil, which was certainly evolved in shallow water and now lives in very deep water; will it have a porphyropsin or a long wavelength rhodopsin?

Pedler: The eye of *Latimeria* has been briefly described morphologically by Millot. It appears to be an all-rod retina (Millot, J., and Cavasso, N. [1955]. *C.R. Acad. Sci. [Paris]*, 241, 576).

Klüver: I am wondering about further adaptations to the photo-environment in terrestrial mammals and birds, such as changes in the transmission characteristics of the lens and cornea. Published studies as well as my own observations on mammals have shown that the lens generally does not transmit below 310 nm.; in the nocturnal owl, however, the cut-off in the near ultraviolet is at about 300 nm. Are there any data on the transmission characteristics of the lens in fishes living at great depths? Do you have, as it were, an owl among the fishes?

Dartnall: This is a very puzzling problem. The more one finds out about adaptation to environment, the less one seems to understand it. *Coris julis*, one of the fishes which we collected in our Mediterranean expedition, is common at 250 feet down. This fish has a longish λ_{max} pigment and a yellow cornea. What can one make of that!

Klüver: What about the ultraviolet absorption of the lens? Does it transmit below 310 nm.?

Dartnall: We did not measure the lens. The absorption of the yellow cornea was rather like that for β -carotene, although I am not suggesting that the pigment involved was β -carotene. In other words, its absorption was centred at about 460 nm., just about in the centre of the maximum transmission of the water.

Pirenne: We have been assuming implicitly that the more sensitive the fish's visual system is, the better. Obviously that is not necessarily so; it depends on the behaviour—many of us wear dark spectacles in sunshine. Fish that are near the surface may have too much light, and they may want to cut it off. They may want to cut off the blue to correct

chromatic aberration. It seems to me that the quantitative aspect of the sensitivity of the fish, in relation to the amount of light available, is very important as far as behaviour is concerned. The question is: what do these fishes do at night? Do they just go to sleep in a safe place, or are they hunting?

Munz: Some do and some do not. The measurements by Kennedy and Milkman of the spectral transmission of the cornea showed that in some shallow-water marine fishes the cornea is yellow and there is a definite absorption of the blue (Kennedy, D., and Milkman, R. D. [1956]. *Biol. Bull. mar. biol. Lab., Woods Hole*, III, 375). They did not work with any deep-sea fishes.

Dartnall: In support of what Dr. Pirenne said, we have one or two cases (*Coris julis* and *Phalossoma parva*) where the cornea is yellow just at the top.

Crescitelli: In connexion with Dr. Klüver's question, there are some findings that should be checked with modern methods (Walls, G. L. [1942]. *The Vertebrate Eye*. New York: Hafner). The purely diurnal ground squirrel has a very yellow lens, sometimes almost orange. The tree squirrels and shade-loving squirrels have a light yellow lens and the nocturnal flying squirrels have a colourless lens. One could regard these facts as examples of different solutions to the problem of reducing chromatic aberration in different environments.

Klüver: A study of the transmission characteristics of the lens in squirrels would probably also show that the absorption in the near ultraviolet increases with advancing age. If we turn to an animal as thoroughly nocturnal as, for instance, the night monkey (*Aotes*) we might perhaps expect that transmission measurements would yield values similar to those found in the owl. However, when I measured the transmission of ultraviolet through the lens of *Aotes trivirgatus* I found a sharp cut-off at 310 nm.; that is, I obtained measurements not essentially different from those obtained in my experiments with diurnal monkeys (Klüver, H. [1936]. *J. Psychol.*, 2, 49; [1942]. *Biol. Symp.*, 7, 253). It is now generally agreed, I believe, that night monkeys have become nocturnal secondarily.

DUPLICITY THEORY AND MICROSTRUCTURE OF THE RETINA

RODS AND CONES—A FRESH APPROACH

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THE purpose of this paper is to examine the internal components of some vertebrate photoreceptors using electron microscopy, to relate these to the concept of a "rod" and a "cone" and to consider some of the connexions made by them in the outer plexiform layer.

Twenty-four species including man have been studied (see Table I), and nine major receptor components are considered.

Preliminary results from serial reconstruction of parts of isolated pigeon and monkey foveae are also reported, and the "one-to-one" relationship of foveal receptors and bipolar processes is questioned on a cell-to-cell basis in these species.

From a comparison of this electron microscopic data with the anatomical relationships claimed by the light microscopist it is suggested that the concept of the "rod" and "cone" no longer fits the morphological facts well enough, and is due for replacement. It is also pointed out that some aspects of retinal cytology shown by the Golgi technique are in the light of electron microscopic evidence actively misleading and must be modified as a result of this evidence.

In conclusion, a tentative description of receptors which relates the function of the receptor to the electron microscopic evidence is put forward.

Table I

SPECIES STUDIED BY ELECTRON MICROSCOPY

Two squirrels (*Sciurus carolinensis*, *Citellus lateralis*)
 Six geckos (*Gekko gekko*, *Phelsuma inornata*, *Tarentola mauritanica*, *Aristelliger* sp.,
Hemidactylus naricinus, *Phelsuma madagascariensis longipollicis*)
 Two lizards (*Laerta muralis*, *Laerta viridis*)
 Monkey (*Macaca mulatta*)
 Rabbit (*Lepus cuniculus*)
 Rat (*Rattus rattus*)
 Cat (*Felis felis*)
 Bush baby (*Galago*, unclassified)
 Chicken (*Gallus domesticus*)
 Pigeon (unclassified)
 Bat (*Pipistrellus*, unclassified)
 Owl (*Strix aluco*)
 Mole (*Talpa europae*)
 Mouse (*Mus mus*)
 Dragonet (*Callionymus lyra*)
 Cod (*Gadus morhua*)
 Sailbe (*Gadus virens*)
 Alligator (*Alligator mississippiensis*)
 Grass snake (*Natrix natrix*)
 Spanish water snake (*Natrix maura*)
 Snapping turtle (*Chelydra serpentina*)
 Man

MATERIALS AND METHODS

Retinae for electron microscopy were removed from decapitated and pithed animals and immersed within one minute in either veronal-buffered 1 per cent osmic acid solution or phosphate-buffered 3 per cent glutaraldehyde solution. Contrast was enhanced in the osmic acid fixed material either by block impregnation with alcoholic phosphotungstic acid or by section staining with lead citrate (Reynolds, 1963). In the case of the glutaraldehyde-fixed specimens, staining was by 1.2 per cent aqueous potassium permanganate. Sections were cut in the gold-silver range on a Huxley ultra-microtome and examined in an E.M.6 microscope at accelerating voltages of either 50 or 75 kv. It was found to be important to transfer excised retinae to fixative within the time specified, to avoid tissue damage.

To fix some of the material used for serial reconstruction, phosphate-buffered glutaraldehyde was perfused through the left ventricle for 30 minutes followed by sucrose buffer for approximately 20 minutes. The eyes were then removed and the foveae separated by punching them out with a 2 mm. corneal trephine. The rim of the disk of retina thus obtained was removed with a 1.5 mm. trephine and the ring and the disk were then diced separately in buffered osmium tetroxide. Up to 70 serial sections of one block were studied as an intact sequence.

Separation by trephining makes it possible to assume that all the synaptic pedicles in a section come from a region immediately surrounding the foveal pit and therefore belong to the receptors next to the foveal centre. The annulus of retina obtained by trimming the first disk was processed separately and is referred to as parafoveal.

Other trephined foveae were fixed in veronal-buffered osmic acid, dehydrated with graded alcohols, stained with lead citrate and embedded in araldite.

All serial sections were of gold or silver colour.

RECEPTORS AND DUPLICITY

To explain duplex retinal properties it is assumed that there are two morphological varieties of receptor intimately associated with the phenomena of photopic and scotopic vision. The words "rod" and "cone", however, were first used by the light microscopist to describe nothing more than two cell varieties which could be distinguished by certain staining characteristics and the overall shape of parts of the cell. Ever since, the terms and their implicit link with duplex retinal function and nocturnal and diurnal habit have been adopted by workers from almost every discipline. The photochemist refers to *rod* and *cone* pigments without having any precise method of referring such pigments to a *particular* cell, at least until the advent of single-cell densitometry. The neuro-

physiologist, studying light-evoked retinal potentials, assumes the type of cell in the retina from which he has recorded his potentials, again without direct correlation between his findings and the actual receptors initiating the potential. Similarly, the electron microscopist describes fine structural distinctions between receptors, again often dividing them into the two types if at all possible. Thus, the rod and the cone have acquired a number of separate identities: they are morphological entities in the light and electron microscopes, they have a photochemical set of references, they are defined electrophysiologically, their presence or absence is predicted by animal behaviour and habit and their nature is assumed from the type of retina in which they are found.

Thus, there is categorization without cross-reference showing a bias towards the division of all phenomena into two classes to fit a concept of two receptor varieties. That photoreceptors cannot be completely divided into two classes is clear from the number of anomalies which exist among the vertebrate family. It will only be possible to consider a few of these here.

Anomalous receptors

There are many photoreceptors where the fit of morphological duplicity is bad. For example, in *Sphenodon* the only surviving Rhynchocephalian, there is a shallow fovea. Moreover its retina (Walls, 1963) contains predominantly rods, although it has cones in addition, and is therefore duplex, although only 20 "cones" may be found in a single, sagittal 10 μ section. Furthermore, the cones do not contain oil droplets, whereas nearly all the rods do. Thus, using classical duplicity we have to account for a retina containing rods and cones, with a fovea constructed mainly from rods, the cones taking no special part in this structure whatever—a situation which is hard to reconcile with the view that cones are associated with acuity and rods with sensitivity. It is more probable that *whatever* cells form the fovea are associated with maximum acuity whether they are rod-like or cone-like to the light microscopist.

A similar situation occurs in the owl retina which contains both rods and cones and a fovea, the cones taking no particular part in the formation of the fovea.

Protopterus, a lung fish, contains apparent rods and cones and yet the "rods" are almost completely cone-like, containing an enor-

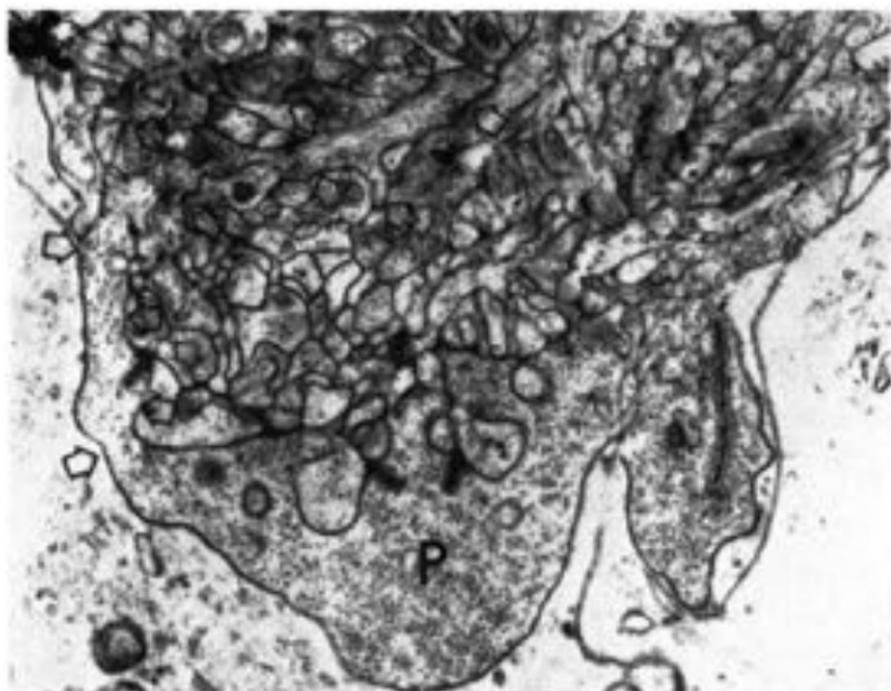


FIG. 1. Multi-channel pedicle (P) from *Alligator mississippiensis* showing a basal filament (arrows). These may have no other function than to increase the surface area of the pedicle. They do not commonly contact other pedicles. Phosphotungstic acid. $\times 14,000$.

mous oil droplet and a well marked paraboloid. The nuclear chromatin is also typically cone-like. The cones contain similar intracellular organelles except that some are rod-like in that they do not show a well demarcated paraboloid (Walls, 1963).

The photoreceptors of the leopard frog, *Rana pipiens*, are divided into rods and cones by the morphology of their inner and outer

segments, yet the nuclei of all varieties are in the "cone" position on the outer limiting membrane. It is reported by Walls that the synaptic pedicles of all varieties have basal filaments (Fig. 1) and although no electron microscopy has yet been carried out on this species, this strongly indicates that the pedicles are of the complex or multi-channel type. We have in fact never seen basal filaments

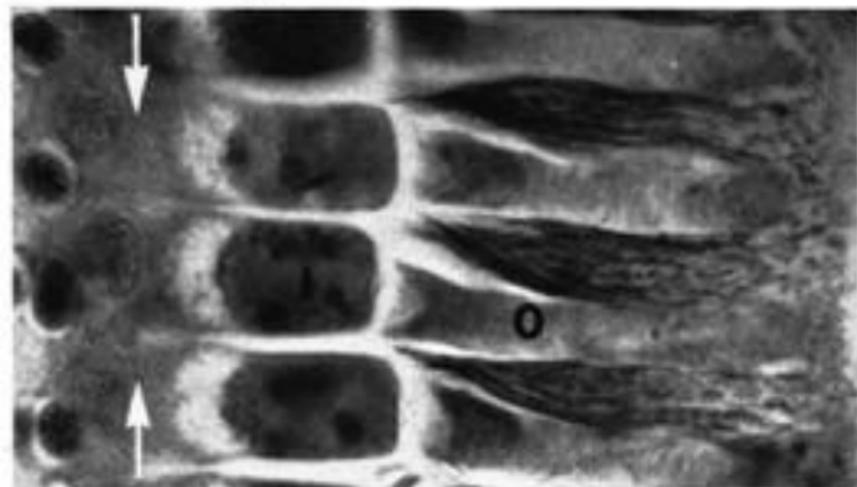


FIG. 2. The receptors of a nocturnal gecko (*Gekko gekko*). These are entirely rod-like to the light microscopist, yet they have a complex pedicle and a "cone-type" retina. These receptors lie within the type B category suggested in the text. Arrows—outer limiting membrane, I—inner segment, O—outer segment. Mallory's phosphotungstic acid haematoxylin. $\times 2,500$.

on a simple synapse in the electron microscope. Again, there is no difficulty in dividing these receptors into two varieties if the pedicles are ignored. But if they are included, dichotomy is impossible.

Another basic tenet of duplicity theory is that rods are connected in convergent array to the bipolars, whereas cones are connected in a "one-to-one" relationship to the bipolars in central areas. This situation is impossible to reconcile with the finding that the pure "rod" retina of the nocturnal gecko (Fig. 2) may have a bipolar surplus relative to the receptors of as much as 5:1 in the central

area, a figure not greatly different from a diurnal member of the same family (Pedler and Tilly, 1964).

At this point it is helpful to compare receptor type with retinal type to determine whether there is any fixed relationship or not. It is obvious that the geckos do not show this relationship for, as we have seen, "rods" are present in an otherwise cone-like retina. In fact, the geckos appear to have achieved a highly economical solution to the problem of evolving effective photopic and scotopic apparatus, for instead of developing two receptor varieties, changes in intracellular components have evolved so that the separate needs of sensitivity and acuity can be met by transmutation of the components in one basic cell variety.

Similarly, in *Alligator mississippiensis*, over 50 per cent of the synaptic pedicles observed in the electron microscope are of the complex or cone-like variety (Fig. 1), whereas cone-like inner and outer segments are present in a maximum proportion of one to every ten rod-like cells, implying that a proportion of the cells with rod-like outer and inner segments possess complex pedicles (Kalberer and Pedler, 1963).

Apart from these anomalies, however, the light microscopist can undoubtedly see two apparent receptor varieties and the "rod" is usually associated with the nocturnal habit and the "cone" with diurnality. But this assumption can now be examined more closely using the electron microscope. What it actually means is that when the light microscopist sees a "rod" he sees a relatively large, cylindrical outer segment attached to a narrow inner segment and when he sees a "cone" he is referring to a small, often conical outer segment attached to a broader barrel-shaped inner segment. In neither case does he commonly see the synaptic pedicle, which is difficult to stain, and if he does he is unable to connect it with a particular receptor body in a retina containing both "rods" and "cones". The reason for this is evident in Golgi preparations and in some electron micrographs where the thin conducting fibre can be seen to follow a tortuous path, so tortuous in fact that in

ordinary stained preparations with all conducting fibres coloured there is no possibility of connecting a given pedicle and receptor in the tangled mass of fibres and nuclei (Fig. 3). The irregular course of the conducting fibre is also a serious disadvantage to the electron microscopist, which is fortunately minimized because he can see all the pedicles and receptor bodies in a given section. An assessment can therefore be made of the type of pedicles most commonly associated with particular receptors even in the absence of complete conducting fibres connecting the two, provided that a large number of receptors in several specimens are examined to reduce errors of sampling. One apparent flaw in this reasoning is that a given pedicle might be connected to a very distant receptor body. This is certainly true in some foveae in the fibre layer of Henle (Fig. 3), but here the lateral displacement of pedicle and receptor body is in one plane only, and can be recognized, measured and allowed for. Golgi preparations show that, extra-foveally, although the conducting fibre is tortuous, the pedicle and receptor body which it connects are usually in line and not displaced laterally.

Thus, light microscopic methods show part of the cell only. Similarly, it is usual to find that workers who use the Golgi method draw the pedicle, conducting fibre and nuclear region only occasionally in conjunction with the inner segment and practically never with the outer segment. Again, only part of the cell is described. It is of no value to categorize a cell leaving out perhaps its most vital feature. Clearly, all parts must be included in any general description.

Furthermore, it is unnecessary to assume that when a receptor evolves in response to environmental need it does so either on a whole-cell basis or into one of two varieties. Now that more is known about the internal components of receptors, it appears that parts of the cell can evolve and transmute as need arises; that an outer segment can become larger and develop more lamellae if sensitivity is demanded; that the mitochondria of the inner segment can alter according to energy requirements and the adequacy of the

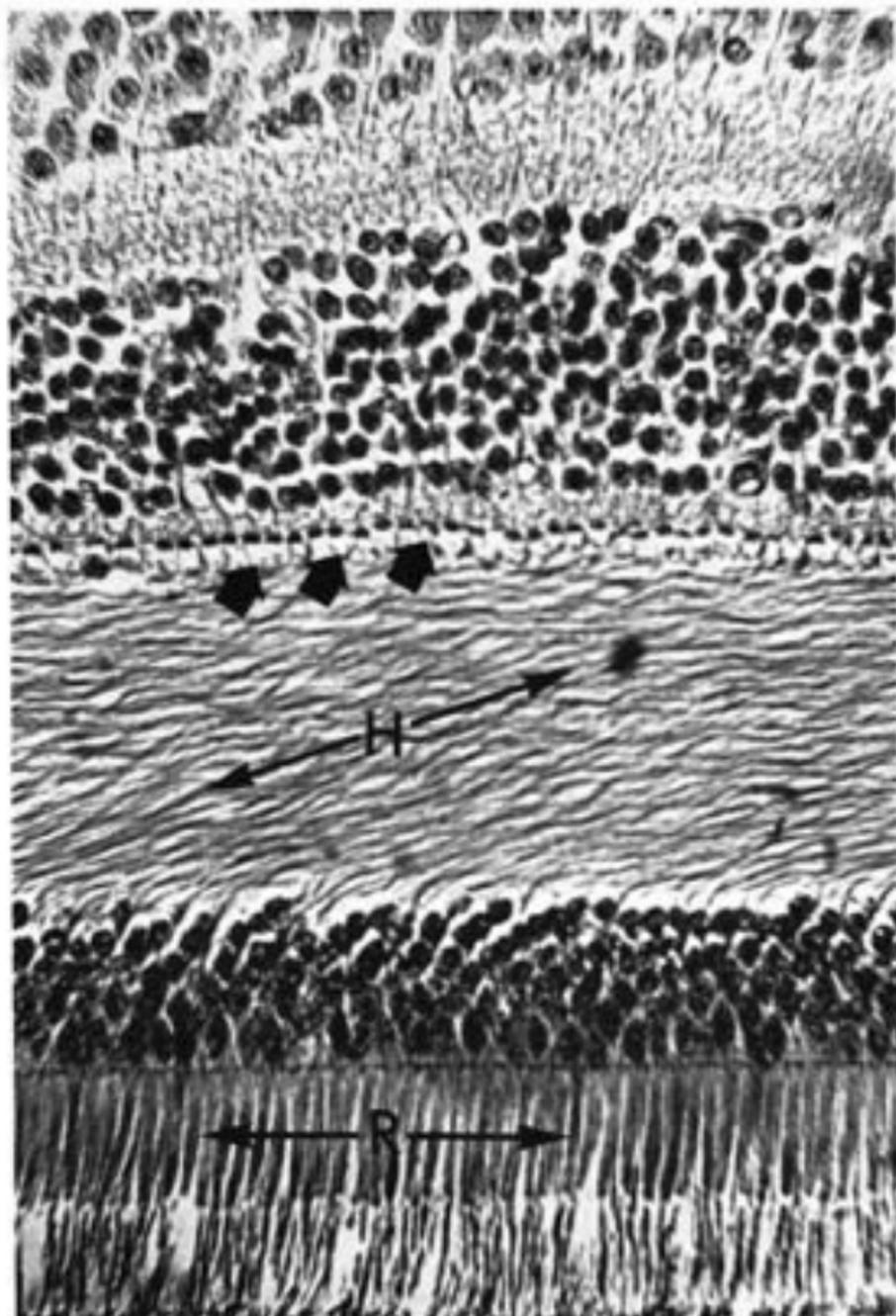


FIG. 3. Vertical section of the parafovea from a rhesus monkey. The synaptic pedicles (arrows) are in a regularly arranged row with little space between them. Compare with Fig. 3. H—Fibre layer of Henle, R—receptors.

retinal vasculature; and that synaptic matrices can change to deal with the quantity and type of data to be processed.

Any new classification must, therefore, account for all varieties of receptor however anomalous they appear from the standpoint of duplicity theory. To construct such a scheme, however incomplete it must remain at present, the results from the classical neuroanatomical methods must first be examined and modified in accordance with electron microscopic data and then the receptor must be broken down into the structural features which are most relevant to function.

THE GOLGI METHOD AND ELECTRON MICROSCOPY

Electron microscopy of the species so far studied shows that nearly all the neurites forming the outer plexiform layer lie in a horizontal plane (Fig. 4) and that the bipolar most closely related to a particular receptor even in the pigeon fovea does not contact the latter with more than an occasional dendrite.

This at once creates a serious discrepancy, since one of the most obvious features of a Golgi preparation in this region is the apparent apposition of receptor pedicles and all the dendrites of the adjacent midget bipolars in line with it. Indeed, this is one of the mainstays of the one-to-one theory. To resolve this difficulty we must first examine the nature of the Golgi technique and then the resolution of the optical microscope as applied to specimens stained by that method.

Under optimum conditions, viewing suspended particles, the best two-point resolution of the optical microscope, using blue light (405 nm.) and a 3 mm. apochromatic objective with numerical aperture of 1.40, approaches 0.15μ (Shillaber, 1947). This is sufficient to see many of the neurites in the outer plexiform layer. Four factors, however, militate against achieving optimum conditions. First, the Golgi technique fails to impregnate the smallest neurites we know to be present. Secondly, some processes are

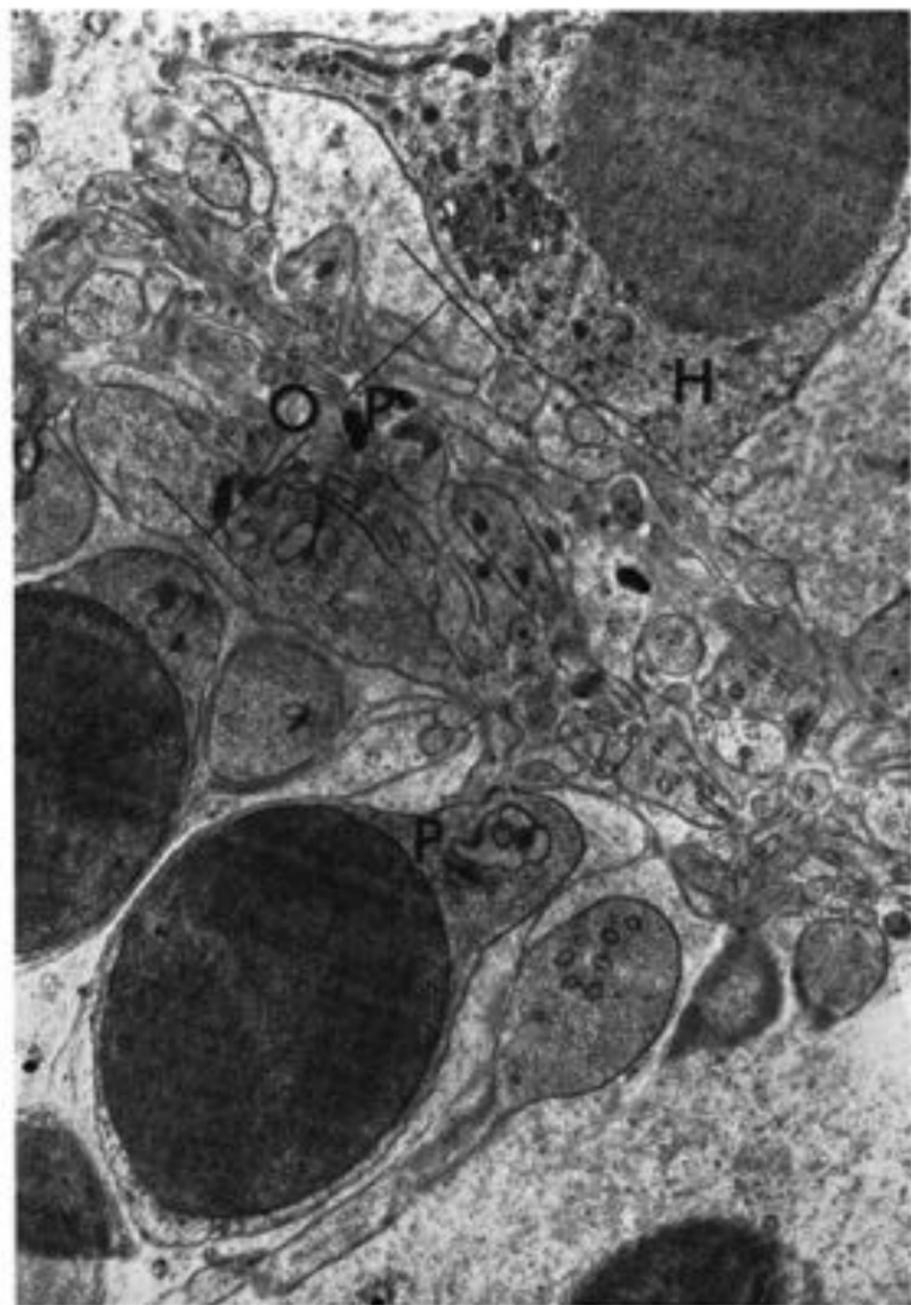


FIG. 4. Vertical section of some single-channel pedicles (P) in the outer plexiform layer of a rabbit (OP). A horizontal cell (H) is included. Phosphotungstic acid. $\times 9,000$.

below 0.1μ in diameter. Thirdly, it is necessary to use sections up to 60μ in thickness since thinner sections preclude the examination of cell lying oblique to the plane of section. Fourthly, the shape of the gap between a synaptic pedicle and an adjacent bipolar dendrite group is concave so that the resolution of an optical section will again suffer from diffraction by the edges of the concavity. All are factors which will seriously affect resolving power. Finally, the Golgi technique, quite apart from its unpredictability and the factors mentioned above, has yet another serious disadvantage: it can be shown to transgress the anatomical boundaries between two adjacent but dissimilar cells in the retina, thus giving a false impression of continuity (Pedler, 1962). It is doubly surprising therefore that Cajal described a gap between synapsing cells which he thought was filled by the "ciment unitif", a unifying material which stretched between all neurone junctions and, at least in his earlier writings, caused him to argue against the neurone theory (Cajal, 1933). Polyak saw a similar gap which he described as "a synapse of reduced contact" composed of spaces between neurones bridged by streams of ions (Polyak, 1941). Thus it seems that the one-to-one hypothesis arose partly because of the light microscopists' inability to see into this gap and observe the complex circuitry it contains. For these reasons the Golgi method alone is actively misleading since it will not impregnate all the processes of a cell and not all the cells of a histologically homogeneous population. This is a virtue, in that the morphology of a single cell can be recorded; but it clearly cannot give any meaningful indication of the actual circuitry in a given cell mass. In this respect the electron microscope is more truthful because, quite apart from its increased resolution, it shows *all* cell interconnexions in a given section. It can be argued successfully that according to the various methods of preparation for electron microscopy different *cytoplasmic* components are either obliterated or enhanced, but from the point of view of cell membranes and processes as a whole, this stricture is irrelevant, since all methods show these with similar clarity.

An example of how the combined approach of silver impregnation and electron microscopy can be of use is the case of the bipolar cells in the parafoveal region. Fig. 5 shows that at least one foveal bipolar cell has a dendritic domain diameter approximately equal to three receptors. Repeated study of many specimens shows this to be a consistent finding, but it could still be argued that it is only those foveal bipolars with the broad dendritic domains that are delineated by the silver and it could still be possible for cells with narrow dendrites in one-to-one relationship with the adjacent receptors to be present but unimpregnated by silver. An exponent of silver impregnation alone would be forced to agree with this objection unless he could state that electron microscopy of a similar region *never* shows all the dendrites from one bipolar and a receptor pedicle in one-to-one relationship and that nearly all the dendrites in the parafoveal outer plexiform layer travel in a horizontal direction. That this is in fact the case supports the conclusion that the gross morphological pattern suggested by the Golgi impregnation is of value only when considered in conjunction with electron microscopic data and can only be used to gain a rough idea of the approximate extent of dendritic domains. Where the actual contacts between cells are concerned, however, it is fundamentally misleading.

These shortcomings of some of the classical neuroanatomical techniques are in part responsible for the "one-to-one" or "private line" hypothesis, which states that each foveal or parafoveal cone is connected to all the dendrites of a single bipolar in direct line with its long axis. In the 24 species which we have so far examined, we have never found this to be the case and in the serial reconstructions of the pigeon fovea so far completed this is also untrue. The situation revealed by electron microscopy in fact requires a drastic revision of this theory.

A consistent finding throughout all the species studied is the correlation between the ratio of bipolars and receptors and the number of neurites making contact with each pedicle. Briefly, the

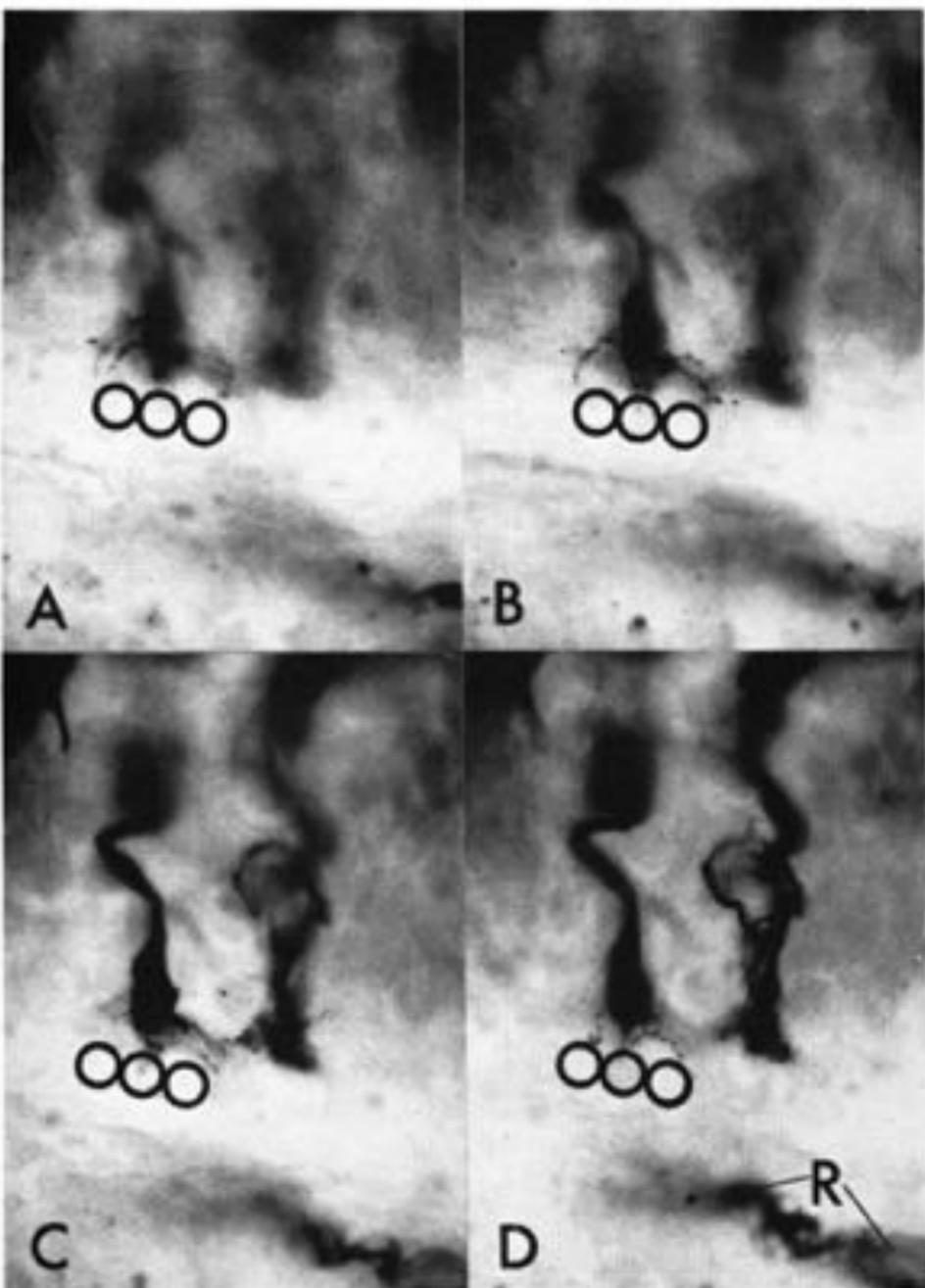


FIG. 5. Through focus series (A-D) of a rhesus monkey foveal bipolar cell. The circles are 5μ in scale diameter and represent the size of receptor pedicles from the same region as measured in the electron microscope. The optical planes of each micrograph are approximately 4μ apart; thus it can be argued that between 5 and 9 receptor pedicles can lie within the dendritic domain of one foveal bipolar. A radial fibre (R) lies obliquely, demonstrating the plane of the fibre layer of Henle which is peculiar to the fovea.

greater the relative bipolar surplus, the greater the number of connexions per pedicle. Conversely, the greater the relative bipolar deficit, the fewer the connexions per pedicle. We have not found a retina with a preponderance of complex pedicles and a bipolar deficit, neither have we found a retina with a preponderance of simple pedicles and a bipolar surplus. At first sight this is unremarkable since the obvious conclusion is that in a convergent retina with a relative bipolar deficit one would expect fewer bipolar connexions per receptor pedicle and in a retina with a relative bipolar surplus, more bipolar connexions per pedicle. This must certainly be part of the truth but two findings are against such a simple answer. First, the greatest recorded relative bipolar surplus is approximately 28:1 (Walls, 1963), whereas it is easy to find complex pedicles with as many as 300 separate neurites making contact with the synaptic surface. Even allowing for horizontal cell connexions, which certainly occur in the pigeon fovea, and branching of bipolar dendrites at their insertions (Figs. 6 and 7) which infrequently occurs, there is a considerable discrepancy here which is hard to explain on any established basis. Furthermore, if a flat section is cut through the outer plexiform layer in a species with a bipolar surplus and with complex pedicles, the neurites enter the pedicles from all points of the compass (Figs. 8, 9, 10). If they were arising from a single bipolar, many of the individual neurites must, at one point, lie in one direction grouped together. Since the reverse is true, these findings, together with the dendritic domain width indicated by the Golgi method (Fig. 5), suggest that in retinæ with a bipolar surplus, the "one-to-one" hypothesis shown by the silver method does not hold on a cell-to-cell basis and in fact there is a "one-to-many" relationship. In alternative terms, a spatially discrete output pulse or other change in state of a receptor is mainly integrated (in the case of a "rod retina") but is differentiated (in the case of the "cone retina") and the total output of information from one receptor in a cone retina with complex pedicles probably never goes to a single bipolar. It has already been

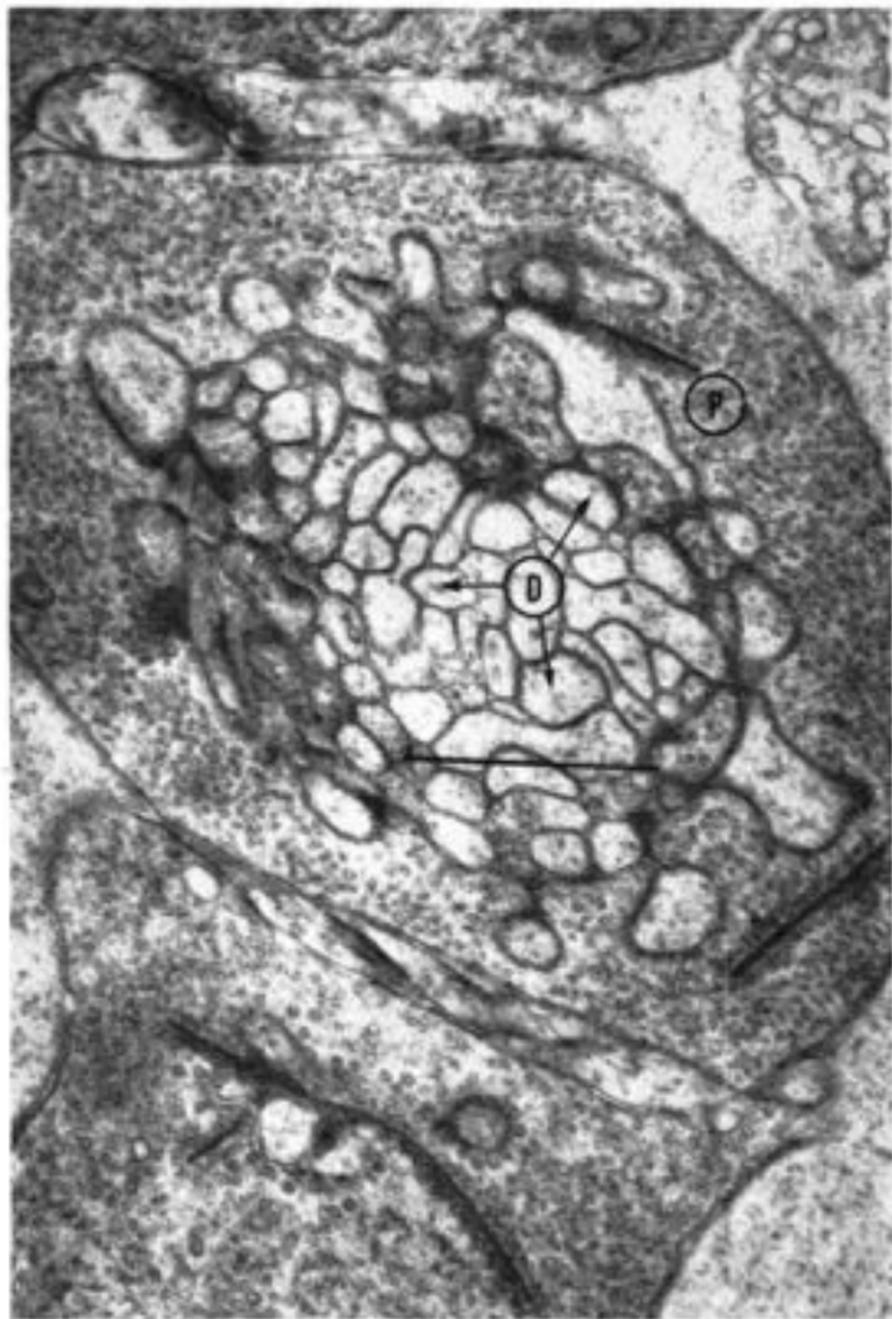


FIG. 6. Flat section of the dendrites (D) making contact with a pedicle (P) (*Lacerta viridis*). Only occasional branching of these processes in the plane at right angles to this section occurs. The profiles indicate that few of these processes would give a false impression of two in the vertical plane. One such process is indicated by arrows, which, if cut in the direction indicated by the line, would appear as two. Phosphotungstic acid. $\times 12,000$.

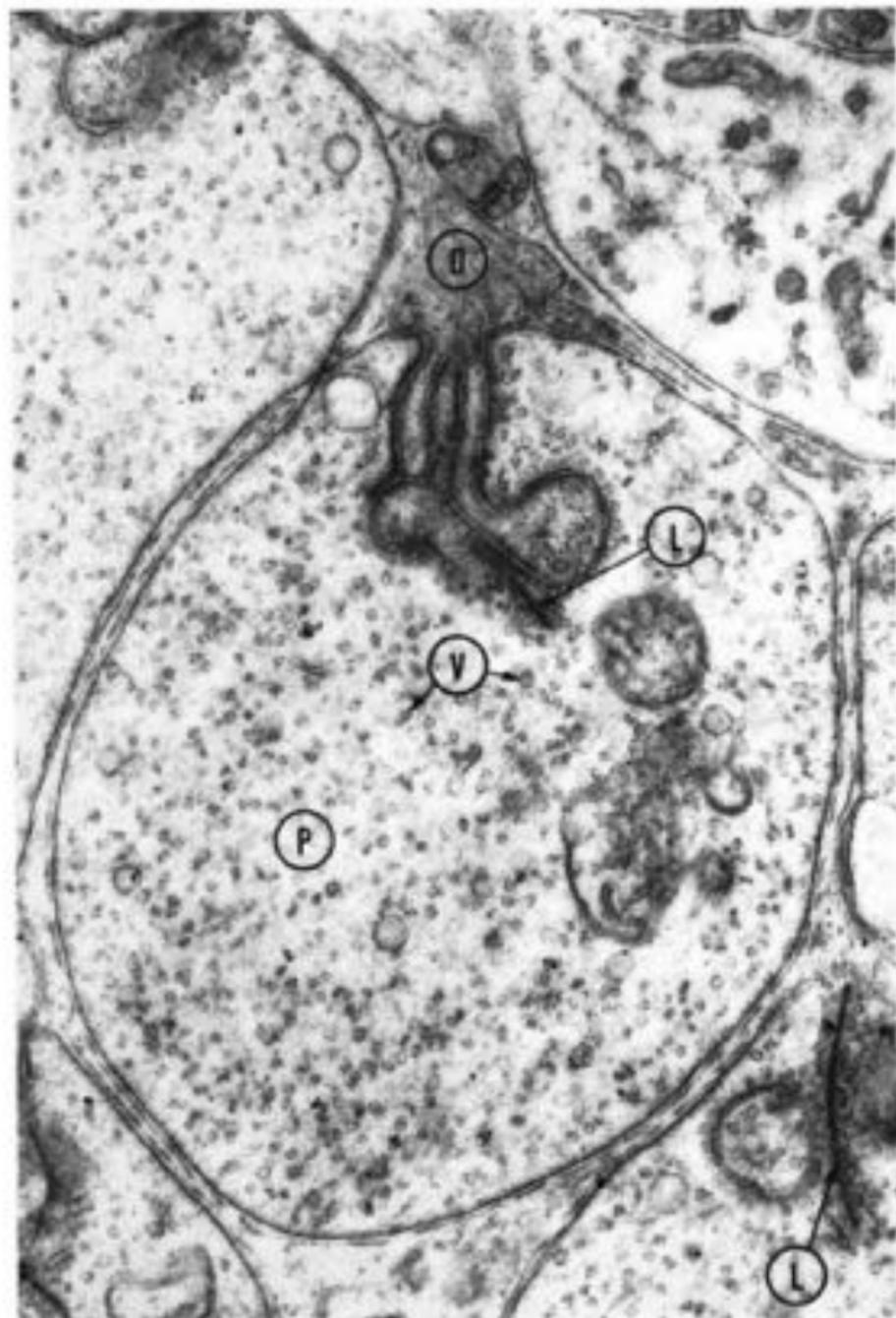


FIG. 7. A simple or single-channel receptor pedicle (P) from the periphery of a rhesus monkey retina. The dendrite (D) branches on entering the pedicle. Various types of synaptic vesicle (V) are present, together with the synaptic ribbon or lamella (L). Lead citrate. $\times 40,000$.

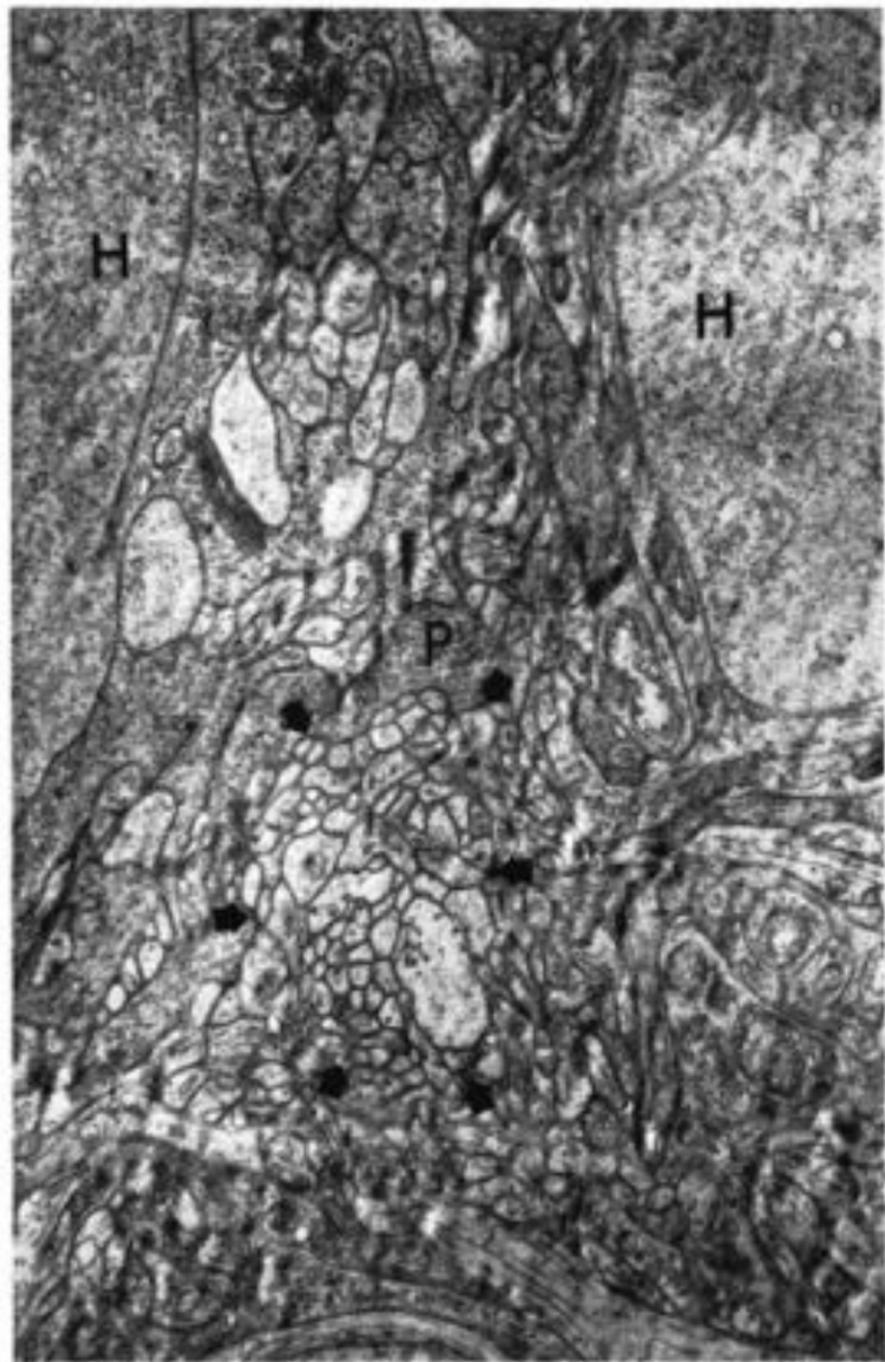


FIG. 8. Flat section of the outer plexiform layer from a pigeon fovea. Processes lie in all directions with no suggestion of regularity. A group of processes are lying at right angles to the plane of section (arrows) just before contact with the synaptic face of a receptor pedicle (P). The cytoplasm of two horizontal cells is shown (H). Lead citrate. $\times 18,000$.

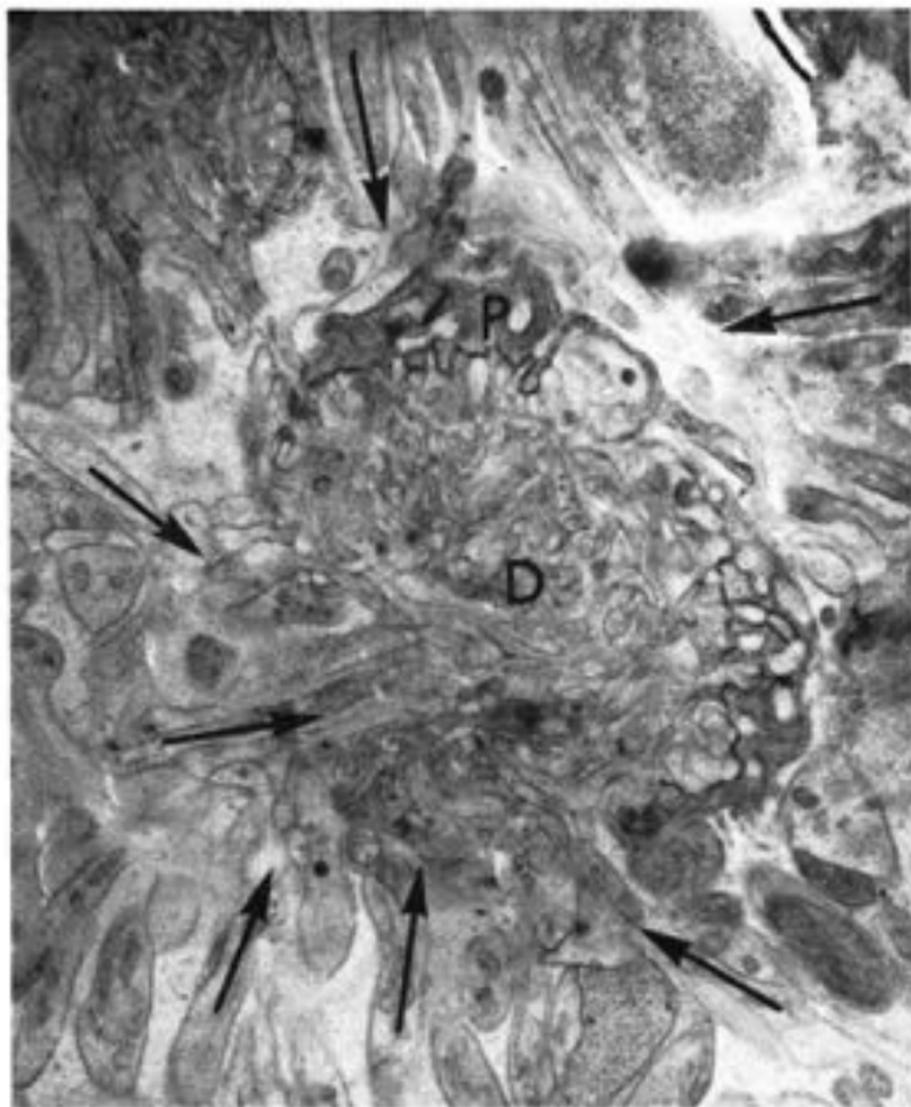


FIG. 9. Flat section of outer plexiform layer (*Lacerta marialis*). The mass of dendrites (D) entering the pedicle (P) are shown. This micrograph illustrates that the processes around a pedicle converge from all points of the compass (arrows). Phosphotungstic acid. $\times 12,000$.

found that the dendrites making contact with a single rod spherule do not originate from the same bipolar cell in the human retina (Missotén, Appelmans and Michels, 1963).

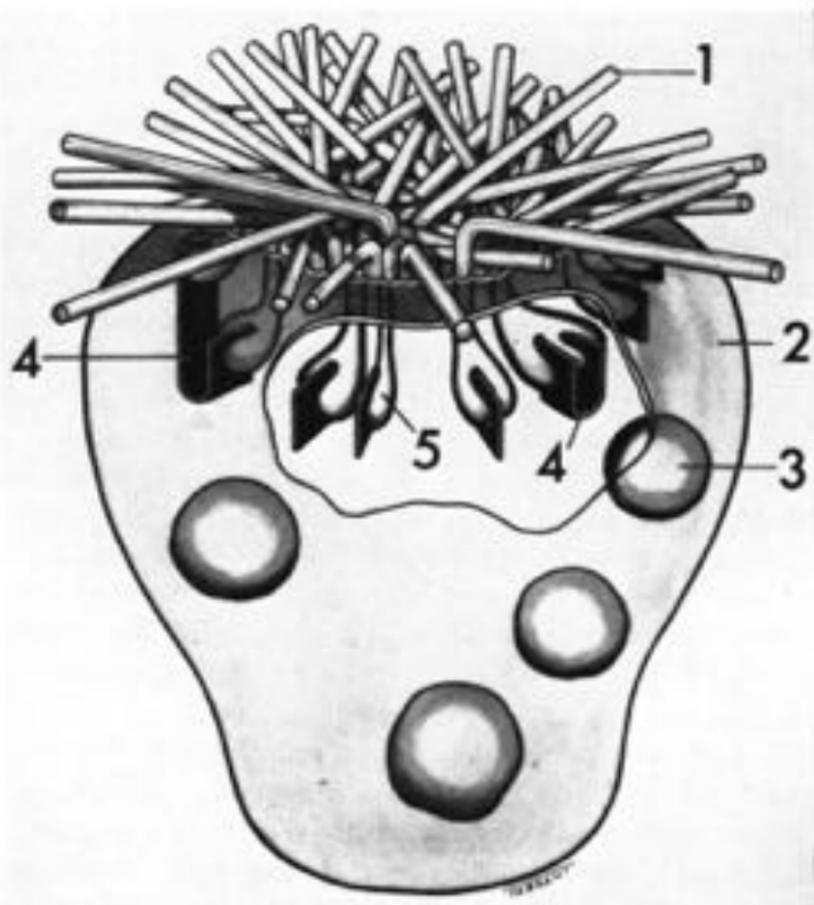


FIG. 10. Schematic illustration to show the relationships between a complex synaptic pedicle (2) and the bipolar dendrites (1). Vacuoles are present in the body of the pedicle (3). The deeply penetrating dendrites (5) enclose the synaptic ribbon (4). The dendrites emerging from the pedicle travel in all horizontal directions in the outer plexiform layer.

Another feature which investigators have used to distinguish between rods and cones is the size and shape of the synaptic pedicle (showing silver impregnations of cones with large triangular

pedicles and rods with small terminal spherules). Again, electron microscopy shows this distinction to be partly incorrect, because receptors with no formed pedicle, but with complex synaptic surfaces, can readily be found which, in Golgi impregnations, could only appear as narrow rod-like terminations. The contacts on their surface, however, are almost as numerous as those of the large expanded pedicles. This is particularly the case with receptors in which the nucleus is close to the outer plexiform layer. In these cells there is no conducting fibre and the cell has a synaptic surface without a terminal expansion containing as many processes attached to it as the complex pedicles. This has been shown in the retina of the alligator (Kalberer and Pedler, 1963). In addition, we have seen that many receptors present some rod-like features to the light microscopist and yet possess cone-like or complex pedicles. But it is the relative bipolar surplus that determines the pedicle type, so that the overall morphology of the pedicle is determined as a net result of surrounding retinal structure as well as being an entity in itself. To regard the receptor in this way helps to minimize another problem—what to call the foveal receptor of man and monkey. We know that in man the absolute thresholds of foveal "cones" and extrafoveal "rods" are of the same order, given a small enough stimulus (Baumgardt, 1949; Stiles and Crawford, 1947). However we now can see that the former have complex or multi-channel pedicles and extra-long outer segments. Yet it is known to the light microscopist that they possess rod-like form and cone-like special staining. It is more relevant to the function of these cells to refer to them as sensitive multi-channel output receptors, rather than as "rods" and "cones".

Similarly, we should now refer to the receptors of the nocturnal geckos and the alligator, the foveal receptors of the monkey and the pigeon and the central receptors of man, together with those of the leopard frog, as "sensitive multi-channel output receptors", instead of getting caught in the semantic web of classical morphological duplicity.

If components of receptors can evolve separately without change occurring in the rest of the cell it will be useful to examine each one to see how it differs from the others and then try to account for some of the differences. Nine are considered: (1) the outer segment; (2) the cilium; (3) the oil droplet; (4) the ellipsoid; (5) the paraboloid; (6) the myoid; (7) the conducting fibre; (8) the synaptic pedicle; (9) the receptor/bipolar ratio.

The outer segment

The differences in form used by the light microscopist to distinguish between rods and cones are easily recognizable in the electron microscope and correlate well with the habit of the animal. In addition, we now find that in some of the species studied, the shorter cone-like outer segments contain reduced numbers of lamellae and that these are incomplete and arranged in relative disorder (Figs. 11 and 12). This is not an absolute difference, as irregular lamellae can be also found close to the cilium in rod-like outer segments (Pedler and Tilly, 1964) and it may be that these irregular lamellae are an artefact. Against this view, however, is the fact that the irregularities are nearly always found in short, conical outer segments and can be seen in the same section adjacent to rod-like outer segments containing tightly packed and regular lamellae. Of interest in this context are the measurements of Sidman (1957), who found by immersion refractometry that in the frog, the salamander and the monkey the rod outer segments contain between 40 and 43 per cent of solids, whereas the corresponding cone outer segments contain only 30 per cent.

That diurnal outer segments are shorter, smaller and incompletely filled by lamellae is at first sight in keeping with their operating conditions, since there are more than enough incident quanta in daylight to make it unnecessary to present either the greatest path length or optimum molecular orientation of photopigment. However, the factor by which human rod sensitivity exceeds cone sensitivity depends considerably on the size of the

test-field employed, and further, when retinal summation is allowed for, either by the use of small fields or by making allowances for inter-neuronal integration, the ratio of rod threshold to cone threshold is approximately independent of the number of receptors stimulated and single rods and single cones may be



FIG. 11. Longitudinal section of a "sensitive" outer segment from the foveal centre of a rhesus monkey. Lead citrate. $\times 40,000$.

equally sensitive (Arden and Weale, 1954). It is difficult to account for a short incompletely filled outer segment being equally sensitive to a larger completely filled outer segment unless some other factor is involved. This apparent discrepancy is probably due to species difference, since Weale's measurements were made on the human retina where the outer segments of the foveal receptors are noticeably longer than in the extrafoveal regions, a fact that is likely to increase their sensitivity by presenting a greater path length

to incoming quanta. The species in which we have so far found incompletely filled and disordered cone outer segments are: a diurnal gecko (Pedler and Tansley, 1963), *Alligator mississippiensis* (Kalberer and Pedler, 1963) the pigeon (*Columba domestica*), the extrafoveal but central area of man, and the foveal centre of the

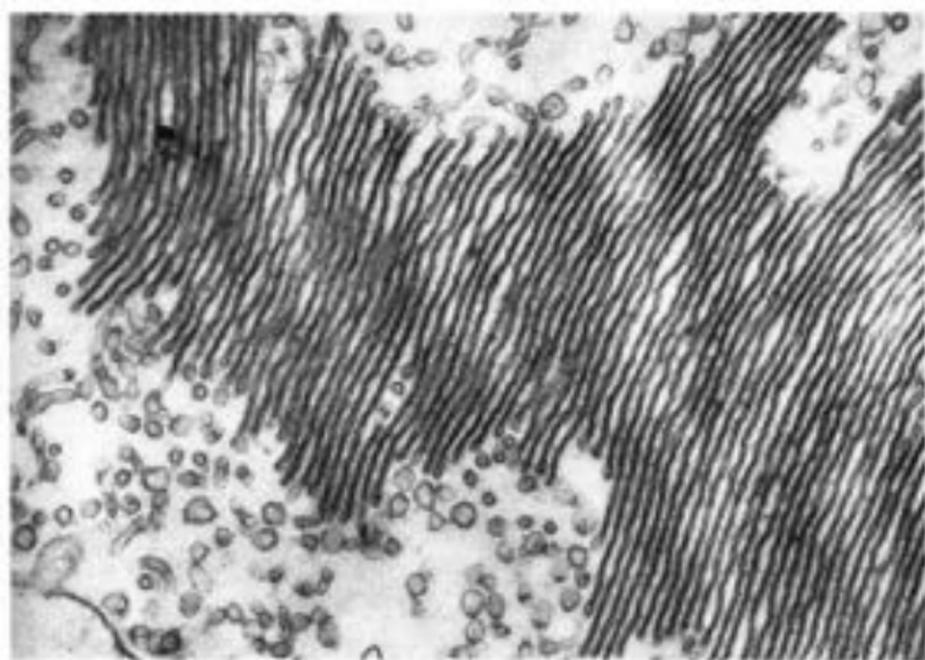


FIG. 12. Longitudinal section of an "insensitive" outer segment from a diurnal gecko, *Phelsuma inunguis*. Phosphotungstic acid. $\times 32,000$.

rhesus monkey. Although there are complicating factors, therefore, it is probably safe to assume that short outer segments with incomplete or irregular lamellae are less sensitive when considered alone than the larger cylindrical varieties.

The cilium

This structure extends between the proximal lamellar incisures of the outer segments and a basal body in the inner segment which

then gives rise to a typical ciliary rootlet. The basal body is commonly associated with one or more centrioles. We have found among the species studied that the cilium is more complete in cells which are rod-like in respect of their inner and outer segment. The most prominent are in human rods where as many as five rootlets have been found in one cell. In receptors with cone-like inner and outer segments it is usual to find rudimentary fragments of these structures only (Pedler and Tansley, 1963), suggesting that the more fully developed structure is associated with high sensitivity. Since the functions of the cilium are not yet known for certain, nothing further can be said about this finding.

Inner segment

According to the species, the inner segment may contain an oil droplet, an ellipsoid and a paraboloid in the region between the cilium and the myoid. The latter contains the Golgi apparatus.

A functional interpretation of these structures is fraught with difficulty for three main reasons: first of all, nothing is known about receptor mechanisms between the absorption of quanta in the outer segment and the release of information from the synaptic pedicle; secondly, at least some of the structural variations encountered among the species probably have no visual significance. Finally, each structure may well have more than one function. With regard to the first point, it is safe to assume that some form of amplification is involved in the receptor, because the single quantum to which a receptor can probably respond almost certainly supplies insufficient energy to activate the receptor. It is also probable that the mitochondria of the ellipsoid are involved in the amplification and that they in turn receive energetic support from the radial fibre complex (Pedler, 1963). Relevant to the second and third points are some of the variations between the nocturnal and diurnal inner segments of the gecko. For example, the main difference between the paraboloids is that the diurnal form is regular and precisely demarcated, whereas the nocturnal variety is

vacuolated, irregular and merges gradually into the surrounding cytoplasm. This, together with the finding that the mitochondria of the ellipsoids are so different, suggests the possibility that a change in emphasis may have taken place in the balance between the local metabolic demands of the visual cell and the need for the condensation of light on to the outer segment (Pedler and Tilly, 1964). In the diurnal form there is a well demarcated paraboloid partially enclosed by the closely packed mitochondria of the ellipsoid. Walls (1963) suggested that this arrangement, as he saw it in the light microscope, resembles an achromatic lens system. If this is the case, the nocturnal forms may have failed to retain this particular feature. Conversely, the local energy turnover in a given time is probably higher in the diurnal species and so, presumably, the need for highly organized mitochondria dominates the need for the condensation of light on to the outer segment. This assumes that the more cristae a mitochondrion possesses, the higher its activity. This is supported by the finding that there is a positive correlation between respiratory activity and density of cristae and further, that cristae as well as the continuous inner layer of surrounding membrane contain most, if not all, of the respiratory enzymes (Palade, 1956). In the nocturnal forms the need for colour correction may have disappeared, but condensation of light on to the outer segment presumably dominated the local need for high energy storage and turnover. Thus the highly organized cristae of the mitochondria were transmuted into the aggregations of amorphous material found in the nocturnal species (Pedler and Tilly, 1964). Also against a strict correlation between the structure and density of mitochondria and visual function is the fact that the foveal receptors of the monkey do not contain dense concentrations of mitochondria although they are presumably in a highly active state for long periods. They are not, in fact, nearly so densely packed or so detailed in fine structure as those in the peripheral receptors of *Lacerta muralis*, a diurnal lizard. A more reliable correlation in this series has been that the structure and density of the mitochondria is

related to the presence of retinal vessels. In avascular retinae the density of mitochondria is greater and *vice versa*, suggesting that in the absence of vessels, receptors may have to be more internally dependent for short-term energy turnover. The fact remains, however, that in light microscopically duplex retinae, receptors with small outer segments do have more mitochondria in the inner segment, so that in addition to one correlation with retinal blood supply there is another with outer segment size.

Similarly, nothing definite can be said about the oil droplets which can be found in receptors with rod-like or cone-like outer segments. They can also be colourless or coloured and have been variously implicated in colour vision, glare reduction and contrast enhancement; but a completely successful theory to account for their function has not yet been established. Apart from the finding that the coloured droplets are more common in diurnal species (Walls, 1963) they are not, in the present state of knowledge, of any vital assistance in differentiating between receptors. The same is probably true of the myoid, the nucleus and the conducting fibre, since there is no electron microscopic indication here of any specialized function other than would be consistent with the transference of the signal towards the synaptic surface.

The two parts of the cell to which function can be ascribed with some certainty are the outer segment and the synaptic pedicle, the one being concerned in the absorption of light and the initiation of a train of events and the other with the release of information to other retinal cells. In the present state of knowledge it is therefore best to base any morphological classification mainly on these two regions, which represent the points of input and output. Whether the interior of the cell operates on the data generated in the outer segment, is at the moment impossible to decide.

The synaptic pedicle

There are at least four varieties of process connecting with the surface of the synaptic pedicle (Pedler, 1965); bipolar dendrites,

horizontal cell processes, processes from the radial fibre complex and filaments from adjacent pedicles. There are, in addition, two morphological varieties of bipolar terminal at the pedicle surface (Figs. 13 and 14).

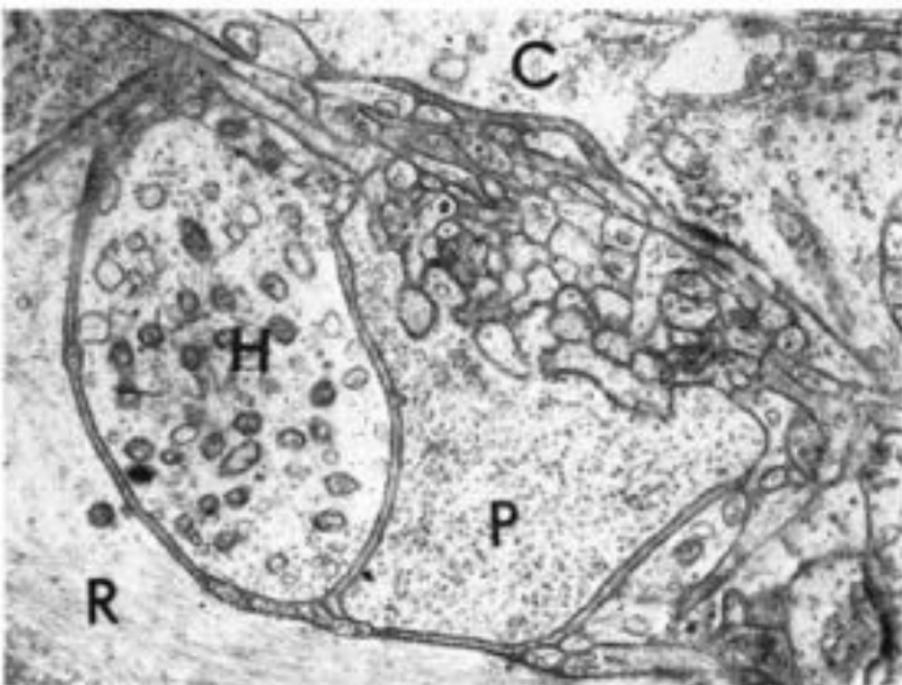


FIG. 13. A multi-channel pedicle (P) and a horizontal cell process (H) from a pigeon fovea. The main horizontal cell cytoplasm (C) and fibrillar radial fibre material (R) are shown. Lead citrate. $\times 21,000$.

Processes from adjacent pedicles are often recognizable since they also contain synaptic vesicles, which are thus found on both sides of the synaptic membranes. The processes from the horizontal cells are also frequently identifiable since they contain cytoplasmic constituents of a form unique to this region. The radial fibre processes can often be traced back to a main radial trunk, so we are left with the bipolar dendrites to account for, which can end in either of the two varieties of terminals. This abundance of con-

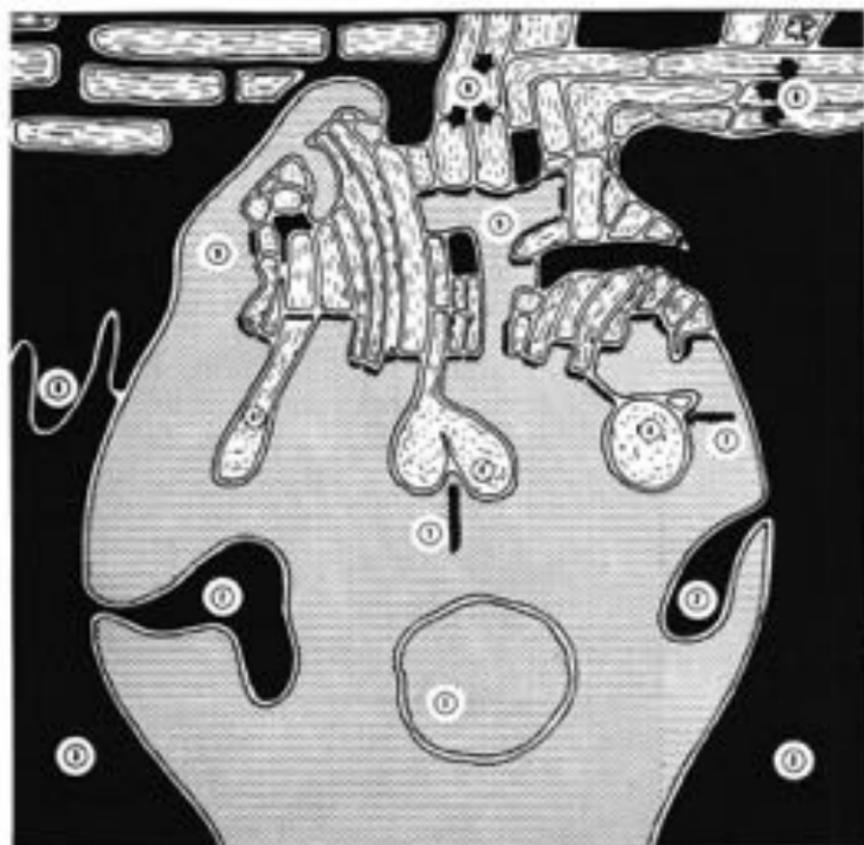


FIG. 14. Schematic illustration to show the main relationships between a complex receptor pedicle and the neurites of the outer plexiform layer. The pedicle body contains several vacuoles surrounded by double membranes (1). The pedicle itself is enclosed by radial fibre material (2, in black), which sends protrusions (2) into the body of the pedicle. Some bipolar dendrites insert deeply into the pedicle (4) and are in close relationship with the synaptic ribbons or lamellae (7). Basal filaments (5) are thrown up on the inner surface and make many contacts with the neurites in the outer plexiform layer. Normally all the latter (6) run horizontally at right angles to the long axis of the receptor.

nexions is found only on the complex pedicles and in the simple variety the type of terminal which inserts deeply into the substance of the receptor is the one most frequently found. However, even allowing for the other three types of contact, the *bipolar* contacts per receptor may number as many as 200. But we also know that

bipolar domain overlap is considerable, even in central or parafoveal regions, and we have also seen that the presumptively bipolar dendrites contacting a given pedicle probably come from many bipolars, so that we have to conclude that a complex pedicle may, even in the fovea, release information to a minimum of 10

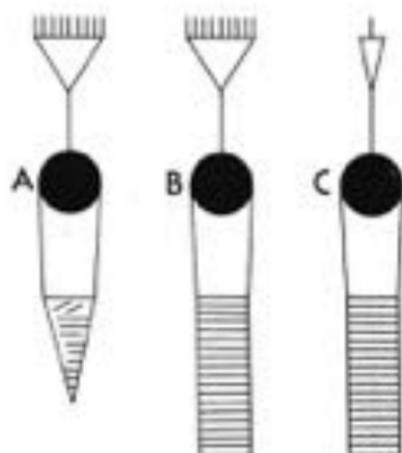


FIG. 15. The three types of receptor proposed. Type A is an insensitive multi-channel output cell, Type B is a sensitive multi-channel output cell and Type C is a sensitive single-channel cell. The classical cone and rod are included in types A and C respectively; type B includes some anomalous cells referred to in the text, together with the foveal receptors of man and monkey.

bipolar cells. Thus, if a pedicle discharges from the whole of its surface, the absorption of a spatially discrete spot of light in one receptor may release information into a large number of channels in many cells in the outer plexiform layer. The receptor with the complex pedicle must therefore be a differentiator and the one with a simple pedicle an integrator. But we have already encountered permutations of this general theme and must therefore complicate matters by speaking of three basic types so far encountered (Fig.

15): an insensitive multi-channel differentiator (type A), a sensitive multi-channel differentiator (type B) and a sensitive single-channel integrator (type C). The fourth logical variety, an insensitive single-channel integrator, will probably not be found, since there is no need for integration in photopic conditions.

This scheme does not exclude the probability that there have been two main lines of cytological development producing many structural features which fall conveniently into two groups. Indeed, types A and C would include the classical cone and rod. The recognition of type B, however, means that we no longer have to force duplicity on the nocturnal geckos, *Sphenodon*, the lung fish, the leopard frog or any of the other anomalous species. Neither do we have to get into semantic difficulties over the rod-like cones of the human and monkey foveae, because the features used in this classification are the ones related to the input and output functions of the receptor and are therefore likely to create fewer problems than the concept of the rod and cone which has, after all, served very well for over a century.

Acknowledgement

This work would have been quite impossible without the enthusiastic help and tireless skill of Mrs. R. Tilly.

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DISCUSSION

Lowenstein: Have light microscopists looked at macerated retinæ and seen these two types of cells in suspension?

Pedler: Yes, they have, but I do not know whether they have seen the types which we now find to be anomalous. Many investigators at the turn of the century looked at such specimens, using intravital methylene blue, and produced beautiful drawings of the classical cone. I am not denying the existence of the classical cone at all, but I am trying to add to it in the light of electron microscopic data.

Willmer: I think we should say that it was to Polyak's credit that he pointed out in 1941 (Polyak, S. L. [1941]. *The Retina*. Chicago: Chicago University Press) that the one-to-one cone-bipolar ratio in the fovea was a myth and that although the midget bipolar existed, which made that connexion, the majority of connexions in the fovea were the multiple ones, as you also believe. Polyak stressed the fact that flat bipolars and brush bipolars are both present in large numbers in the foveal centre. I have preached the same story too; but nobody has listened to us. I am very glad you have confirmed it. I am not sure that I would go the whole way with you, to say that a single connexion *cannot* be made in the fovea. Polyak's data were not sufficient for that. I think it quite likely that there may be *some* unitary connexions in the fovea.

Pedler: I agree with your censure and should have pointed out that Dr. Polyak did see the multiple connexions; I am constantly amazed by how much he did see. I don't know how he accomplished it without an electron microscope. But I suspect that many of the so-called midget bipolars he referred to were simply bipolars in which the body of the cell was impregnated but the processes were not. Neither light microscopy nor silver methods alone are sufficient to demonstrate the finer neurites.

Willmer: He showed that the so-called midget bipolar had a shorter

dendritic tree than the flat and brush bipolars, so there is the possibility that he was indeed dealing with a different category of bipolar.

Pedler: Yes. I would not say that the electron microscope has altered the picture completely, because I am perfectly aware that its sampling error is bigger than that of the light microscope. We simply have a number of very unpleasant discrepancies to explain and I have attempted to provide a basis for such an explanation.

Wolbarsht: Part of the difficulty with the earlier idea of a one-to-one correspondence between the number of receptors and the number of bipolars was the concept of the way visual acuity was made up. All workers found a one-to-one correspondence because they were looking for it. Now we know that receptive fields are, or seem to be, larger with much overlap, and we expect there to be some mechanism for providing for convergence of a large number of receptors on one ganglion cell. You histologists have very obligingly supplied it!

Cresitelli: Have you ever examined twin cones or twin rods? I wonder whether the twins converge on the same bipolar or the same bipolar system, or whether they are independent.

Tansley: Dr. Pedler and I examined the retina of the diurnal gecko, but they are doubles, not twins. I have not looked at this in twins. Doubles certainly do not converge.

Pedler: Most of the cells are in close apposition, with the exception of the synaptic pedicles which are separate.

Cresitelli: There are repeated claims in the literature, which I am not able to assess critically since I am not a histochemist, that it is possible to differentiate by staining properties the two members of the twins, in fishes for example. This would make them not twins at all.

Tansley: I would not agree with that. I have never seen this in twins, although I have not studied twins very much.

Cresitelli: There are even suggestions that cones are transformed into rods during ontogeny. Doubles differ morphologically from twins—double cones are not identical—and the question is whether there is a physiological correlate to this morphological difference.

Wolbarsht: The difficulty is that they are found in different animals; in fishes you find what you would call twins.

Sjöstrand: In twins the two members of the pair are apparently identical in the light microscope, and in doubles they are not.

Wolbarsht: In the fish twin cones Dr. Marks has found by microspectrometry that each cone contains a different pigment. I do not know what that means functionally. He has small samples, of course. But it is difficult to think of a good chemical reaction to distinguish between the two, to make them stain differently, on the basis of the pigment alone. It is possible that if they have a different protein, you might find a stain that would hook on to one and not the other.

When we examine the receptive fields of a single ganglion cell in the goldfish retina we find that the spatial distributions of the sensitivities of opponent colour processes connected with this ganglion cell do not change in the same way. This difference in sensitivity of the two processes presumably reflects a differential distribution of the receptors of each process connected to that ganglion cell. Thus, twin cones would not necessarily be connected to the same ganglion cell and there would not seem to be any functional reason to have the pigments in twin cones rather than separate cones. That the twin cones have a rôle I do not doubt, but what it is I cannot really say.

Pedler: The other point about the double cells is that one must beware of attributing all morphology to a visual need. This is why I did not discuss the inner segments. If you look at the average double, or twin for that matter, the pedicles are separate and the nuclei are opposed, and the inner segments are closely aligned. In fact the two plasma membranes are interdigitated in this plane, but the outer segments are separate. Also the outer segments are separated by pigment, which suggests that they are optically separate.

Piroune: May I go back to visual acuity? As I recall, the diagrams published by Polyak in his book (Polyak, S. L. [1941]. *The Retina*. Chicago: Chicago University Press) and also in later papers show that in the fovea of man or monkeys there are private lines to the brain for the cones, and also other lines which go to the brain and which connect with several of the same cones. The point is that there are enough small bipolars to connect with every cone, and there are also bipolars which each connect to several cones. That does not raise any great difficulty with regard to the visual acuity; at low intensities probably only the bigger units function, and the small ones function at high intensities. I wonder, Dr. Pedler, whether quantitatively you have found that there are *fewer* lines than cones in the central fovea? This would really

create a problem. If there are too many or just enough lines, one can always, for the time being, assume that things work on the basis of one-to-one connexions. If there are not enough, one will have to assume quite a different mechanism.

Pedler: Yes. There is ample physiological evidence to suggest that there is a one-to-one pathway functionally. I merely suggest that this is not on a *cell-to-cell* basis. There may well be a subcellular dendritic pathway within which unit information from a receptor can pass towards the ganglion cells. The circuitry is more complex than would be suggested by single cells plugging into one another. This does not occur.

Pirenne: Anatomically this means that in the fovea of the monkey one cone connects with several bipolars, and one bipolar with several cones; but that if one makes the assumption that physiologically single lines can nevertheless be obtained, there are enough cells to give one-to-one connexions.

Pedler: There are enough cells for that, at least in the monkey fovea.

Pirenne: The situation might be similar to that in *Limulus* where there clearly are single line connexions, as well as nervous interactions producing the inhibitory effects studied by Hartline (see Ratliff, F., Miller, W. H., and Hartline, H. K. [1958]. *Ann. N.Y. Acad. Sc.*, **74**, 210).

Pedler: It is very interesting that you raise this; we are starting to study the region between the ommatidial base and the first optic ganglion in the fly. Fascinatingly enough, we have found that the eight axons emerging from each single ommatidium do precisely the same thing between the ommatidial bases and the first optic ganglion. The axons spread out into different columnar channels in the first optic ganglion. Again this is purely speculation, but you might say that by convergent evolution the same process has arisen in both the invertebrates and the vertebrates.

Wolbarsht: There is one difficulty with that. It is very nice to talk about bipolar cells, but they do not go back into the optic nerve. You have to go through the ganglion cell. I am quoting the work of D. H. Hubel and T. N. Wiesel ([1960]. *J. Physiol. [Lond.]*, **154**, 572) here, and as far as they have told me, the smallest visual field found in the monkey was a quarter of a degree, and that had a surround. It was certainly not due to a single visual cell.

Weale: Are we trying to rescue a phantom? Is there any visual function which requires the possibility of a one-to-one relation, and which cannot be explained on any other basis?

Tansley: It depends what you mean by a one-to-one relation. It is not necessary to have a one cell-to-one cell relation.

Swaetichin: I noticed some horizontal cells in your sections, Dr. Pedler. How do you classify them? Are they neurones or not?

Pedler: There are several factors against them being ordinary neurones. They have a cytoplasm which is distinguishable from that of other retinal neurones, in that it has a much more granular endoplasmic reticulum. They rarely contain any synaptic apparatus (pre- or post-synaptic thickening or vesicles), and glutaraldehyde shows their cytoplasm to be paler. Another, more interesting feature is that the horizontal cells we have observed terminate around bundles of bipolar dendrites. I cannot explain this—one can obviously suggest ideas of how this might work. They do occasionally send a process right round the receptor surface, but the majority of the processes appear to terminate on bundles of presumptively emergent bipolar dendrites, from the pedicle. I know that you yourself have suggested that they have a modulating function; this, structurally at least, would fit in with this idea.

Klüver: In considering the electron microscopy of tomorrow, I wonder how much it will contribute towards a better understanding of the physiology and psychology of visual functions by elucidating the operation of corticofugal systems. The external world with its properties is not simply "projected" into the organism. To achieve an approximate constancy—a homeostasis of the external environment, as it were—there occurs an elimination, a modification, and a transformation of certain properties, or, as it recently has been expressed, a "property filtration". Centrifugal mechanisms, particularly in the visual system, play a great rôle in such a filtration. Unfortunately, we know little about their workings. Polyak, for instance, described a "centrifugal bipolar cell". Can electron microscopy be expected to provide information on the centrifugal bipolar cell which will be helpful in our understanding of processes that act corticofugally?

Pedler: One possibility here, Professor Klüver, is that somebody will develop a method of specifically delineating degenerating neurones in the electron microscope. If this is possible, people can make tectal or

cortical lesions and follow the tract down into the retina where the corticofugal system terminates. There is not yet any such method that I know of. You see, we have no means of distinguishing morphologically between an afferent and an efferent neurone; they have the same cytoplasm, the same membranes, the same nucleus, even the same synapses, so I do not see how we are going to do this unless we can find a neurone which is available from the cortical end.

Mounier: Have you any evidence on the problem of centrifugal control of the retina?

Pedler: I have not studied this at all. There is some excellent evidence from Dr. Cowan (Cowan, W. M., and Powell, T. P. S. [1963]. *Proc. roy. Soc. B*, **158**, 232), who did some beautiful degeneration studies on the pigeon by making pretectal lesions and following the degeneration down into the amacrine cells of the retina. This is the best evidence available for centrifugal fibres in the retina.

DUALITY IN THE RETINA

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IN investigating how an unknown mechanism could work, it is always advisable to study the nature and properties of its constituent parts. Thus, in attempting to solve the problems of physiological function in the organs of higher animals, the ontogenetic and phylogenetic development of the cytological units concerned may offer important clues. In this paper, therefore, I wish to consider the nature and properties of the layer of cells which constitute the primary optic vesicle, because the whole of the retina develops from this epithelial layer. A knowledge of its behaviour and potentialities is therefore of cardinal importance. Moreover, since the optic vesicle grows out from the neural ectoderm near the lateral margins of the neural plate, the cells of the optic vesicle, and hence of the retina, are likely to have many properties in common with those of the rest of the nervous system.

The first question which it is necessary to ask is whether the epithelium of the optic vesicle constitutes an initially uniform population of cells, or whether it contains cells of more than one class. Although the phylogenetic origin of the cells of the neural tube is lost in obscurity, it is very likely that the cells originally formed part of the general body surface of the animal. Since the animal in which the neural tube first began to develop was probably both primitive and aquatic, it is likely that it had a surface epithelium composed of ciliated and goblet cells, and that the water and ionic balance of the animal was at least partly controlled by these cells. This essential duality of epidermal cells is very fundamental, and even in certain epithelia of higher animals, where the cells

normally keratinize, it can sometimes be re-induced by treatment of the cells with high doses of vitamin A (Fell and Mellanby, 1953), a property which is probably not without its significance in relation to the visual epithelia, whose content of vitamin A is abnormally high.

In many species, the cells of the neural folds have been shown to have somewhat different properties from those of the neural plate itself. For example, the neural folds of the frog are ciliated while the plate is not (Assheton, 1896), though in the chick and higher animals the whole of the neural tube is at one stage ciliated. This, of course, does not necessarily mean that every cell is ciliated; indeed the cells in the ectoderm of the frog gastrula form a mosaic pattern of ciliated and non-ciliated cells. In the chick, it is well known that spinal cord tissue can act as an inducing agent for the development of other tissues, and it is interesting, in the present connexion, to note that the powers of induction of the lateral (and subsequently dorsal) parts of the neural tube are very different from those of the more ventral neural plate (Grobstein, 1955; Grobstein and Holtzer, 1955). This presumably reflects the preponderance of different cell types in the two regions. Moreover, motor nerve cells, conducting from centre to periphery, tend to develop chiefly from the neural plate, while sensory cells, oppositely orientated and conducting from periphery to centre, are mostly developed from the neural folds and alar plate (Holtzer, 1951). The cells which emigrate to become the autonomic ganglia are now believed mostly to arise from the ventral part of the tube and they again conduct away from the central system. It is clear that the orientation, or polarity, of the cells in the original epithelium may be of fundamental importance to their subsequent development and function. The cells of the neural crest in the frog are certainly not a uniform population, and the observations of Holtfreter (1947) on the effects of hypertonic solutions on these cells are of special interest. When frog neurulae are placed in a hypertonic solution a number of large polarized amoeboid cells emerge from the neural folds and become free cells

while the rest of the neural epithelium remains relatively unchanged. The fact that hypertonicity is the necessary and sufficient stimulus for this response of one group of cells in the epithelium reminds us again that the neural ectoderm was once the normal ectoderm of the organism and as such was probably concerned with the maintenance of the ionic equilibrium between the organism and its surroundings. This function of the preservation of the ionic equilibrium must presumably be present when the neural folds grow together and fuse along the dorsal surface, thus enclosing a fluid-filled cavity, namely that of the neural tube and optic vesicles. The contained fluid is now of course the cerebrospinal fluid.

The raw material from which eyes are made is an epithelium almost certainly adept at the maintenance of ionic equilibrium between the external fluid, which after its enclosure is the cerebrospinal fluid, and the underlying tissues. In such boundary epithelia a mixture of two types of cells is of such frequent occurrence as to suggest that the equilibrium is maintained by the balanced activities of opposing or oppositely orientated types of cells. In the skin of numerous primitive invertebrates and in the respiratory passages of higher animals ciliated cells are almost invariably mixed with goblet cells; in the alimentary tracts of numerous animals "brush-border" cells alternate with mucous cells. In both cases the production and location of mucopolysaccharides is characteristic and interesting and it will be seen also to have some significance in relation to retinal cells. PAS-positive material is almost invariably present amongst the cilia and microvilli of ciliated and brush-border cells, while in goblet cells this staining reaction is given by globular contents of the cell lying between the nucleus and the external surface. The suggestion has been made elsewhere (Willmer, 1960) that this characteristic distribution of polysaccharides could be related to the direction of ionic or water movement through the cells in question.

To return again to eyes, the first point to bear in mind is that it is extremely likely that they develop phylogenetically and onto-

genetically from an epithelium which has within it the capacity for producing cells versed in the maintenance of ionic equilibrium between an outer fluid and the inner fluids of the body. Let us therefore make this assumption and examine the development of the epithelium of the optic vesicle into the various components of the eye and see what traces may be left of these primary properties.

Figure 1 shows the main derivatives of the optic vesicle after it has invaginated and of adjacent parts of the neural tube. The outside of the vesicle gives rise to the pigment epithelium. At first sight this appears to be a uniform epithelium, but it is worth recording that, in eyes which have both rods and cones, the pigment cells specially associated with cones have fine processes surrounding the cones and containing numerous pigment granules, while those associated with rods have far fewer processes and seldom any pigment granules. There are obvious functional advantages in such an arrangement, but it must also be remembered that there must first be the necessary physiological mechanism behind any such beneficial adaptation. Myeloid bodies are present in the cytoplasm of the pigment cells in cone eyes (Yamada, 1961), while in those of rod eyes probably somewhat different lamellated bodies are present in a very vacuolated or reticulated cytoplasm (Yamada, 1958; Dowling and Gibbons, 1962). These bodies, which in some cases are dependent upon an adequate supply of vitamin A (an ingredient commonly present in fat droplets in the cells of the pigment epithelium) may conceivably be concerned with the photosensitivity which pigment cells often display by altering their shape and pigment distribution in response to illumination. In some animals the pigment epithelium develops into a retinal tapetum in which the cells contain guanin crystals. Both guanin crystals and melanin granules may be present in the pigment epithelium and may tend to move in opposite directions in response to illumination. It is, however, not clear whether in these cases the two substances are housed within the same cell, or whether there are guanin and melanin-

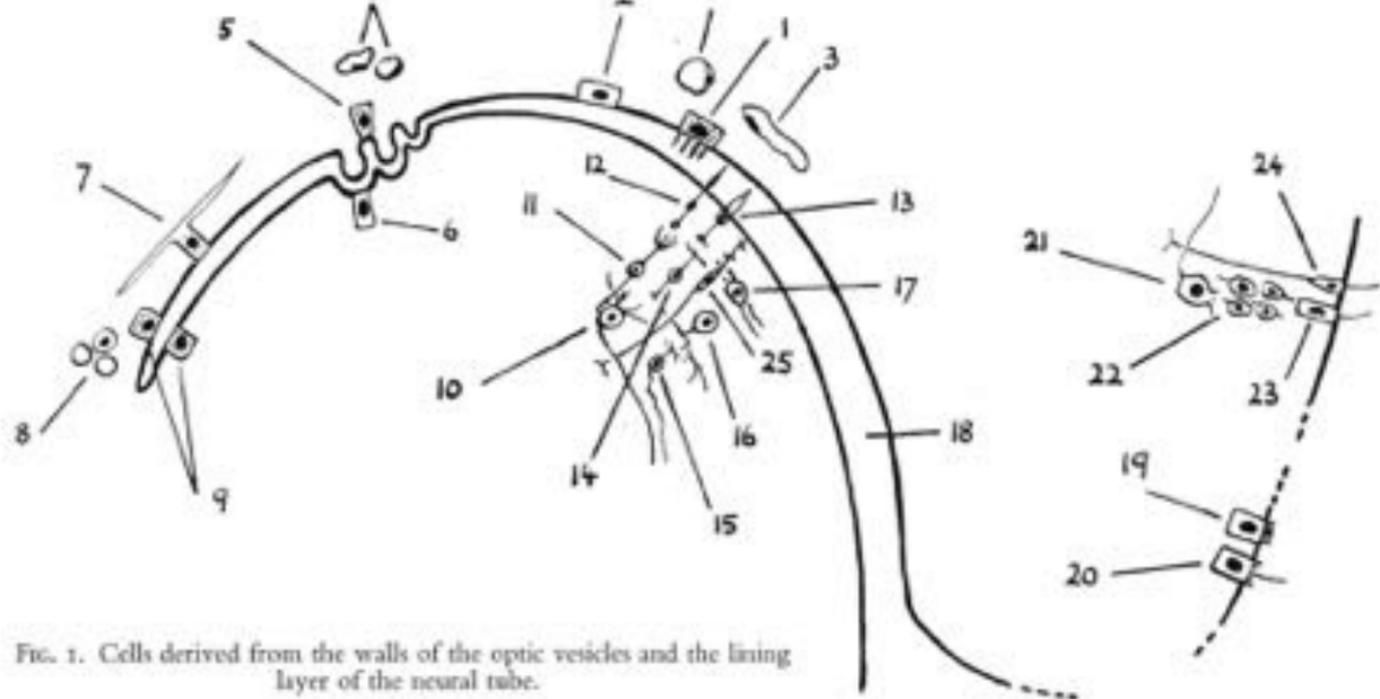


FIG. 1. Cells derived from the walls of the optic vesicles and the lining layer of the neural tube.

1. Pigment cell with processes and pigment, particularly associated with cones.
2. "Pigment" cell, without pigment and with no processes, particularly associated with rods.
3. Blood vessels of choroid (derived from mesenchyme).
4. Blood vessels of ciliary processes (derived from mesenchyme).
5. Pigmented cell from region of ciliary processes.
6. Non-pigmented, "secretory" cell from region of ciliary processes.
7. Epithelio-muscular cell of dilator pupillae muscle.
8. Epithelio-muscular cells of sphincter pupillae muscle.
9. Double layer of pigmented cells from the posterior surface of the iris.
10. Large ganglion cell.
11. Rod bipolar cell.
12. Rod.

13. Cone.
14. Cone bipolar.
15. Small ganglion cell.
16. Amacrine cell.
17. Horizontal cell.
18. Cavity of optic vesicle, continuous with that of neural tube.
19. Cell of choroid plexus, with microvilli.
20. Cell of choroid plexus, with cilium and microvilli.
21. Large nerve cell, in central nervous system.
22. Nerve cells and neuroglia.
23. Ependymal cell.
24. Ependymal cell, with process crossing the wall of the neural tube.
25. Muller's fibre (cf. 24).

containing cells. The guanin-containing cells are often very localized in their distribution, as also are the melanin-containing cells.

If we follow the pigment epithelium towards the ciliary processes it is found to cover these processes as the outer and pigmented of the two covering layers which separate the ciliary mesenchyme and blood vessels from the aqueous humour. After the epithelium has been reflected back on itself, as it lines the back of the iris and at the margin of the pupil, it again comes to cover the ciliary processes as the inner, unpigmented and columnar epithelium generally held responsible for the formation of the aqueous humour. This is of course an activity which is in line with its presumed initial function of preserving the ionic equilibrium and which also remains a function of the neuroepithelium, at least in the choroid plexus, if not also in the rest of the ependyma. An electron microscope picture of these epithelia shows that the cells in the two layers are quite different (Pappas and Smelser, 1961). It is always said that it is the columnar layer which does the secreting, but since the material for the secretion must come either from the remnants of the cavity of the optic vesicle, or, more probably, from the blood vessels which are separated from the columnar layer by the other pigmented layer, this may not be altogether a justifiable conclusion and the cells of both types may be involved. In this connexion, too, it may be well to point out that the rods and cones of the retina probably get most of their nutrition from the vessels of the choroid and, hence, the pigment cells may well contribute actively to this process, since they bar the way of any direct connexion. In both these cases it should be remembered that there is a potential space between the two layers of epithelia, namely the original cavity of the optic vesicle occupied initially by cerebrospinal fluid or something very like it, and now by a metachromatic mucopolysaccharide (Sidman, 1958).

When the pigment epithelium at the back of the iris is examined, there is nothing particularly noteworthy with respect to the present

discussion, except for the extremely important fact that in development it gives rise to the sphincter and dilator pupillae muscles. The importance of this is twofold. First, the sphincter pupillae may be directly sensitive to light, as it is in the elasmobranch fishes (Young, 1933), while the dilator is usually controlled by nerves or hormones. Secondly, these muscles are antagonists; in higher animals the dilator contracts in response to adrenaline, while the sphincter is activated by acetylcholine. In these muscles, therefore, there is evidence for both light sensitivity and for mutual antagonism between two classes of cells. A somewhat similar antagonism, together with photomechanical responses, is found in the rods and cones of certain species. In the catfish (*Ameiurus*) for example, both the rods and the cones change their shape and position in response to light (Detwiler, 1943). The cones shorten and move nearer the lens, while the rods lengthen and move away from the lens. In the frog, adrenaline (like light) causes the cones to contract and the pigment in the pigment epithelium to extend, but it appears to have no action on the rods.

These comments bring us to the retina proper, which is the part of the optic vesicle that behaves much more like the neuroectoderm elsewhere, in that it does not remain as a single layer of cells but buds off first ganglion cells, and then bipolar cells, horizontal cells and amacrine cells, away from the cavity of the neural tube (i.e., the optic vesicle). The cells of Müller's fibres also migrate inwards though still maintaining contact with the original surface. Obviously the nature of these derived cells must depend at least in part upon the nature of the cells in the original epithelium from which they take origin. The layer of cells which remains in contact with the lining of the optic vesicle, and thus corresponding to the ependymal cells surrounding the neural tube elsewhere, is of course the layer of rods and cones. It is tempting therefore to think that these two groups of cells may still reflect something of the duality which we have assumed to be inherent in the original epithelium. We have seen that in the other derivatives of this layer

there is evidence for something of the original duality and mutual antagonism. It might be expected therefore that rods and cones might in consequence show similar features and (1) be concerned with the maintenance of ionic equilibrium (their most primitive function) possibly by being oppositely polarized and transferring substances in opposite directions; (2) be capable of photomechanical responses in opposite directions; (3) store vitamin A and/or carotenoids; (4) store or eject mucopolysaccharides; (5) be adrenergic or cholinergic; and (6) have cilia, flagella and/or microvilli. Certainly the rod and cone cells do show many of these properties, and can often be distinguished from each other along these lines. Perhaps the most spectacular example is the mutual antagonism of the rods and cones in the catfish, which has already been mentioned, and in which light causes movement of the two in opposite directions. Incidentally, the mechanism whereby the myoids of the rods lengthen in response to light is a matter of some interest. How does light inhibit these rods? Directly or indirectly?

Table I

SOME PROPERTIES OF RODS AND CONES

<i>Rod</i>	<i>Cone</i>
Process derived from flagellum by vesicle formation	Process derived from flagellum by formation of flattened macrovilli
Oil globules not generally present	May store oil globules May store carotenoids (e.g. macular pigment)
Polysaccharides not present, except in certain amphibia	May store polysaccharides, e.g. in parabolooids
May lengthen in light	May contract in light May contract with adrenaline, e.g. frog
Nucleus with several nucleoli	Nucleus with single large nucleolus
Quickly damaged by iodoacetate	Relatively insensitive to iodoacetate
Synaptic knob	Synaptic foot
Contains rod "opsin"	Contains cone "opsin"
Refractive index 1.41	Refractive index 1.38

Some of the properties of the rods and cones relevant to this discussion are shown in Table I, and it is quite clear that we are deal-

ing with two different sorts of cells and that the differences between them run much deeper than the simple traditional belief that cones have a high threshold and mediate colour vision, while the rods are for colourless vision in dim light.

Ionic equilibrium may still be one of their major functions, for it is interesting to note that when cultures are made of retinal tissues some groups of cells arrange themselves around fluid filled vesicles (rosettes) while others may form solid clusters of rather large cells, so-called lentoids (Moscona, 1960). The nature of the cells taking part in these phenomena is unfortunately not yet clear, but it is interesting to note that when cultures are made of the choroid plexus, large vesicles are also formed (Lumsden, 1958). Curiously enough, in the latter, the cilia remain on the outside of the vesicle, whereas in growths of ependymal epithelium (Cameron, 1953) the cilia point inwards into vesicles and in the retinal rosettes there is also some suggestion of the presence of flagella pointing inwards into the vesicles. The direction of secretion in the tissue cultures of the choroid plexus is the same as that of the columnar layer of cells over the ciliary processes which produce the aqueous humour, that is, from the external (cerebrospinal) fluid to the "tissues". It must be remembered that during development the optic vesicle collapses and the fluid disappears from between the opposing layers except for minimal quantities of the mucopolysaccharide-containing fluid already mentioned (Sidman, 1958). This change of morphology may be brought about simply by change of shape and method of packing of the cells concerned. Nevertheless, most of the fluid disappears somewhere and, in any case, maintenance of the proper composition of the remaining fluid, which bathes the photosensitive receptors, might seem to be a very important requirement for their exceptionally high sensitivity and stability throughout life. The balancing action of rods against cones on one side of the cavity and of two sorts of pigment cells on the other could be a very efficient way of achieving this end.

Since the rods and cones, as the surviving members of the original

neuroepithelium, are thus so manifestly different from each other, it is logical to look for a similar differentiation into two corresponding classes among the cells of the other groups to which the neuroectoderm has given rise in the retina.

Excluding minor morphological variations of unknown significance, there are at least ten different groups of cells in the retina in addition to the rods and cones. These include at least two types of ganglion cells, probably three main classes of bipolar cells, the horizontal cells, amacrine cells, and the cells of Müller's fibres and some glia cells, all of which probably spring from the neural ectoderm. Finally, there are the microglia which seem to have the properties of macrophages and may have a different origin.

The problem would seem to resolve itself into the discovery of which retinal cells, in emerging from the primary neuroepithelium, have been derived from the same stock as the rods and which from the cone stock. With the answer to this problem will go that of another major question; namely, in the transmission of information along a neuronal chain does "like" stimulate "like" or do the neurones of different origin alternate? If the latter occurs and stimulation follows a chain of alternating neurones, does inhibition result when "like" plays on "like"?

Nerve cells can presumably be considered as extremely elongated epithelial cells, and, since the latter must inevitably have possessed a definite polarity, because epithelia normally separate two different media, the derived nerve cells may well retain this original polarity. Cells migrating directly out of a columnar epithelium to form secondary layers probably retain their original polarity. If, however, the second layer arises by mitosis from the first layer a change of polarity of its cells is quite likely. In the retina it is not known what happens, though Glücksmann (1940) has shown that in the tadpole's eye 82 per cent of the mitoses in the retinal neuroepithelium are orientated with their axes parallel to the surface, so that the daughter cells might be expected to maintain their original polarity. In the other 18 per cent the axes are at right angles to the

surface, so that one daughter cell moves away from the epithelium and is likely to have the opposite polarity to the one which is left behind. It is also known, however, that considerable degeneration of cells goes on in the retina during development, though it is not known which cells are involved. Little can therefore be said with certainty about the polarity of the cells in the inner retinal layers. In the organization of the cells of the retina, then, each bipolar cell theoretically arises either from rod stock or from cone stock, and in addition, could be orientated as in the original epithelium or in the reverse way. If the cell was originally polarized, then presumably its two ends would continue to show different properties. Since the nerve tissues elsewhere are produced in much the same way as the retina these are, of course, problems which are not confined to the retina, but must apply to the organization of all neural tissues.

In the retina, the bipolar cells are broadly divisible into those with a restricted and flat dendritic tree, which Cajal described as the cone bipolars, and those with a more branching and "vertical" dendritic tree, the rod bipolar of Cajal (1904), whose axons make direct contact with the cell bodies of the ganglion cells while the cone types relay on to the dendrites of the ganglion cells. The so-called midget bipolar (Polyak, 1940) is probably a specialization of the flat-top cone bipolar but with an even more restricted dendritic tree. There are certainly several forms of nuclei in the bipolar cell layer but it is not known which nucleus belongs to which Cajal type (Villegas, 1960).

It is thus not very difficult to make out an *a priori* case for the duality of the rod and cone layer being continued into the bipolar layer, though not enough is at present known about the properties of the various groups of cells for it to be possible to say whether or not the cone cell transmits through a bipolar derived from a rod-forming cell of the neuroectoderm while the rod transmits through the derivative of the cone-forming cell or whether like plays on like and inhibits the unlike.

On the basis of morphological studies, again there are certainly

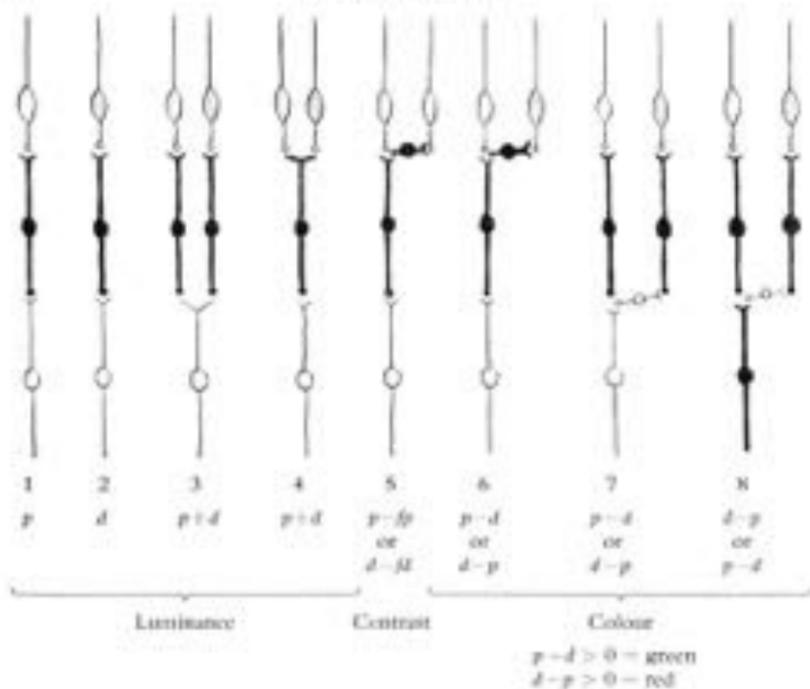
two main types of ganglion cells with many variations. First, there are those with large nuclei containing a single large nucleolus; these cells have extensive and "chunky" Nissl's granules in their abundant cytoplasm, and possess widely branching dendrites. There are also the smaller and more numerous cells, with more restricted dendritic trees, more diffuse Nissl's substance, less cytoplasm, and nuclei with less distinct nucleoli. Incidentally, it seems probable that most of the electric recording from ganglion cells has been carried out on the large type and may thus give a somewhat distorted view of events in the retina.

If the hypothesis which has been outlined is essentially correct and the neural retina is indeed composed of cells derived from an epithelium in which there were originally two main types of cells, the problem of tracing that duality among the various types of cells becomes of cardinal importance, not only with respect to retinal histology but to neurology in general, and to colour vision in particular.

If a potential rod cell leaves the primary epithelium to become a bipolar cell, how does it orientate? Is it stimulated only by a rod? Does it relay only on to a ganglion cell of the same origin, or does it only relay from a cone and pass its information on to a ganglion cell of the cone class? In other words, if nerve cells in a chain alternate, do they do so for purposes of stimulation or inhibition? The hypothesis of antagonism between the original parents suggests that spontaneous activity in one should stimulate the other to opposing activity, because if activity in one suppressed activity in the other, then the *status quo* would be upset rather than preserved. If, however, activity is induced by change of external conditions and the cell responds in such a way as to put those conditions right, then this would be achieved more quickly and efficiently if the opposing cell were inhibited. Whichever arrangement actually exists, it is probable that activation results from one arrangement and inhibition from the other. Similarly, it is necessary to know to which class of cells the horizontal and amacrine cells respectively belong.

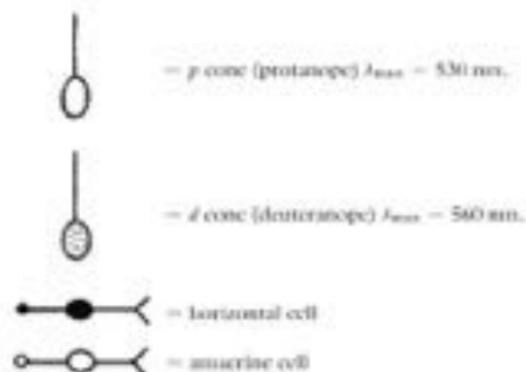
There is a great deal of evidence from direct recording in the retina by Granit and his school (Granit, 1947), by Kuffler and others (e.g. Kuffler, Barlow and Fitzhugh, 1957), by MacNichol and Svaetichin (1958) and also from recording in the lateral geniculate body by De Valois (1960) and others, that summation and inhibition go on freely in the retina, and it would seem that the idea of two opposing families of cells could provide exactly the sort of interaction which is being shown to occur.

For example, there is good evidence that in the colour vision of the human central fovea the information from both the *p* (green) receptors and the *d* (red) receptors can be pooled for purposes of assessing luminance, while for colour analysis the one must antagonize the other so that only the unsuppressed information initiated by the more actively stimulated cone is transmitted (Willmer, 1955). If, for example, the arrangement of cells were as shown in the diagram (Fig. 2) and the amacrine cells (or horizontal cells, if present) acted as cross-links, then activity in the various ganglion cells could signal brightness, greenness, or redness, as indicated on the figure. Several alternative schemes to this one could also be envisaged and it remains to be seen how far future research can identify and define the synaptic and cellular activities and allow a definitive diagram to be drawn. It is perhaps a clue that in birds, at least, cholinesterase is associated with the amacrine cell to ganglion cell junctions (Francis, 1953), suggesting that amacrine cells liberate acetylcholine and, if this is inhibitory, the foveal ganglion cells are not cholinergic, and therefore on the proposed scheme are probably of cone stock (the cones in frogs are activated by adrenaline). Alternatively, the direct path may be inhibitory, and excitation of the ganglion cells may be conveyed through the amacrine cells, in which case the ganglion cells would be of rod stock. Complex and obscure as the organization of the central fovea is, it is probably one of the simplest and most accessible of neural mechanisms, and the position for the whole eye is presumably much more complex than that of the foveal centre only; rods,



In arrangements 5-8 the types of cone may be reversed, yielding the alternatives shown.

FIG. 2. Some possible pathways involving summation and inhibition in the human central fovea, and the information which they could provide.



horizontal cells and further classes of both ganglion and bipolar cells are all added to the mechanism; perhaps "blue" cones also make their contribution.

SUMMARY

The argument which has been developed is based on the assumption of a fundamental duality of the primary neuroectoderm, a duality initially imposed upon it by the necessity of preserving ionic and fluid balance, first, between the external and internal environments and, later, between the fluid in the neural canal and optic vesicle on the one hand and the tissue fluids and blood on the other. If the constituent cells are thus primitively divided into two mutually opposing stocks, it means that the retinal cells which are derived from them may still retain traces of this inherent antagonism and be divided into two distinct stocks whose activities would probably continue to oppose each other. On these assumptions, the essential problems of retinal physiology are therefore to define which type of cell belongs to which stock and to determine the orientation or polarity of each cell group. If these problems can be solved, the respective stimulatory or inhibitory effects of the various cells may then be elucidated, and perhaps the mechanism of colour vision will then emerge on a solid physiological foundation.

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DISCUSSION

Lowenstein: Is it in fact true that the morphological differences between the rods and cones are more pronounced in more primitive eyes than in more highly sophisticated eyes?

Willmer: It is very difficult to know exactly what one means by primitive and highly organized. Fish, for example, could be as highly organized as we are. By a more primitive eye I merely meant an eye which has not in fact developed very far along the evolutionary pathway. If it belonged to an animal where the external environment was still having a big influence on the internal environment, you might expect that the rod and cone difference would be great. As the internal environment became more and more constant, you would expect the difference to get less.

Crescitelli: The cyclostome eye is of interest here. Not many people have studied this eye, but Gordon Walls did at one time. He saw two types of cells but he did not call them rods and cones; he called them

short cells and long cells, and tried to argue that one was the rod-type and the other was the cone-type, but it did not come out very convincingly. Walls studied the adult cyclostome. What would be interesting is the examination of the ammocoete larva of the cyclostomes, to see what kind of visual cells they have.

Pedler: The lamprey is interesting because the first person to work on it was Heinrich Müller. He wrote two papers on the visual cells of the lamprey and changed his mind completely between the two. In the first he called the short receptors cones and the long receptors rods, and in a paper five years later he put them the other way round. But I think that it is fruitless to attempt this sort of differentiation at this level. The most likely explanation in the primitive vertebrate eye is that the inner segments are globular and the outer segments are quite narrow and cylindrical. Now, if the animal had found it necessary to align its outer segments fairly close together, he could not get them closer together than the width of one inner segment, so he staggered the receptors; therefore one is short and the other is long, in order, if you like, to provide a retinal mosaic with a higher resolving power.

Tansley: Or it might be in order to accommodate more visual cells and so obtain higher sensitivity.

Cresitelli: Dr. Willmer, how do you fit the invertebrate eye, which has a different embryological origin, into your system? Biochemically it fits very well; the invertebrate eye has the retinene₁ system, and you can do a lot of things with it that you can do with the vertebrate eye. Is there one type of invertebrate visual cell, and what relation has it to the vertebrate visual cells?

Willmer: As far as I know there is mainly one type of visual cell in the eyes of invertebrates and its origin is rather different from that of the vertebrate cells. It really adds point to my story that vertebrate eyes have all been derived from the one, particular, neural structure, where there were originally two sorts of cells, and these two sorts of cells have persisted. Invertebrate eyes, on the other hand, have not all developed in the same way; I would not really make the comparison.

Pudgham: Dr. Willmer, what exactly do you mean by rods and cones opposing one another to maintain the stability of their environment? Stability of what?

Willmer: Let me first say why I am interested in this problem at all

in this sense. I have recently been working with an amoeba which changes from a flagellated form to an amoeboid form and back. It changes into the flagellated form when the fluid surrounding it is diluted, and it reverts to the amoeboid form when the medium is concentrated. If you consider a sponge blastula which has flagellated cells at one end and phagocytic cells at the other, its cavity remains stable, and I have a pretty good hunch that that stability is brought about by the relative activity of the two halves of the blastula. If it was not like that, the blastula would either behave like the isolated choroid plexus, which when put in an artificial medium blows right up into vesicles, or like the proximal tubules of the kidney, which in the same conditions of tissue culture also distend their cavities enormously, or like the distal tubules which collapse. In other words, the stability which I was discussing may be concerned with the passage of water across the cell, or it may be with the passage of ions and other metabolites across the cell. In the case of the amoeba, it is ions.

Lowenstein: Dr. Willmer, are we to understand that the light-sensitive elements are either flagellate cells or goblet-cell-shaped in origin?

Willmer: It looks as though the two sensitive elements might be related to microvilli on the one hand or to flagella on the other. In the choroid plexus, the cells have cilia and microvilli, or microvilli only. Now, there is no doubt at all that the rod comes from a cilium or flagellum by a process of vesiculation, whereas the cone seems to grow by the covering membrane of the flagellum forming what could be considered as broad and flattened microvilli.

Sjöstrand: In the electron microscope, rods and cones appear to develop in exactly the same way, by invagination of the plasma membrane. There are no vesicles that can be correlated with the formation of the structure.

Willmer: But the nature of the folding of the outer membrane is surely quite different in the two cases.

Sjöstrand: No; it is the same. You cannot distinguish a developing cone from a developing rod, except on indirect evidence.

Klüver: It has been reported that pigment migration may occur in the absence of changes in illumination or even in total darkness. I wonder whether in the case of so-called photomechanical responses we are not often dealing with hormonal factors that have been ignored?

Willmer: I think that is very likely, and that what is going on at the synapse may be the production of hormonal substances like adrenaline or acetylcholine. The frog's response to adrenaline (and/or light) that S. R. Detwiler spoke of ([1943]. *Vertebrate Photoreceptors*. New York: Macmillan) was a response both of the cone layer and of the pigment cell layer.

Poller: In our survey of vertebrate species we have found that the cilium is much more apparent in the "rod" type of cell. But where I would part company with you, Dr. Willmer, is where you try to divide up visual function. I am prepared to accept that there have been two phylogenetic roots, but where I would not agree with you is that this has anything to do with visual function now. The duplex nature of receptor morphology is entirely to do with this phylogenetic root; it is not necessarily connected with function.

Willmer: This is rather what I wanted to say. Features like the paraboloid, and whether the mucoprotein collects inside or outside the cell, should, I believe, be related to ionic movements or cellular metabolism, and they may have nothing particularly to do with vision.

Rushton: I would like to emphasize the point you made about vision, because although a great deal has been said about the antagonistic function of rods and cones I do not know a single instance of it. Stiles' work, and particularly the paper of Aguilar and Stiles (Aguilar, M., and Stiles, W. S. [1954]. *Optica Acta*, 1, 59) where the function of the rods was traced right up to the intensity at which they saturate, shows a complete independence, as far as increment threshold measurements are concerned, between the rods and cones. We know that the rods and cones can add their function, because they can both contribute to brightness at mesopic levels, but can you think of a single example where the functional *antagonism* of rods and cones is seen?

Willmer: Before hearing of Dr. Marks' results I had thought that an antagonism culminated in blue vision.

Pickford: The antagonism of red and green vision is very marked in a great many psychological experiments; but could it be linked with the physiological and morphological contrast between rods and cones? And could the blue and yellow contrast be due to rod and cone antagonism?

Rushton: I believe that there is no antagonism between rods and cones.

Crescitelli: Dr. Willmer made one point that has interested me and I think is still true, that you can differentiate a rod and a cone by the opposing photomechanical actions of the myoids. It is true that there are rods that do not have such photomechanical action and there are cones that do not have it. This may be simply because they have lost this function in the course of evolution. But are there any examples where this is not true, where the rods and cones move in the same direction? I have never seen an example of this, and one definition of rods and cones might then be in terms of this photomechanical action.

Weale: Dr. Willmer, would you agree that the photochromatic interval in the fovea for long wavelengths is zero? In other words, we see red light when we see. From that point of view, one would question whether any marked differential comes into this story. The second point, that there is no significant difference between rods and cones, is one that I have defended for a long time.

You pointed out that the melanin content of the fundi of various species can be correlated with dominant rod and cone function respectively. Of the few fundi that I have examined, there are dark fundi in frogs, monkeys, and the cat, where there is no tapetum, and I think functionally these eyes would not be classed as similar; the pigeon and man can also be grouped together; if you look at a negroid fundus, you would not say it was the same as the average fundus found in this room. I question whether the division put forward on those grounds can be defended.

Willmer: I would not like to press the division of the pigment layer very far, except to draw your attention to the fact that it is not by any means uniform. One can find, in the same eye, batches of cells which are heavily pigmented next to batches which are unpigmented. What the significance is there, I do not know at all. With regard to the photochromatic interval, both red and violet can be seen as such at threshold level, but that can conceivably be got over on a different basis. There are, after all, two types of receptors present, so that if one of these types is not signalling anything and the other is responding actively, the difference (recorded by a cell in the next layer) is likely to give rise to strong colour effects. If a and b are the responses from the two types of visual cell and if when $a = b$ there is no sensation of colour, then when $a > b$ there would be a sensation of colour even if $b = 0$.

Monnier: If there is antagonism between rods and cones it should be regulated neurohumorally. You said that adrenaline contracts the cones: have you further data on a possible neurohumoral antagonism and on other substances which would act at this level?

Willmer: There are very few data, but I think that that is a line along which one might expect to derive much information, except that the cell processes are probably so intermingled that it will be extremely difficult to separate them neurohistologically.

THE SYNAPTOLOGY OF THE RETINA

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OUR studies of the retina are concerned with two different aspects of retinal structure. On the one hand we are interested in analysing the structure of the receptor cells at the highest possible resolution that can be obtained, and on the other hand we are intrigued by the possibilities that electron microscopy offers with respect to an analysis of the synaptology of the retina.

MOLECULAR STRUCTURE OF THE OUTER SEGMENT DISKS

In the first case we want to record those structural features that can give clues to the molecular organization of the disk structure in the outer segments. These disks must be of prime importance for the excitation of the receptor cells by light.

In the first study revealing the disk structure of the outer segments (Sjöstrand, 1949), each disk was shown to consist of two membranes or "membrane elements". This observation, which was made on fragmented outer segments, was confirmed by an analysis of ultra-thin sections through the retina (Sjöstrand, 1953*a, b*). In this latter case, each disk appeared as a "double membrane" disk with two opaque layers separated by a light interspace. With techniques allowing a more complete staining of the disk structure, it was possible to demonstrate a five-layered structure of the disks—three opaque layers were separated by two light layers, as shown in Fig. 1 (Moody and Robertson, 1960; Sjöstrand, 1960). The middle opaque layer can appear split in the middle and the existence of two membrane elements then becomes apparent. Such membrane elements showed a triple-layered structure, and

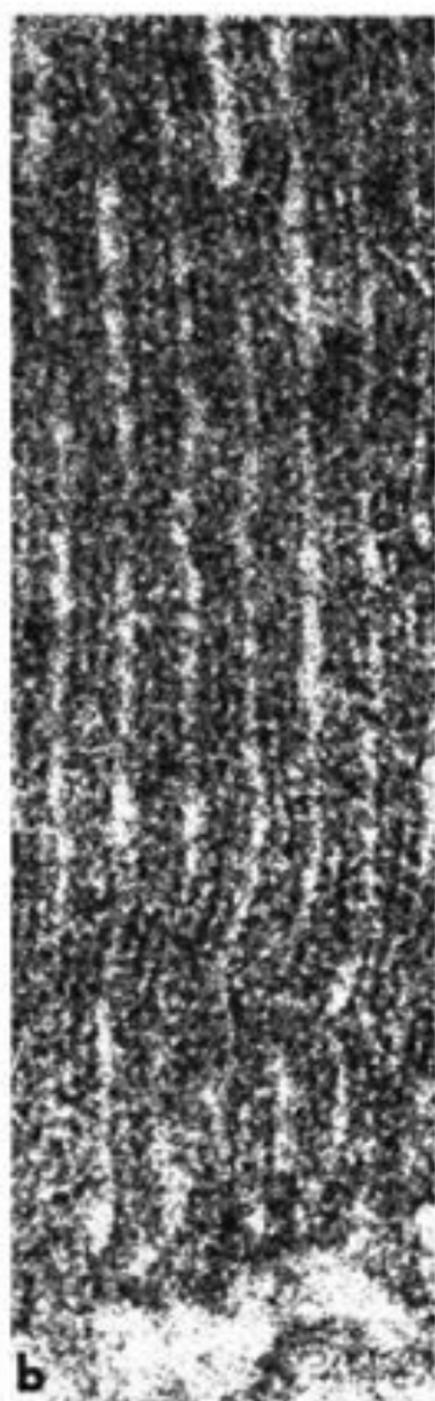
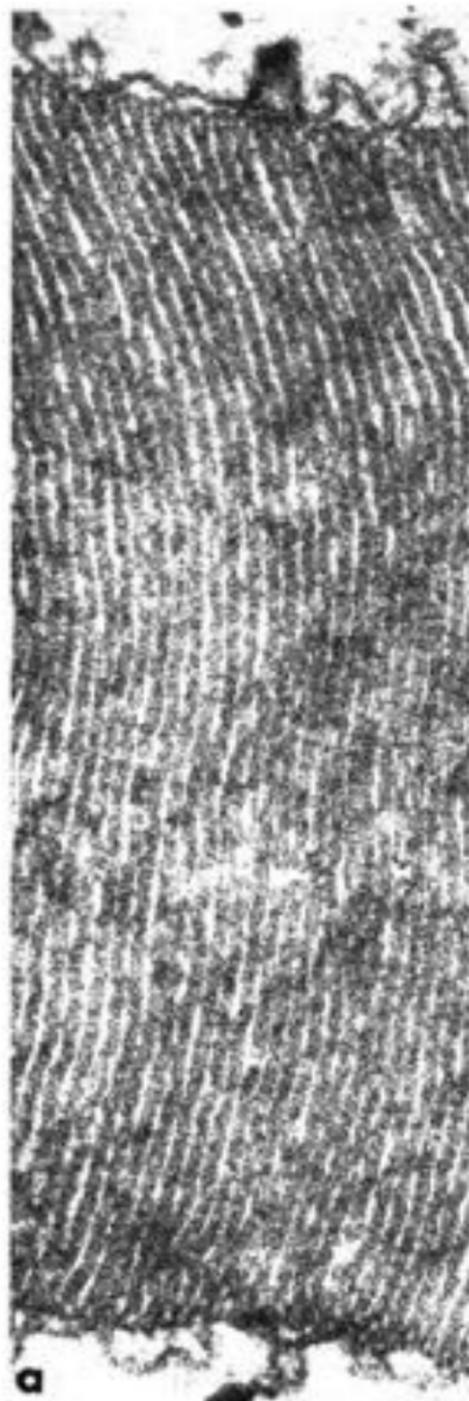


FIG. 1a and 1b. Different magnifications of cross sections through outer segment disks in the rod cells of the rabbit retina showing the five-layered structure of each disk. Magnifications: 1a: $\times 120,000$; 1b: $\times 400,000$. (From Sjöstrand and Nilsson, 1964.)

the five-layered disk structure appeared when the two triple-layered membrane elements were closely packed, causing a fusion of two opaque layers in the middle of the disk.

The triple-layered appearance of the membrane element of the disks depended on the staining of an additional layer of the membrane element which had remained unstained in the osmium-fixed, unstained material investigated earlier. The interpretation of the disk as a composite multilayered structure (Sjöstrand, 1949, 1953*b*) was supported by these observations. The thickness of the disks in the guinea pig retina was identical when the triple-layered disks observed in osmium-fixed, unstained specimens were compared with the five-layered disks seen in potassium permanganate-fixed or osmium-fixed, stained material. This fact made it obvious that the new stained layer was located in the middle of what appeared as a light middle layer in the material investigated previously.

The triple-layered pattern of the two membrane elements of the disks was interpreted to mean that each membrane element consists of a bimolecular leaflet of lipid molecules sandwiched between two layers of protein (Sjöstrand, 1960), in agreement with the Danielli-Davson model for the plasma membrane, an interpretation that Robertson (1957) had proposed for the corresponding pattern observed in the plasma membrane as well as in other cytoplasmic membranes.

A more complex membrane structure has been indicated in recent studies of the membrane elements in mitochondria and of cytoplasmic membranes (Sjöstrand, 1963*a, b*). The two opaque layers appeared connected by opaque septa extending across the light middle layer. The membrane element, therefore, appeared split up into small subunits. This pattern was interpreted to reveal the existence of a globular subunit of the membrane elements (Sjöstrand, 1963*a, b*).

A similar substructure has recently been observed in the membrane elements of the outer segments of retinal receptor cells (Fig. 2) in our laboratory by Dr. S. E. Nilsson (1964*a, c*). On the other

hand, no such structure could be observed with certainty in the plasma membrane, even when the plasma membrane was located side by side with cytoplasmic membranes and mitochondrial membranous elements (Sjöstrand, 1963*c*). A similar substructure,

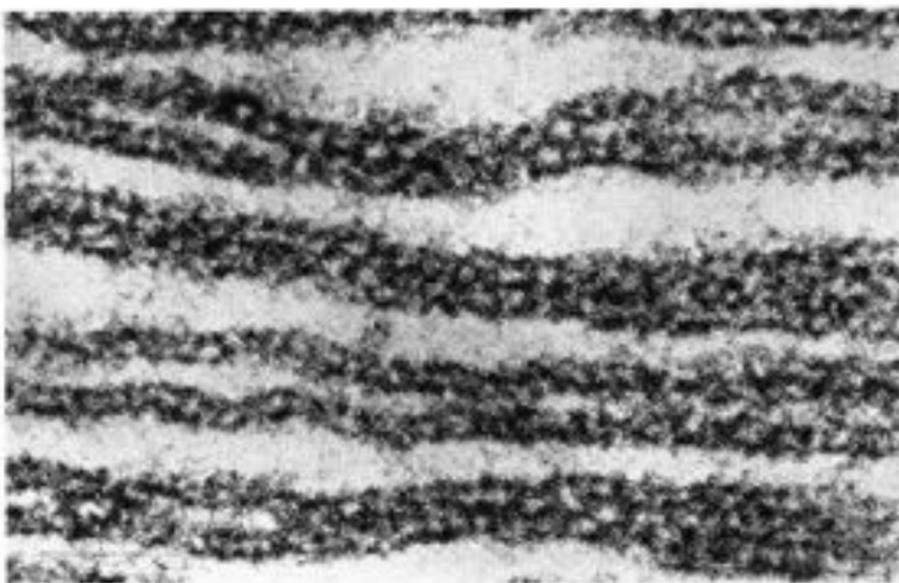


FIG. 2. Globular substructure of the two membrane elements of the outer segment disks. These two membrane elements are here partially separated by a space of varying width which is due to an artificial swelling of the disks. The globular substructure is easier to observe in the individual membrane elements of swollen disks although its presence is independent of such separation of the two membrane elements. $\times 800,000$. (From Nilsson, 1964*b*.)

although more geometrically regular, appeared also in frozen-dried material (Sjöstrand, 1963*a*; Sjöstrand and Elfvin, 1964). The fact that this structural feature could be observed in material prepared in such different ways made it appear reasonable to propose that this substructure was real and not an artefact introduced by the fixation and embedding techniques. The latter possibility cannot, however, be ruled out at present. If this pattern were due to an artefact produced in lipoprotein membranes, it appears

strange that this distortion does not appear in the plasma membrane.

Assuming that this structure is real, it can be interpreted as showing that the lipids and proteins of the membrane elements are arranged in small globular or cylindrical units (Fig. 3). Lipid molecules in the centre of the globule would be surrounded by protein molecules. In the outer segment disks the opsin of the rhodopsin molecules would then be located in the peripheral protein shell and the retinene would extend into the lipid central part of these globules. Such globules containing rhodopsin could

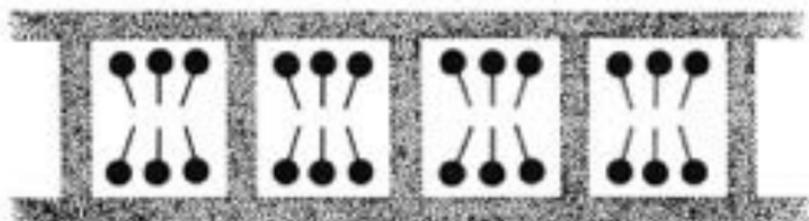


FIG. 3. A simple schematic presentation of a possible interpretation of the molecular arrangement in the individual membrane elements, with each globule consisting of a lipid core surrounded by a protein shell.

then represent the elementary structural unit of the outer segments and the primary excitatory reactions would take place in these units.

From an ultrastructural point of view there is a definite difference between rod and cone cells. In the cone cells the disks of the outer segment form a continuous structure and appear as invaginations of the part of the plasma membrane which covers the outer segment (Fig. 4). In the rod cells, on the other hand, the disks are free from the plasma membrane and possibly free-floating without any connexions between adjacent disks.

The thickness of the disks is identical in rod and cone cells, but the distance between the disks is larger in cone than in rod cells (Nilsson, 1965). In the double cones of the retina in the leopard frog eye the spacing of the disks was identical in the principal and the accessory member (Nilsson, 1965).

THE LATERAL CONNEXIONS BETWEEN RECEPTOR CELLS

The synaptology of the retina is studied in our laboratory with the intention of describing in as precise a way as possible the contact relations between the various retinal elements. Light microscopic studies have revealed a variety of neuronal elements in the retina,

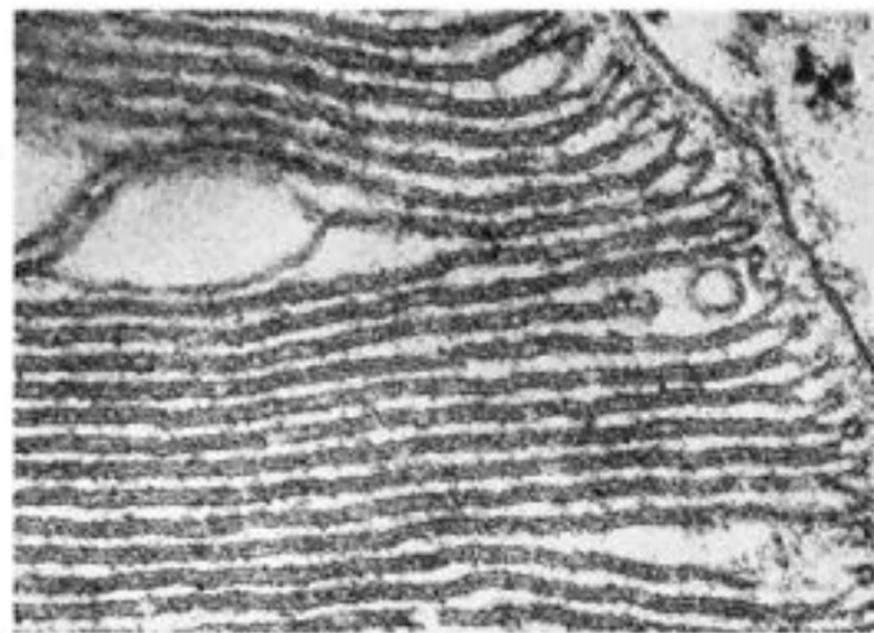


FIG. 4. The disks in the outer segment of a cone cell are connected at part of their circumference to form a continuous structure along the whole outer segment. Notice partial disintegration of one disk which results in the appearance of isolated vesicles. $\times 80,000$.

but the diagrams of retinal structure presented by light microscopists are composite schematic drawings in which these elements have been incorporated in an arbitrary manner. In contrast to such schemes we aim at working out a rather complete circuit diagram for the retina in which the number of elements, their morphological characteristics, their contact relationships with respect to the number of contacts as well as the surface area of each contact, are all represented.

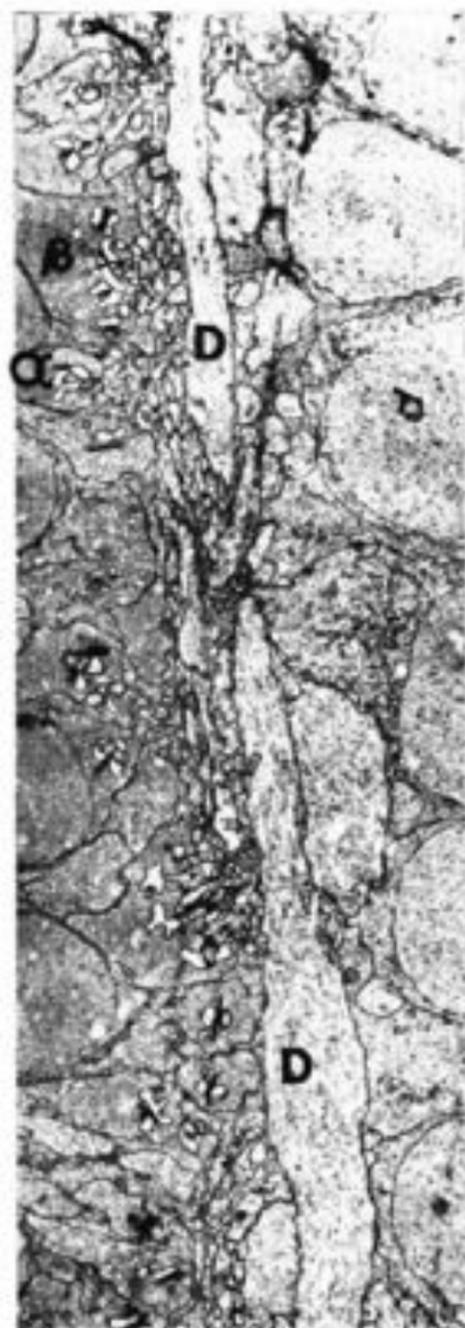
The construction of such a circuit diagram is important for understanding the function of the retina. However, the behaviour of the retinal circuits cannot be deduced directly from the circuit diagram, since we lack knowledge of the physical characteristics of the various retinal elements and of the various contacts. The electrophysiological analysis of the retina, on the other hand, can only give information referring to certain points in the circuit which are available for recording with this technique. Such points, if their position is known, can be considered as test points in the circuit. If the behaviour of the circuit is known at a sufficient number of test points, it seems plausible to deduce a limited number of alternatives with respect to the function of the circuit.

It seems justifiable to assume that a detailed understanding of the function of nervous centres like the retina depends upon such combined information.

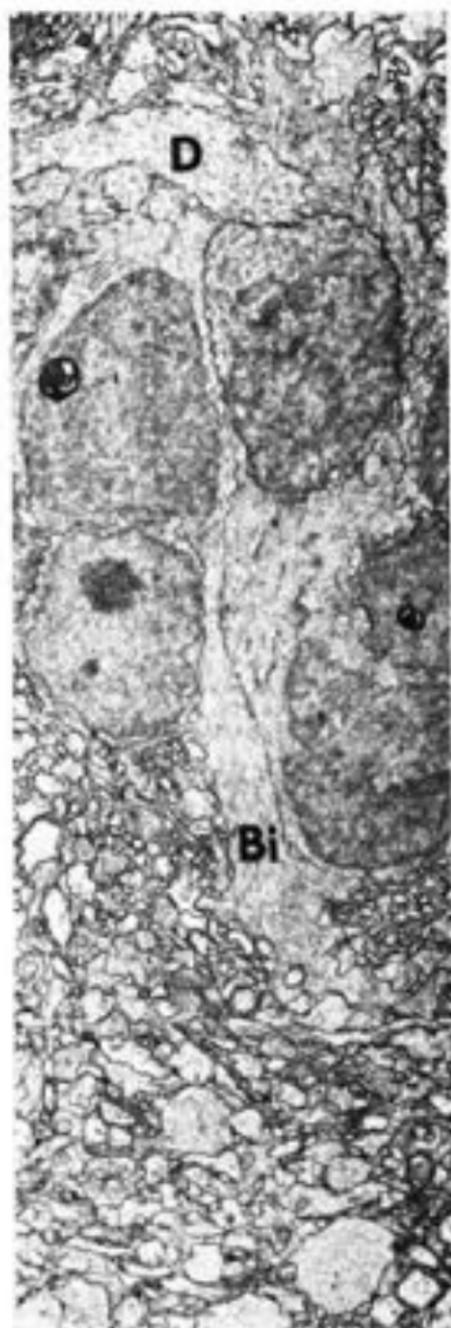
Our studies are so far fragmentary. This is explained by the technical difficulties encountered in such a study and the complexity of the synaptology of the retina (Figs. 5 and 6). The analysis is based on three-dimensional reconstructions from long series of sections. Series consisting of 100-300 sections are analysed by means of the electron microscope. Drawings on transparent cellophane are prepared from the electron micrographs and these drawings are then stacked on top of each other, allowing a three-dimensional representation of the synaptic connexions. Three-dimensional models are prepared either graphically or by means of plastic reconstructions from the stacks of sheets of cellophane.

The first observation that was made by applying this technique revealed lateral connexions between the receptor cells (Fig. 7) at the level of the outer plexiform layer (Sjöstrand, 1958). These interreceptor contacts were unknown until then. They appeared to furnish a structural background for at least certain phenomena involving lateral inhibition in the retina and were interpreted to play a rôle in contrast enhancement (Sjöstrand, 1958).

Further studies of these contacts (Sjöstrand and Mountford,



(a)



(b)

FIG. 5(a). Outer plexiform layer of the rabbit retina showing its limited thickness and the presence of processes (D) from horizontal cells. The two types of receptor cell synaptic bodies are seen, the small α -type and the large β -type. Receptor cell layer orientated to the left side of picture. $\times 5,000$. (From Sjöstrand and Nilsson, 1964.) (b). A bipolar cell (Bi) can be seen extending from the cell body down into the inner plexiform layer. $\times 5,500$. (From Sjöstrand and Nilsson, 1964.)

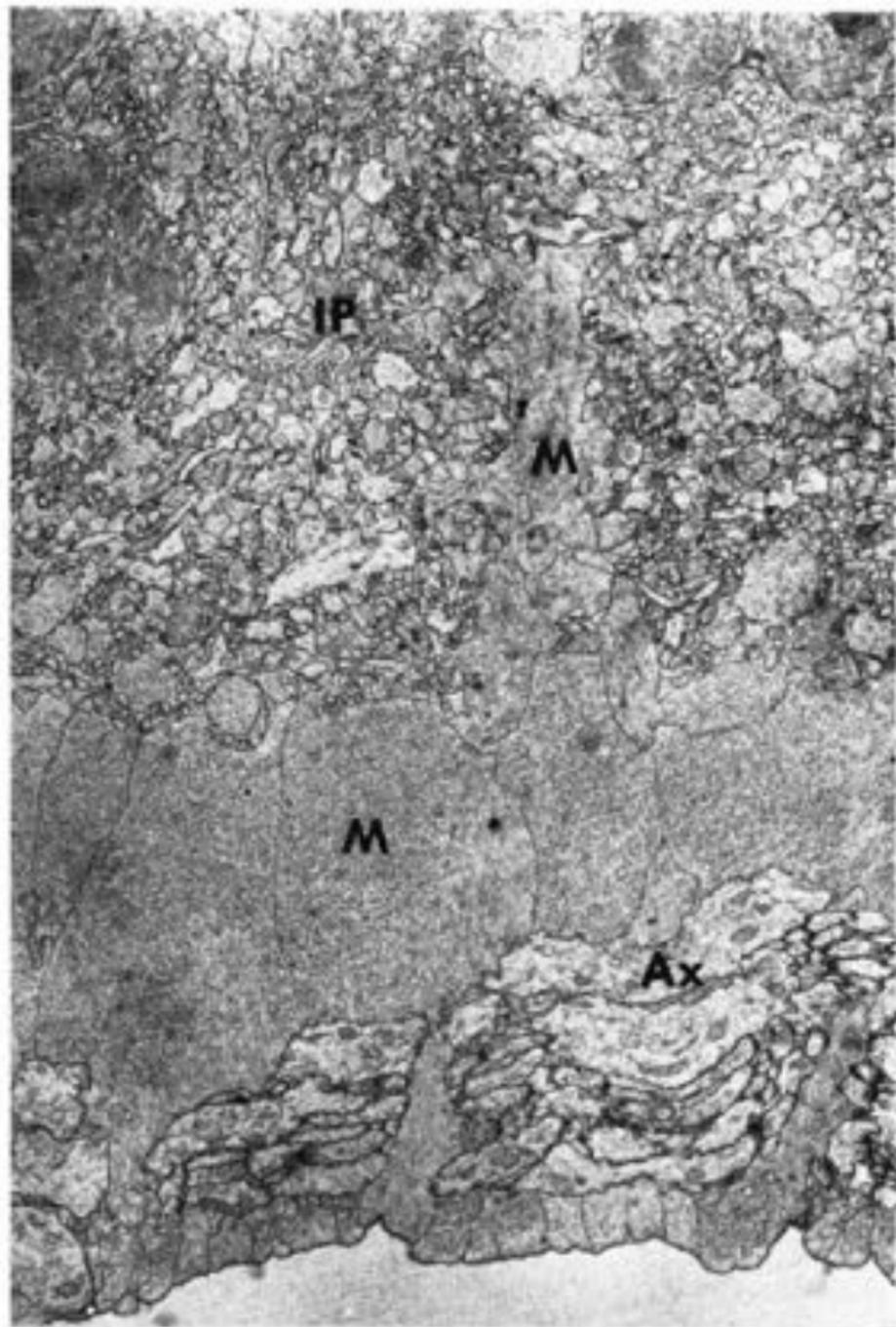


FIG. 6. The inner plexiform layer (IP) and the layer of the ganglion cell axons (Ax) in the rabbit retina. M = Müller's cells. $\times 5,000$. (From Sjöstrand and Nilsson, 1964.)

1965) have shown that we can distinguish between short and long processes extending laterally at the receptor cell level. The short processes end at adjacent receptor cells (Figs. 7 and 8), while the long processes (Fig. 9) pass by one to three rows of receptor cells before they end in contact with the synaptic body of a receptor cell. Long processes measuring 7-10 μ have been traced.



FIG. 7. Short lateral connexion (X) between a β -type receptor cell (β) and an α -type cell (α_1) in the guinea pig retina. PV_1 and PV_2 = proximal vacuoles; SR = synaptic ribbon; M = Müller's cell processes. $\times 26,000$. (From Sjöstrand, 1958.)

In the guinea pig retina these lateral processes extend only from the β -type receptor cells (Sjöstrand, 1958), that is, the receptor cells that end with a conically shaped body. They end at the surface of the synaptic bodies of both α - and β -type receptor cells. In the shape of their synaptic body these β -type cells exhibit one structural feature which is characteristic of cone cells. In other respects these receptor cells cannot be distinguished from the second type of

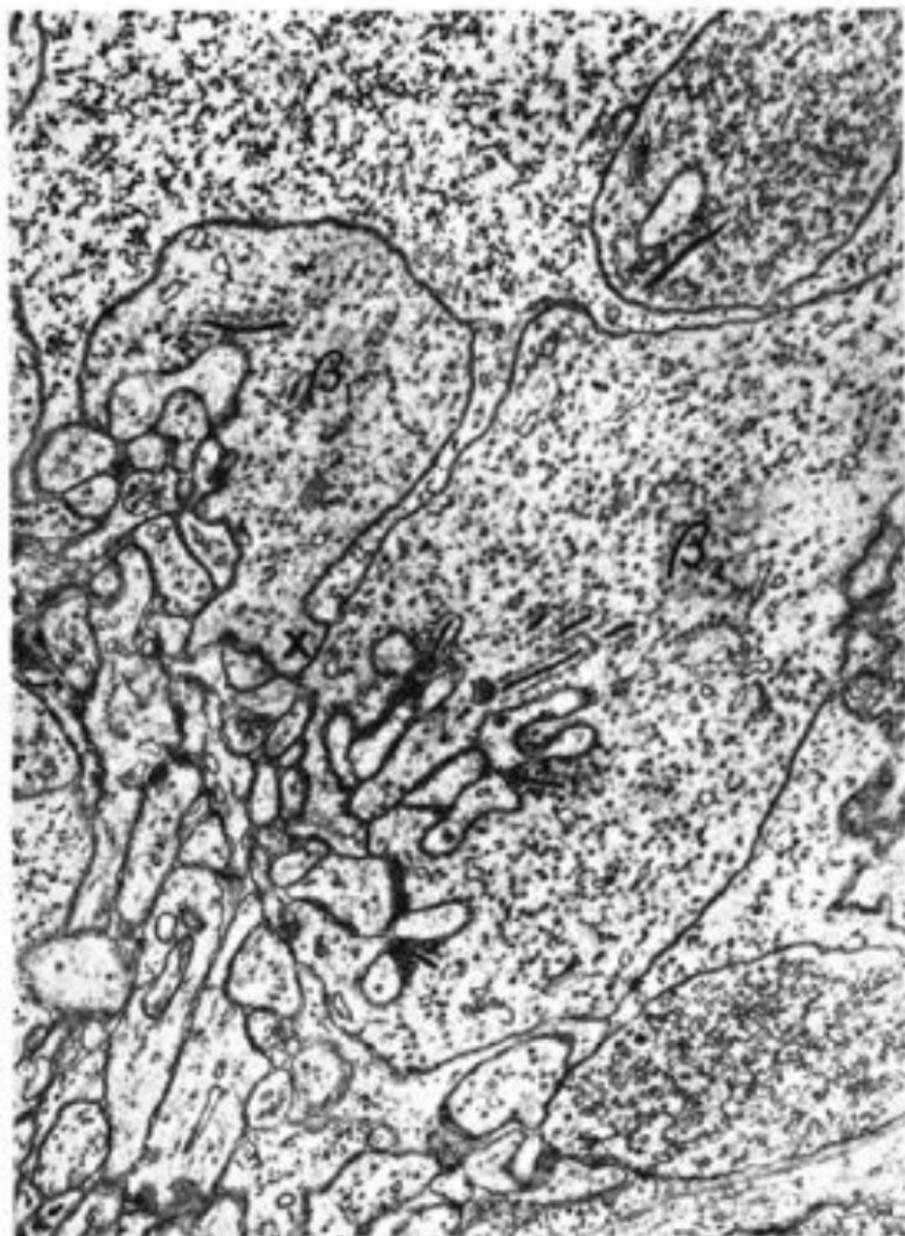


FIG. 8. Short lateral process (X) from β -type receptor cell (β_1) contacting an adjacent β -type cell (β_2) in the guinea pig retina. $\times 24,000$. (From Sjöstrand and Mountford, 1965.)



FIG. 9. Long lateral connexion (X) between two β -type receptor cells (β_1 and β_2) in the guinea pig retina. M—Müller's cell process. $\times 13,650$. (From Sjöstrand and Mountford, 1965).

receptor cells, the α -type cells. The synaptic body of these receptor cells is smaller and has an ovoid shape.

It appeared of interest to analyse further whether the short and long processes extending from the β -type receptor cells were arranged in a systematic way. Preliminary observations indicated that the long processes might be arranged to form a square or triangular network. If they were orientated in some preferred direction, this would mean that the interaction between receptor cells due to these connexions could be polarized. This could furnish a structural basis for the perception of movement, since the inhibitory or excitatory interaction between receptor cells would then be timed differently, depending on the direction in which a light stimulus moved over the retinal surface.

Extensive reconstructions involving as large areas of the retina as the present technique allows us to analyse with long series of sections have not yet given any conclusive support for any such systematic arrangement of the long interreceptor contacts (Fig. 10), although over a certain limited area the long processes from the β -type receptor cells appear to be preferentially orientated in one particular direction.

To do further work on this problem we must have access to an electron microscope which has been modified to allow a considerably larger range of movement of the specimen. At present the useful range is about 2 mm., which means that each section in a series of 100 sections cannot measure more than 0.02 mm. in width. With such a limited width of the sections the region of the retina that can be reconstructed becomes very limited. We are quite confident that this technical problem will be solved by the introduction of a specimen stage that allows scanning of an area of the specimen which measures 2×15 to 2×20 mm.

DIFFERENT TYPES OF SYNAPTIC CONNEXIONS OF RECEPTOR CELLS

The three-dimensional reconstruction of the synaptic bodies of the α -type receptor cells revealed four main patterns. In an earlier

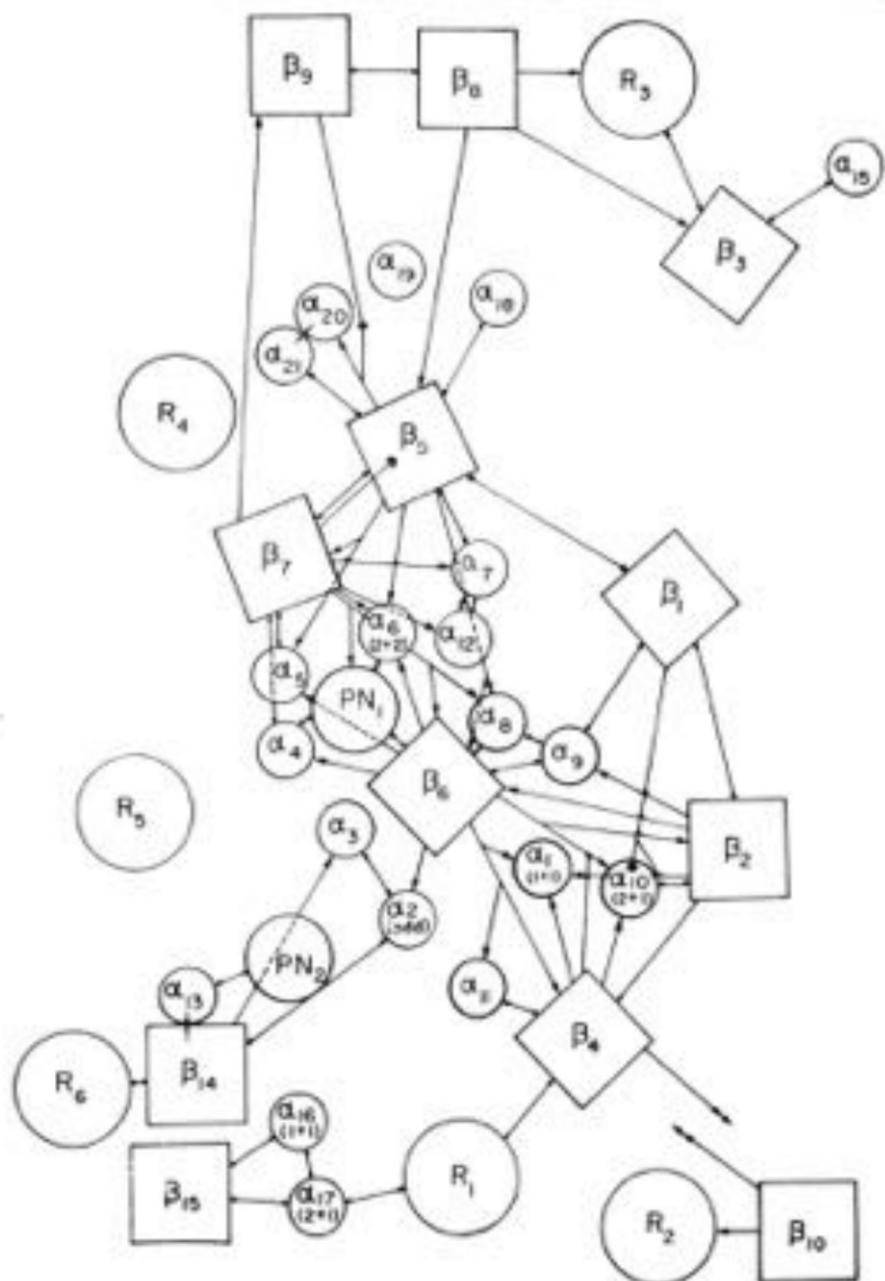


FIG. 10. Schematic presentation of lateral interreceptor contacts in a fair-sized area of the outer plexiform layer. Only the central part of the scheme is complete with respect to such connexions. (From Sjöstrand and Mountford, 1965.)

study (Sjöstrand, 1958) it was demonstrated that dendritic processes from the bipolar cells extend into an invagination of the plasma membrane covering the synaptic body (Figs. 11 and 12).

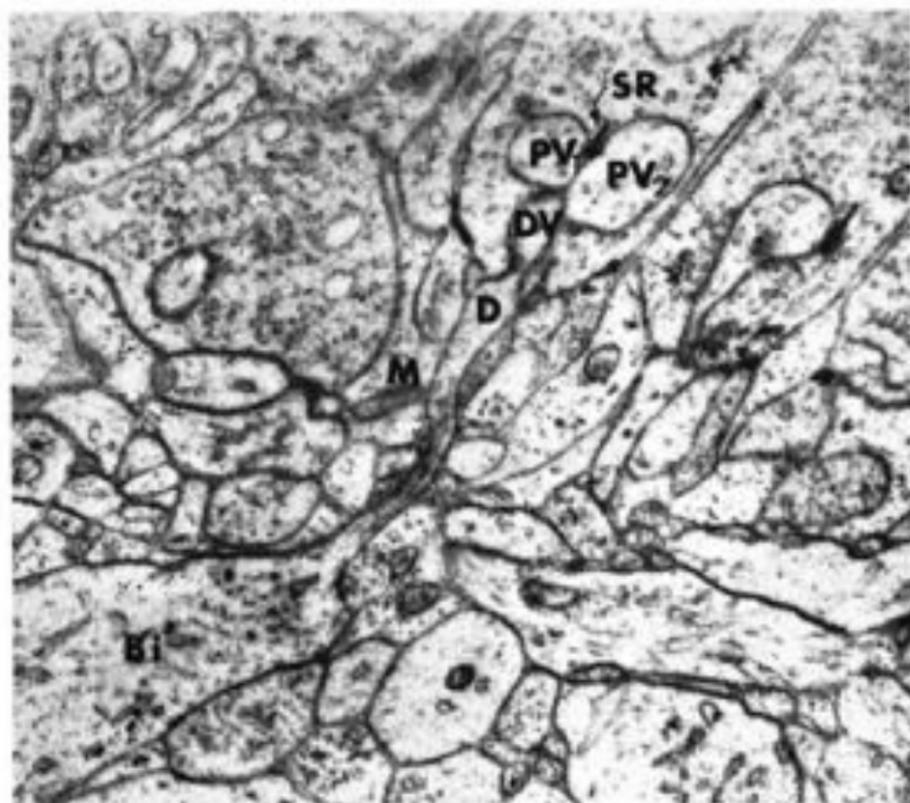


FIG. 11. Dendritic process (D) from a bipolar (Bi) all ending in the invagination of the plasma membrane of the vitreally orientated pole of an α -type receptor cell, the end of the process forming a distal vacuole (DV). PV₁ and PV₂ = proximal vacuoles; SR = synaptic ribbons; M = Müller's cell process. $\times 18,000$. (From Sjöstrand, 1958.)

Two such dendritic processes, very probably originating from different bipolar cells, formed a pair of dendritic end-branches extending into this invagination. At their ends these branches were in contact with large vacuoles located more sclerally in the synaptic body. In these early reconstructions these large vacuoles could be

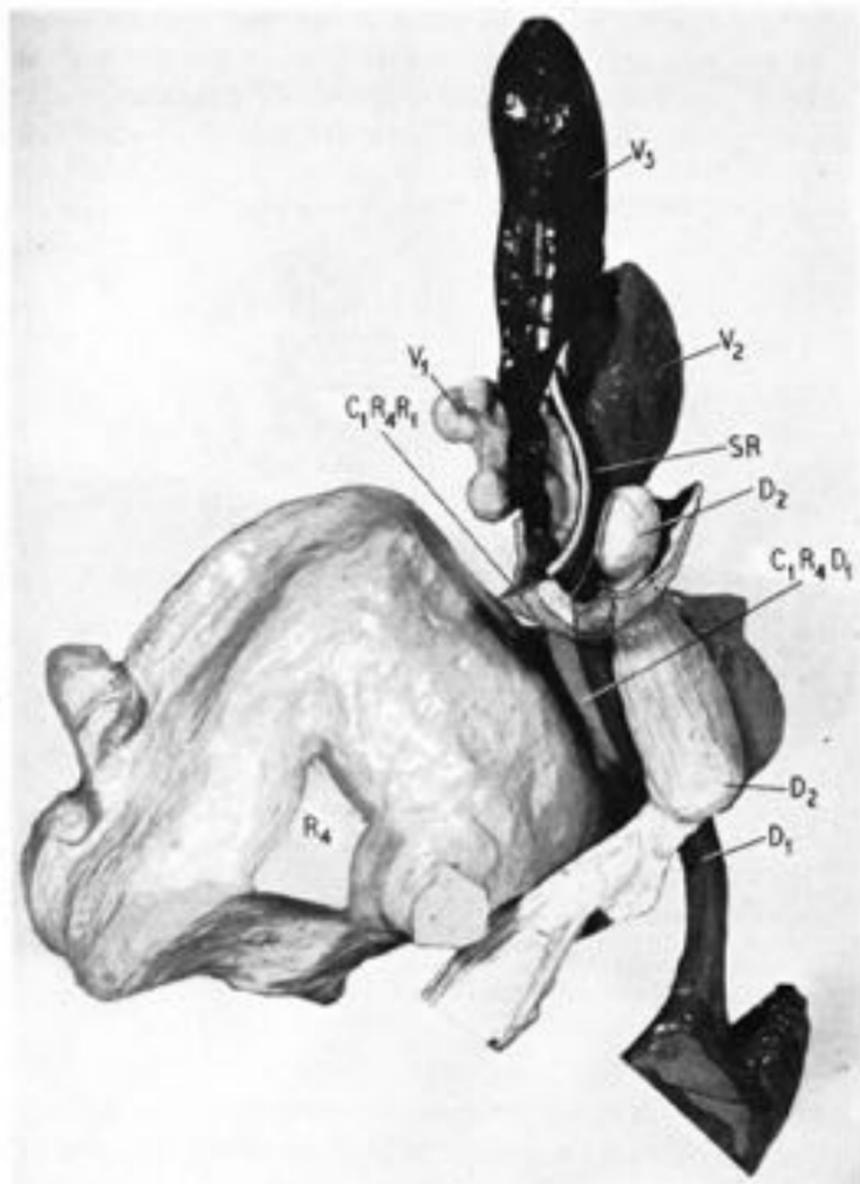


FIG. 12. Photograph of the plastic model of the synaptic body of an α -type receptor cell and its connexions. The model is a three-dimensional reconstruction from serial sections, two of which are presented in Figs. 7 and 11. V_1 and V_2 = proximal vacuoles; V_3 = an accessory vacuole continuous with V_1 ; D_1 and D_2 = processes, D_1 identified to originate from a bipolar cell (see Fig. 11); D_2 is presumably a process from another bipolar cell. One β -type receptor cell (R_4 , shown in Fig. 7) is included in the reconstruction. (From Sjöstrand, 1958.)

traced to extend to the vitreally located pole of the synaptic body of the receptor cells, but no further. In the present material (Sjöstrand and Mountford, 1965) this situation has been recorded in a few cases. In the majority of the synaptic bodies these vacuoles

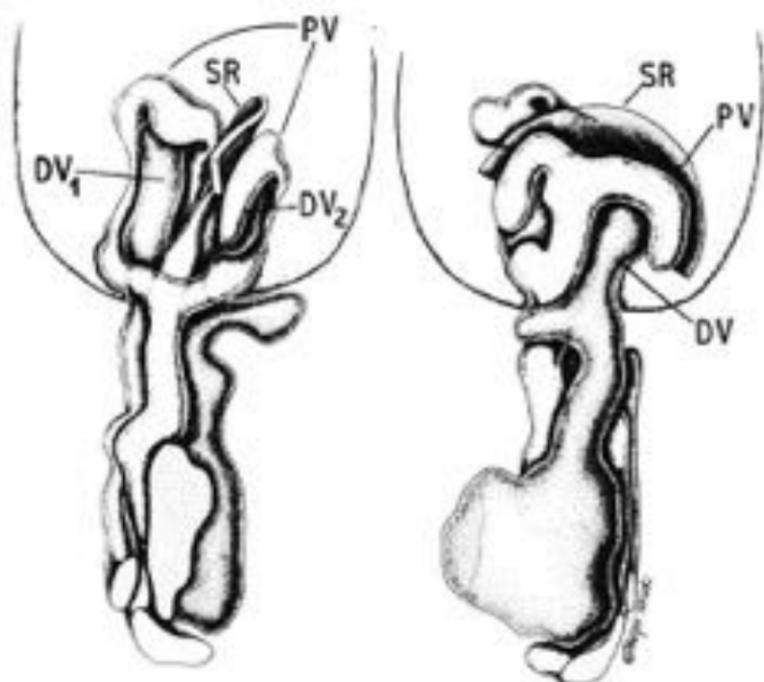


FIG. 13a. Three-dimensional reconstruction of synaptic contacts of α -type receptor cell, the second type described in the text. Proximal vacuoles (PV) are branches of a single process entering through the vitreally oriented pole of the synaptic body. Two other processes enter independently and end as distal vacuoles (DV_1 and DV_2) in contact with the proximal vacuoles. SR = synaptic ribbon. (From Sjöstrand and Mountford, 1965.)

could be traced out into the outer plexiform layer but until now we have not, with one exception, been able to identify from which type of retinal element they originate. In one case such a vacuole could be identified as a process originating from a Müller's cell. These vacuoles we can classify as the *proximal vacuoles* in contrast to the *distal vacuoles* which have been repeatedly identified as dendritic

end-branches from bipolar cells, and in one case as the end of a process originating in a β -type receptor cell.

The proximal vacuoles appear paired from an analysis of the sections (Fig. 11). They are characterized by their close topographical relations to the synaptic ribbon, an intensely stained ribbon-shaped structure located in the furrow between the

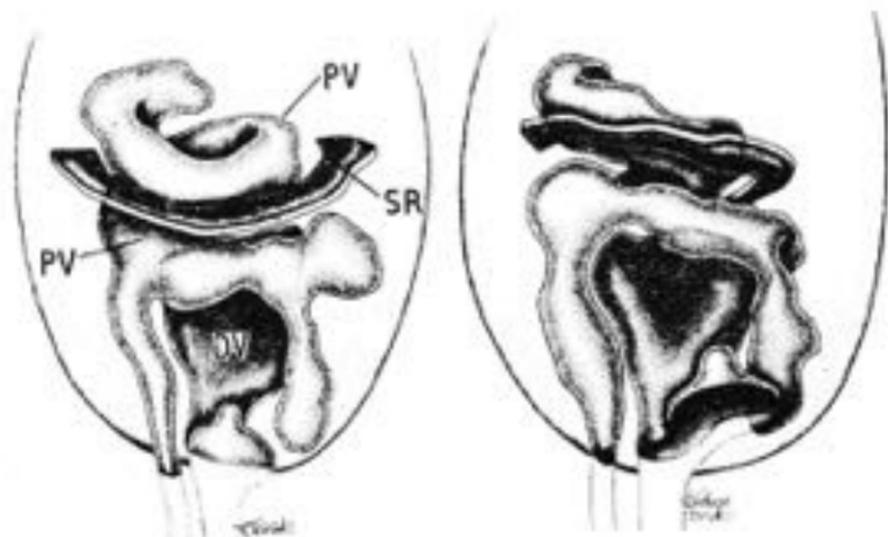


FIG. 11b. Three-dimensional reconstruction of an α -type receptor cell synaptic body of the third type described in the text. The proximal vacuoles (PV) are formed from a process entering the vitreally oriented pole of the synaptic body and assuming a spiral course. One single process of a different type forms a single distal vacuole (DV). (From Sjöstrand and Mountford, 1965.)

proximal vacuoles sclerally to an area over which the vacuoles are in close apposition.

The number of processes ending in the invagination of the plasma membrane extending into the synaptic body varies in the four main types of synaptic bodies. In one type the two proximal vacuoles can be followed separately out through the invagination and are in contact within the invagination with two separate dendritic end-branches forming a pair of distal vacuoles. In a second type two distal vacuoles can be traced out into the outer

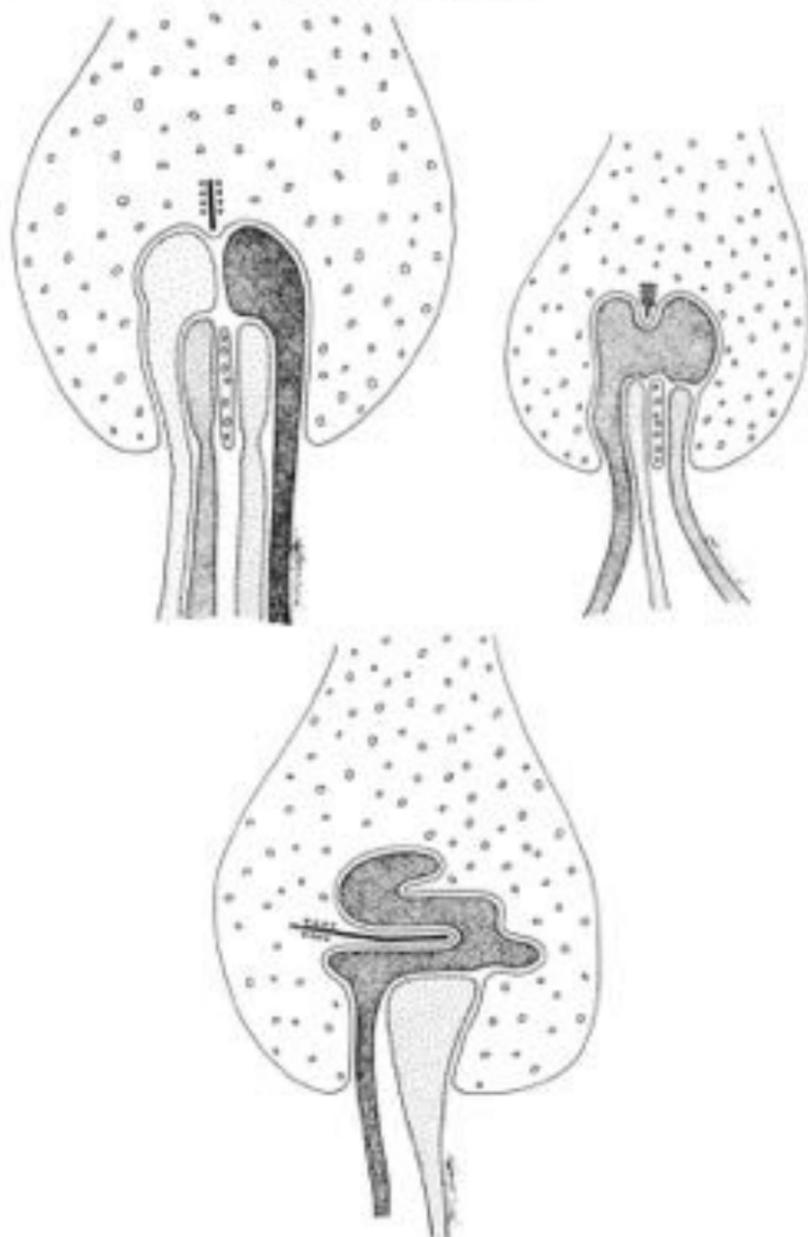


FIG. 146. *Top left*: the 2+2 type with two separate processes forming a pair of proximal vacuoles and two separate processes forming a pair of distal vacuoles. *Top right*: the 1+2 type with one process contributing to both the proximal vacuoles and two separate processes forming a pair of distal vacuoles. *Bottom*: the 1+1 type with one process forming the proximal vacuoles and one process contributing a single distal vacuole.

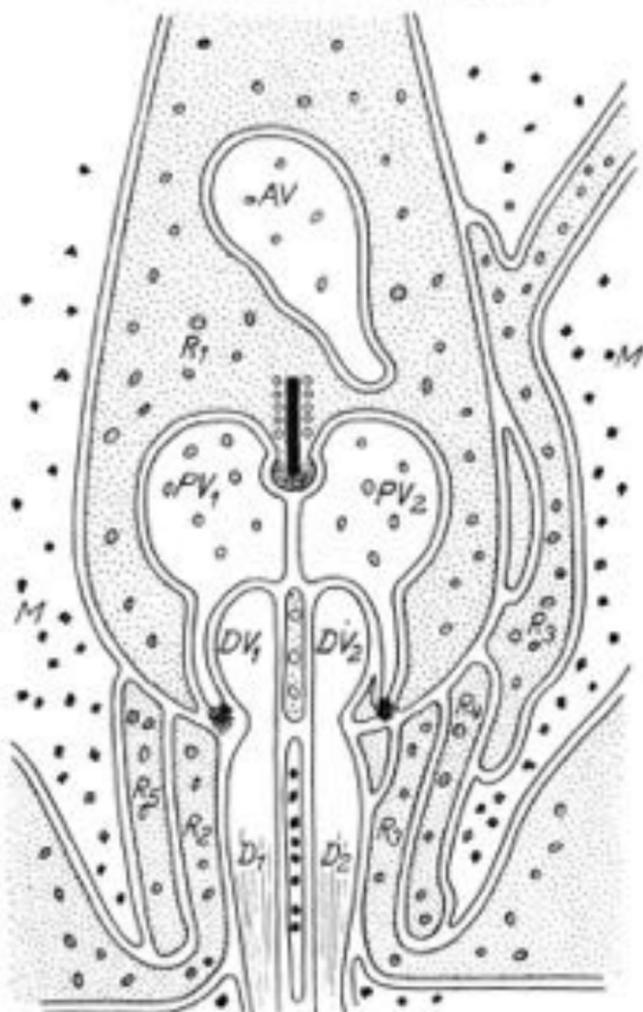


FIG. 14^b. The fourth type of synaptic body with the proximal vacuoles (PV₁ and PV₂) possibly not associated with any process in the outer plexiform layer and a pair of distal vacuoles (DV₁ and DV₂) representing the endings of two separate dendritic processes (D₁ and D₂). In this figure the lateral connexions from four different β -type receptor cells (R₁, R₂, R₃ and R₄) are included. (From Sjöstrand, 1958.)

FIG. 14. Schematic presentation of relationships between synaptic structures of α -type receptor cells and processes from the outer plexiform layer entering the synaptic invagination.

plexiform layer, while the two proximal vacuoles are branches from a single process entering the invagination at its vitreally orientated pole (Fig. 13*a*). In one such case one distal vacuole could be identified as a process from a β -type receptor cell.

The third type is characterized by only one distal vacuole and a single proximal vacuole forming a spiral proximally to the distal vacuole (Fig. 13*b*). In this case the synaptic ribbon is orientated in a plane approximately perpendicular to the long axis of the receptor cell and located between the basal and the apical turn of the spiral. In the fourth type it has not been possible to trace the two proximal vacuoles out through the invagination. Fig. 14 illustrates schematically the four types of synaptic contacts of the α -type receptor cells.

In rare cases the synaptic bodies of the α -type cells differed from those four main types of synaptic organization. Some synaptic bodies were lacking any invagination at the vitreally orientated pole and contacts with processes in the outer plexiform layer were only located at the surface of the synaptic body. In such cases no synaptic vacuoles or synaptic ribbons could be observed.

The β -type receptor cells are characterized by their large synaptic body which has a conical shape and faces the outer plexiform layer with a rather broad base. At the periphery of this broadened base the synaptic body extends processes in a lateral direction. The synaptic body of the β -type receptor cell contains several synaptic ribbons associated with groups of synaptic vacuoles (Fig. 15). Each such group appears similar to the set of synaptic vacuoles observed in the α -type receptor cells with paired proximal vacuoles and a single distal vacuole. The synaptic bodies of the β -type receptor cells, therefore, appear far more complex than do the smaller synaptic bodies of the α -type cells. However, three-dimensional reconstructions have made it likely that all the proximal vacuoles in these synaptic bodies are branches from one single process. Since these reconstructions have been partial reconstructions only of β -type synaptic bodies and no such body

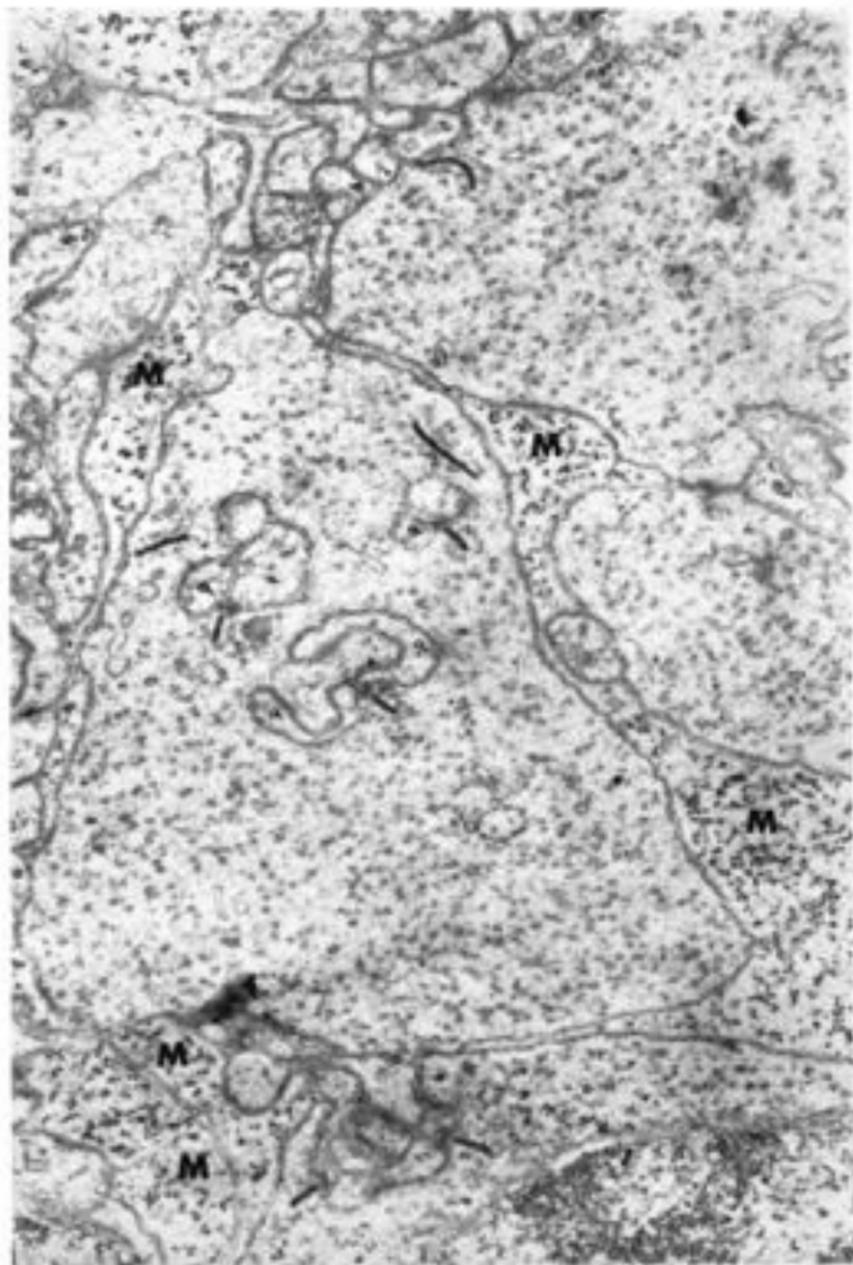


FIG. 15. Cross section through a β -type receptor cell showing several groups of proximal vacuoles (a), which in three-dimensional reconstruction were found to be part of a continuous tubular structure. M = Müller's cell cytoplasm. $\times 20,000$. (From Sjöstrand and Mountford, 1965.)

has been completely covered by our serial sectioning, this interpretation is tentative. However, in all the large number of partial reconstructions made up to now, all proximal vacuoles contained

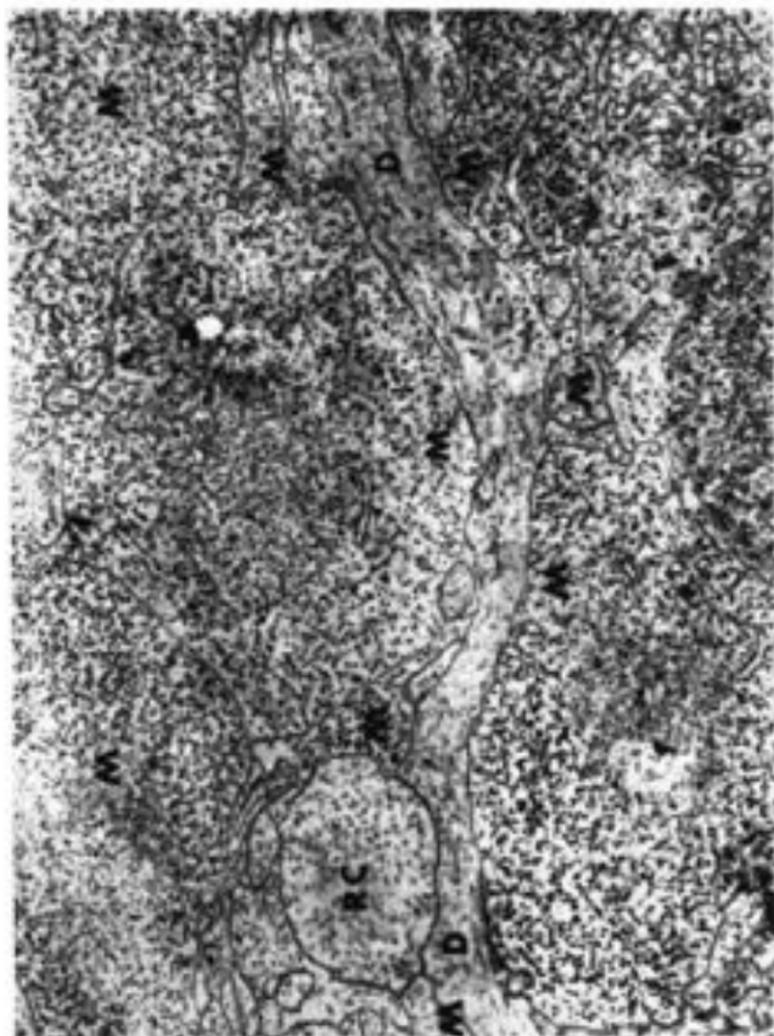


FIG. 16. Horizontal cell process (D) in the guinea pig retina showing contacts between this process and receptor cell synaptic bodies (RC). The horizontal process is surrounded by large sections of Müller's cell protoplasm (M). The contact between the horizontal process and the receptor cell is structurally indistinguishable from a synaptic contact. $\times 17,500$. (From Sjöstrand and Mounmford, 1965.)

in the series of sections have been found to be part of one continuous, irregularly shaped tubular process.

In each group of vacuoles associated with one synaptic ribbon

there appears to be one distal process associated with the proximal vacuoles.

The processes observed in the outer plexiform layer are of various kinds and it is not possible to identify them with certainty in randomly chosen single sections. Only tracing by means of three-dimensional reconstruction can reveal whether they are dendritic processes extending from bipolar cells, processes originating in horizontal cells, or Müller's cell branches. Processes from horizontal cells have been traced over very long distances in the outer plexiform layer (Fig. 5*a*) without revealing their endings. We do not know, therefore, to what extent processes from horizontal cells are contributing to the synaptic structure of the receptor cell. They contact a large number of receptor cell synaptic bodies as well as dendritic processes from the bipolar cells (Fig. 16).

The synaptic bodies of the receptor cells are generally separated by a thin layer of Müller's cell cytoplasm but direct contact between adjacent synaptic bodies is found fairly frequently. Such contact between the cells in the layer of the bipolar cells is also frequently observed.

In the retina of the leopard frog (Nilsson, 1964*b*), the interreceptor contacts are mainly through such direct contacts between the synaptic bodies of adjacent receptor cells. Lateral processes from the synaptic bodies are exceptional. By means of extensive serial-sectioning through the layer of the receptor cells, Dr. Nilsson in our laboratory could trace the various types of receptor cells of this retina (Fig. 17) all the way from the outer segments to the synaptic bodies (Nilsson, 1964*b*). This allowed him to relate the various types of synaptic bodies to the different types of receptor cells and to reveal the pattern of contacts between the latter. In this retina we can distinguish between red rods and green rods (Schwalbe's rods), single cones and double cones with one principal and one accessory member.

It is characteristic that extensive lateral contacts between red rods and single as well as double cones occur but that the green rods differ distinctly in this respect, having no such lateral contacts

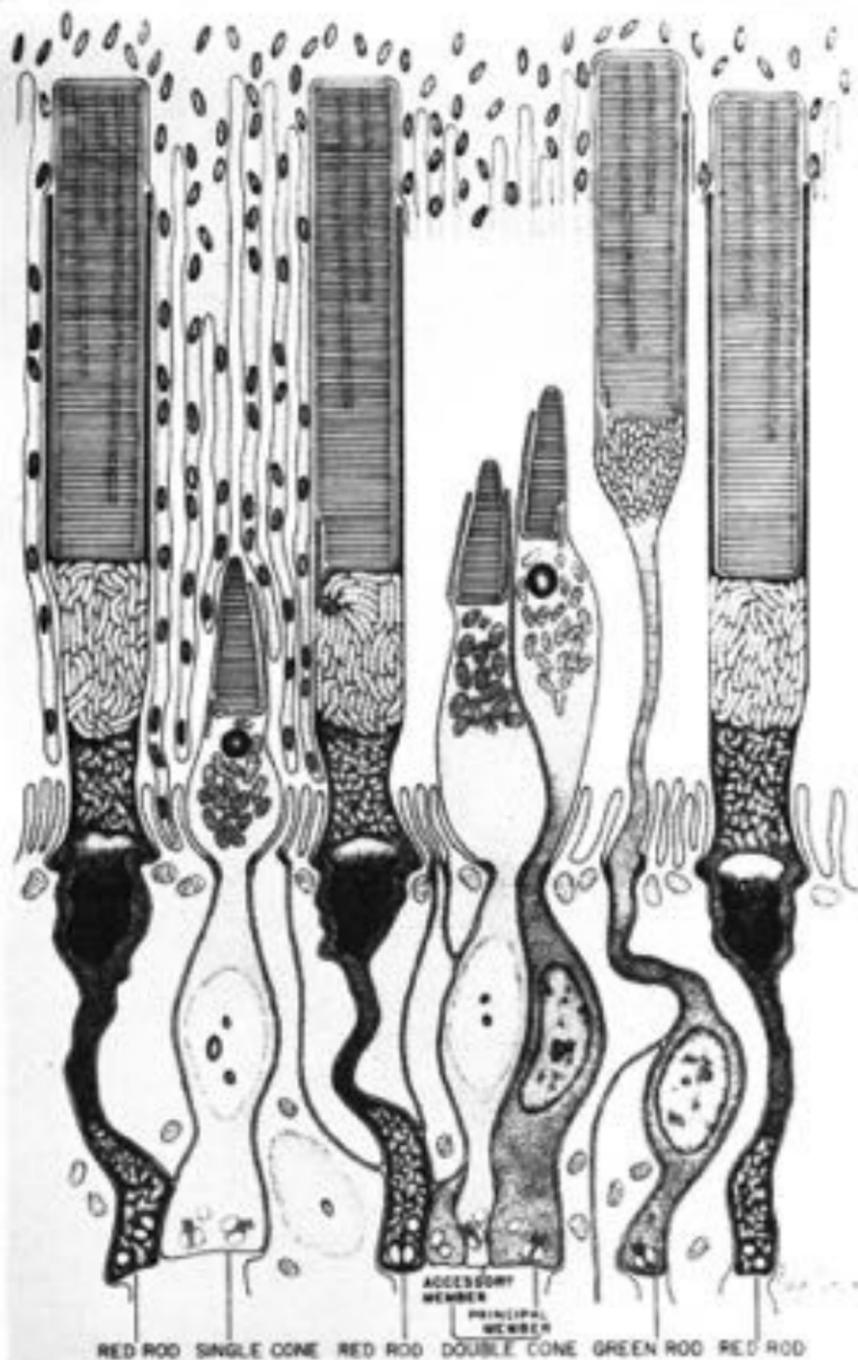


FIG. 17. Schematic drawing showing the different receptor cell types in the frog retina. (From Nilsson, 1964b.)

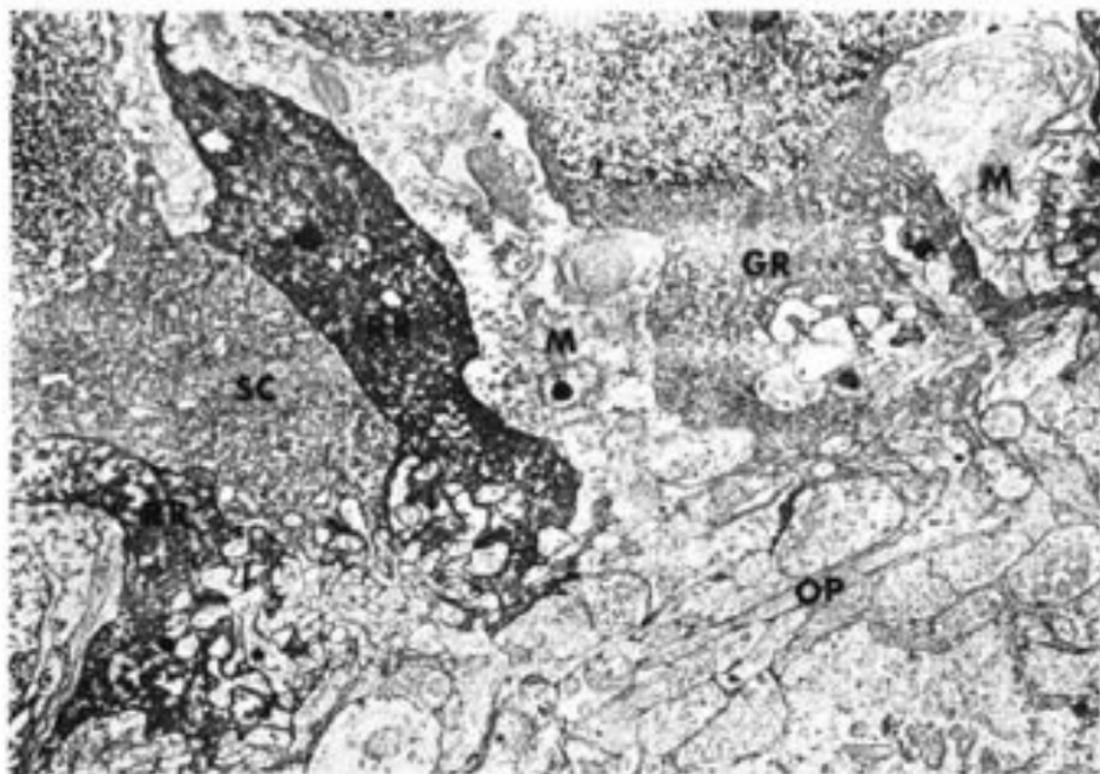


FIG. 18. Single cone receptor cell (SC) surrounded by two red rod synaptic bodies (RR) and a green rod synaptic body (GR) surrounded by Müller's cell protoplasm (M) in the frog retina. OP = outer plexiform layer. $\times 14,000$. (Unpublished picture by S. E. Nilsson.)



FIG. 19. Drawing showing interreceptor contacts in the frog retina. R = rod rods; C = single cones; D = double cones; black with figures = green rods; white = Müller's cells. (From Nilsson, 1964b.)

(Fig. 18). Müller's cell processes surround the synaptic bodies of the green rods, completely shielding them from surrounding receptor cells. The pattern of lateral contacts between the various types of receptor cells is illustrated in Fig. 19 and accounted for in Tables I and II.

Table I

INTERRECEPTOR CONTACTS (LARGE AREA TYPE) IN A REGION CONTAINING 215 RECEPTORS, DISTRIBUTED AS SHOWN IN TABLE II

Type of contact	Number of contacts (%)							
	0	1	2	3	4	5	6	7
Single cone-red rods	—	—	22.2	46.7	28.9	2.2	—	—
Principal member of a double cone-red rods	—	—	10.0	86.7	3.3	—	—	—
-single cones	—	—	—	—	—	—	—	—
Red rod-principal members	—	8.3	19.3	39.4	20.2	9.2	2.7	0.9
-red rods	—	—	—	—	—	—	—	—
-single cones	—	—	—	—	—	—	—	—
Red rod-principal members	—	17.4	56.0	21.1	5.5	—	—	—
Red rod-single cones	8.3	53.2	33.9	3.7	0.9	—	—	—
Red rod-principal members	31.2	57.8	11.0	—	—	—	—	—
Red rod-red rods	31.2	45.9	16.5	6.4	—	—	—	—

Table II

DISTRIBUTION OF THE DIFFERENT RECEPTOR TYPES IN AN AREA FROM THE POSTERIOR POLE OF THE FROG RETINA (*Rana pipiens*)

Parameter	Red rods	Green rods	Single cones	Double cones
Number	109	31	45	30
Percentage	50.7	14.4	20.9	14.0

THE INNER PLEXIFORM LAYER

The tracing of the bipolar cell processes into the inner plexiform layer has started in our laboratory. Dr. R. A. Allen (1965) has been able to show that the endings of the bipolar cell processes in the inner plexiform layer are in contact with a very large number of synaptic endings (Figs. 20 and 21). Synaptic ribbons were found to be present rather frequently at these contact regions. In other cases the cytoplasm showed a dense zone close to the plasma membrane at the surface of contact. Even when analysis was restricted

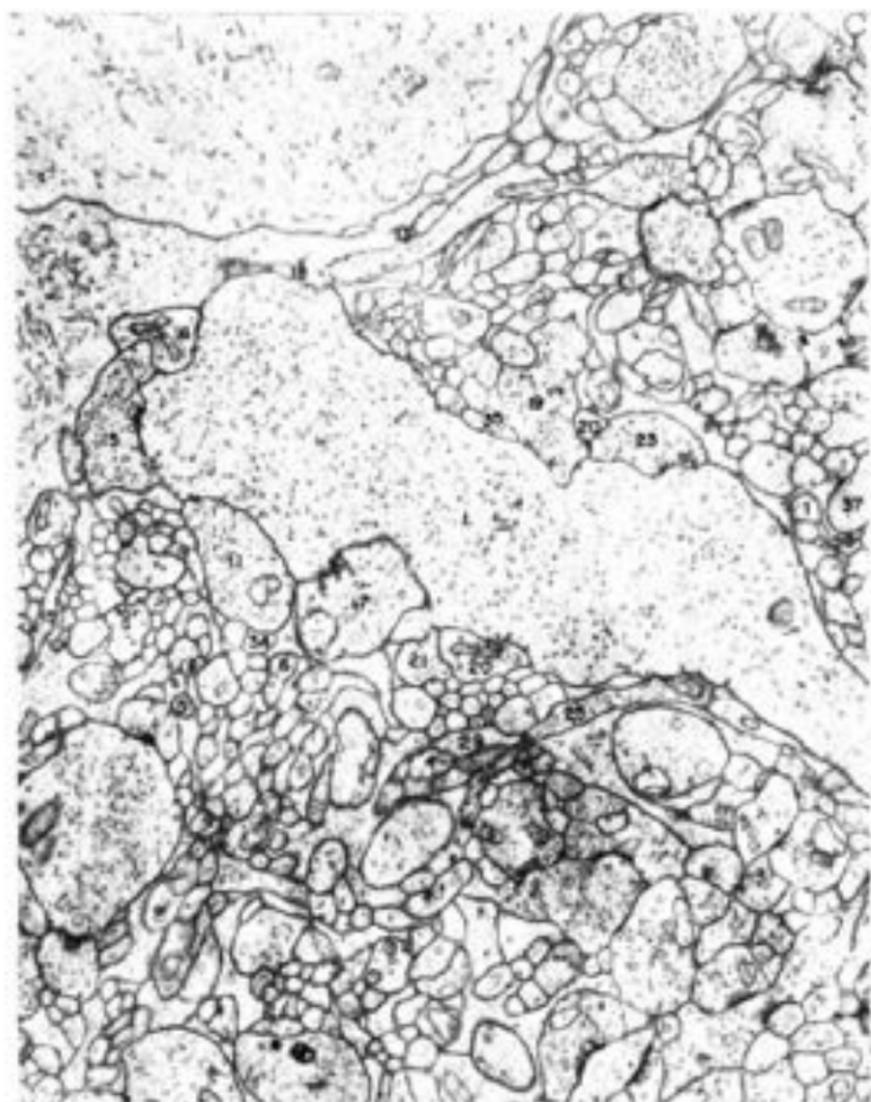


FIG. 20. Synaptic connexions of one bipolar cell in the inner plexiform layer. Fig. 20b shows a drawing of this bipolar cell as reconstructed from the inner nuclear layer to the inner plexiform layer. Numbers refer to processes in the inner plexiform layer that make synaptic contact with the bipolar cell (1) with specialized structures at the surface of contact. Magnification: $\times 21,650$. (From Allec, 1965.)

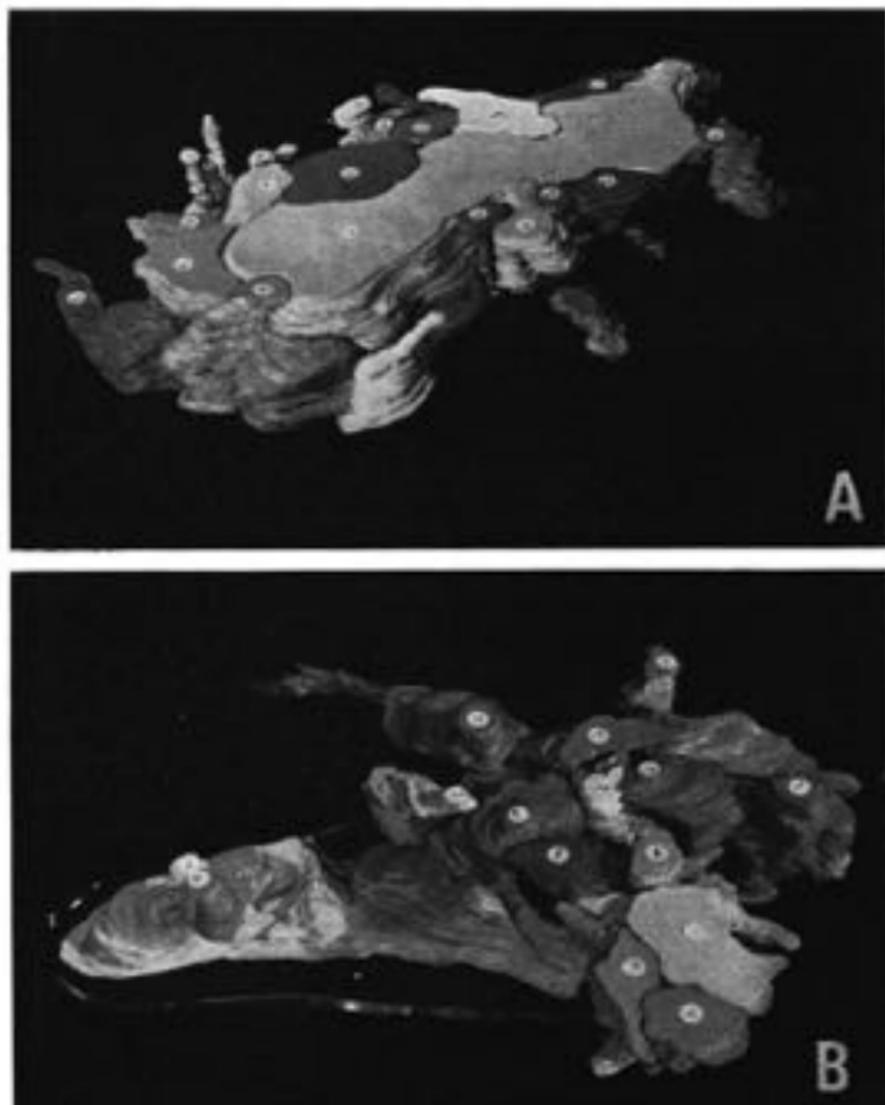


FIG. 21 *a* and *b*. Photograph of three-dimensional model prepared from serial sections of the ending of the same bipolar cell as shown in Fig. 20. Fig. 21*a* shows the model as viewed from a plane corresponding closely to that illustrated in Fig. 20*a*. Fig. 21*b* shows the model turned 180° with only half the structures making contact with the surface of the ending of the bipolar cell. Half of these structures were removed to make part of the surface of this ending appear. (From Allen, 1965.)

to contacts characterized by any such specialized structure, it appeared that each bipolar cell can be estimated to make about 70 such contacts. If all other contacts were counted, this figure might be almost doubled.

This shows the degree of complexity that is characteristic for the inner plexiform layer. It was furthermore possible to distinguish between bipolar cells ending at different levels in the inner plexiform layer. At least three different types of bipolar cells could be distinguished from this point of view. The cell body of the bipolar cells ending nearest to the layer of the ganglion cells was located furthest in a scleral direction. The layering of the cell bodies of the bipolar cells therefore corresponds to differences in the extension of the cell processes into the inner plexiform layer.

A considerable amount of work remains to be done before the contact patterns in the inner plexiform layer will start to appear. It seems likely, however, that several parallel connexions exist between many of the receptor cells and the inner plexiform layer, mediated by separate bipolar cells. It also appears quite possible that these bipolar cells end at different levels in the inner plexiform layer.

FUNCTIONAL INTERPRETATION OF SYNAPTIC RELATIONS

From the point of view of functional interpretation of the fragmentary information we have on retinal synaptology, it seems justifiable to propose that the lateral connexions at the receptor cell level are of considerable importance as one structural basis of lateral inhibition phenomena. Whether structural conditions are fulfilled which suggest that such contacts are also involved in the perception of motion is as yet uncertain. The β -type receptor cells stand out as a particular type of receptor cell by the extensive system of lateral branches extending from the synaptic bodies to make contact with the α -type cells. The shape of the synaptic bodies of these cells is determined by these processes extending

laterally from the base of the synaptic body. The large size of these synaptic bodies is certainly determined by the branching and large extension of the proximal vacuole, which has the form of a tubule of irregular shape, and by the separation in space of the presumably dendritic endings forming the distal vacuoles.

The β -type cells, therefore, appear either to affect the activity in the α -type receptor cells, or to be affected by the activity in those cells. It seems, however, unjustifiable to classify these cells as cone cells because of the similarity in shape between their synaptic bodies and those of cone cells. The presence of lateral connexions at the receptor cell level might be characteristic for cone cells as well as for the β -type cells, and this would explain this similarity. But this structural feature does not need to be linked with any other characteristics of cone cells. Cone cells are defined by the conical shape of their outer segments, which are also smaller in size than rod outer segments. These structural features might indicate that the cone cells have a higher threshold for light stimulation than the rod cells. The considerably smaller total surface area of the outer segment disks in cone cells presumably reflects a higher threshold. This particular shape of the outer segments, however, does not need to be directly related to colour discrimination. The receptor cells in the human fovea, for instance, are functionally characterized as colour-discriminating elements. Their outer segments are not conically shaped and morphologically they are not cone cells.

I think it is important to stress that we do not know of any particular structural feature at a microscopic or ultrastructural level which determines the functional property of colour discrimination. It is very unlikely that the structural properties of cone cells as demonstrated today can be crucial in determining the spectral sensitivity of the cells. These properties are most probably directly responsible for functional characteristics other than spectral sensitivity. However, these functional characteristics might frequently be associated with colour discrimination, but not necessarily so, and therefore the cone cells will frequently be identified

correctly as associated with colour vision on the basis of structural features not directly related to this function.

It seems futile at the present time to try to describe the structural basis for colour discrimination at the retinal level. It appears more justifiable to warn the physiologists about any preconceived ideas regarding functional properties of cells that, for one reason or another, have been classified as cone cells by classical morphologists.

The situation in the inner plexiform layer lends itself to speculation only. There is a considerable convergence towards the ganglion cells, which are few in comparison with the receptor cells. The information from each single receptor cell furthermore appears to be transferred over several paths to the inner plexiform layer. It therefore seems obvious that the information fed into this layer must be subject to a rather elaborate process of selection and coding before a condensed set of information is transferred to the brain through the limited number of channels in the optic nerve.

The fact that certain ganglion cells in the rabbit retina can discriminate between image movements shows that this kind of information has been coded and fed into particular channels of the optic nerve.

It is proposed that the main function of the inner plexiform layer is to code the information submitted by the receptor-bipolar cell system and that coding for different qualities takes place in different circuits in the inner plexiform layer. These circuits are located at different levels in this layer. Information from the receptor cells can be fed into several such circuits in parallel, and while one circuit might code for shape or colour, another circuit might code for movement. In the schematic Fig. 22, this concept has been illustrated by the three "black boxes" drawn in the inner plexiform layer.

The function of the outer plexiform layer, on the other hand, seems to be to allow extensive short and medium distance (inter-receptor contacts) as well as long distance (horizontal cells) lateral

interaction. This lateral interaction could be of prime importance for the enhancement of contrast and possibly furnish some basis for the perception of movement and shapes.

Unfortunately, this survey of what we have been doing in our laboratory with respect to retinal synaptology does not help us in

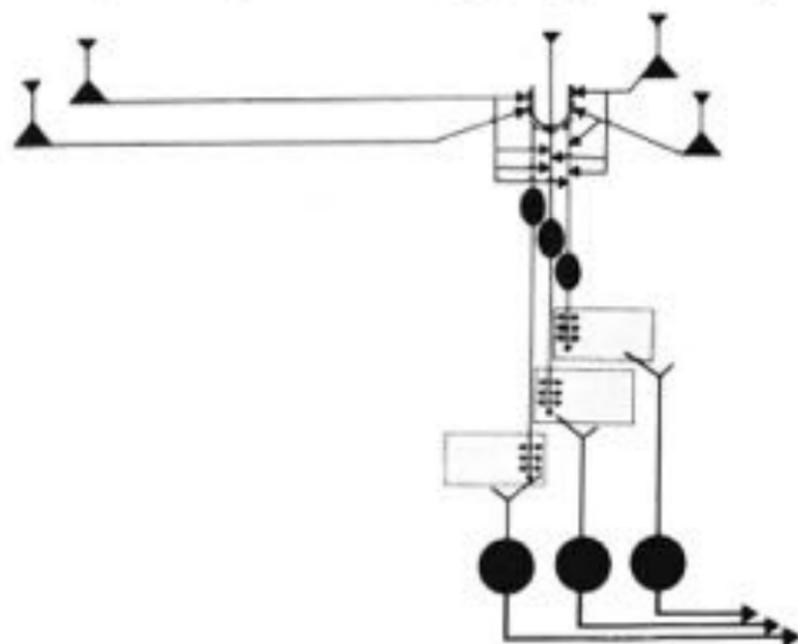


FIG. 22. Schematic representation of interreceptor contacts and possible receptor-inner plexiform layer connections. The three rectangles are assumed to represent circuits assigned to code the information received from the receptor cells. (From Sjöstrand, 1964.)

understanding the retinal mechanism underlying colour vision. There is little hope, in fact, that a slight variation in the structure of the photopigment of the receptor cells would be associated with any great structural differences at a microscopic or ultrastructural level. Any of the possible photopigment molecules could fit into the outer segment disks without any modification of their ultrastructure as observed in the electron microscope. The possible correlation between cone cells and colour discrimination might

well be one of mere coincidence which does not exclude the association of colour discrimination with entirely different structural features. On the other hand, certain structural features of cone cells reflect other functional properties than colour discrimination and can therefore be found in cells lacking this capacity. It would be useful, I think, to talk about colour-discriminating and colour non-discriminating cells, basing the definition entirely on the functional properties of the cells, and at the present time to reject morphological criteria for such a classification as unreliable. A cone cell, according to definition, must have a conical outer segment. When other cells are classified as cone cells, the precise morphological definition has been twisted and the word "cone" has lost its obvious meaning. The duplicity theory is being upheld by artificially adjusting morphological classification to physiological observations.

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DISCUSSION

Pickford: Professor Sjöstrand, may I confirm that none of the animals you have mentioned has colour vision?

Sjöstrand: I have not been concerned with colour vision; I have chosen the guinea pig because it has a pure rod retina, and I wanted to work on a simple retina. It may be that the animals that we studied do not have any colour vision or, as Granit puts it, they might have colour sensitivity but not make any use of it. It would be nice to do this kind of study on animals that are known to have colour vision, but first of all we want to learn as much as possible on a simpler system. The situation with respect to the frog retina is at the present time too complex to analyse this way.

Svaetichin: The guinea pig probably does not have colour vision, and I am inclined to believe that the organization of intersynaptic contacts you have described represents a mechanism for the detection of movement and pattern.

Crawford: Do we actually know that the guinea pig has no colour vision, and if so, how has it been tested?

Tansley: The guinea pig has been tested behaviourally and shown not to have colour vision by such tests.

Meyer: R. C. Miles, P. Ratoosh and I made that study ([1956], *J. Neurophysiol.*, **19**, 254). We used the discriminated operant technique, and found that the animals apparently cannot distinguish green from either red or blue. This was after many thousands of responses. That is the basis for the current conclusion that the guinea pig has no colour vision.

Land: What was the guinea pig looking at? And what was the background? That might be important.

Meyer: The animal was looking at a food hopper which was completely surrounded by one colour or the other. The animal was asked to press a lever placed beside the hopper. The animal compartment's walls were painted white, and the coloured light projected on to the hopper was reflected on to the walls.

Weale: Kolmer stated many years ago that the guinea pig retina contains as many cones as the human periphery (Kolmer, W. [1936]. *In* *Handbuch der mikroskopischen Anatomie des Menschen*, ed. von

Möllendorff, W. Berlin: Springer), and as recently as 1947 O'Day published a paper to the effect that the statement that the guinea pig retina is pure rod is erroneous (O'Day, K. [1947]. *Nature [Lond.]*, **160**, 648). I am not in a position to judge this, but I do not think the answer is absolutely clear.

Sjöstrand: It depends how you define a cone. If you accept a β -type synaptic body as sufficient evidence to classify these cells as cone cells, there are plenty of such cells in the guinea pig retina. As I have already pointed out, I do not consider such a criterion sufficient to identify a cell as a cone cell. If you look with the electron microscope at definite cone cells, as defined by light microscopists, you find that the outer segment disks are continuous with the plasma membrane and appear as invaginations of the plasma membrane. No receptor cells in the guinea pig retina show that, and the only difference between α and β cells is the shape or size of the synaptic body and the localization of the cell nucleus.

Mounier: I know of investigations with micro-electrodes which suggest that some elements of the guinea pig's retina are sensitive to 460 nm. and to 520 nm. light. This indicates some responsiveness to colour.

Sjöstrand: Yes, but I cannot define a cone cell on that evidence. Dr. Pedler is quite right in saying that we should not force ourselves to associate colour with cone cells. We might end by defining cells using a morphological description which is not relevant.

Klüver: I am impressed by the tremendous number of contacts you have found microscopically in the bipolar cell. Will variations in water-content ever influence the number of contacts at the surface of such a cell?

Sjöstrand: No. I think you have in mind the classical picture of the central nervous system, according to which neurones and glial cells are more or less freely floating in a large extracellular aqueous space. In fact, electron microscopic studies have made it justifiable to question the existence of any such extracellular space and to propose that the neuroglia represent what has formerly been referred to as extracellular space (Sjöstrand, F. S. [1958]. *J. Ultrastruct. Res.*, **2**, 122). The arrangement of neurones and glia is very intimate and comparable to the neurone-neurone relationship at the synapses. Shrinkage is rather minute with proper technique and it is more likely that it affects the

volume of the cells than that it eliminates the extracellular space, at least if shrinkage due to osmotic movement of water is concerned. The Müller's cells separate the receptor cells all the way except at these contact areas, and it is unlikely that the Müller's cells would shrink preferentially to produce artificial neurone-neurone contacts; all experience points to the opposite. Bad fixation is associated with swelling of glial elements in the nervous system.

Svaetichin: Are the intersynaptic contacts between the receptors typical synaptic contacts or just membrane-to-membrane contacts?

Sjöstrand: They are membrane-to-membrane contacts with synaptic vesicles on both sides. The short branches are filled with vesicles all the way, but the long branches contain synaptic vesicles only at the end where the contact is. Furthermore, the plasma membrane at the contact is frequently more intensely stained, just as at what is considered a regular synaptic contact.

Wolbarsht: Professor Sjöstrand, you have answered one problem I have always had. Everyone compares lateral inhibition in the vertebrate eye with lateral inhibition in the *Limulus* eye but I have always felt that these were completely different things. In the *Limulus* eye one finds what is called mutual inhibition, a phenomenon of disinhibition by which you can put light on two spots which will interact in such a way as to release a third spot from inhibition. But this is a phenomenon which I have never seen in the vertebrate retina, although it exists in the vertebrate visual system, so it must occur beyond the retina. The connexions that you have shown are not for mutual inhibition but merely for inhibition; that is, the fibre goes from one cell to another but does not come back. That would agree with the kind of inhibition we have seen in the vertebrate eye and not with the kind we have seen in the *Limulus* eye.

Pedler: I would like to take issue with you, Professor Sjöstrand, in your interpretation of these circuits. You are looking at them as if the retina is a deterministic engine in which one cell can fire significantly. But one should construct circuit diagrams in the way that the retina probably functions; that is to say, it is a probabilistic device in which information is caused to travel over the same time-base time after time in order to reach significance. I do not believe there is much point in trying to draw deterministic flow diagrams. I agree with you that there are many

channels which probably subserve different information pathways, but the logical place for these would be in the outer plexiform layer, not in the inner plexiform layer, because the retina is a noisy system with continuous spontaneous activity and the logical place to generate information would seem to be before the noise has a chance to reduce the significance of the signal. The multi-channel system of differentiation, integration and lateral inhibition is therefore more likely to occur first in the outer plexiform layer. I would suggest that one of the functions of the inner plexiform layer might be to act as a delay-line system, if one must have a black-box analogy at all. The inner plexiform layer may well, in these terms, hold up messages solely from a need to change spatial into temporal resolution. Impulses are being fed sequentially into the ganglion cells and so somewhere they must be held up so that they can be transferred in temporal sequence.

Sjöstrand: Since we know so little about the retina from this point of view, everyone has the freedom to choose his own concept. I am not looking at the retina as if it were a deterministic engine: I am not approaching the problem with such generalizing concepts in mind. We are trying to introduce into the discussion some facts with respect to the synaptology of the retina, and the reason why we start out with single receptors and follow paths associated with single receptors is simply because this is the only practical way to do research of this kind. When our analysis along these lines has progressed a great deal further, we can start to think about mass-action circuitry.

I do not agree with the idea that the outer plexiform layer could do the coding, because the outer plexiform layer in the guinea pig retina is not complex enough. There are the branches from the bipolar nerve cells which are limited in number, and then the horizontal cells. We have not yet been able to trace these to the end of their branches, because it is not possible, below a certain diameter of the branches, to distinguish between horizontal cell processes and the dendrites of the bipolar cells. I do not agree with you that it has been established that the horizontal cells do not have any synaptic structures, because no one has traced horizontal cells to the end of their branches where there might be synaptic structures. Anyhow, with a fairly simple system of horizontal cells, which can reach over very long distances in the guinea pig retina, and with very few dendrites from the bipolar cells, I cannot see how

your mechanism could be accounted for structurally. In contrast, the inner plexiform layer is the dominating synaptic layer and reveals the complexity of contacts that could account for the coding.

Lennox-Buchthal: I want to support Professor Sjöstrand in his idea of the black box, because we certainly know with respect to colour vision that by the time information has reached the geniculate it has already been divided into three black boxes; that is, the cells in the three layers of the geniculate respond differently, as R. L. De Valois and his co-workers have shown. That division must have originated in the retina, and it is interesting to have an idea of where it might have happened.

Klüver: Professor Sjöstrand mentioned coding for "colour" and "movement" in connexion with the bipolars and the inner plexiform layer. Suppose we consider, instead of such complex phenomena, a seemingly "simple" phenomenon, such as a white dot on a homogeneous black ground which has no boundaries. How will electron microscopic analysis, if it can do so at all, cope with even the simplest visual *Gestalten* or the most primitive "figure-ground" organizations?

Sjöstrand: This could possibly be accounted for by the regular geometry of the interreceptor contacts, for instance, with these contacts being arranged in a hexagonal pattern with chains of interacting receptor cells. The primary response of the receptor cells would be modified by the surrounding receptor cells in this hexagonal network and the signals fed into the coding circuits would therefore already be based on integration of the activity within groups of receptor cells associated in a geometrically regular fashion in the plexus of the receptor cell layer. Wouldn't the subdivision of the receptive surface into a regular mosaic of territories make it easier to code for *Gestalten* than a system of randomly arranged spots represented by individual receptor cells?

Louenstein: The field concept is a very difficult one to discuss philosophically, because there is the question of atomism versus continuity. It is always being raised in such connexions, but in the end if we want to analyse our fields we shall probably have to go back to unit analysis.

Sjöstrand: We have been trying to find something that could be responsible for the Stiles-Crawford effect. Could the fact that the cone cells are asymmetric with respect to their outer segment disks be responsible for your effect, Dr. Stiles?

Stiles: Unfortunately I have not made any progress in thinking of the suggestion you made. The trouble with trying to use structures of this kind has been the very short spacing of these disks and layers. You are thinking of some kind of interferometric principle, which was, as you know, proposed in a very simple form by E. Ingelstam ([1956]. *In Problems in Contemporary Optics*, pp. 640-668. Istituto Nazionale di Ottica: Arcetri-Firenze) and there are objections to his analysis because of the very high refractive index of the layers that would be required. But now you are raising a new point, in that a hint might be got from the difference between the open-ended structure of the cones and the closed plates of the rods. It is an interesting clue, but it has not suggested anything to me yet.

Willmer: Can we infer from that that they are all orientated in the same direction? Because the Stiles-Crawford effect works over numbers of these units, so they would all have to be orientated in the same way.

Sjöstrand: We do not know yet whether there is any systematic orientation of the asymmetric structure within populations of cells. In each cell the connexions between the disks are all orientated in the same direction.

Marks: Would you comment on the theory that the rhodopsin molecule when excited changes its shape and makes a leak, a monomolecular hole, in the membrane of the receptor? Have you any ideas whether the currents could actually form a complete circuit, ionically?

Sjöstrand: I think that with this model the structural unit to be affected would be the globules observed in the disks. One rhodopsin molecule associated with a globule would have some local effect confined to this globule. But what would the hole do? What is the next step? This is a free-floating membrane; there is no contact with the plasma membrane, so one cannot think of any excitation being conducted anywhere. This sort of thing would be happening very locally in the outer segment, perhaps 200 disks away from the inner segment. Since lipid-aqueous systems are labile and can easily be subject to phase changes it seems reasonable to speculate that the photo-rhodopsin interaction might produce a phase change in the lipoprotein structure of the disks which could lead to secondary effects.

Lowenstein: Is it true that there is no contact or continuity with the plasma membrane?

Sjöstrand: According to recent studies, by Dr. A. Cohen for instance, there is no contact. The contact I have described might well be an artefact.

Cresitelli: Since a hole has been mentioned, I should like to mention some work that Dr. Trevor Shaw and I have been doing. We find that in three different visual pigments which we have studied, the chromophore, which is optically inactive of itself, has optical activity induced in it by the protein. That is, there is a conformation induced by the opsin. We determined this by measuring the circular dichroism in the region of the absorption. There is circular dichroism in the three visual pigments which we measured: frog rhodopsin, gecko visual pigment, and the visual pigment of the conger eel. We have examined the dichroism in three different wavelength regions and in all cases the circular dichroism disappears, as if the conformation is lost when the visual pigment is exposed to light.

THEORIES OF COLOUR VISION

SKETCH OF THE PRESENT POSITION OF THE YOUNG-HELMHOLTZ THEORY OF COLOUR VISION

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IN so far as the Young-Helmholtz theory suggests a minimum of three selective primary receptors as the basic requirement for human colour vision, it is almost axiomatic. The Hering concepts have been regarded recently (e.g. Marriott, 1962), not as an alternative to Young-Helmholtz, but as relating to grouping of the signals from the primary receptors on their way to the final perception centres. One of the principal interests in work on the eye and vision has naturally been the endeavour to follow up the Young-Helmholtz theory by actually finding the sensitive substances of the primary receptors. Searches by many methods have been rewarded by an encouraging measure of success and we stand at the interesting point of confirming and extending our knowledge of the photopigments concerned in colour vision and of welding the results into a quantitative specification of colour vision. This work includes, and may well be greatly helped by, investigation of the colour vision of animals other than human.

It was in 1802 that Young suggested that colour vision depended on the presence in the retina of the eye of three sorts of receptor, the messages from which, when integrated in appropriate proportions, produced the whole gamut of colour sensations. The idea was then forgotten until Helmholtz revived it fifty years later. He drew three

qualitative spectral sensitivity curves for the three sorts of receptor and in due course the idea became known as the Young-Helmholtz theory of colour vision. Quantitative estimates of the shapes of the spectral sensitivity curves followed at intervals and have continued to follow in bewildering variety of form. A brief modern survey of the more important stages in this development is given by Marriott (1962). The very wide variety exhibited by the results, for example, between the curves of Hecht (1930) and of Thomson and Wright (1953), shows how necessary it is to attempt to investigate the sensation curves by some objective method. The first method, historically, was the extraction of photolabile pigments from excised retinæ. These pigment extractions have been carried out for over 100 animals, both vertebrates (mammals, birds, amphibians and reptiles, fishes) and invertebrates. They have yielded spectral absorption curves which agree, in many cases, very satisfactorily with the scotopic sensitivity curve of vision. So far, however, no entirely satisfactory correspondence with photopic sensitivity or colour vision has been found (Dartnall and Tansley, 1963). This is in spite of the fact that an almost undoubted cone pigment has been extracted from the retinæ of squirrels, which contain only cones, since an apparently identical pigment has been extracted from the pure rod retina of the hamster (Fig. 1) (Dartnall and Tansley, 1963), from which one can only conclude that the photopic or scotopic nature of vision is a function of receptor structure and interconnexion rather than of receptor pigment. The tentative explanation put forward by Dartnall (1960) should be noted in this context (Fig. 2). Similar considerations apply to the iodopsin isolated by Wald, Brown and Smith (1955) from fowl retinæ. On the whole, however, it cannot be said that a completely satisfying clarification of the Young-Helmholtz theory of colour vision has yet come from the *in vitro* examination of visual pigments.

The *in situ* examination of visual pigments has yielded rather more information, in spite of the apparently greater difficulties in the way of exact experimentation. The *in situ* condition may be

conveniently subdivided into *in vivo* and *in mortuo*. The work in the *in vivo* division is associated chiefly with the names of Rushton and of Weale, both with various collaborators. In broad outline, they measure an effect by comparing light emerging from the eye

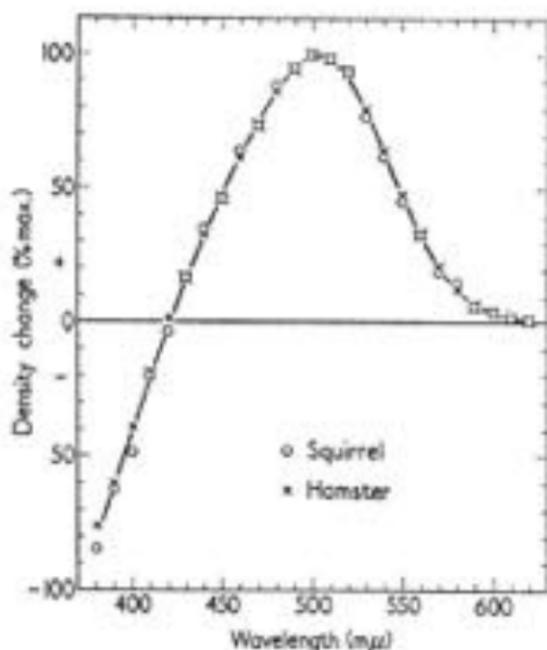


FIG. 1. Comparison between the difference spectra, in alkaline solution, of the visual pigments of the squirrel (pure-cone retina) and hamster (pure-rod retina). Squirrel data at 20°C; hamster data (Bridges, 1959) at 25°C. (From Dartnall, 1960, by permission of the author and the Editor of *Nature*.)

with light entering. Some of the emergent light will have traversed some of the photolabile pigments, if any, contained in the retinal sensory elements. By partially or completely bleaching the pigments a change in the measured effect will be apparent. The variables in the experiment are the intensity, wavelength and temporal variation of the bleaching light and the wavelength and time at which absorption is measured. For discussion of the detailed

reasoning according to which the results are interpreted, reference must be made to the original papers (Rushton, 1963*a,b,c* and 1964; Ripps and Weale, 1963, 1964). It appears that there has been

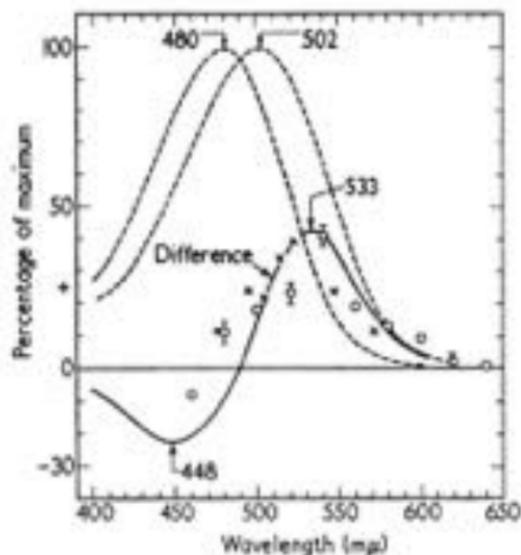


FIG. 2. Interpretation of *in vivo* measurements of the squirrel pigment and of the spectral sensitivity function. (From Dartnall, 1960, by permission of the author and the Editor of *Nature*.)

Circles (with bars indicating standard deviations, are Weale's (1955) results for the density changes on bleaching the squirrel retina; crosses are Arden and Tamley's (1955) mean measurements of spectral sensitivity (equal quantum-intensity spectrum, corrected for lens absorption). Both sets of data are scaled so that the maxima agree with that of the difference curve, that is, the difference between the 502 and 480 nm. curves.

definite isolation of a pigment absorption curve related to green-perceptive receptors (chlorolabe in Rushton's nomenclature), with detection of a red-perceptive pigment (erythrolabe). Rushton isolates the chlorolabe curve by using a protanope as observer; no erythrolabe is present and the presumed cyanolabe is not apparent

(Fig. 3). Ripps and Weale show apparently pure difference spectra which may correspond to chlorolabe, and mixed difference spectra

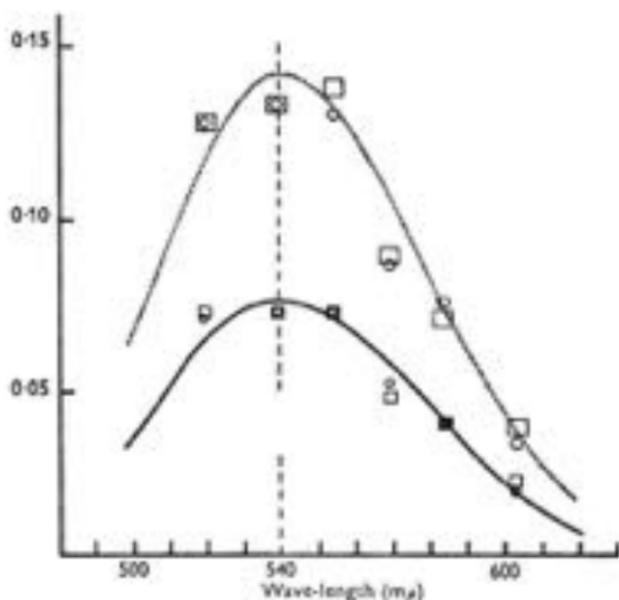


FIG. 3. The circles plot at six wavelengths the change in double density when the dark-adapted fovea was bleached by the red light transmitted through an Ilford 205 gelatin filter (small circles), followed by bright white light (large circles). Small squares show a similar experiment where the bleaching light passed through an Ilford 623 (bright spectrum blue-green) filter; large squares, bright white light. The white lights were strong enough to bleach fully; the coloured lights were adjusted in intensity to bleach 50 per cent. Since red and blue-green result in the same difference spectrum, only one photosensitive pigment is present. Curves (Pitt, 1944): protanope spectral sensitivity. (From Rushton, 1963a, by permission of the Editorial Board of the *Journal of Physiology*.)

which may indicate that the effects of chlorolabe and crythrolabe are blended (Fig. 4*a,b*). In the latter case, Fig. 4*a*, the proportion of the blend varies with the intensity of the bleaching illumination and so produces a shape of difference spectrum which changes corre-

spondingly. In the former case, Fig. 4*b*, however, the absence of change of shape of the difference curve does not necessarily mean that its shape is entirely due to chlorolabe; as Ripps and Weale show, the shape may still be the result of the composite absorption of chlorolabe and erythrolabe together. Ripps and Weale also

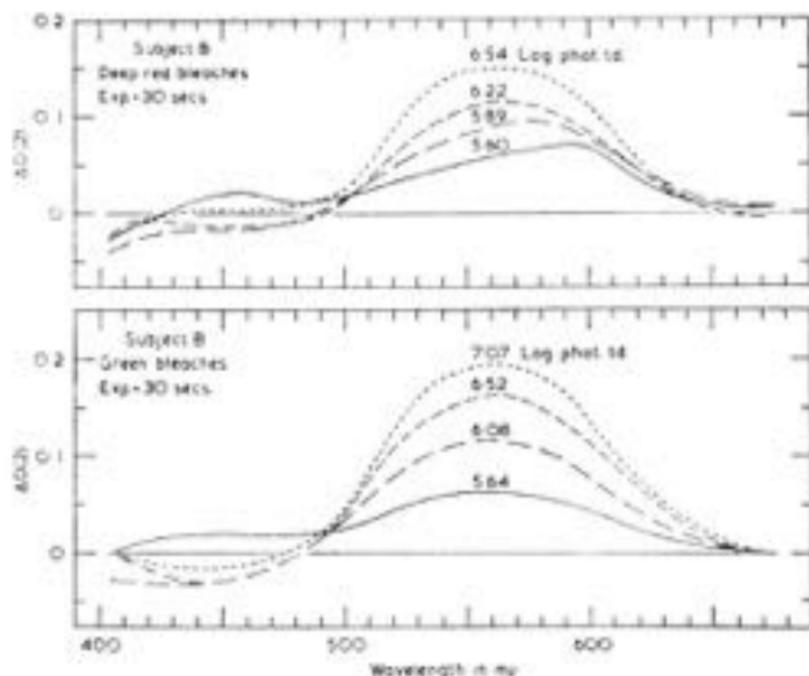


FIG. 4. Difference spectra showing (a) the change in shape and shift in λ_{\max} with red bleach of varying intensity, and (b) the constancy of λ_{\max} for green bleaches. (From Ripps and Weale, 1963, by permission of the Editor of *Vision Research*.)

deduce action spectra from their basic measurements of difference spectra which agree very satisfyingly with the shape of the curve of foveal spectral sensitivity (Fig. 5).

In the *in mortuo* division of the *in situ* measurements, visual cone pigments have been detected and measured by Marks, Dobbelle and MacNichol (1964) and Brown and Wald (1964). The principle of their method is to illuminate a single retinal receptor in an excised

retina with a specially designed microdensitometer and determine difference spectra by the bleaching technique. Carefully designed precautions were necessary against stray light, the influence of the measuring light on the pigment and deterioration of the retina, and in favour of the economical use of a retina, once obtained. Difference spectra of three main varieties were obtained with maxima at

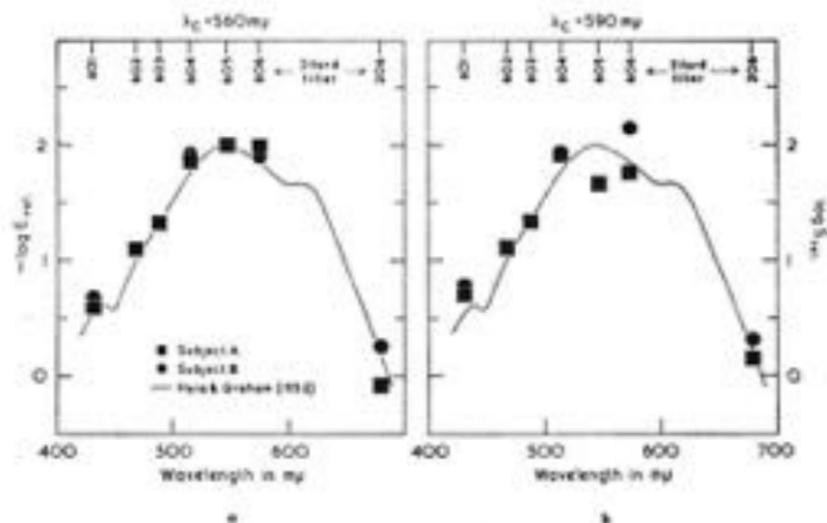


FIG. 5. Action spectra (source calibrated for an equal energy spectrum) obtained for criterion density changes of 0.1 at (a) 560 nm. and (b) 590 nm. The curve represents the logarithm of the relative foveal sensitivity function (A^* 601, 602, 603, 606; B^* 604). (From Ripps and Weale, 1963, by permission of the Editor of *Vision Research*.)

approximately 445 nm., 535 nm. and 570 nm. (Fig. 6; Marks, Dobbelle and MacNichol, 1964) and at approximately 450 nm., 525 nm. and 555 nm. (Fig. 7; Brown and Wald, 1964), which are presumed to belong respectively to blue, green and red receptors—cyanolabe, chlorolabe and erythrolabe in the Rushton nomenclature. The shapes are not improbable, although the figures for maximum absorption given by the various groups of workers vary rather widely, from 0.012 (Brown and Wald, 1964) to 0.35 (Rushton, 1963*b*). Considering the difficulties of the measurement,

due to the smallness of the individual cones and the complexity of their structure and assembly, these discrepancies are not surprising. In this connexion, the analysis given by Rushton (1963*b,c*) should

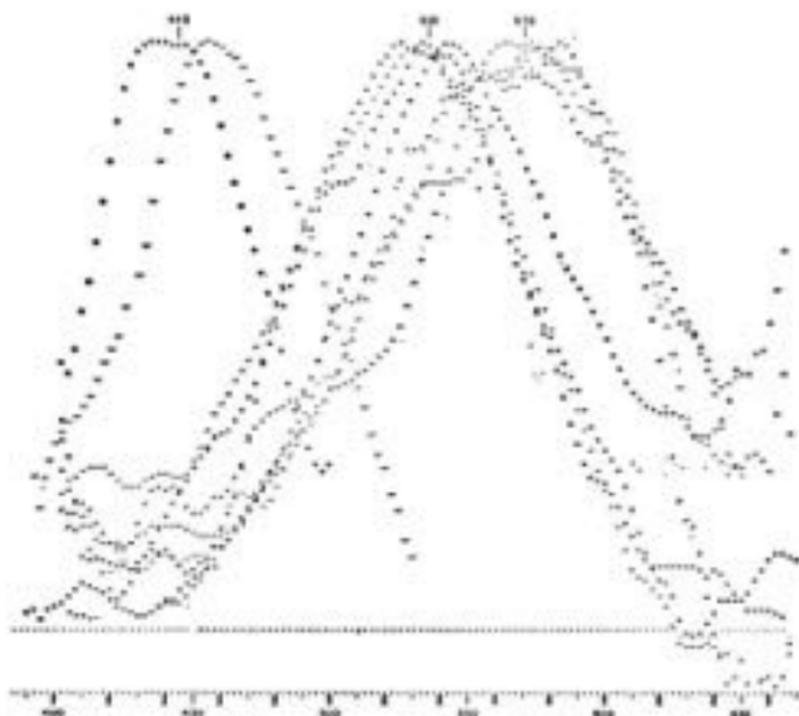


FIG. 6. Absorption difference spectra from ten individual primate cones, corrected for bleaching by the measuring beam. (From Marks, Dobbie and MacNichol, 1964, by permission of the authors and the Editor of *Science*.)

Curves recorded from monkey cones (*Macaca nemestrina* and *M. mulata*) are represented by numbers, those from human cones by open parentheses. Maximum absorption, 3 to 6 per cent, except the human blue, 0.4 per cent, is decreased by light scattered past the receptor. This figure is a photograph of the original record plotted automatically by a digital computer.

be carefully noted. It must also be remembered that all these *in situ* measurements are comparatively new; subsequent refinements of technique may soon diminish or remove the discrepancies.

In connexion with the question of interpretation, the case of *Xenopus* should be noted (Denton and Pirenne, 1951, 1954; Dart-

nall, 1954, 1956), in which a behavioural spectral response curve has been shown to be nearly congruent with the modular difference between the density spectra for two retinal pigments (Fig. 8). The double-peaked response curve shows what complications may be

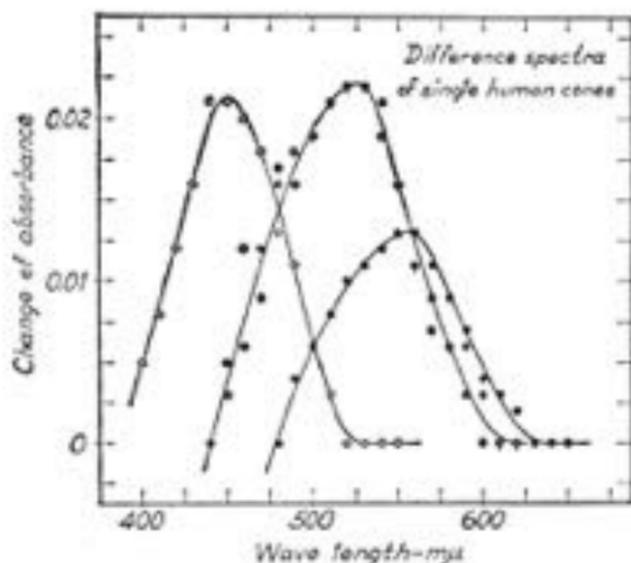


FIG. 7. Difference spectra of visual pigments in single cones of the parafoveal region of the human retina. (From Brown and Wald, 1964, by permission of the authors and the Editor of *Science*.)

In each case the absorption spectrum was recorded in the dark from 650 to 380 nm., then again after bleaching with a flash of yellow light. The differences between these spectra are shown. One of these cones, apparently a blue receptor, has λ_{max} about 450 nm.; two cones, apparently green receptors, have λ_{max} about 535 nm.; and one, apparently a red receptor, has λ_{max} about 555 nm. In making these measurements light passed through the cones axially, in the direction of incidence normal in the living eye.

produced by an apparently simple array of primary chromolabes in the retinal receptors.

The method of chemical indication of retinal response (Enoch, 1964) should not, perhaps, be considered in this review as it has only been applied so far to predominantly rod vision and so, presumably, to the detection of rhodopsin, which is doubtfully connected with

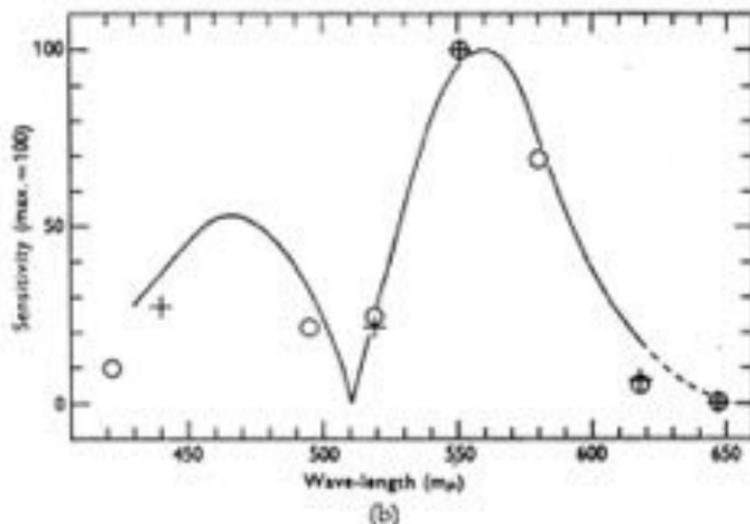
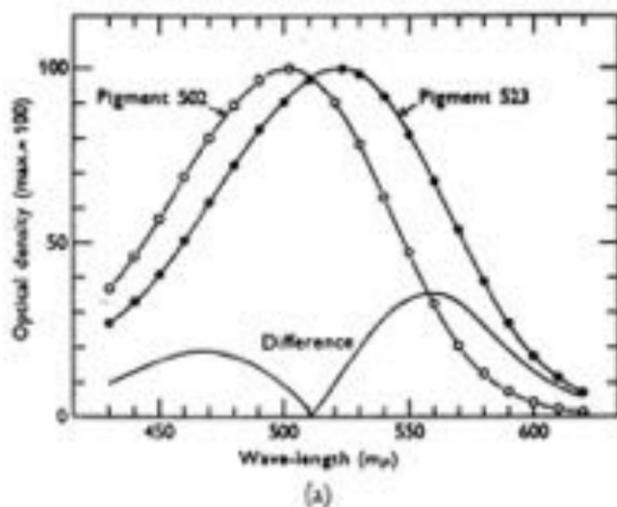


FIG. 8. (a) The density spectra (max. = 100) of pigments 523 and 502 of *Xenopus*, and the difference curve formed by subtracting one from the other, all differences being counted as positive. (b) Interpretation of the spectral sensitivity of *Xenopus*. (From Dartnall, 1956, by permission of the Editorial Board of the *Journal of Physiology*.)

+ , ○, relative visual sensitivity (max. = 100); +, from thresholds for expansion of skin melanophores; ○, from thresholds for the seeking of shade (Denton and Pirene, 1951). The bipartite curve (cf. Fig. 8a) is the modulus of the difference (max. = 100) between the density spectra of the two visual pigments of *Xenopus*.

colour vision. I believe it deserves passing mention, however, as a new method which is likely to be applied very soon to cone vision and which should be watched for results bearing on the mechanism of colour vision.

Turning now to the electrical indications of visual activity, mainly those originating in the retina, recent work here also has demonstrated responses which are fairly closely parallel to the spectral measurements, *in vitro* and *in situ*, on retinal cone pigments. De Valois (1960) worked with a primate (*Macaca mulata*), but recordings were taken from microelectrodes in the lateral geniculate nucleus and so do not provide direct evidence regarding the nature of the primary retinal receptors. Due, presumably, to nervous interconnexions between the retina-brain pathways, the electrical responses to stimulation of the eye by light show a complex pattern of narrow-band, broad-band and on-off responses varying with the location of the exploring electrode. It may be remembered, however, that on-off responses appear to be an essential feature of nervous conduction, so that a transformation from a Young-Helmholtz behaviour at receptor level to a Hering behaviour at all higher levels is merely the inevitable consequence of nervous conduction. De Valois' determinations of receptor response depend on differential bleaching by selected spectral bands and so are only directly comparable with experiments such as those of Rushton and of Weale, referred to earlier; equation with true receptor response is more problematical. In one instance, however, (De Valois, Jacobs and Jones, 1963) there is a striking and very significant coincidence of results, namely in the comparison of hue discrimination determined from electrical response ($\Delta\lambda$ for change of 1 spike/sec. in response rate) with Wright's hue discrimination data (Fig. 9).

A number of other workers, among them MacNichol and Svaetichin (1958) and Wagner, MacNichol and Wolbarsht (1960), have experimented with fish retinæ, obtaining results which obviously have some relation to the requirements of the

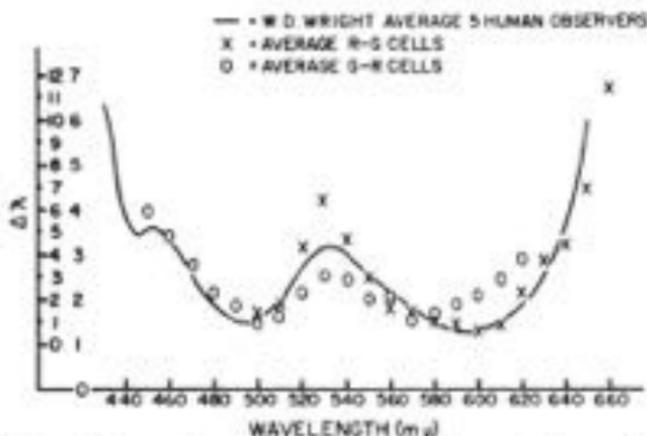


FIG. 9. Comparison of the differentiation of the equal luminosity curves with Wright's hue discrimination function. (From Wright and Pitt, 1934, by permission of the Editor of the *Proceedings of the Physical Society*.)

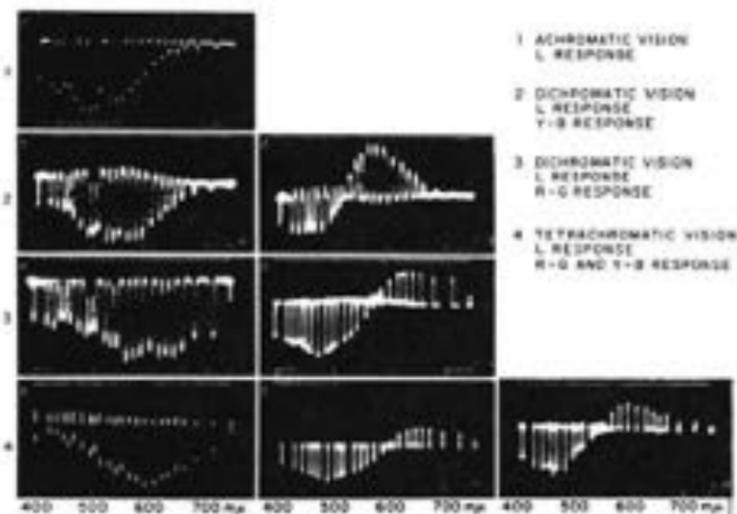


FIG. 10. Responses to light, passed through interference filters, from retinæ of fishes belonging to different families. (From MacNichol and Svaetichin, 1958, by permission of the authors.)

- (1) *Lutjanidae*, which live deeper than 30 m., gave a response of fixed polarity in every wavelength. Size of deflection varied to indicate one single function of wavelength likely to be brightness or luminosity, L.
- (2) *Serranidae* gave the previous response plus one characterized by opposite slow potentials in short and long wavelengths (blue and yellow).
- (3) *Centropomidae* gave responses as in (2) but stated to be antagonistic with respect to red and green.
- (4) *Mugilidae* gave all three types of response.

Young-Helmholtz theory, but more to the Hering opponent colours scheme (Fig. 10).

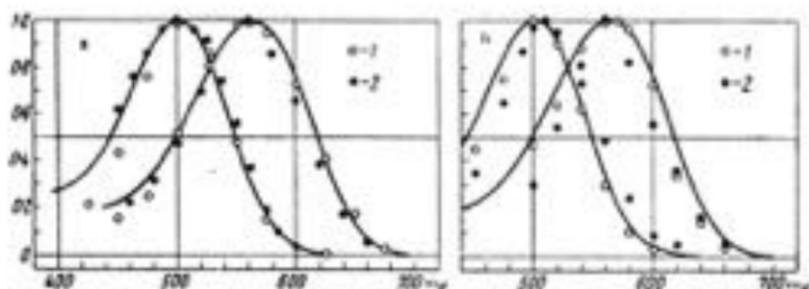


FIG. 11. Receptors of dichromate animals and pigments of retinene, system-rhodopsin and iodopsin. (a) Spectral characteristics of receptors of lake frog determined by the colorimetric method (1) and from threshold data of Granit (2); (b) Spectral characteristics of toad receptors (1) and grey mullet (2). (From Mazokhin-Porshniakov, 1960, by permission of the Editor of *Biophysics*.)

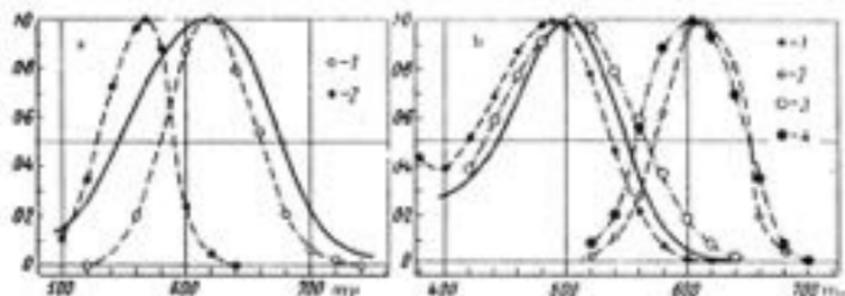


FIG. 12. Spectral characteristics of receptors of some animals which are unlike those of known visual pigments. (a) Longwave (1) and shortwave (2) receptors of tortoise and absorption spectrum of cyanopsin. (b) Rhodopsin and receptors of insects; (1) and (2) house fly receptors, (3) and (4) dragon-fly receptors. (From Mazokhin-Porshniakov, 1960, by permission of the Editor of *Biophysics*.)

A somewhat different approach to the exploration of vision via the electrical manifestations from the retina has been made by a number of Russian workers, for example, Mazokhin-Porshniakov (1960) and Orlov (1961). The colorimetric properties of an eye are determined by a matching process analogous to that of normal colorimetry; a field of illumination containing light of only one

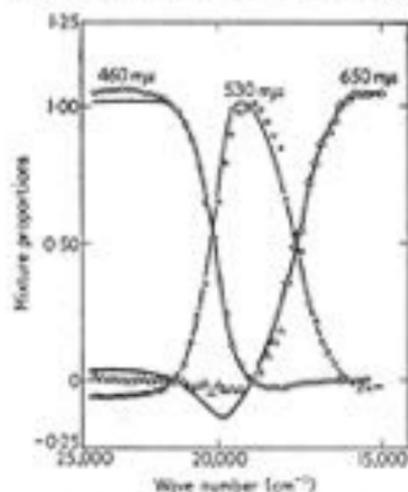


FIG. 13. Colour matching curves based on the mechanisms π_1 , π_2 , and π_3 . The curves are the spectral colour matching curves of a normal subject (Wright, 1946). (From Stiles, 1953, by permission of the author.)

They show the proportions of three primaries of 460, 530, and 650 nm. which gave a match with monochromatic lights of different wave numbers (negative proportions appear in the same half of the field as the test light). The points give the results which would be obtained by a subject with fundamental trichromatic sensitivities corresponding to the spectral field sensitivities of π_1 , π_2 , and π_3 .

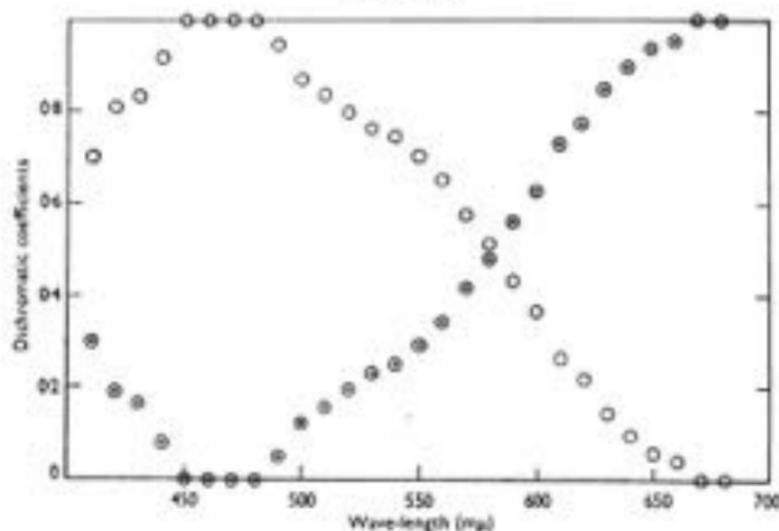


FIG. 14. Dichromatic coefficient curves for the spectrum in the state of artificial tritanopia. ○: amount of B (480). ◐: amount of DR (680). Units based on a match on λ 582.5 nm. (From Brindley, 1953, by permission of the Editorial Board of the *Journal of Physiology*.)

waveband alternates with another containing light of a mixture of two (or more) wavebands, the criterion of match being the absence of change in the electrical activity of the retina. The human subjective sensation of equality is thus replaced by an objective electrical "sensation" of equality. The latter can be applied to a non-human organism and so opens the way to a very wide range of

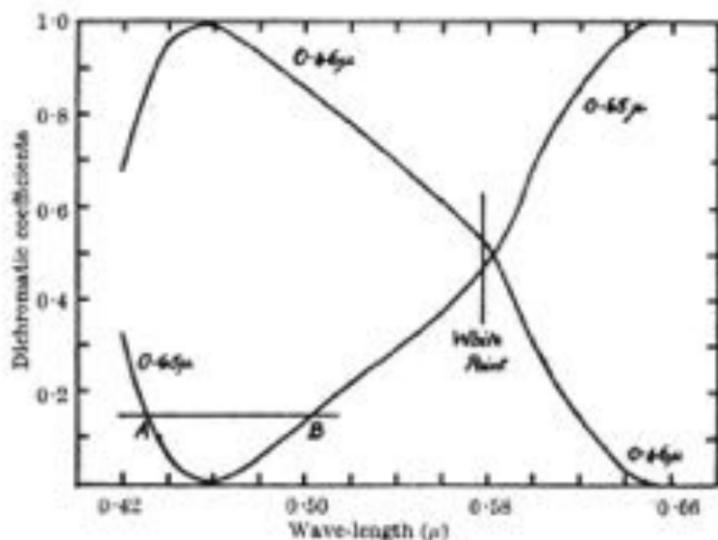


FIG. 13. Dichromatic coefficient curves for W. D. Wright. White point at 0.578 μ (578 nm.). A and B, spectral colours which match each other. (From Willmer and Wright, 1945, by permission of the Editor of *Nature*.)

colour matching investigations, within the limits, of course, of this particular procedure. The results appear, in many of the cases in which comparison is possible, to be closely parallel to sensations as judged by other methods, such as Granit's threshold measurements (Figs. 11, 12). It would seem too early to come to any sort of final conclusions, but it may be expected that further work on these lines and comparative study of the results will advance our knowledge.

The deduction of basic theorems of the mechanism of colour

vision from psychophysical observation has recently been most ably discussed by Brindley (1957) and it would be a waste of time to attempt another one here. Walraven (1962) has also discussed the matter at some length. A few examples may be given. The

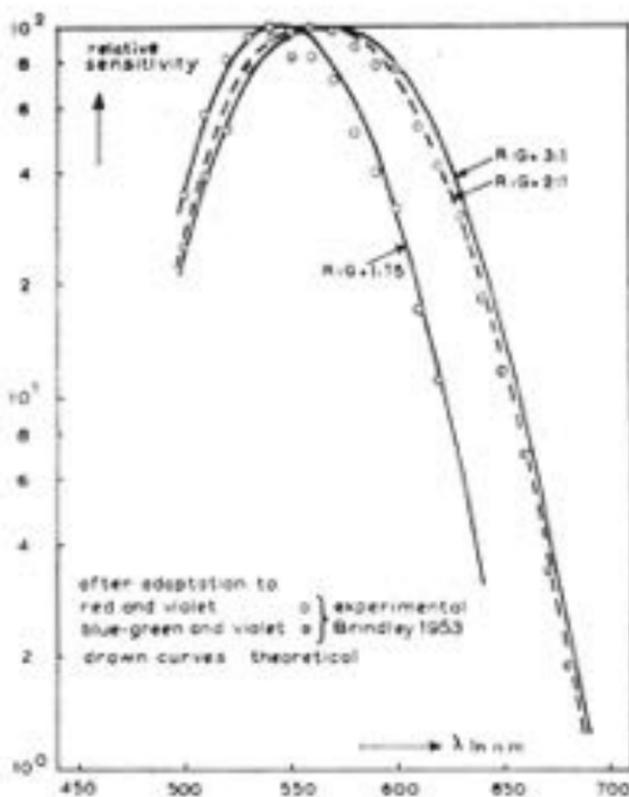


FIG. 16. The eye sensitivity curves after strong adaptation, compared with the theoretical predictions as described in the text, using Pitt's (1944) fundamental curves. (From Walraven, 1962, by permission of the author.)

deduction of the colour matching functions from Stiles' retinal mechanisms π_1 , π_4 and π_5 is only approximately correct (Fig. 13; Stiles, 1953) and Brindley points out that the disagreement may be due to straightforward experimental error, or to observer differences between the π determinations and the colorimetric work.

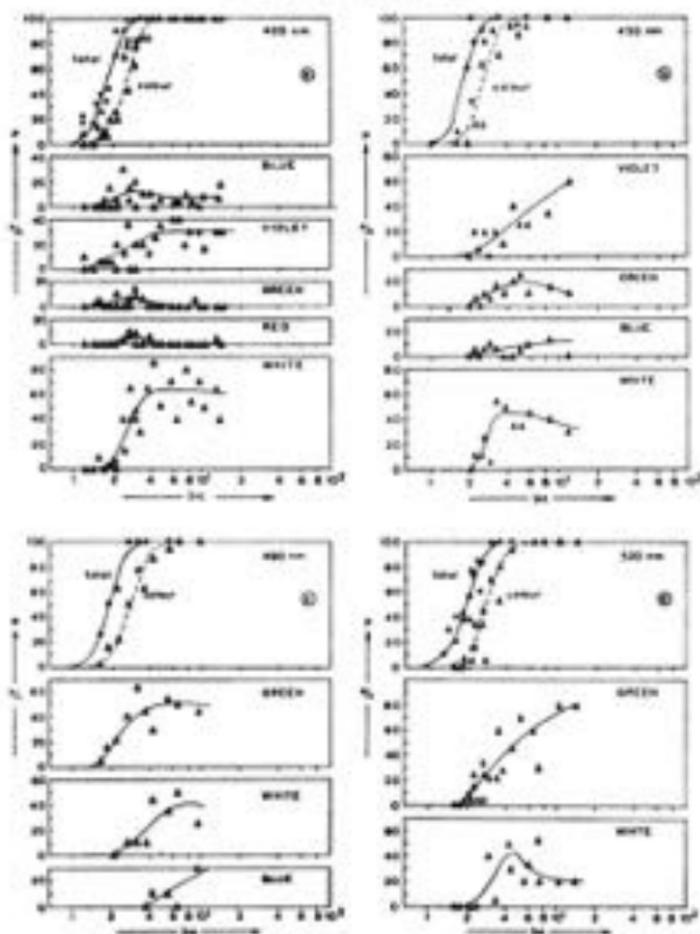


FIG. 17. Frequency of seeing curves as a function of intensity at the indicated wavelengths, for flashes of small visual angle ($3'$) and short duration (0.05 sec.). The upper graph represents the frequency of seeing at all (total) and the frequency of seeing colour at all (colour). The other graphs represent the frequencies of seeing of the particular colours as indicated. (From Walraven, 1962, by permission of the author.)

Both Brindley and Walraven discuss the behaviour of the eye in colour matching (Brindley, 1953) and in spectral sensitivity (Walraven, 1962) after adaptation to very high levels of illumination. Brindley finds the colour matching properties of the eye under

these circumstances (artificial tritanopia) to be closely similar to the small-field tritanopia demonstrated by Willmer and Wright (1945) (Figs. 14, 15). Walraven uses Pitt's curves for the sensitivity of the colour vision mechanisms (Pitt, 1944) to deduce theoretical spectral sensitivity curves which fit satisfactorily the experimental curves of Brindley under conditions of artificial tritanopia (Fig. 16).

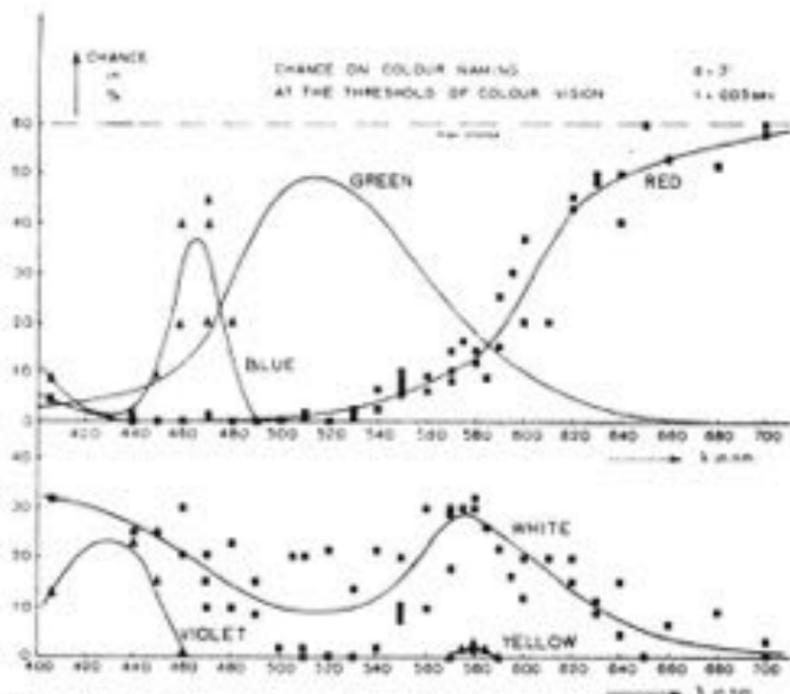


FIG. 18. The frequencies of seeing particular colours as indicated, at the threshold of colour, as a function of wavelength. (From Walraven, 1962, by permission of the author.)

Walraven (1962) shows that the Stiles-Crawford hue shift effect can be predicted by the use of sensation curves such as Pitt has determined, but not by the curves proposed by Hurvich and Jameson (1955) on the basis of their opponent colours (Hering) theory. All these results are strong evidence in favour of the Young-Helmholtz theory as far as the retinal receptor layer is concerned.

Walraven (*loc. cit.*) also discusses another aspect of colour vision, the appearance of coloured fields at intensity levels near the threshold of perception. Under appropriate conditions of exposure, the perceived colour is inconstant, as shown in Fig. 17, and it is supposed that this is connected with the manner of absorption of quanta in

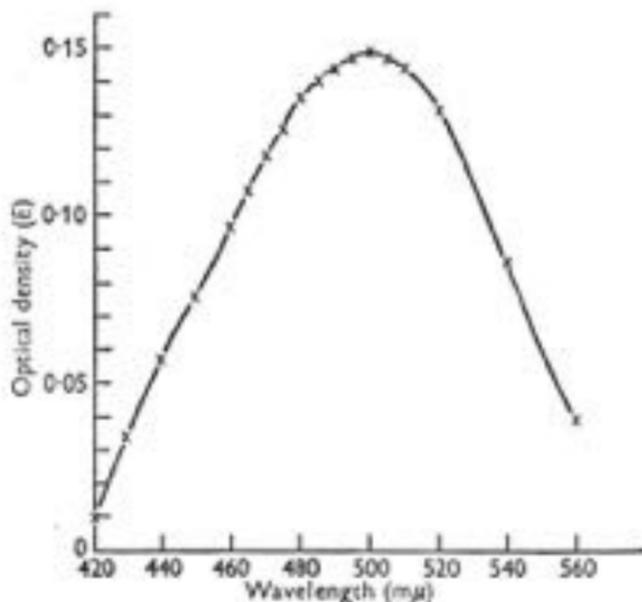


FIG. 19. Absorption characteristics, shown as optical density E ($\log_{10} \frac{\text{intensity transmitted after bleaching}}{\text{intensity transmitted before bleaching}}$), of retinal pigment extracted from dark-adapted herring eyes. (From Blaxter, 1962, by permission of the Company of Biologists Limited.)

the receptors. Herein lies the importance of this method of investigation: the quantum relations definitely locate the effects in the receptor end-organs. No formal relationship with the Young-Helmholtz theory has yet been proposed, but the shapes of the curves relating frequency of seeing a colour with its wavelength are suggestive (Fig. 18).

The advantage of non-human animals for the investigation of

the mechanism of colour vision is, naturally, the much greater freedom with which their eyes may be subjected to electrode insertion or to excision and pigment extraction. The disadvantage is the language barrier, but the increasing flow of experimental study of psychophysical response from non-human animals is an indication that the barrier is not absolute. The results may be typified by

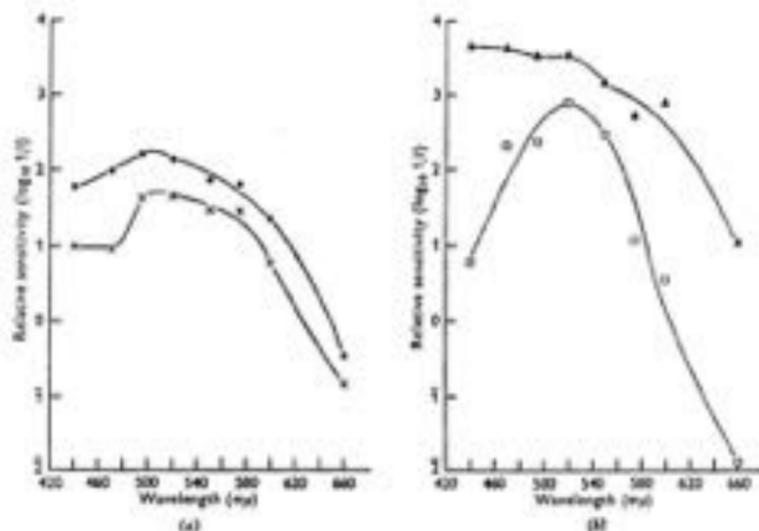


FIG. 20. The relationship between relative sensitivity ($\log_{10} I/\text{threshold intensity}$) and wavelength in the herring. (a) Feeding as a group, \times — \times ; singly, \bullet — \bullet . (b) Phototaxis, \square — \square ; perceiving a barrier, \blacktriangle — \blacktriangle . (From Blaxter, 1964, by permission of the Company of Biologists Limited.)

those of Blaxter (1964) on the spectral sensitivity of the herring. The spectral sensitivity may be determined using several aspects of behaviour as criterion. Retinal pigment can also be extracted from retinæ and its absorption characteristics measured (Fig. 19). The curve for phototaxis is closely similar to that for extracted pigment (Fig. 20b); the curves for group feeding and barrier avoidance are vastly different (Figs. 20a,b). The tempting, naive conclusion is that the herring may have a cone colour vision mechanism for

which the photopigments have not yet been found; the modest and sober fact is that beginnings have been made in a very promising line of investigation.

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DISCUSSION

Weale: Dr. Crawford did us the honour of showing one of our bleaching results, and implied that the green bleaches were intended to show the spectral characteristic of chlorolabe. I am sorry if we conveyed that impression. It was not our intention: the green bleaches are not believed to have isolated chlorolabe. They were intended to show that when you bleach a human fovea with green light of various intensities the difference spectrum which you obtain is to a first approximation independent of the intensity of the bleaching light, whereas the contrary is true of a red bleaching light.

Crawford: I have obviously misinterpreted your paper as definitely implying that these constant shape curves represented chlorolabe alone. The whole history of the Rushton-Weale retinal bleaching experiments, as revealed in the totality of their papers, shows how tricky is the technique and also the interpretation. It may well be that the true interpretation still awaits further experimental developments, partly or wholly new techniques.

Rushton: Have you recorded from the geniculate, Dr. Lennox-Buchthal?

Lennox-Buchthal: No, we have not measured from the geniculate but we have recorded from the cortex of monkeys and have found a picture quite different from that in the geniculate: the spectral sensitivity of single units is much narrower, in that the "colour-coded" units respond only to blue, green or red no matter how high the intensity, and at threshold they may respond to 10 nm., possibly less. At the time of this original work (Andersen, V. O., Buchmann, B., and Lennox-Buchthal, M. A. [1962]. *Vision Res.*, **2**, 295) we did not draw the spectral sensitivity curves because there was a good deal of variation from one trial to the next in any one unit, though still within the same range of the

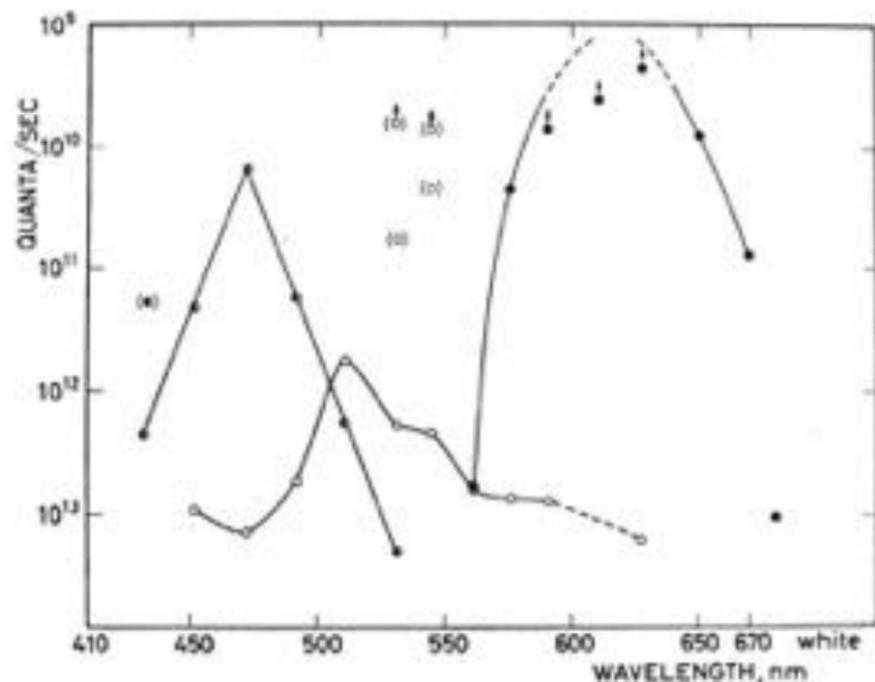


FIG. 1. (Lennox-Buchthal). Combined spectral sensitivity curves of units in the cortical area corresponding to central vision in the monkey (*Mangabey—Cercopithecus torquatus atys*).

Responses to at least a third to a half of the flashes of each wavelength, at the indicated and all higher intensities; fewer responses at lower intensities.

	sensitivity		
	● blue	○ green	● red
Number of units	8	8	8
Number of presentations	17	23	23
Criterion number of responses	7	7	11

The single symbol on the right indicates the one response to white with the criterion used.

spectrum. Since then we have drawn the combined spectral sensitivity curves of all the blue, all the green and all the red-sensitive units, using different criteria of threshold—for example, responses to a third to a half of the presentations of that wavelength at a given intensity and at all higher intensities, and fewer responses at all lower intensities (Fig. 1).

When this is done, the blue and red-sensitive units have clearly defined spectral sensitivity curves, the blue-sensitive units showing a sharp peak at 470 nm. and the red-sensitive units showing a somewhat broader curve but clearly most sensitive, in our limited number of units, to about 630 nm. The green-sensitive units showed more variability and sometimes responded in a vaguely opponent colour fashion to about 510 and 590 nm. Thus the colour code in the cortex seems to be clearly trichromatic. It looks as if the activity of single cells in the cortex is very much influenced by inhibition, presumably in the cortex itself. In order to test that we have recently put d.c. currents across the single cells to see if we could influence their spectral sensitivity. I must say that I cannot quite see how one can get away from the cortex in trying to work out colour vision mechanisms; I cannot see how one can explain everything on the retina.

Craford: I do not want to give the impression of trying to get away from the cortex. I do want to find out what each does. If the cortex and the retina do different things, then something has happened on the way, and this is what we want to know. Colour response must at least have started in the retina.

Weale: An important point arises which may partly account for differences between work on the cortex and on other parts of the visual pathway. In the first place, De Valois and his collaborators succeeded in matching the human wavelength discrimination curves, not sensitivity curves, from results obtained on the lateral geniculate body. The more important technical point is that they have really come to grips with stimulus relations. This is a point which has eluded some electrophysiologists, and not only electrophysiologists. De Valois and his co-workers are quite aware that if you stimulate the eye with equal energy spectra you find one sort of response, but if you stimulate it with equal luminosity spectra you find an entirely different response. They used the human luminosity curve to calibrate the spectrum in energies which gave a constant luminosity response. It is those energies which they applied to the monkey eye in order to measure the response. Their computed data, which match the human wavelength discrimination curve, are obtained from an equal (human) luminosity stimulus. We have emphasized equal energy stimuli but they are neither here nor there physiologically; they are useful to physicists because we know then that

one part of the spectrum does not give you an advantage from a stimulatory point of view over another part before it reaches the eye, but as far as the eye is concerned an equal energy spectrum is useless.

Crawford: A physicist would, perhaps, say that an *equal quantum energy* spectrum is the most fundamentally significant.

Wolbarsht: Dr. Weale, this does not help when you are working on a single unit, because all you can do there is to work out the energy necessary to give a constant response from that one unit. You have then to put these curves together to get the equal *luminosity* curve for the total of vision, because an equal luminosity curve is the combination of all the receptor types. When you are working with only a single, or two, receptors you cannot use the whole curve and expect that one receptor to give a constant response. If there is any non-linearity in the system you will not be able to interpret your results.

Weale: I am aware of that difficulty, but in fact when you measure human wavelength discrimination curves you measure on the basis of equal luminosities. You are going to get quite a different curve if you do not equate your luminosities.

Wolbarsht: One cannot define hue discrimination with single units; but you are talking about De Valois' work on the lateral geniculate.

Weale: The energy relations have to apply at the level of the receptors.

Wolbarsht: I agree, but what energy relations? How should we equate the value of one receptor-type with another in the overall luminosity curve with electrophysiological methods?

Klüver: More than twenty years ago Judd pointed out that practically all colour theories have reference to one type of colour only, namely aperture colour with a dark surrounding field, thereby implying that an adequate theory for surface colour does not exist (Judd, D. B. [1940]. *J. opt. Soc. Amer.*, 30, 2). What he had in mind was the distinction introduced by David Katz half a century ago between "aperture" or "film" colours on the one hand and "surface" colours (which generally represent "object" colours) on the other hand. According to Judd, aperture colour can be expressed as a function of three variables, whereas a surface colour (which implies the idea of a surface reflecting light in the presence of other surfaces in an illuminated space) requires *at least* six variables. No doubt it is chiefly surface colours that determine the directions and

turns of behaviour whenever man or animals respond to colour in the external environment.

Crawford: There is not any real distinction between the two; it is simply a matter of comparing an inner field with an outer field, or a smaller field with a larger one. The larger field, whatever its form, tends to be an adapting or controlling field, and according to its intensity level relative to the smaller field, the smaller field may be a surface colour or an aperture colour. This can be demonstrated with quite simple apparatus; surface colours can be made to appear as aperture colours, or *vice versa*. If paper of a certain tint of red, for instance, is surrounded by successive grades of grey paper going from white to black, one's impression of the red is of a surface colour at one end and an aperture colour at the other. I should have thought that the perception of those psychologically different types of colour could be just as obvious to an animal as it is to us. I don't really see any fundamental difference between them.

Klüver: The question would then seem to be whether the immense amount of experimental work done in the fields of sensory psychology and physiology has really justified the criteria used by Katz and others for distinguishing surface colours and film colours. For instance, it has been maintained that it is only in the world of surface colours that an "approximate constancy of colour", the perception of "object" colours, becomes a problem.

CELLULAR MECHANISMS OF A YOUNG-HERING VISUAL SYSTEM

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THE following statements by Marriott (1962) in his recent review of colour vision provide in a nutshell an introduction to the problem: (p. 314) "... The Hering theory offers only the most nebulous advantages over the classical Young theory, and has several disadvantages..." (p. 315) "... the zone theories, however comprehensive and ingenious, are based more on intuition than on experimental fact."

Young's three-receptor theory

Colour matching and mixing indicate that three independent and linear receptor mechanisms are involved, in agreement with the original proposal by Young (1802), which has received further support from cone pigment studies by Rushton (1959), Ripps and Weale (1963), Brown and Wald (1963) and Marks, Dobelle and MacNichol (1964). The term trichromatic (three colour) theory is not appropriate for Young's proposal, and in this paper we shall use the name "the three-receptor theory". The idea of Young, as expressed in its original form, was to have "the number limited" of the specific "sensitive points" or receptors of the retina, instead of having one for each hue, without pretending to explain how colour is seen. The number three was a good guess at that time, without precise colour matching data.

Hering's opponent colour theory

The opponent colour theory of Hering (1872; see Hering, 1878), which indeed deals with the seeing of colour, is deduced from psychological facts. Hering paid attention to the functional coupling of our four unique colour qualities (B: blue, G: green, Y: yellow, R: red) into opponent pairs (B-Y and R-G), the intermediary hues being combinations of one member from each pair, while the opponent, mutually exclusive colours do not mix, and bluish-yellowish and reddish-greenish hues do not exist (see Linksz, 1964). These fundamental characteristics of colour vision find no explanation in the three-receptor theory. Hering did not specify any receptors for colour or brightness; he suggested only the existence of opponent retinal processes, catabolic and anabolic, corresponding to the opponent colour and black-white sensations. It is of interest to mention that our interpretation of a non-neuronal (glial) control of excitability and inhibition is discussed in terms of catabolism and anabolism respectively (Svaetichin *et al.*, 1961, 1963).

A Young-Hering system

Having four unique colour sensations, but three different cones, indicates that the three cone-pigment spectra by no means correspond to any of the four unique colour sensations. Hering's opponent processes are compatible with the standard neurophysiological principle of reciprocal excitation-inhibition, which is operative for instance in a flexor-extensor control system. An excitation-inhibition mechanism can, at one point, deal simultaneously with a maximum of two antagonistic information qualities. From this point of view, it is obligatory to transform, at the retinal level, the three-receptor information from one point into two parallel opponent processes, which then simultaneously each provide only one information quality for the intermediary hue processing at the cortical level. With this reasoning, the opponent

colour system is a consequence of the functional design of the nervous system, as pointed out earlier (Svaetichin, 1956).

Three is the minimum number of receptors on which the Hering opponent system can be based; four would also do, but nature designs economically. This is the only magic of the number three in vision. The finding of a fourth cone in the periphery of the human retina would not at all reduce the value of Young's idea.

The failure of an opponent mechanism leads of course to the lack of the corresponding unique colour pair. Normal vision is not trichromatic but rather tetrachromatic three-receptor vision, and defective vision is either dichromatic or achromatic. The famous discussions of yellow were caused by misleading deductions from Young's theory. The yellow colour sensation is related to the cone pigments in the same way as the blue, green and red sensations.

Existing neurophysiological data

By now, abundant electrophysiological evidence indicates that wavelength discrimination is based on a Hering type of opponent system, probably with a three-receptor input. Motokawa and Svaetichin independently demonstrated wavelength-dependent opposite polarity potentials in the fish retina (S-potentials from non-neuronal controller elements), and subsequently, numerous papers have been published on the same subject from Tomita's, Motokawa's and other laboratories (see Tomita, 1963; Motokawa, 1963; Svaetichin, 1956; Svaetichin *et al.*, 1961, 1963). The wavelength-dependent opposite polarity responses were discussed extensively in terms of a Hering opponent colour theory by Svaetichin (1956) and by Svaetichin and MacNichol (1958). Evidence indicating a three-receptor input was presented by Svaetichin and co-workers (1961; Fig. 5).

De Valois and co-workers (1958) demonstrated in the lateral geniculate body of the monkey spike firing patterns reflecting opponent colour coding, and a corresponding behaviour of neurones

of the fish retina was observed by Wagner, MacNichol and Wolbarsht (1960).

In their early papers, De Valois and co-workers (1958) described in addition to the opponent cells, findings of geniculate neurones with a firing pattern corresponding to the narrow-band modulators of Granit (see Granit, 1962). However, in a recent report at the Fourth International Photobiology Congress in Oxford, De Valois, Jacobs and Abramov (1964) state the following: "Two classes of cells can be distinguished in the primate retino-cortical pathways on the basis of their responses to stimulation of the eye with various monochromatic lights: (a) spectrally non-opponent cells, which respond with inhibition to all wavelengths, or with excitation to all wavelengths, and (b) spectrally opponent cells, which show excitation to some wavelengths and inhibition to others. That the non-opponent cells are concerned with brightness vision is indicated by the fact that their spectral sensitivity curves coincide with the luminosity curves of the monkey (and man). The opponent cells appear to be concerned with colour vision." This valuable statement shows that their recent findings agree with the studies in Motokawa's laboratory (see Motokawa, 1963), and with our work on the monkey cortex and geniculate body (M. Verzeano, E. Vallecalle, M. Laufer and G. Svætichin, unpublished data, 1959). Also in the monkey cortex, a typical, spectrally opponent firing pattern is seen in recordings from small neurones of the superficial layers, provided the cortex is in good condition, otherwise the "off" firing will be abolished, and only the narrow-band modulator type of firing is observed. Similarly, if the fish retina is in a poor functional state, the inhibitory "off" firing is lacking, as well as the depolarizing component of the chromatic S-potential. Consequently, when the "off" firing of a spectrally opponent cell deteriorates, the "on" firing simulates a narrow-band modulator. This conclusion agrees with Dartnall's (1962) suggestion that the narrow-band modulators are due to an interaction between the information from two different receptors (cf. Fig. 10). Thus the

photopic and scotopic dominator of Granit agrees with recent experimental results, while the modulator concept does not.

Receptor potentials

Isolation of the electrical response recorded across the receptor layer was achieved by exposing the isolated fish retina to a small amount of ammonia, sufficient to block the function of all other retinal elements (Mitarai *et al.*, 1961). The large (2–5 mV), graded square wave response to light stimulation, and the increased d.c. level (5–15 mV) of the transretinal recordings obtained after ammonia treatment, were shown by two-microelectrode differential recording to originate across the receptor layer only (within 80 μ from the exposed receptor surface, Fig. 1A). The receptor response was restricted to the illuminated area only. The response to light was related to the d.c. level across the receptor layer, and corresponded to a graded decrease of this d.c. potential, which response is linearly related to the light stimulus intensity (Fatehchand, Laufer and Svaetichin, 1962). Moving one microelectrode of a differential pair placed on the receptor layer surface into the receptor layer caused a proportional increase of the d.c. level and the light-induced response (Fig. 1A).

Altering the d.c. level by an externally applied potential did not influence the response to light, neither could the receptor response be evoked by electrical pulse stimuli. Anoxia, carbon monoxide with 5 per cent oxygen, azide and cyanide abolished the responses and the d.c. potential within seconds. Gamma irradiation from a cobalt source, in doses which had no influence on the neuronal spike, abolished the receptor potentials and also the S-potentials (K. Negishi, G. Svaetichin and J. A. Velandia, unpublished data, 1964). Gamma irradiation destroys the mitochondrial membranes (Portela, Perez and Stewart, 1963). Temperature changes showed a pronounced influence on the receptor response, the d.c. potential changing by about 1 mV per $^{\circ}$ C, the optimal temperature range for the response being 18–25 $^{\circ}$ C, indicating an intimate

coupling to enzymic processes. The receptor potential shows a behaviour in this respect very similar to that of the non-neuronal controller cells.

A retina which was dark-adapted before the ammonia treatment gave slow responses (Fig. 1B) showing two spectral maxima at 470 and 542 nm. (Fig. 1D), while the previously light-adapted retina

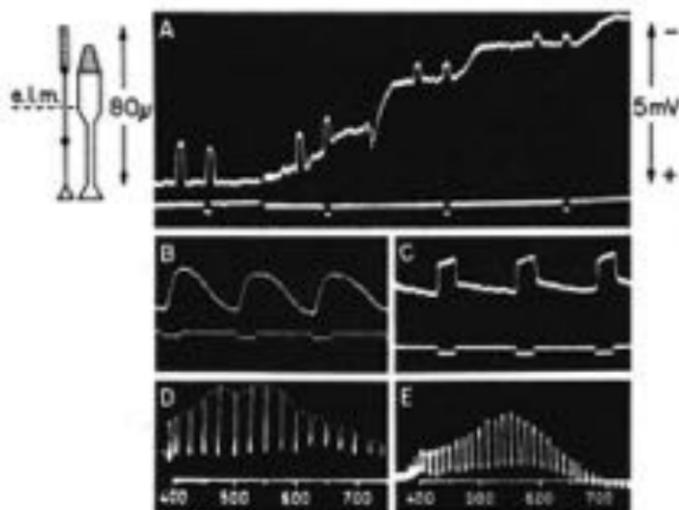


FIG. 1. Recordings of receptor potentials (Centropomidae). In A, one electrode of a differential pair moved into the receptor layer during the recording (read from left to right). *e.l.m.*: external limiting membrane. See text. (Unpublished data, M. Laufer, G. Mitarai and G. Svaetichin, 1959.)

produced fast square waves with a single spectral maximum at 542 nm. (Fig. 1D, E). The spectral maxima indicate cone activity (cf. Fig. 4, Centropomidae). Selective bleaching did to some extent, but not clearly, reveal the different spectral characteristics of the cones.

When inserting a superfine microelectrode (200–500 megohm) carefully into the exposed receptor layer, we obtained potentials within the receptor layer (40μ from the exposed receptor surface) showing characteristics similar to the "so-called cone action

potentials" described by Svaetichin (1953). These membrane potentials, which apparently are to be regarded as recordings across the plasma membrane of the cone myoid or ellipsoid (also from frog rods), were difficult to maintain for any length of time. Tomita (1963) has earlier reported similar findings using the Naka type of electrode, which we also appreciate. These findings probably correspond to the recordings showing no area effect, described by Oikawa, Ogawa and Motokawa (1959), when using electrodes below 0.2μ . The electrodes used by Svaetichin (1953) had a tip diameter well below 0.1μ (see the electron micrograph by H. Fernández-Morán in Ottoson and Svaetichin, 1952). Thus at least some of the "so-called cone action potentials" were in fact recordings from cones, whereas certainly most of the recordings by Svaetichin in 1953 were of a non-neuronal origin. (It is of interest to mention that the maxima given in 1953 were at 450, 540 and 600 nm.)

Absorption spectra of cone and rod pigments

Precise information regarding the cone pigments of the fish retina was lacking until the remarkable demonstrations by Marks and MacNichol (1963) and by Marks (1963) of pigment spectra of single cones of the goldfish retina. Pioneering experiments by Hanaoka and Fujimoto (1957) gave different results. We decided to carry out similar experiments and determine the cone pigment spectra of the species of fish which were used in our comparative electrophysiological, neurohistological and biochemical studies.

In our pigment spectra studies we made use of a photostimulator and microspectrophotometer unit, employing interference filters, which was built in 1959 (described in Mitarai *et al.*, 1961). We would like to express our gratitude to Mr. W. Krattenmacher and Mr. W. Weber for the earlier construction work, and particularly to our present collaborator, Mr. C. Muriel, for his devoted and skilful work. The methods used in our pigment spectra studies are in principle the same as those described by Hanaoka and Fujimoto

(1957) and by Marks (1963). In all our spectral absorption measurements plane polarized light was used (Nicol prism), focused by an apochromatic objective into a circular spot of 2-3 μ diameter or a slit, at the level of the isolated cone. Our single-beam microspectrophotometer is rather simple, and does not have the accuracy and spectral resolution of the ingenious machinery of Marks and MacNichol. However, for these comparative studies the method proved sufficient, having the advantage of giving a spectral run in two seconds, and causing little bleaching of the pigment. The peak transmissions of the interference filters used are given at the bottom of Fig. 4. The responses of the photomultiplier to the different spectral lights passing the interference filters were approximately equalized (using neutral filters), the noise and error varying between 1 and 2 per cent. Thus, equal downward deflections of the oscilloscope tracings correspond to equal absorption over the spectrum used.

Absence in cones of photoproducts within the visible range

The records of spectral absorption in Fig. 2 illustrate the effect of bleaching on the spectral characteristics of the receptor pigments. The measurements on fish rods (Fig. 2 G-I, small adjacent rods in parallel) show that the photoproduct absorbs in the short-wave end of the spectrum (I), in agreement with the behaviour of rhodopsin in solution (see Dartnall, 1962). However, we never observed in cones photoproducts produced by bleaching, within the spectral range used. The cone records A-C and D-F show that after bleaching the absorption is reduced (B and E), additional bleaching making the absorption close to zero. When the measuring light beam was moved outside the cone into a cell-free area, the same zero line of absorption was obtained, while measurements within the ellipsoid, for example, showed a slight absorption in the short-wave end. Some of the fish cones used had a laminated outer segment large enough for several independent measurements to be made in different parts of the same cone. Bleaching of the pigment com-

pletely in one part of the receptor did not influence the absorption of a neighbouring unilluminated region, indicating that the photopigment molecules are fixed to the structure. The lack of photoproducts in cones within the visible spectrum is probably important for colour vision.

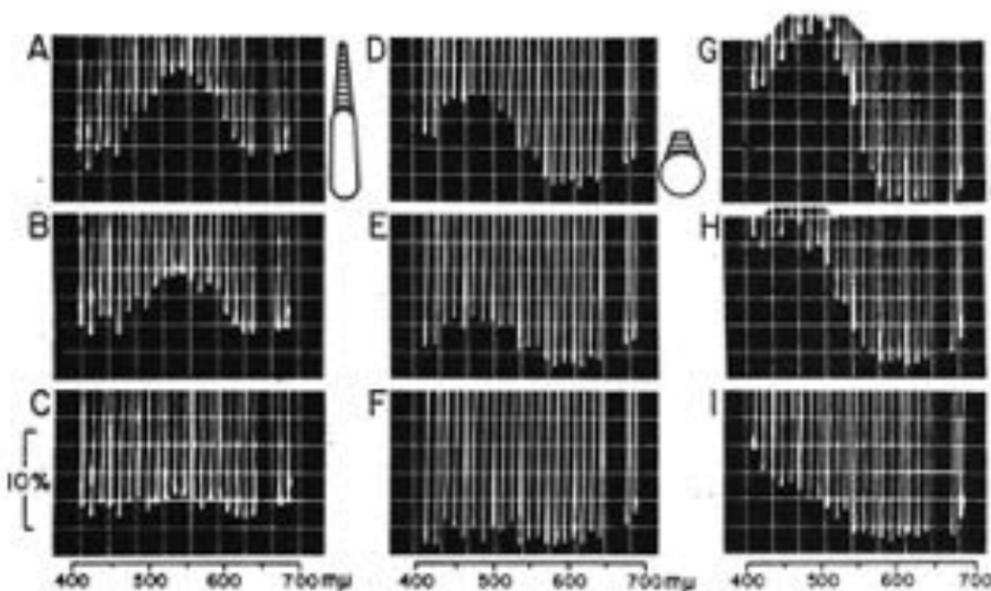


FIG. 2. Oscillographic recordings of spectral absorption of isolated receptors and photopigment bleaching in *Haemul pomadoury*. Ten per cent absorption given to the left. See text.

Regular orientation of the cone pigment molecules

The oscillographic recordings of absorption spectra shown in Fig. 3 illustrate the effect of plane polarized light, when passing through the laminated portion perpendicularly to the receptor axis (all our experiments were done this way). The black two-way arrows indicate the main vector of the polarized beam of light in relation to the receptor axis; A, B are absorption spectra of fish rods (adjacent rods in parallel), and C, D shows the spectral absorption of an "additional short single cone", while E, F is the absorption of a

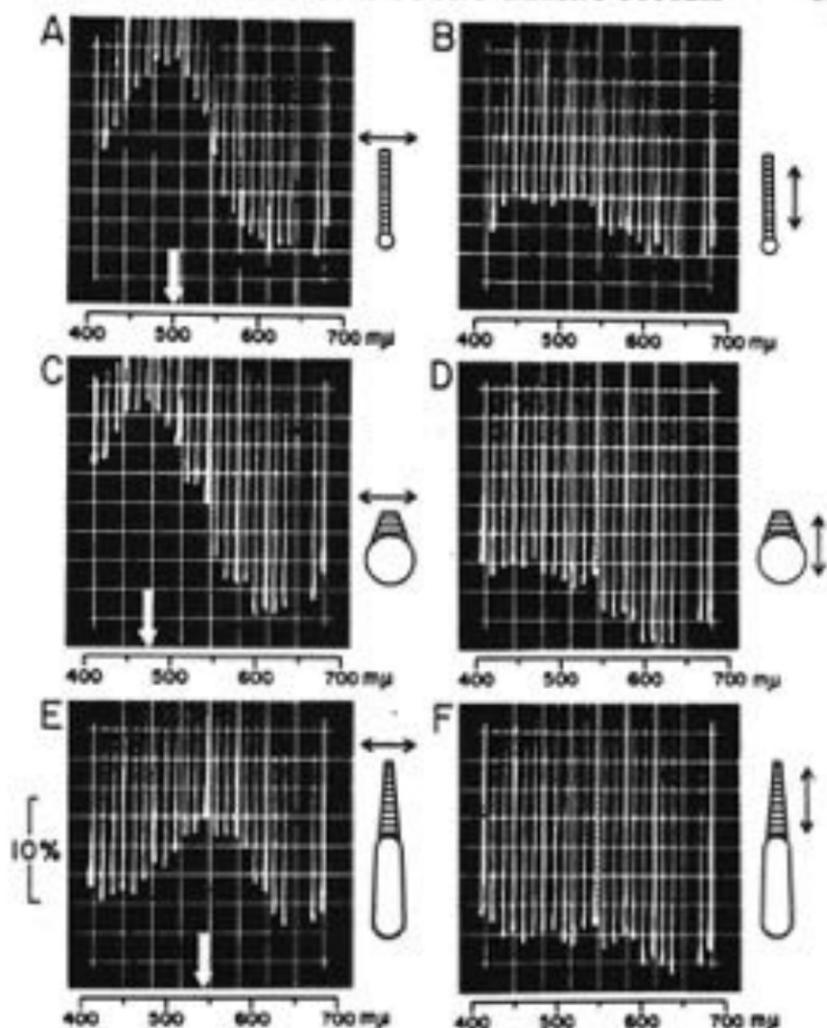


FIG. 3. Spectral absorption recordings of isolated receptors of *Haemul pomadoury*. Effect of plane polarized light. Two-way arrows indicate plane of polarization of light beam passing perpendicularly to receptor axis through laminated segment. See text.

"central long single cone" (cf. Fig. 4). The maxima of absorption are indicated approximately by the vertical white arrows.

The experiments demonstrate that the orientation of the plane of polarized light has a pronounced effect on the efficiency of

absorption, when the light beam is at right-angles to the receptor axis. This effect has been previously shown to be true for rods (Schmidt, 1938; Denton, 1954) and to depend on a semicrystalline regularity of the photopigment, the axis of the prosthetic group molecules being orientated in a plane perpendicular to the receptor axis.

Spectral distribution of the receptor pigments

In the present studies the spectral absorption of more than a thousand individual cones from nine different species of fish was measured. The results are summarized in Fig. 4. Generally, the cones of the fish retina form a regular pattern, and this was true for all the fishes studied, except *Mugil braziliensis*. The mosaic is built of different types of single and double cone, and it was easy to identify each cone type in the microscope in conjunction with the measurements. Of importance was the collaboration with Dr. A. Selvin De Testa of our laboratory and Mr. V. Partés of the University of Carabobo, Venezuela, who carried out parallel morphological studies. The extensive work by Engström (1963) offers data concerning the cone types and patterns of a large number of fish species. In the present paper we use Engström's naming of the different cone types: (a) the additional short single cone, which has an eccentric location, (b) the central long single cone, which is in the centre of the mosaic unit, (c) the equal double cones, a large and a small type, and (d) the unequal double cones (goldfish). The different mosaic patterns formed by the cones in the different fish species are indicated to the right of Fig. 4.

The maxima of the spectral absorption of one type of cone, in a given fish, showed surprisingly small scatter. Fig. 4 shows the maxima of the spectral absorptions of the different types of cone observed in the nine different fish species. The maxima of all the additional short single cone pigments are grouped around 465 nm. (Fig. 4, filled circles). The maxima of the rods are at 500 nm., except the 531 pigment rod of the goldfish (Fig. 4, rods indicated by rod-

like bars). The central long single cones showed maximal absorption either in the 500 nm. region or at about 540 nm. (Fig. 4, indicated by dotted or striated circles). The large and small equal double cone absorptions are distributed in the regions 500, 540 and

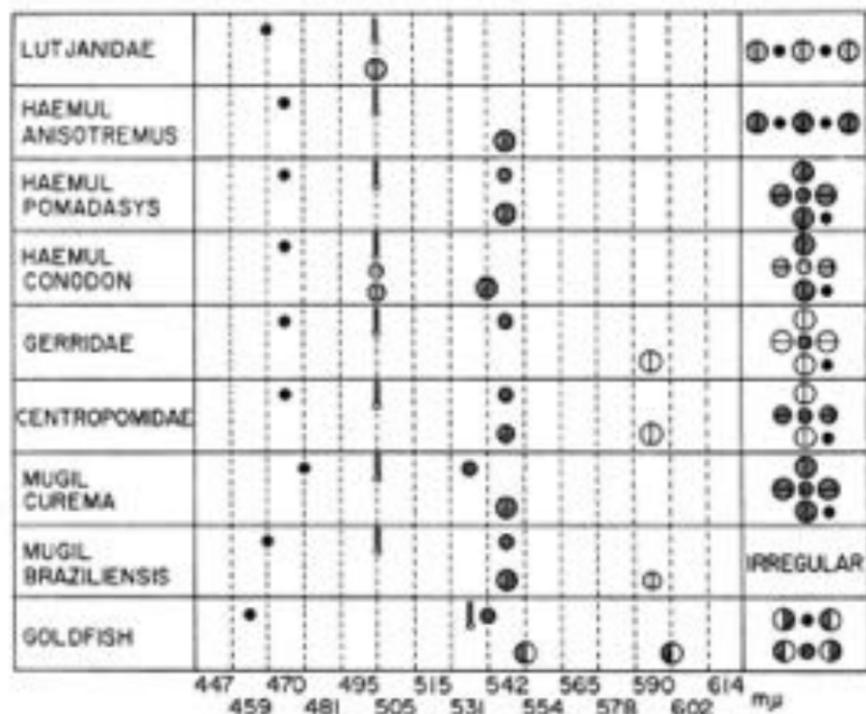


FIG. 4. Summary of the receptor pigment data. See text.

590 nm., while the pigments of the two members of the unequal goldfish double cones are located at 540 and 590 nm. respectively (Fig. 4, double cones indicated by divided circles).

The goldfish inhabits freshwater, whereas all the other fish studied are from lagoons and river mouths of the Venezuelan coast. The rod pigment of the goldfish is apparently a retinene₂ pigment, while the others are probably (rhodopsin) retinene₁ pigments (see Dartnall, 1962). It is worth mentioning that during some periods of the year the retina of the fish from the lagoons show a colour

different from that of a frog retina (see Dartnall, 1962). The absorption maxima of the goldfish cones agree with the data given by Marks (1963). It could be suggested that the cones grouped around 500 nm. contain rhodopsin; however, the lack of a photoproduct is against it. We never observed "empty" cones without a bleachable pigment, in a carefully prepared retina. Hence, no other types of cone pigment are expected in these fishes. Three different cone pigments was the maximum number found in one retina, but some had only two (Fig. 4). We suspect that some fish from deep water have only one pigment in the region between 450 and 500 nm., in agreement with our earlier electrophysiological data (Svaetichin and MacNichol, 1958).

The photopigment studies are in good agreement with our electrophysiological studies, and will be discussed below. It is interesting to note that the fish which has electrophysiologically and structurally the most advanced retina, *Mugil braziliensis*, has an irregular pattern of cones. It seems reasonable to suggest that the regular pattern and the equal double cones are adaptations for pattern and movement recognition. The grouping of cone pigments at about 465, 540 and 590 nm. agrees approximately with the pigments found in the primate and human retina (Rushton, 1959; Marks, Dobelle and MacNichol, 1964). The spacing of approximately 35, 40 and 50 nm. between the different cone pigment groupings of Fig. 4 is possibly due to a stepwise structural difference of the pigment molecules.

Electrical responses of neuronal and non-neuronal elements of the retina

To the left of Fig. 5 is reproduced a stage theory diagram according to Judd (1947). The three curves above to the left, labelled P_1 , P_2 and P_3 , correspond to the proposed three retinal input processes (König type fundamentals), while, below to the left, the opponent processes indicated by b-y and g-r represent transformations from the curves above. To the right of Fig. 5 recordings are shown of spectral response curves obtained from different non-

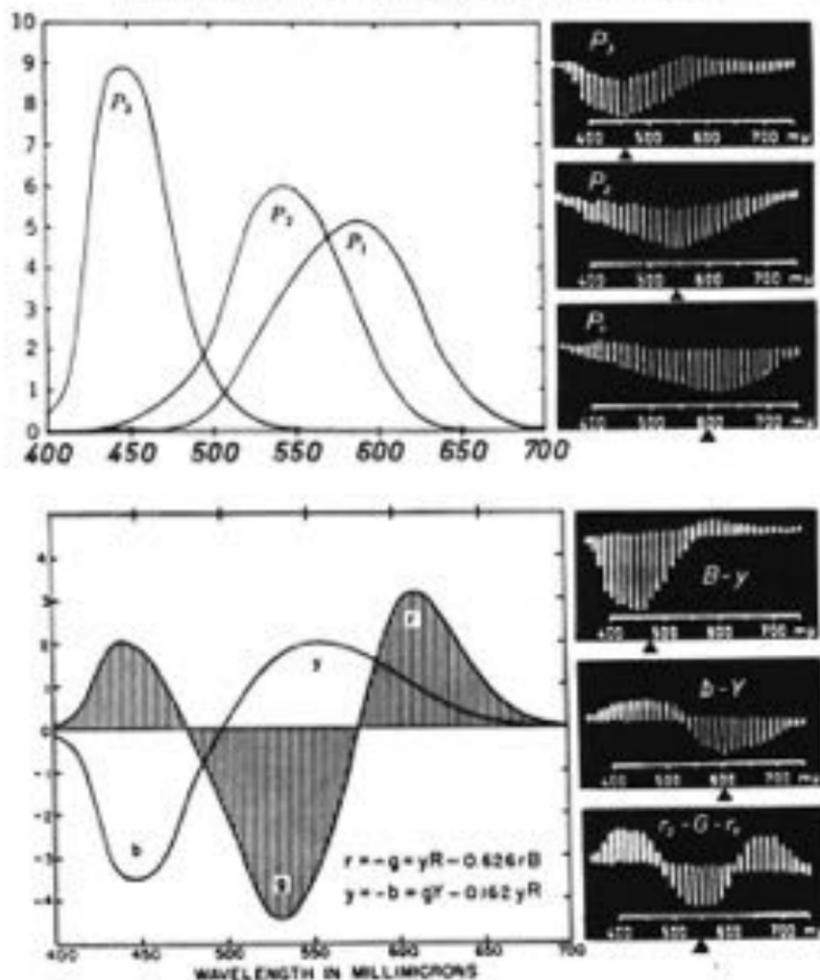


FIG. 5. To the left, stage theory diagram reproduced from Judd (1947). To the right, spectral response recordings from non-neuronal elements of fish (*Mugil braziliensis*). Hyperpolarizing potentials downwards. See text. (From Svetschichin *et al.*, 1961, by permission of the Editors.)

neuronal retinal elements of fish (*Mugil braziliensis*). The black triangles below each recording give the spectral absorption maxima of the three different cone pigments determined in the same fish species (Fig. 4). The luminosity (L-type) spectral response curves indicated by P_1 , P_2 and P_3 are intracellular recordings of hyper-

polarizing potentials obtained from the three different horizontal cell layers, while, the chromatic (C-type) spectral response curves indicated by B-y, b-Y and r_2 -G- r_1 are intracellular recordings of wavelength-dependent, hyperpolarizing or depolarizing potentials, obtained from non-neuronal elements located in the amacrine layer (B-y and b-Y), and at the level of the ganglion cells (r_2 -G- r_1). The cone pigment spectra agree well with the spectral response curves of the L-type, and also approximately with the hyperpolarizing (downwards) components of the C-type, but not with the depolarizing ones. On the basis of the surprising similarity between these spectral response curves, the stage theory diagram, and the curves measured by Hurvich and Jameson (1955, 1960), it was concluded (Svaetichin, 1956; Svaetichin *et al.*, 1961) that the basic mechanism of vision of fish and man is the same, the recordings from the horizontal cells reflecting the three-receptor input (Svaetichin *et al.*, 1961).

The scheme of the retinal structural framework shown in Fig. 6 is combined with electrophysiological and pigment absorption data (cf. Figs. 4 and 5). P_1 , P_2 and P_3 indicate recordings of the three cone-pigment absorption spectra, and P_r that of the rod. L_1 , L_2 and L_3 are horizontal cell spectral response curves, which agree with the three cone-pigment spectra. The bipolar cells B_1 , B_2 and B_3 have their hypothetical connexions and interactions with other cells indicated by two-way arrows. The chromatic responses indicated C_{3-1} and C_{-3+1} originate in the amacrine cell layer. There are several reasons to suggest that these two opponent, chromatic type of responses, which are approximately mirror images of each other, are created by an interaction between two adjacent non-neuronal elements. We believe that when the cell membrane of one element hyperpolarizes, this induces a depolarization of the neighbouring cell, and *vice versa* (this interaction being indicated by one-way arrows). The depolarizing response is always delayed with respect to the hyperpolarizing one (Svaetichin, 1956; Svaetichin and MacNichol, 1958). This pair of non-neuronal cells

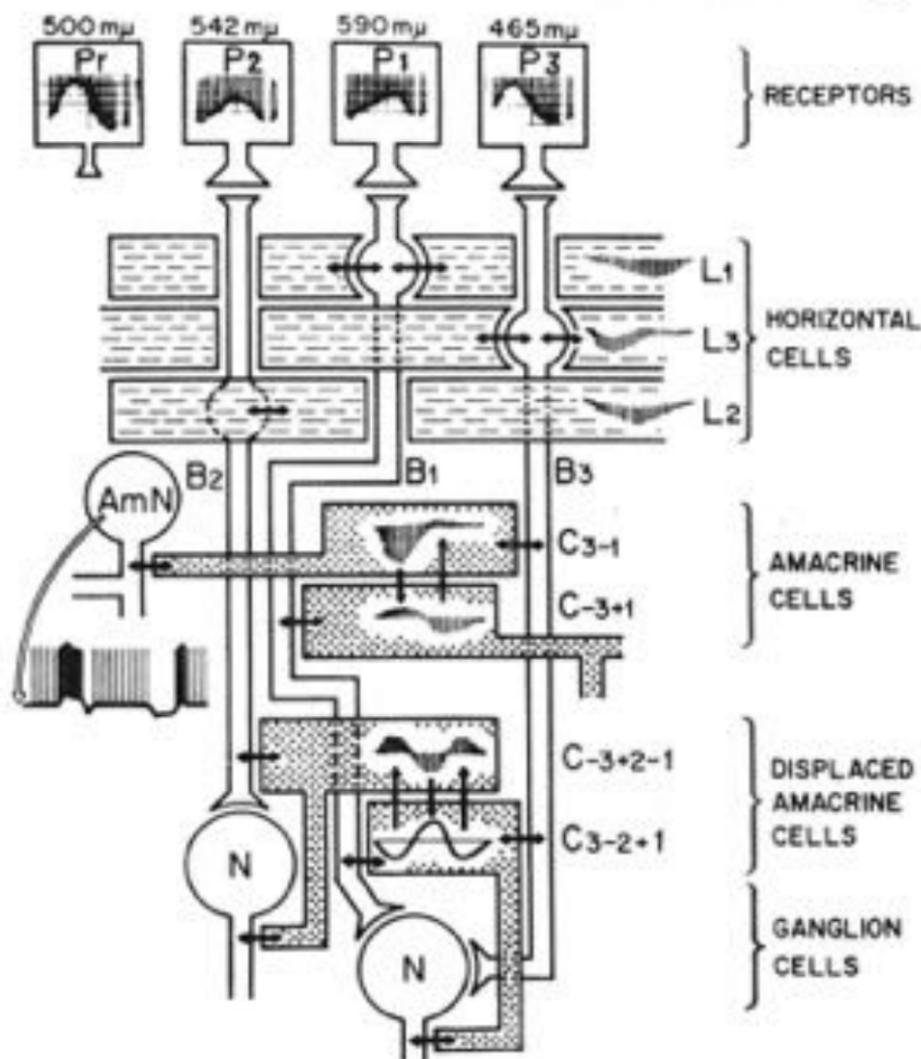


FIG. 6. Scheme showing structural framework of fish retina, combined with electrophysiological and photopigment data (*Mugil braziliensis*). Hyperpolarizing potentials downwards. See text.

seem to form part of a reciprocal excitation-inhibition system, which controls the firing pattern of the spectrally opponent amacrine neurones and ganglion cells. In the scheme, contacts at

the axon hillock region between the controller cells and the neurones, and also at the pre-synaptic region of the nerve fibres, are tentatively shown. Intracellular recordings obtained from an amacrine neurone (AmN) have been redrawn, the firing at "on" corresponding to hyperpolarization of the controller element C_{3-1} , while inhibition and firing at "off" correspond to controller cell depolarization. A corresponding reciprocal system for excitation and inhibition operates at the level of the ganglion cells (C_{321}). Studies by Tomita (1963) indicate that this mechanism at the ganglion cell level is more complex, and in some way related to the amacrine layer system. The neuronal and non-neuronal elements of

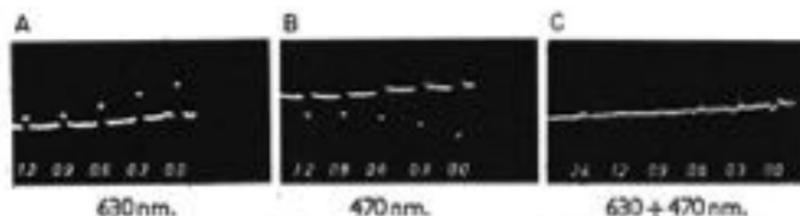


FIG. 7. Cancellation of opposite polarity responses (*Centropomidae*). Hyperpolarizing potentials downwards. See text. (Unpublished data, M. Laufer, G. Mitarai and G. Svaetichin, 1958.)

the amacrine layer have a tangential orientation (studied by Selvin De Testa), offering a structural and functional basis for colour induction, margin contrast and the spectacular Land phenomena (Land, 1965).

Cellular mechanisms for colour matching and mixing

The records shown in Fig. 7 are of the chromatic type, similar to B-y of Fig. 5. In these experiments the retina was illuminated by different intensities of light, the density of the neutral filters used being given at the bottom of each recording. The depolarizing responses of record A were produced by the stimulus light 630 nm., and the hyperpolarizing ones of record B by light of wavelength 470 nm. However, in record C, the two different wavelength

stimuli were applied simultaneously, in a ratio of intensities giving cancellation of the opposite polarity responses, except for the transients at "on" and at "off". The cancellation was still achieved in record c when the intensity of the two light stimuli was changed, provided the ratio was kept constant. This cancellation produced by the two spectral lights corresponds, of course, in a C-type of spectral response curve, to the cancellation reached by one spectral light of a wavelength in the middle part of the spectrum (see Fig. 5).

The records of Fig. 8 are also of the same C-type, producing hyperpolarizing and depolarizing potentials by alternate short and

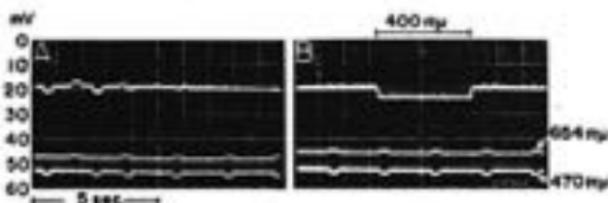


FIG. 8. Cancellation of opposite polarity responses (Centropomidae). Alternate and subsequently simultaneous 470 and 654 nm. stimuli indicated by square waves of lower tracings. Hyperpolarizing potentials downwards. See text. (Unpublished data, K. Negishi, G. Svætichin and P. Witkowsky, 1963.)

long-wave stimuli, as seen in the beginning of record A, while cancellation was achieved when the two spectral stimuli, of a certain ratio of intensities, were applied simultaneously. In record B the cancellation by the two superimposed stimuli was still maintained when the membrane potential was hyperpolarized by the application of a third spectral stimulus of 400 nm. The opposite polarity responses are both nonlinearly related to the light intensity (see Fig. 7A, B), and their sensitivities depend in an inverse and opposite way on the membrane potential level; hyperpolarization of the membrane potential causes an increase of the depolarizing response, but a decrease of the hyperpolarizing one, and *vice versa*. Thus an imbalance would be expected by the hyperpolarization caused by 400 nm., shown in record B. However, the match was

still maintained, showing that the cancellation must depend on a linear mechanism for matching at an earlier stage. This retinal mechanism for linear matching of the linear receptor information (Fatehchand, Laufer and Svaetichin, 1961) has an unknown location, but it is peripheral to the amacrine layer.

Exposing a fish retina, without pigment epithelium, to normal room light bleaches the rod pigment but does not abolish the electrical responses evoked by additional light stimuli. Apparently, the bleaching and recovery remain for a while in equilibrium in the cones under aerobic conditions, without the pigment epithelium.



FIG. 9. Bleaching experiment with L-response (*Centropomidae*). See text. (Unpublished data, M. Laufer, G. Mitarai and G. Svaetichin, 1958).

In our studies of the pigments of isolated cones, the cones were enclosed between two cover glasses in anoxia and no recovery of the cone pigment after bleaching was observed. Probably recovery of the cone pigment is dependent on aerobic systems of the large cone ellipsoid, which is filled with mitochondria. Studies on isolated receptors in aerobic conditions have not been made.

The record of Fig. 9 is from a horizontal cell. After the three initial responses to flashes of light, a very strong light stimulus was applied for about 30 seconds from the photostimulator, furnished by a 1,000 Watt xenon high pressure lamp). Probably due to bleaching of the cone pigments, the subsequent responses to light flashes were reduced in amplitude, without an accompanying membrane potential change. The bleaching was repeated in the same recording, causing an additional reduction of the responses to

flash stimuli. The full response amplitude was recovered in about 5 minutes. A substantial reduction of the pigment concentration seems to alter the linear characteristics of the cones.

Subsequent to the recording of a chromatic type of spectral response curve shown in Fig. 10A, a strong light of a wavelength band indicated by the white bar in B was applied. After removing the bleaching light the spectral response curve B was obtained, displaying a reduced component of hyperpolarization. The record

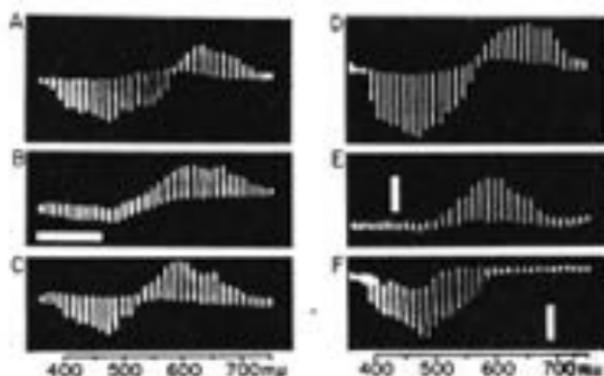


FIG. 10. Selective bleaching with C-response (Centropomidae). See text. (Unpublished data, M. Lauffer, G. Mitarai, G. Svaetichin and J. Villegas, 1959.)

C was obtained before full recovery of the short wave component was reached. A shift of the cancellation point of the spectral response curve can be seen, probably due to nonlinear receptor behaviour at a low level of pigment concentration (cf. A and C).

In the experiments illustrated in Fig. 10 D-F, bleaching was by strong spectral lights of short (E) and long (F) wavelengths, indicated by the white bars, previous to the recording of the spectral response curves shown in E and F. The experiment shows that after intensive bleaching a spectral response curve can be obtained which lacks one or the other of the opposite polarity components. The recordings shown in this figure were obtained from the Centropomidae species (cf. Fig. 4). In this fish we have only

observed this type of chromatic response, displaying hyperpolarizing potentials to short-wave stimuli. The bleaching experiments described indicate that only cones containing the 470 and 590 pigments are involved in the chromatic response, while the 542 and 590 pigments are reflected in the only two different horizontal cell responses observed in this fish, and which are possibly connected with information on brightness (cf. Mitarai *et al.*, 1961). Note that the depolarizing component of the normal spectral response curve in D does not agree with any of the cone pigment absorptions. However, when the 470 cone has been bleached (E), the depolarizing component does agree with the 590 pigment, indicating that the shift of the depolarizing component in normal conditions is due to a process of interaction (see Dartnall, 1962). This observation explains the lack of correspondence between the cone pigment spectra and the spectral distribution of the inhibitory "off" firing described by Wagner, MacNichol and Wolbarsht (1960).

Since the opponent colour code is reflected in the spike firing patterns obtained from the fish tectum (Villegas, 1961) and monkey geniculate body and visual cortex, it can be concluded that the processing of the intermediary hues takes place in the optic centres. Of the four unique colour sensations, one sees only three as pure in a spectrum, corresponding to the three cancellation points of the opponent colour processes (see Fig. 5). Red will always be contaminated with yellow, the short-wave end being a mixture of blue and red (see Linksz, 1964). The so-called invariant hues correspond to the three cancellation points. The three invariant hues do not change with variation in light intensity, because they depend on the linear matching process of the retina, which is peripheral to the amacrine level (Fig. 7). The intermediary hues depend on the ratio between two different information qualities transmitted to the cortex from the opponent colour processes. This opponent colour information is nonlinearly related to the light intensity (Fig. 7), and therefore the ratio and the intermediary hue will change with a variation of the light intensity.

Non-photochemical components of adaptation

Simultaneous recordings of the transretinal d.c. potential, the ERG, the receptor potential and intracellular recordings from non-neuronal controller elements indicate (as suggested by Vallecalle and Svaetichin, 1961), that the nonlinearity of responses obtained from structures subsequent to the receptors (Weber-Fechner behaviour; see Rushton, 1963) is due to a feedback control system, possibly consisting of (a) linear receptor input, (b) neuronal forward conductor line and (c) non-neuronal feedback elements. The transients seen at "on" and "off" in the ERG, which often oscillate, seem to reflect the feedback interactions, brain waves and evoked potentials possibly being of similar origin. In light adaptation the excitability control is reflected in an increased resting level of the controller cells and a decreased transretinal d.c. potential; similarly in arousal, the transcortical d.c. level is reduced and a desynchronization pattern is observed (Negishi *et al.*, 1963; Mitarai *et al.*, 1961).

Explanation of directional sensitivity of the retina and polarized light analysers of the insect eye as effects of molecular orientation of photopigments

The retinal directional sensitivity known as the Stiles-Crawford effect probably depends on the regular orientation of the cone pigment molecules. Since light entering in parallel with the receptor axis has electrical vectors optimal for absorption by the orientated prosthetic group molecules, a change of the direction of the light will reduce the absorption and the sensitivity. The rods are possibly less regularly orientated in the retina than the long outer segments of the cones; however, other possibilities exist to explain the difference of rods and cones in respect to the directional effects.

The ability of many insects, like the honeybee, to analyse polarized light (Autrum and Stumpf, 1950), probably also depends on the regular orientation of photopigment molecules. The observed perpendicular orientation of the tubular ultrastructure

(Goldsmith, 1962) and possibly also of the photopigment molecules of the two "opponent" pairs of rhabdome units is a suggestive basis for an "opponent" polarized light analyser mechanism.

Possible molecular mechanisms for interpretation of conduction along electrically inexcitable membranes and of nonsynaptic cellular interactions

Many phenomena described in the present study are difficult to explain on the basis of the classical synaptic and ionic theories. In central nervous tissue, where the electrical insulation provided by myelin sheaths is largely lacking, the distribution of current flow as determined by Kirchoff's law offers insufficient specificity for the delicate cellular interactions on which brain function is based. The electrical response to light of the photoreceptors is not influenced by externally applied current flow. The membrane potential and the conduction (for example, along horizontal cell networks, 0.35 m./sec.) of the non-neuronal controller cells show similar properties, and like the receptor potentials are closely dependent on aerobic metabolism. The following suggestions are made towards an interpretation of these and similar nervous phenomena (see also: Arvanitaki and Chalazonitis, 1949; Green, 1956; Jahn, 1961; Kasha, 1962; and Svaetichin *et al.*, 1963).

It is reasonable to assume that the plasma membrane of some cell types, by analogy with the mitochondrial membranes, contains as a structural component the respiratory chain. The respiratory chains can be considered as energy dipoles, orientated, with say 25 Å spacings, across the lipoprotein membrane and moderating the reaction of hydrogen with oxygen. In a cross-connected respiratory chain network of a cell membrane a tendency exists towards an equal rate of transfer of all chains. A local rise of the energy level, induced, for example, by an absorbed light quantum or a free hydrogen radical from the Kreb's cycle, will cause (a) a transfer of energy across the membrane along the respiratory chain (e.g. Förster resonance transfer between the non-identical molecules) and (b) a

lateral dispatch of energy within each layer of identical molecules of the cross-connected respiratory chains (e.g. exciton transfer, Coulomb interaction). Such a disturbance at one point of a cell membrane possibly induces the formation of additional free radicals or a release of energy from phosphate bonds, compensating for the energy degradation of the lateral spread. Conduction along electrically inexcitable membranes may possibly depend on such chain reactions of a respiratory chain network. Nonsynaptic interactions between adjacent cells, based on aerobic intercellular energy transfer systems, may possibly explain the phenomena of excitability control and inhibition described above and the accompanying active ion transport. Further evidence in favour of the concept of nervous function outlined above is presented in recent publications from this laboratory (Svaetichin *et al.*, 1963, 1965).

Concluding remarks

We expect that our oscilloscope tracings will help to do justice to the visionary predictions by Young and by Hering, which were further crystallized in the abstractions of the stage theories (von Kries, Adams, Müller, Schrödinger and Judd; see Judd, 1947). It is hard to see what further evidence can be required, when the stage theory diagrams and the measurements by Hurvich and Jameson predict in detail the spectral response recordings from individual cells of the visual system.

SUMMARY

Evidence is presented from electrophysiological and cone pigment studies showing the essential correctness of the original proposals by Young and by Hering, which are in agreement with the stage theories and with the measurements of Hurvich and Jameson.

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COMMENT

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Dr. Svaetichin has asked us to comment on the problem of colour adaptation and contrast in human colour vision, particularly on the way

* Dr. Hurvich was invited to the symposium but was unable to attend. He and Dr. Jameson were invited to comment on Dr. Svaetichin's paper for this publication.

we handle it theoretically, and to point to parallels that we may see in his work on the visual mechanisms of the fish retina.

In our analysis of human colour vision, exposure to a coloured background must have two effects. One of these effects alters the balance of sensitivities in the three-variable system; the other produces incremental (and decremental) changes in the responses. The alteration of sensitivity balance among the three variables may occur at any level in the receptor-optic nerve-cortex system, and in our analysis we have assumed that it occurs at the receptor level and is brought about by selective bleaching of the cone photopigments (Jameson and Hurvich, 1956). The change in balance of sensitivities is assumed to follow what is known as the von Kries coefficient law. That is, each of the three selective spectral distributions is itself unaltered in form, but each is reduced uniformly by a different coefficient or multiplicative factor whose magnitude depends on the colour of the background light. In Dr. Svaetichin's picture of the fish retina we find such a coefficient change in the selective bleaching of the cone photopigments and in the horizontal cell recordings that seem to reflect the three photopigment absorptions. The paired, opponent response functions that in the human are correlated with the colour perceptions are, of course, altered in their spectral distributions as a result of these controlling sensitivity changes (Jameson and Hurvich, 1956), as are Dr. Svaetichin's recordings of opposite polarity that he attributes to the glial cells in the fish.

It is at the level of the opponent response activity (and not photopigment absorption) that the second kind of change caused by the coloured background seems to occur in the human. This change does not follow a coefficient law as does the balance of sensitivities, but rather, it is an incremental (and decremental) effect. Thus, for example, a yellow-red background induces in the test area a fixed increment of blue response (decrement in yellow) in the yellow-blue response pair, and a fixed increment of green (decrement in red) in the red-green response pair. There is also a blackness increment in the white-black response pair. We have documented elsewhere the variety of data that point to such an incremental effect in addition to the coefficient changes. We have also called attention to the difficulties in analysis that arise when these incremental effects are ignored in studies of colour adaptation (Hurvich and Jameson, 1958, 1965; Jameson and Hurvich, 1959, 1964).

We find a parallel for these incremental effects again in the fish retina in the shifts in baseline that occur when the S-potentials of opposite polarity are measured against backgrounds of different colours. A shift in baseline is of course exactly equivalent to a constant increment in the response amplitudes of one polarity and a constant decrement of the same size in the responses of the opposite polarity. Both kinds of change, the coefficient and the incremental ones, must be understood in order to comprehend the phenomena of colour adaptation and contrast (this includes the so-called Land effects) and to account for them quantitatively. Both kinds of change must also be understood by those who would determine the spectral absorptions of the cone photopigments by measuring visual thresholds against backgrounds of strong, coloured bleaching lights.

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DISCUSSION

Donner: In some of your recordings showing receptor sensitivities, your baseline, which I imagine would be expected to be straight, shows a deviation in those spectral regions where you have the greatest sensitivity; in other words, for some light exposures your potential does not return to the original value. This may shift the sensitivities a little, depending on the order in which the exposures were given. In what direction did you work—in random directions from the blue to the red end and *vice versa*?

Swaetichin: Yes; we use a reversible-motor gearbox for driving the interference filter wheel of the photostimulator. The oscillographic spectral response curve recordings are identical for both directions of spectral scanning, provided the sweep velocity does not exceed the

limitation due to the rise and decay times of the responses to the spectral light flashes.

Donner: But is this lack of return to the baseline a normal phenomenon?

Svaetichin: When the retina is dark-adapted the lack of return to the baseline is a normal phenomenon, since the light adaptation produced by a strong illumination changes the equilibrium level of the membrane potential of the non-neuronal controller cells, which is low (5–10 mV) in dark adaptation and high (30–40 mV) in light adaptation (Mitarai, G., Svaetichin, G., Vallecalle, E., Fatchchand, R., Villegas, J., and Laufer, M. [1961]. *In* *The Visual System: Neurophysiology and Psychophysics*, p. 463, ed. Jung, R., and Kornhuber, H. Berlin: Springer; Svaetichin, G. [1962]. *In* *Information Processing in the Nervous System*, International Congress Series No. 49, p. 352, ed. Gerard, R. W., and Duyff, J. W. Amsterdam: Excerpta Medica Foundation). In a light-adapted retina the rise and decay of the response is fast, while in dark adaptation or an old preparation the response is very slow.

Louvenstein: I may have misunderstood you, Dr. Svaetichin, but did you imply that an electrically inexcitable membrane is incompatible with a sodium-potassium mechanism?

Svaetichin: Your question is not easy to answer. Our ideas in this respect are presented in the last part of our paper. We believe that the sodium-potassium mechanisms are to some extent related to a functional, metabolic interaction between the neurone and the non-neuronal, glial elements. Not only the neurone, but also the non-neuronal cells, and their networks, show a propagated plasma membrane disturbance. The local current flow theory provides an explanation for conduction along the electrically excitable neuronal membrane; however, new concepts are obviously needed for making understandable the conduction along the electrically inexcitable plasma membranes of the non-neuronal elements. (For further details, see Svaetichin G., Negishi, K., Fatchchand, R., Drujan, B. D., and Selvin de Testa, A. [1965]. *In* *Progress in Brain Research*, vol. 15, *Biology of Neuroglia*, ed. De Robertis, E. D. P., and Carrea, R. Amsterdam: Elsevier.)

Monnier: On your schema, the last amacrine cell layers act on the axon hillock of the ganglion cell. Why do you suggest that? Do you postulate that the axon hillock is more sensitive to inhibition?

Svaetichin: That is correct. We assume that the initial segment of the

axon, which is a critical trigger region for the spike firing, is influenced by the non-neuronal controller cells. Experiments by Dr. K. Negishi and others in our laboratory (1964, unpublished observations) indicate that the Schwann cell also influences the axonal excitability.

Padgham: One of your figures showed the potential across the receptor layer in response to a flash stimulus. Was the decay exponential?

Svaetichin: The electrical responses recorded across the receptor layer and also the responses of the non-neuronal elements seem to have an exponential decay.

Wcale: Dr. Svaetichin, you mentioned that you bleached your preparations. How do you know that you bleached them? What were the intensities of the lights you used? After bleaching the preparation you got an effect, and I took it that you attributed it to a receptor phenomenon, but have you shown that they bleach or have you an intensity measurement which leads you to believe that in fact you bleached them?

Svaetichin: The cone pigment bleaching was not measured simultaneously with these electrophysiological experiments since, as you know, this would not be easy to do on the whole intact retina. We know, on the other hand, from our photopigment studies on isolated cones that the cone pigment is bleached by this same illumination intensity, and that the pigment recovers in aerobic but not in anaerobic conditions. We have no absolute values for the intensity of the light used.

VISUAL PIGMENTS OF SINGLE CONES

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THOMAS YOUNG suggested in 1802 that colour vision depends on perhaps three visual pigments whose excitation is distinguishable by the nervous system. This paper suggests the view that the actual situation in the retina is almost the simplest possible one compatible with Young's theory. Namely, that in the goldfish and man there are only three visual pigments, that these are almost completely, if not completely, segregated into separate cones, and that all three pigments are of the rhodopsin type, having the same shape when plotted against wave number, but displaced to three points on the wavelength axis.

The data on which the argument depends are mainly recordings of the bleaching difference spectra of single cones of goldfish, the macaque monkey (*Macaca nemestrina* and *M. mulatta*), and man.

The microspectrophotometer with which our work was done is described by MacNichol and Marks (1965). The results for the goldfish and the technique are described by Marks (1963, 1965) and MacNichol (1964). Our data for the human and monkey, which are preliminary in nature, were gathered by Marks, Dobbelle and MacNichol (1964).

The technique consists, in brief, of mounting pieces of goldfish or primate retina in a gelatin-saline solution between two cover glasses. Single receptors therein are illuminated with a microbeam of monochromatic light formed by demagnifying a small aperture with an inverted microscope objective. A similar reference beam passes through a clear area. The two beams, which are chopped so that they appear alternately, are detected by a sensitive photo-

multiplier, amplified by a stable feedback circuit, and the ratio of their intensities is formed and plotted by a chart recorder. This ratio is the fraction of light transmitted by the receptor, and when plotted automatically against the wavelength of the light, gives the absorption spectrum of the receptor.

The principal pitfalls of the method are (a) chromatic motion of the measuring beams, which causes the light to pass through different amounts of pigment at different wavelengths, and (b) distortions due directly or indirectly to photon noise. The usual response to photon noise is either (i) to turn up the light intensity to reduce it, distorting the spectrum by bleaching the pigment, or (ii) to increase the smoothing time constant of the instrument, which also distorts the spectra. Too great intensity displaces the spectral maximum toward earlier wavelengths, and too great smoothing time displaces the peak toward later wavelengths.

Fortunately, the number of photons required to reduce the noise and smoothing time constant in goldfish cone spectra to acceptable levels bleaches only about 5-10 per cent of the pigment during a scan (MacNichol and Marks, 1965).

P. A. Liebman (personal communication), recording spectra of single goldfish cones by a similar technique, finds a similar result.

Noise figures of the same order can be obtained with primate cones, provided the receptors are illuminated "end on". In this case, the light traverses several microns of pigment, as it does with the thicker goldfish cones lying on their sides.

RESULTS

The photosensitivities and absorptions per micron thickness of the pigment of goldfish cones and frog rods lying on their sides were found to be approximately equal, indicating that within the ratio of the quantum efficiencies of bleaching of the two pigments, their concentrations must be the same. This concentration was calculated, using the known properties of frog rhodopsin, to be

about 2 mm. If all of the cones of a goldfish eye were extracted and suspended in 1 ml. of water, their absorption should be about 2 per cent per cm. path length. This should be easily detectable, especially if the rods are first removed from the suspension.

Measurements of different regions of goldfish outer segments lying on their sides showed that the pigment was distributed uniformly. Such measurements could not be performed with the primate "end on" preparation. However, work with human rods and cones gives us the impression that the properties of the rod and cone pigments are similar.

The number of red and green receptors in the goldfish eye is about five times greater than the number of blue receptors. In the primate 13 red, 15 green and 2 blue receptors have been found, suggesting that in the primate parafovea there are about seven times as many red and green receptors as blue receptors.

The mosaic of goldfish cones is made up of rows of symmetrically joined twin cones separated by one or two single cones. The twin cones are almost always composed of a red and a green receptor, and the single cones are mainly red and green receptors, more greens than reds. The blue receptors seem to have more rounded ellipsoids, and cones satisfying this description have been seen in histological sections made by Mrs. Werner Sickel, separated rather systematically by several pairs of twin cones.

Only the parafoveal region of the primate retinal mosaic has been studied. The cones here are separated rather uniformly from each other by about five rods, and are separated from the rods by clear areas formed by the cone ellipsoids. On two occasions two or three cones have been found to be identical to their near neighbours, in one case two green receptors, in another three red receptors. This suggests, perhaps, that the primate mosaic is not rigidly organized.

In order to discover the number of kinds of goldfish receptors, the absorption maxima of 113 goldfish cones were plotted in a histogram, after correcting the maxima for dislocation caused by bleaching. All but two of these absorption maxima clustered into

one of three rather close-knit groups. Twenty-eight spectra plotted together after correction for distortion by bleaching also formed three groups. These two results are the basis for the conclusion that there are just three kinds of goldfish cones.

The result for the primate retina is similar but less compelling. Spectra corrected for bleaching and plotted together fell into three groups. The conclusion was similar: there are probably only three types of human cones. Though the result depends on only 30 spectra, it is supported by the trichromacy of human colour vision. If we grant only three human pigments, a fourth human cone type must be based on a mixture of pigments. Spectra containing such mixed pigments were not obvious. Most of the diversity in the spectra could be explained by known instrumental factors.

The absorption maxima of the three groups of goldfish spectra averaged 455 nm., 530 nm., and 625 nm. The primate absorption maxima were near 445 nm., 535 nm., and 570 nm. Recent measurements with dimmer lights put the goldfish green maximum nearer 535 nm.

Most of the well-characterized visual pigment spectra, when plotted against wave number, have the shape of rhodopsin (Dartnall, 1953, 1962). The goldfish cone pigments showed this relationship fairly well, and the small differences were within the range of possible error caused by chromatic motion of the measuring beam and by photoproducts. The goldfish red-sensitive pigment may be similar to cyanopsin (Wald, Brown and Smith, 1953), for both are retinene₂-based cone pigments. Cyanopsin is a pigment of rhodopsin type.

Stiles (1959), by testing the human increment colour threshold, found a blue mechanism (π_2) and a green mechanism (π_4) which fit the rhodopsin shape quite accurately. Our primate spectra and the human blue receptor of Brown and Wald (1964) as well as the green-sensitive pigment which they measure in whole foveas (Brown and Wald, 1963) furnish evidence that Stiles' π_2 and π_4 mechanisms are visual pigments, since their spectral maxima,

440 nm. and 540 nm., are within 5 nm. of those of the primate spectra. π_4 also corresponds with chlorolabe (Rushton, 1959) and with psychophysical measurements.

The spectrum of Stiles' red mechanism π_5 , however, does not have the shape of rhodopsin, and neither does erythrolabe (Rushton, 1959). Hence, it is gratifying to find that the 570 nm. primate red receptors of Marks, Dobbie and MacNichol (1964) fit the shape of rhodopsin to within 2 per cent width error, as does the 565 nm. pigment measured in whole human foveas by Brown and Wald (1963).

Hence the combined data of Stiles; Rushton; Marks, Dobbie and MacNichol; and Brown and Wald suggest that the human visual pigments are of the rhodopsin type, three in number, absorbing maximally at about 440, 540, and 567 nm. (the latter being a compromise between our result and the result of Brown and Wald, 1963), and are segregated into separate receptors.

SUMMARY

Microspectrophotometric studies on single cones of goldfish, macaque monkeys, and man have shown that each of these has three types of cones, and that to a good approximation each cone contains only one visual pigment. In both the goldfish and the primates, blue receptors are about one-fifth as numerous as red and green receptors. About half the goldfish cones are continuous twin cones. Almost always one of these is a red receptor and the other a green receptor. The goldfish pigments absorb maximally at about 455, 535, and 625 nm. and the primate pigments at about 445, 535, and 570 nm. When plotted against wave number, each of these absorption spectra has the shape of that of rhodopsin. The photosensitivity and pigment concentrations are also similar to those of rod pigments and the distribution within the receptor seems uniform, as in rods.

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DISCUSSION

Rushton: It is two and a half years since I first saw these experiments being done at the Biophysical Laboratory at Johns Hopkins University, and I would like to stress the difficulty and the beauty of this achievement. Many of us have tried in a slight way putting light through cells and seeing what we could get. Two and a half years ago this was being done with some success and what is most impressive about this achievement is the part of which we have heard only a little, the difficulty with the physics, the programming and so forth. This is a tremendous technical achievement because of the limited amount of pigment in a single receptor and because every piece of information involves the bleaching away of some of the pigment to be measured. By the time you have as much accuracy as you would like, there is no pigment left to do the measurements on. The earlier work though very exciting was full of snags but the authors have not rushed prematurely into print; they resolutely held up publication until they were sure of the answer.

Ripps: Dr. Marks, is the comparison you made between Dr. Rushton's most recent data and your own measurements a comparison between two sets of difference spectra? That is to say, are your measurements difference spectra as well, Dr. Rushton?

Rushton: Mine are difference spectra also. If you are doing reflectance measurements, you have to define what you mean by difference spectra. We were measuring the difference in intensity of the reflected light, before and after bleaching, and dividing by the intensity of reflected light in the totally bleached retina.

Weale: As regards this comparison between your paper, Dr. Marks, and so-called psychophysical curves, numerous workers have shown that the difference spectrum *in situ* is displaced significantly along the wavelength scale from the parent absorption spectrum. I am not clear what photoproducts you believe that your cones contain; I gather there are some. If this is so, then the data you have shown us, while agreeing very nicely with the other data, are not the correct data to compare. Can you give an estimate of the actual absorptions or, alternatively, can you assure us that the photoproducts are absolutely negligible in their effect?

Marks: The two main photoproducts of rhodopsin-like pigments are retinene and indicator yellow. The effect of these is to tend to cause an increase in absorption in the blue on bleaching, so that the difference spectrum there tends to be low or negative when they are present. Our goldfish cone difference spectra are flat and positive in the blue region at about 20 per cent of peak absorption, in agreement with the spectra of rhodopsin-like pigments. The effect of other photoproducts such as the meta-pigments is usually negligible at these temperature and time scales. What happened to the blue-absorbing photoproducts I don't know; perhaps they diffused out of the small outer segment. In larger frog rods at pH 5.5 we see a hint of an absorption increase in the blue on early scans, but later scans through the spectrum reverse this, giving positive difference spectra like those of the cones. The primate cone difference spectra, though less accurate, are also positive at all wavelengths.

Ripps: Have you any suggestions as regards the difference between your density measurements and those measured in the living eye?

Marks: Yes. In the first place, we do not know at what level the 2-micron beam is focused. We try to adjust it for maximum bleaching.

Secondly, the numerical aperture is too great for the ellipsoid and outer segment to contain all the light; light is lost from the ellipsoid and probably all along the outer segment. In the fovea where the receptors are packed together this spilled light would go through other receptors, giving a higher optical density than we find.

If the primate pigment density is the same as in the goldfish cones and frog rods, about 0.008 log units per micron, a 30-micron outer segment would have an optical density of 0.24 log units.

Stiles: The great merit of using the difference spectrum method in this case is, then, that when you bleach, everything else remains unchanged, and in particular the light paths are assumed to remain exactly the same.

Welbarsht: Dr. Marks has pointed out that these twin cones exist side by side in the goldfish, one with red pigment and the other with green. We recorded in the ganglion cell layer and also in more distal places in the retina in the goldfish, and obtained the same types of curves that Dr. Svætichin finds. This indicates that there is an interplay between the red and the green pigments; but the difficulty arises when you start to plot the spatial distribution of these receptors which are connected to the same ganglion cell. A plot of an equal response curve for the sensitivity of the ganglion cell, or the amount of energy necessary to give an equal response from, let us say, the green pigment, is not equal to that from the red pigment of the same ganglion cell. So the sensitivity is quite different. Since these are equal response curves, it would imply that the concentration of pigment, or the number of receptors connected to the ganglion cell, is quite different for the green receptor and the red receptor. It is very difficult even to think of our results being due to scattered light or any instrumental difficulties. (See General Discussion, pp. 278-9, for more complete description of these experiments.)

Padgham: Dr. Marks, on the question of technique, you spoke of scanning the receptors from one end of the spectrum to the other; in what light do you set up the apparatus? Presumably you needed to look down the microscope to align a single receptor on the aperture.

Marks: When we were aligning a receptor in the beam we illuminated the microscope field with deep blue or deep red light, or infrared light visualized with an image converter. The red background light bleached

the red receptors. The blue light bleached the blue receptors, and also, since they are rhodopsin-like pigments, bleached the other two receptor types as well, more slowly. When infrared background light was used all the cones contained one of the three pigments.

Mimz: I'd like to make a comment and ask a question. My comment concerns the products of bleaching, and in this I feel somewhat like a convert to a certain dentifrice: I wonder where the yellow went! My question is: have you studied the regeneration of pigments in the individual cones?

Marks: I ran regeneration experiments on all three receptors but unfortunately all the good data came from green receptors, and in that case the result was simple; if you shine green light of constant wavelength through the receptor (in the presence of 11-*cis* retinene₂) you find a certain pigment density; if now you reduce the intensity of the light, the density of the pigment increases to a new steady level, and if you reduce the intensity further, the density increases again; if on the other hand you increase the intensity to the original value, the density goes back to the old level. The spectrum of the regenerated pigment is the same as that of the original pigment.

Willmer: Dr. Marks, I believe you said that the blue cone of the human retina was a bigger piece of machinery than the red or green cone?

Marks: My impression is that the ellipsoid of the blue receptor of the *goldfish* is rounder than that of the red and green receptors; this would be a very useful fact if it is borne out, but at present it is an impression only.

Willmer: There is the interesting observation of E. H. Leach ([1963]. *J. roy. micr. Soc.*, **82**, 135), and I have seen it myself recently on monkey retinae, that the cones in the foveal centre are all fairly uniform in size, and we are pretty certain that these are the red and green ones; but there are also receptors which in fixed preparations blow up to relatively enormous size, and they have a less regular distribution and seem to be much less frequent in the foveal centre. Perhaps those are the cones you are looking through.

Swaenichin: In the fishes we have studied, the receptors with the short-wave absorption are exactly as you described them, Dr. Marks; they all belong to the type called "additional short single cone".

THE RETINEX

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SINCE the time of Newton, colour vision has been approached starting from the properties of the eye in viewing small areas and working from there to explain how colour in everyday life comes about. More recently, Hering introduced the idea that the regions surrounding the areas in question are also very significant. Nevertheless, I think all of us still tend to inhibit the recognition of the *overriding* importance of the *whole* area that is being viewed. Because of the meticulous measurements that can be made in colour-matching, one has attempted to start from the colour-matching laws and, by adding terms to their equations, to describe what happens in extended areas.

Here, I want to talk about the consequences of coming at the problem of colour the other way around. I want to suggest that there is a set of important laws which involve the whole area that is being viewed: laws which describe the functioning of the eye. Let me say first of all, that it has been possible to construct an heuristic system, a mathematically functional system, which describes a large variety of phenomena correctly. It is not my purpose to suggest that this heuristic system is real or unreal, but rather to use it as a very simple hypothesis for containing an elaborate group of visual phenomena. The first experiments we should be looking at, if I had my demonstration equipment, would involve a large board made up of many rectangles after the fashion of the artist Mondrian, selected from a group of grey papers, so that there were white areas, and grey areas and black areas, miscellaneously distributed. We would then look at that board in a

pulse of light—lasting only a millionth of a second—and see that most of it was visible. We would see the greys, the white and the black, and the chief effect of seeing it by the light of a pulse would be to heighten the vividness and the contrast. (This is the antithetical experiment to that of Ditchburn and Ginsberg, 1952, and Riggs and co-workers, 1953, in which stabilizing an image continuously makes much of it fade away.) In the time that it takes an electron or a proton to move in the retina, there can be imprinted on the visual system all the information necessary for establishing a lightness scale. (The remarkable thing is, incidentally, that in the one-tenth of a second that it takes to process that imprint, one is not free to impose another imprint. In that period the information, as it were, seems to progress down channels which confine it, because the edges stay sharp during the whole one-tenth of a second that it takes for the processing to be completed.) So in that millionth of a second we can establish a lightness scale.

A second property of this imaginary Mondrian is as follows. Illuminate the board by a projector in which has been placed either a neutral wedge which is light on one side and dark on the other or a slide composed of miscellaneous mottled areas with non-sharp edges. If I then used a cadmium-sulphide cell as a light-sensitive probe over the surface of the board so that I could read the light coming from the board to the eye, I might find an area which looks black in one spot and an area which looks white at another, but which both send the same number of photons to the eye. Alternatively, I could use uniform lighting and, by varying the intensity of the projector, show that sequentially coming from one area which persists in looking black is the same number of photons as in another instant came from another area which persists in looking white.

I regard this competence of the eye to discern a lightness scale under uneven lighting conditions as a great biological accomplishment, because it means that in a world of changing illumination, with moving shadows and with changing sunlight falling over

objects, entities keep their form. If this were not so, the world would dissolve away from us as we looked at it. There is nothing new about all this; indeed, it is rather so old that I feel it needs special and extra emphasis. It is so old that it does not impress a child, but it is also so old that, when you do an experiment such as described above, even the most experienced physicist will want to come up and hold the light-meter himself, because, intuitively, one simply cannot believe that a good black can be sending the same radiation to the eye as a good white.

Imagine now an animal with a one-pigment receptor system, rather than with the three pigments which Dr. Marks has described for us. Let us then assume that this animal thereby has the ability to discern a lightness scale. Suppose further that the animal develops another pigment. From the point of view of evolutionary adaptation to its photoenvironment, this second pigment may do the animal harm. If you recall the early days of black and white photography, you remember that the most attractive films were orthochromatic, whereas panchromatic films greatly reduced the contrast within a picture. In the same way, the animal would be far better off with only one of Dr. Marks' pigments. The interacting pigment curves of the world multiplied by such a retinene-type curve would result in a series of different greys: a lightness scale. But if the animal evolved for himself a second retinene and added another curve to this first retinene curve, he would start getting into trouble, because he would get as much response from red leaves as from green leaves, and from the ground as from the sky. The animal's lightness scale, instead of getting better and more resolved, would get worse, because he would have lost some of the revealing contrast among its steps.

Given an additional retinene, what was needed was an *orthogonal* system which would allow each pigment to act separately in the same way as did the first single pigment system. Let us take a series of numbers which might represent some of the steps on the lightness scale which the animal would perceive with a single

pigment: 3, 5, 100, 80, 1, and so on. If he simply added another retinene, some of these steps might be lost from the lightness scale entirely, or, at best, no additional information would be added. But now suppose he evolved another retinene into an orthogonal system. Then the step which was 3 for the first retinene might be 96 for the second; step 5 for the first might be step 20 for the second; step 100 might be step 4, and so on. Remember what we saw at the beginning with our black-grey-white "Mondrian". It did not matter how bright the light was that illuminated the "Mondrian" the black stayed black and the white stayed white. Similarly here, it would not matter whether it were noon or moonlight, because for one particular retinene whatever was step 3 on a lightness scale would stay step 3, whatever was 5 would stay 5; for the other retinene what was step 96 would stay step 96, what was 20 would stay 20. If the animal pools this information, he is in trouble with the two retinenes. If he keeps the information separate and keeps track of the two scales simultaneously, then instead of having one scale of 40 steps, for example, he has 40×40 combinations or a possibility of 1,600 components of information rather than the mere 40 he would have had from the scales pooled.

But the animal would still need some way of keeping track of the results of these orthogonal systems. My proposal is that he has a way of keeping track: he has a sense for that purpose, and the name of that sense is colour. The sense of colour exists to give a report on the orthogonal combinations. The point represented by the steps 3-96, for example, is always the same colour. Colour then, from this point of view, is the sense produced by correlating the steps of the various orthogonal lightness scales, whose positions stay the same, independent of the relative amounts of light at any given wavelength over very considerable ranges.

Now suppose we replace the "Mondrian" made of grey strips with a board made with coloured papers. (I recommend papers for which the reflection density for either component is not greater than 1.0. They still give plenty of colour, and it keeps one from

being involved in the problems of surface light.) Using such papers, with matt finishes, we made up a "Mondrian" comprising yellows, reds, greens, browns, blues, whites, pinks, in a gorgeous display which one may recommend to any pedagogue because it will hold the class's attention even if the lecture does not. We then light the coloured "Mondrian" with two or three projectors, each fitted with a band-pass interference filter. We tried to select these filters so that there is a minimum amount of colour showing in the "Mondrian" when only one projector is on (see Land, 1964). Other than a wash of the coloured light passed by the given filter, the board seems to display only a series of varying neutral strips.

To start with, we turn on only two projectors to light the coloured "Mondrian". It is interesting that even though it is not a natural image, it will be quite richly coloured, with whites and yellows, reds, greens and greys. Now we turn off one projector, lighting the whole of the board with only the long wavelength. We pick one area—say a lime-green area—and measure the amount of light of long wavelength coming from it: suppose the light-meter is reading 18. Then we turn off that projector, turn on the other projector and obtain a light-meter reading from the same lime-green area for medium wavelength light of, say, 12. Now you could pick any other area on the board—brown, grey, red or whatever; we turn on only one projector and adjust its brightness so that the light-meter reads 18 for the long wavelength light coming from that area to your eye. Turning off that projector, and turning on the other projector, we adjust its brightness until the light-meter reads 12 for the middle wavelength light coming from this second area to your eye. Both readjusted projectors are turned on: this second area will be brown, grey, red or whatever it was before, even though it is sending to your eye the same number of quanta of the same frequency that the lime-green area formerly did. So you could amuse yourself going from place to place on the board with this null experiment, making the same light fall on

the photometer and, of course, on your eye, irrespective of the area you are examining. It does not matter if the area you select is surrounded by browns or reds or greens. You can pick the white area or the yellow area: as each region holds its position in the orthogonal lightness scales, it holds its position in the hue scale— independent of what light is coming from it.

The colour-forming process is entirely systematic if the orthogonality of the retinene system is accepted. Suppose that all Dr. Marks' long-wave receptors are interconnected, and also his middle and short-wave receptors—perhaps cortically, or retinally, or in the visual pathway—and call these each interconnecting system with its cerebral liaison a "retinex". The function of the retinexes is first, to produce three independent lightness scales, and then to correlate the positions in the three scales of each visual area. It is this correlation that determines the colour of any area.

Finally, one should have a test for this hypothesis. One prediction that this scheme offers is that there should be situations when uniform colour will appear: those situations when the steps in the lightness scales being correlated fall in the same order. That experiment can be done very readily. Suppose we take three identical black and white photographs of the chairman and put one in one projector while we put the other two in superposition in the second projector—two photographs on top of each other, so that they multiply each other's fractional transmissions. We project the two sets of images in superposition. Now where we have one-fifth of the light coming from one projector, we have one-twenty-fifth coming from the other; where we have one-tenth from the one, we have one-hundredth from the other: a wide variety of ratios of energies. Retinex theory predicts that the positions of an area in the lightness scales or the set of correlation numbers determines an object's colour, rather than the ratio of energies. Since these scale positions for each area in the slide of the chairman are unchanged by superimposing two identical images, the theory predicts that whatever filters are

used, the entire image will appear as a uniform colour wash. The fact is that it does.

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DISCUSSION

Stiles: Most of us have been brought up in the cramped trichromatic tradition which corresponds rather to the case of the kinetic theory of gases, where one tries to follow the laws of motion of the individual molecules, whereas Dr. Land is trying to arrive at rules analogous to the thermodynamic principles which apply when there is a certain randomness in the system. Yet I think that one would also agree that anything that can be deduced from thermodynamic principles could ideally also be worked out, more painfully no doubt, if one knew all the laws governing the individual reacting particles. These laws, in the cases Dr. Land is speaking of, govern the interaction not just between intimate neighbours but over the whole field. This interaction may be cerebral, of course; one doesn't know where it occurs. The actual quantitative laws Dr. Land referred to in the latter part of his paper, where he spoke of lightness scales for different systems, one for a red pigment system and another lightness scale for a green pigment system, require some kind of direct experimental test. The kind of test that a trichromatic man might think of would be something like binocular matching, say, of various individual elements of the complex "Mondrian" system in one eye to a very much simpler system viewed in the other eye. I don't know how far your theory, Dr. Land, rests on a quantitative concept of lightness scale or whether you are thinking of it in general terms. Do you think forty steps are actually involved?

Land: I gave forty as an example.

Stiles: I see. In your earlier work in your wonderful coloured demonstrations one felt that the conclusions to be drawn could only be expressed by verbal descriptions of the colours seen. To go a little further you would have to reduce this to quantitative terms. The traditional colour worker would go about this by making a binocular matching

system, and he would then try to tie up his quantitative results in an elaborate scheme such as you have suggested. Would you agree with that?

Land: Yes, I agree. There is one precaution, however. The amount of binocular interaction is still uncertain. But one thing which is definite is that perception covers the whole range with the same colorimetric measurement, from something that looks roughly like this brown wood to something that looks roughly like that green leather, so the gross changes are indisputable. The question is, using, say, red and green filters, what is the range between white and yellow that would be covered? Or in another situation, what is the range between, say, this brown and that brown? Your proposal would come down experimentally, as I understand it, to using a white surround with a colorimetric centre viewed by one eye and the "Mondrian" by the other eye. I think that would work and would be very satisfying.

Stiles: And then when you have the quantitative results for such a complex field, you would be able to derive your "thermodynamic" principles on the basis of this set of data.

Land: Except for one thing. If the comparison of ranks is a vital part of the mechanism for seeing colour, I don't see how you can rank unless you have enough elements to rank. Consider the experiment which I described in which three identical images of the chairman were projected in red and green light. The argument says that if the rank orders are the same, independently of what wavelength filters we use in the projectors, at all ratios of red and green the whole thing will be colourless. Now I don't see how you can arrive at that particular result by building up from colorimetry. Continuing with your analogy, one can predict many thermodynamic conclusions from the properties of single particles but many of the concepts of thermodynamics themselves could not have come into being to be tested if one had not already had this kind of theory of sets. In your case, if one took a white surround and put in the coloured centre, and then looked at the chairman with one eye, while one looked with the other eye at the centre, your experiments would show that every part of the chairman would be the same hue, tested this way. But you could not have hit upon that test if you had not approached it from the theory of sets.

Klüver: Psychologists and even some physiologists have always

found it a puzzling problem that, when one looks at objects of the external world, that is, when one looks at different surfaces reflecting light in an illuminated space, colours appear which should really not be there. A green surface, for instance, may remain approximately green under a wide range of chromatic illumination. Hering introduced the idea of "memory colours" to account for this. Although the spectral analysis of a reflecting surface may not indicate that it will appear green, "memory" transforms the colour data in such a way that it does appear green. As you know, this is one of the earliest explanations of "colour constancy".

Land: In respect to memory colours, in one experiment we take some oranges, paint them many different colours, and project them on the screen with arbitrary stimuli, so that the observers don't know what the wavelengths are. Everybody agrees about which one looks orange, although some look green, some look brown, and some look pink, and in fact which one looks orange is determined by the relative lightnesses rather than by the relative energies at the two wavelengths. If you reverse the filters the one that looked orange will look green. While memory has a small influence, it has no more influence than it has anywhere else in life. What we think we are talking about is a systematic statement of the basis of colour constancy. Colour constancy, far from being a mere illusion, is the basic phenomenon of vision. But how can one have colour constancy on one hand and yet have everybody knowing what colour he is seeing? Our scheme tells you that one can have colour constancy, as far as relative amounts of different wavelengths go, and yet the law of correlation numbers can predict what the colour will be.

Klüver: Does your theory extend to moving objects? For instance, does it attempt to account for the Fechner colours?

Land: Our theory does cover ordinary motion in everyday life, although it doesn't cover Benham's top.

Marks: Dr. Land, let us suppose that one of your arrays of coloured paper (the "Mondrian" board) is irradiated by three projectors—a long-wave, a middle-wave and a short-wave—projected to give gradations across the surface of the screen. An object seen at the right-hand side might be third in order for the short-wave light, it might be the brightest with the middle-wave and it might be second in order for the long-wave.

Land: Your supposition is right but not your conclusion. I began by saying that with the grey "Mondrian", if I put a wedge into the projector, I could have the same amount of radiation coming from an area coloured black as from an area that looked white. The remarkable phenomenon is our competence to classify them in the lightness scale, which is not a measurement scale. Now take the coloured Mondrian. If in the projector with the long-wave filter we put a wedge such that the amount of light coming from a black area is equal to the amount of light coming from a white area, it continues to look black; you are keeping it in the same place in the lightness scale. If we now turn on the green projector and put another wedge in the green illuminant, the colours persist and you can have simultaneously instead of successively a green pear and an orange school-house, say, sending the same numbers of quanta of light of the same frequency to your eye. They retain their colours because they keep their positions in the lightness scale independently of the amount of radiation coming to your eye.

Marks: This brings me to my question. Suppose you slide the green pear across to the other side of the picture. The green pear has constant ratios of reflectances for two colours, so that if there is a lot of red on the left and less on the right-hand side, as you slide it across the amount of red that it reflects back will cross the amount of red something else reflects and the rank order will change.

Land: No! You are quite right up to the final point; its rank order will stay the same; that is the miracle of the eye. Let us take the lightness scale for each pair of receptors separately. In the grey Mondrian, as you move across the screen the pear stays the same lightness in spite of the fact that the light from it is changing. We have been taught that a white surround will make a small grey area in the centre look dark. But note that if you take a piece of paper and move it around in the room, although the room is brighter in one place than in another, you are sure of the paper's position in the lightness scale; you keep your knowledge of its position in the lightness scale independently of the surround. So that as the pear is moved across the *coloured* board, it will keep its position in the lightness scale as it goes from a place where there is a lot of light to where there is a little light. You are right about the crossing-over in the ratios of the amount of light, but there is *not* a crossing-over in the position of the lightness scale.

Turn off one projector; have light in the other two. Consider a pear which was light in that projector. If you move it all the way across the screen to where there is only one-tenth as much light, it continues to look light. In the other projector the pear was dark in one position, and it continues to look dark wherever you put it.

Marks: It seems that you must be computing the lightness scale only over a local region and not over the whole board.

Land: I am not computing the lightness scale; I am looking at it. The miracle of the eye is its ability to hold objects in their position in the lightness scale independently of the fluctuations in the illumination—and to do that, in our opinion, independently for each retinex.

HUMAN COLOUR VISION

THE GENETICS OF COLOUR BLINDNESS

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ACHROMATOPSIA, or total colour blindness, was described in the seventeenth century by D. Turberville. A pedigree, in which one woman and five men were affected by red-green blindness in three generations, was published by J. Scott in 1778. It is well known that Dalton, who gave his name to red-green blindness, described his own defect (protanopia) in 1794 with admirable objectivity. Another kind of red-green blindness, now called deuteranopia, was described by A. Seebeck in 1829.

In 1881 Lord Rayleigh invented an instrument now called the anomaloscope. It can be employed to detect and measure degrees of red and green colour vision defect intermediate between protanopia or deuteranopia and normal colour vision, called anomalous trichromatism. The anomaloscope is the only practical instrument by means of which the distinctions of type and extent of defect can be readily determined.

DEGREES AND KINDS OF DEFECT

The Rayleigh Equation is used in the Nagel anomaloscope. Red (670.8 nm.) and green (546.0 nm.) are mixed and form a metameric match for yellow (589.3 nm.). Rayleigh worked with filtered lights, but in the Nagel anomaloscope spectral lights are used. In this instrument it is also possible to carry out many other equations, and the most useful of them is that in which bluish green (518.5 nm.) and indigo (464.5 nm.) are mixed to match a cyanide blue

(486.1 nm.). Pickford (1951) has worked with a yellow-blue and other equations. In the Pickford-Nicolson anomaloscope (Pickford and Lakowski, 1961) a red-green, a green-blue and a yellow-blue equation are used.

Those subjects who have no red-green discrimination are called protanopes if the red end of the spectrum is shortened, and deuteranopes if it is not shortened. Those in whom there is some red-green discrimination, but who have a very wide range of colour matches for the yellow, including the normal mid-matching point, and with or without a large deviation to the red or the green side, are called extreme anomalous. If the red end of the spectrum is much shortened, they are called extreme protanomalous; if it is not shortened, extreme deuteranomalous. If there is a very large deviation of the mid-matching point, and little increase of matching range, so that it does not include the normal mid-point, then the subjects are called simple anomalous. If the deviation is towards the red side they are protanomalous, and, if it is towards the green side, deuteranomalous.

All these are major defectives and will be failed by any adequate colour blindness test. Excluding them, if the matching ranges are somewhat enlarged, above twice the normal modal range, the subjects may be called colour weak. If the deviation is outside the limits of $\pm 2 \times$ sigma for normal subjects, they may be called deviants. A person may be colour weak and a deviant at the same time. In general the deviants and colour weak, who are minor defectives, pass ordinary colour blindness tests, but they tend to make more errors than the perfectly normal.

In the red-green equation, tests on a large random sample of the population tend to show a small number of major defectives, about 7 or 8 per cent of males among Caucasian whites, and their variability is discontinuous with that of the normal group, the remaining 93 or 92 per cent of males.

In the yellow-blue and blue-green equations there are equally extreme cases as the major defectives in the red-green equation, but

they are very much more rare, and the frequency distributions, in consequence, are continuous.

CLASSIFICATION OF COLOUR VISION DEFECTS

The following classification of colour vision defects has been widely adopted, although some people are not confident of the distinctions between extreme and simple anomaly, between rod and cone achromatopsia, or between tritanopia and tetrartanopia.

I MAJOR DEFECTS

A. *Total (Achromatopsia)*

Rod achromatopsia

Cone achromatopsia

B. *Red-green blindness*

Protanopia (P), dichromatic

Extreme protanomaly (EPA), trichromatic

Simple protanomaly (PA), trichromatic

Deuteranopia (D), dichromatic

Extreme deuteranomaly (EDA), trichromatic

Simply deuteranomaly (DA), trichromatic

C. *Yellow-blue blindness*

Tritanopia (dichromatic)

Tritanomaly (trichromatic)

Tetrartanopia (dichromatic)

Tetrartanomaly (trichromatic)

II MINOR DEFECTS

A. *Deviants*

Deviation in the anomaloscope equation tests more than $2 \times$ normal sigma (trichromatic)

B. *Colour weak*

Small but enlarged matching ranges in the anomaloscope tests, greater than twice the modal range (trichromatic)

There is not complete agreement about the importance of the matching range. Waardenburg (1963), following Hartung, thinks of the matching range as due to a separate defective gene, also found in normal subjects (colour asthenopes), which accounts for the difference between PA and EPA, or DA and EDA. Kalmus (1965) suggests that every anomalous trichromat has a preferred anomaloscope match, perhaps not simply the mid-point of his matching range. In consequence he tends to treat the matching range as a range of tolerance, depending on the intensity of the yellow, and lays little or no stress on its importance. The present writer is not convinced of the satisfactory identification of colour asthenopes, and regards the proper determination of the matching range as of fundamental importance in colour vision testing.

For anomalous trichromats there is the following relationship between deviations and matching ranges (Willis and Farnsworth, 1952; Pickford, 1958). As deviation increases through simple anomalous subjects, either PA or DA, the matching range increases but little. As we approach the extreme anomalous subjects (EPA or EDA), however, matching range increases and the deviation decreases again until dichromats are reached (P or D), and then, of course, it disappears entirely. It is not true that deviation increases steadily from the normal to dichromacy. For some EPA and EDA subjects the deviation is even slightly to the unexpected side of the normal mid-matching point. These facts are shown in Fig. 1.

THE GENETICS OF COLOUR BLINDNESS

Sloan (1954) has suggested that achromatopsia might be a partially sex-linked recessive with the locus of its gene on the homologous parts of the X and Y-chromosomes. Kalmus (1955) suggested that tritanopia might be due to a dominant autosomal gene, but Walls and Mathews (1952) treated it as a sex-linked recessive. Probably not enough pedigrees have been studied of achromatopsia or tritanopia for certainty on their inheritance as yet. It is generally agreed that all forms of red-green blindness,

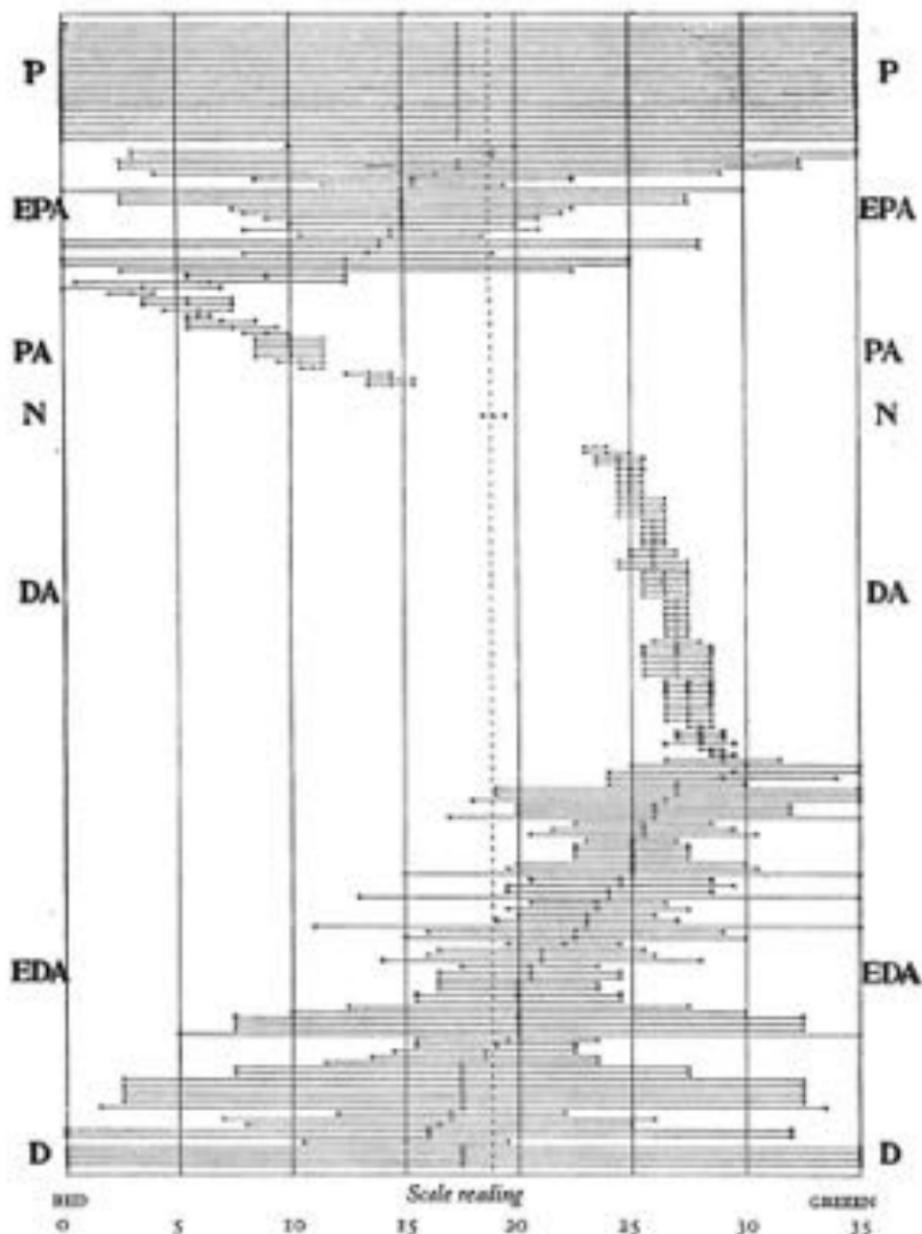
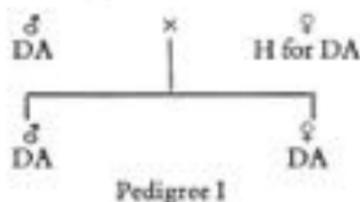


FIG. 1. Matching ranges and mid-points for 191 major red-green defectives on the Pickford anomaloscope. (By permission of the Editor of *The Advancement of Science*.)

One normal subject is shown in the middle for comparison. P = protanope; EPA = extreme protanomalous; PA = protanomalous; D = deuteranope; EDA = extreme deuteranomalous; DA = deuteranomalous; N = normal. Crosses = men; circles = women (crosses and circles visible with a lens).

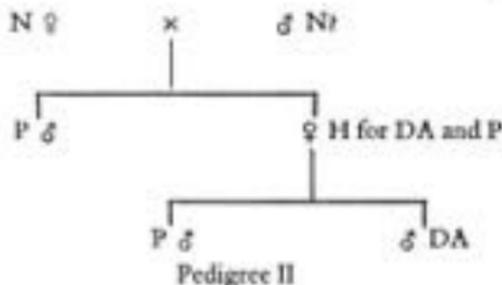
excluding minor defects, are inherited as sex-linked recessives. Minor colour vision defects might be due to the action of additive and subtractive autosomal genes.



Pedigree I shows the typical pattern of inheritance of simple deuteranomaly. In this pedigree the daughter, who was the proposita, was deuteranomalous, and her father and brother had defects almost exactly the same as her own (DA). Her mother, who showed a large enough matching range to be classed as red-green weak, was doubtless a heterozygote (H) for the defect manifested in her daughter.

The analysis of the pedigree is that the proposita must have had two genes for DA, one coming from her father, a defective hemizygote, and one from her mother, while her brother had one gene for DA coming from the mother.

Most pedigrees, of course, involve but one gene, which is manifested in male members of the family, according to the sex-linked pattern, such as men and their maternal uncles. In such cases the defects are almost identical. Not only is the kind of defect, such as P, EDA or PA, inherited true to type, but the precise magnitudes and characteristics of the anomalous defects are closely reproduced.



The two-locus theory

New problems may arise where pedigrees for red-green blindness include two or more types of defect. For example, Pedigree II, from Waaler (1927, p. 304) shows a woman, herself not a major defective, who had two sons, one of whom was a protanope and the other deuteranomalous. If a normal woman has a defective son she must be a conductor, or heterozygote, for that defect. If she has two sons with different defects, she must have one defective gene on each X-chromosome, unless they could be on the same chromosome with crossing-over.



Pedigree III

Pedigree III, also from Waaler (1927, p. 304) shows a deuteranomalous woman who had three sons, two being deuteranomalous and one a deuteranope. Similar pedigrees might be found for other genes. For instance, two sons of a protanomalous woman might be a protanope and a protanomalous, and here again, the mother must have two defective genes and would manifest the defect found in one of the sons.

The essential problem for such pedigrees is whether all these heterozygotes are to be regarded as allelic or as non-allelic compounds. The solution of this problem depends on whether we accept the theory of one locus for all red-green blindness genes, or the two-locus theory. When Waaler published his data on the testing of 18,121 Norwegian children (Waaler, 1927), two important facts were established beyond reasonable doubt. The first was that a normal woman could have two sons with different defects, if one was a protan and the other a deutan. The other was that the proportion of colour-blind females in the population was

less to a statistically significant degree than the square of the proportion of colour-blind males in the same population.

The first of these facts was almost certainly incompatible with the single-locus theory, on which the woman in question would be an allelic heterozygote, and should manifest the defect of one of her sons. The second fact indicated that there was in some way a loss of colour-blind women in comparison with the number expected on the single-locus theory. Waaler claimed that these women must be compound but non-allelic heterozygotes, who have two defective genes in different loci, each being neutralized by an appropriate but different normal gene.

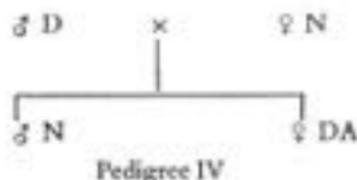
Both these facts are compatible with the two-locus theory which he proposed, and they have been adequately substantiated by the work of subsequent investigators, such as Franceschetti (1928, 1949, 1960) and others. Kherumian and Pickford (1959) have shown that throughout the world, the frequency of colour-blind women is lower than the chance expectation based on the single-locus theory with but few exceptions.

There has been much scepticism about the two-locus theory, but Kalmus (1962) has discussed the whole problem recently and concluded that the two-locus theory is the more likely. He considers that the two loci are not very close together. In view of the statistical evidence and the detailed pedigrees now available for study, the single-locus theory would seem most unlikely, or even quite impossible.

Multiple alleles and dominance orders

Franceschetti (1928) claimed that the various forms of red-green blindness are due to two series of multiple allelomorphs. For example, a woman who has the defect DA herself may have two sons one of whom is a deuteranope, D, and the other deuteranomalous like herself. In another pedigree a daughter may manifest a defect different from that of her father, but the same as that of her maternal uncle, although her mother is normal. A typical

example of an allelic relationship is given in Pedigree IV (Pickford, 1951). This and similar pedigrees fit the hypothesis that the two groups of defects, P, EPA and PA, and D, EDA and DA, correspond to two series of multiple alleles, each of which has its own normal gene, its own locus on the X-chromosome and its dominance order, P and D genes being most recessive and the normal genes most dominant.



The multiple allele theory would be compatible with the single-locus theory, but then a dominance order covering all the six defective alleles and a single normal gene would be expected. In this case we should find many pedigrees like Pedigree V, in which a defective woman has several sons, some deutan (like herself) and others protan. Pedigrees of this kind are, however, conspicuously rare, and if a woman has one protan and one deutan son, her colour vision is usually normal. This supports the two-locus theory, because each defective gene must be neutralized by a different normal gene dominant to it. There is no evidence that any gene of the protan series is dominant or recessive to any of the deutan series.

Table I

OBSERVED AND CALCULATED FREQUENCIES OF DEFECTIVE WOMEN

Defect	PA	EPA	P	DA	EDA	D	Total
Observed frequency	4	5	2	18	8	0	37
Calculated frequency $\times 100$	2.69	3.11	0.66	18.00	9.56	0.23	34.25

More than the study of individual pedigrees is needed to support the two-locus and multiple allelomorph theories. Among 34 (now 37) colour-blind women in the West of Scotland, the observed frequencies of the six types of defect were closely in accordance

with the frequencies predicted on these theories by calculation from the observed frequencies of the same types among men in the same geographical area (Pickford, 1959a). These data are given in Table I. The greater incidence of protan women over the predicted frequencies might be explained by a tendency for the protan genes to have more penetrance than deutan genes, resulting in the inadvertent inclusion of some protan heterozygotes as defectives. It would be especially valuable to have efficient data of this kind for racial groups in which the frequencies of the various defects were different from those in our own country.

Manifestation among heterozygotes

Waalder (1927) and later observers (Kherumian and Pickford, 1959) have found heterozygous manifestations of red-green blindness among women who are conductors of the defects. Schmidt (1955) showed that heterozygotes for protanopia often have a diminished sensitivity to red, corresponding to the shortening of the red end of the spectrum in protanopes. Minor red-green defects are more frequent among heterozygotes than among homozygous normal women, and tend to correspond to a statistically significant extent to the major defects of relatives. This correspondence is shown in Table II (Pickford, 1951, p. 336).

Table II
77 WOMEN WITH MAJOR DEFECTIVE RELATIVES

Grouping of Relatives	Grouping of Women				Totals
	Normal	R-G Weak	G Deviant	R Deviant	
P; EPA	6	9	1	5	21
D; EDA	7	20	4	0	31
DA	6	1	12	1	20
PA	2	1	0	2	5
Totals	21	31	17	8	77

In a sample of 71 simple heterozygotes about 50 per cent had distinct minor defects (Pickford, 1959a) but the other 50 per cent were not distinguishable from the homozygous normals who have

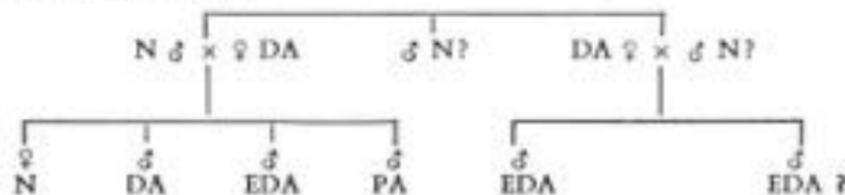
no minor defects. Even when the anomaloscope is used, heterozygotes cannot be detected with certainty, because the minor defects they often exhibit are not distinguishable from those of homozygous normals who may also show minor defects.

Waler (1927) and Pickford (1959a) have shown that heterozygous manifestation may occur in compound allelic heterozygotes, as, for instance, between EPA and PA, just as it may occur between either P, EPA or PA and normal (N).

The writer (1957 and 1959a) showed that what may be heterozygous manifestation can be found among the parents of the totally colour blind, and takes the form of a general minor colour vision weakness. If, as Sloan suggested, achromatopsia is a partially sex-linked recessive, or if it is an autosomal recessive character, then we should expect both parents of an achromat to be heterozygous for this defect, and this would accord with the possibility of both showing slight general colour weaknesses.

Double hemizygotes for red-green defects

One man in 833, according to Franceschetti (1960), might be a hemizygote with two defective genes in his X-chromosome. Pickford (1962) suggested 1 in 728, and Kalmus (1962) 1 in 1,000. It is surprising that these individuals have not been found more frequently, and their colour vision described. Walls and Mathews (1952) gave an account of two brothers believed to be double hemizygotes for protanopia and deuteranopia. Kalmus (1962) mentioned other cases due to Jaeger, and to Vanderdonck and Verriest (1960). Pedigree V from Pickford (1962) gives an illustration of the problem.



Pedigree V

The deuteranomalous woman, who had a normal daughter and three sons with different defects, must have had genes for three different forms of colour blindness. Her daughter must have been a heterozygote for at least one of them who did not manifest any minor defect. Her sister was also DA, and had an EDA son. Her own defect and that of her sister were identical, as were the defects of their EDA sons. The propositus was her PA son, and his colour vision was unusual. He had little darkening of the red end of the spectrum, but a very large red deviation on the anomaloscope when first tested. After 13 years he was recalled, and then he had a fluctuating sensitivity and was classified as apparently EDA. There is little doubt that he was a double hemizygote, and combined the characteristics of the PA and EDA defects. In order to account for this pedigree we have to assume crossing-over of genes for the EDA son.

The writer (1962) made tentative predictions of the colour vision of the possible kinds of double hemizygotes and their frequencies. These predictions are shown in Table III.

Table III

POSSIBLE PREDICTIONS OF THE COLOUR VISION OF DOUBLE HEMIZYGOTES

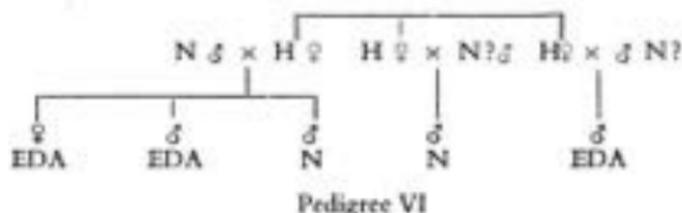
	DA	EDA	D
PA'	N	PA' (or EDA)	PA' (or EDA)
PA	N (or PA)	PA (or EPA)	PA (or EPA)
EPA	PA (or EPA)	EPA	EPA
P	P	P	P

PA' = protanomaly with little darkening of the red.

Sloan (1950) and other workers have reported small numbers of subjects with unusual defects. It is likely that double hemizygotes will be found among such subjects, if they can be identified, but this is not possible unless they are found as members of pedigrees which include a colour-blind woman and other subjects whose defects can pin-point the genes combined.

Colour blindness in Turner's and Klinefelter's syndromes

Interesting patterns of inheritance are found in pedigrees in which there are sex-chromosome abnormalities (Franceschetti, 1960). Since the Klinefelter has two X-chromosomes (XXY), his pattern of inheritance for colour blindness follows that usually found in women, and the frequency is as low as for normal women. For women affected by the Turner syndrome, when there is only one X chromosome (XO), the pattern and frequency of inheritance are as found in normal men. Pedigree VI (from Pickford, 1958) illustrates this point for a case of the Turner syndrome which was examined by Dr. John S. S. Stewart.



Here a father who had normal colour vision and a mother who was also normal but who must have been a heterozygote for EDA, had an EDA daughter affected by the Turner syndrome. Since she had only one X-chromosome, the gene for EDA on it was fully manifested, as would be expected in a man. The problems are much more complex, and cannot be dealt with fully here, but complete details are given by Franceschetti (1960) and Stewart (1961*a*). The latter (1961*b*) has shown how the study of a colour-blind patient with chromatin-positive Turner's syndrome may suggest that the colour blindness loci are situated on the short arm of the X-chromosome.

Inactivation of one X-chromosome

Lyon (1962) has put forward the interesting hypothesis that one of the two X-chromosomes in female mammals may be inactivated in cells in different parts of the body, with the resultant tendency to

form mosaics. This might affect colour vision in women heterozygotes for defective colour vision in such a way that one eye had colour sensitivity different from that of the other. Colour blindness might be produced in this way in one eye in a woman, or one eye might show more heterozygous manifestation than the other for defective colour vision. A comparison of the colour vision of the two eyes in women known to be heterozygotes for defective colour vision, or homozygous defectives, would therefore be of considerable interest, and the writer intends to carry out such a research. The great variability of heterozygous manifestation of defective colour vision, and the apparently complete heterozygous expression in some cases which has been claimed by several authors, might be due to varying degrees of inactivation of the X-chromosome carrying the normal gene in both eyes.

NATURAL SELECTION AND COLOUR BLINDNESS

An important discussion of the parts played by colour vision in the evolution of the primates and of man has been given by Polyak (1957). The frequency of red-green blindness among European and American whites (about 7 to 8 per cent) is greater than that among Asiatic Indians and others (about 5 or 4 per cent), and among American Indians and Australian aborigines (about 3 or 2 per cent) (Pickford, 1958, 1959*b*). Civilization might have brought about a relaxation of natural selection against colour blindness. Civilized groups have developed food hygiene to a high degree and depend less directly on the hunting and gathering of food, or upon direct colour judgments about its under- or over-ripeness or degree of decay. It is clear that to be colour blind might be a disadvantage in hunting and the choice of food by colour, and that more colour blind than normal individuals in tribes at the hunting and food-gathering stages of civilization might die of food poisoning or be unsuccessful in the chase. Selection might be increased again by the importance of coloured road signals and motor rear lights, with the

dangers of a child or adult being killed or maimed for life in a road accident.

Post (1962) has given full details of the possible relaxation of natural selection against colour blindness owing to removal from direct hunting and food gathering during the period of about 120 generations since Neolithic times. This might, he thinks, be reflected in the varied frequencies of colour blindness found among 123,414 men in the British Isles, and published by Vernon and Straker (1943). The greater frequencies in the South and West may have been due to waves of immigration in which the more primitive peoples were pushed away by peoples of more advanced culture from Europe. Colour blindness is most frequent in those parts of Britain removed longest from direct food gathering.

Post interprets the distribution reported by Kherumian and Pickford (1959) for France, among 6,635 men and 6,990 women, in a similar way. The two areas of highest frequency for colour blindness in France are the Mediterranean and English Channel littorals. These are associated with the two greatest waves of immigration of more advanced cultures, the Megalithic and Chalcolithic respectively. The areas of lowest frequency are on poor soils where hunting habitats may have lasted longest (Post, 1963 and private communication; see also Neel and Post, 1963).

The frequency of colour blindness in smaller groups may also support the same hypothesis. Post points out that the frequency of colour blindness among Brahmins is greater than among lower caste Indians, as do Kherumian and Pickford, and this may accord with the greater removal of the Brahmin population from direct hunting and food gathering than the lower castes. If all the data on colour blindness frequencies for Indians are pooled, we can compare 1,194 Brahmins with 4,031 Indians of other castes and tribesmen. Then the difference is strongly significant in the statistical sense (Pickford, 1963).

It is clear, however, that in hunting and food seeking, where camouflage of an animal depends on colour differences splitting up

the creature's form and making it blend with the environment, the colour blind might sometimes have an advantage, as found during war time when colour-blind men were used to identify camouflaged buildings and other objects and installations.

SUMMARY

The inheritance of red-green major defects is by the sex-linked pattern, and it is reasonably established that there must be two loci on the X-chromosome, one of which may be occupied by a defective gene of the protan group, and the other by one of the deutan group. In addition, the genes of each of these two groups may be arranged in a dominance order, which is the reverse of the order of magnitude of defect.

Heterozygous manifestations of colour blindness are found in about half of the women conductors, and take the form of minor defects corresponding in most cases to the major defects of their colour-blind relatives.

About one man in 700 to 1,000 may be a double hemizygote, carrying two defective genes on his X-chromosome.

Among men of the Klinefelter syndrome (XXY) and women of the Turner syndrome (XO) a reversal of the usual pattern of inheritance is found.

Red-green blindness is most frequent among Caucasian whites, and least frequent among Negroes and Australian aborigines. There may have been a relaxation of natural selection since Neolithic times, owing to decreasing importance of hunting, food-gathering, and the choice of fruit which is not under- or over-ripe or rotten. At the present time the importance of red-amber-green signals for life and safety might lead to an increase in selection against the colour blind because road accidents might cause the deaths of more colour blind than normal persons.

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DISCUSSION

Laud: Can you tell us what would happen if the selective process stopped? I gather that the normal phenotype is dominant. If we all stopped hunting and being hunted by automobiles, would the amount

of colour-blindness level off, when there was no selective process occurring at all?

Pickford: There can rarely be no selective process—mostly there must be some selection. I suppose the mutation rate would simply make colour-blindness steadily increase, but over a very long period; it would be very slow.

Welbarsh: Another possibility is that these mutants may not be stable genes, so that they would revert back to normal. Colour-blindness would then actually die out. You have the information in genetics available to deal with any set of data.

Land: What then happens to a characteristic that does not matter?

Welbarsh: It can increase, remain the same, or decrease!

Mounier: Is it true that colour-blind people are killed by cars in greater number? Are they not twice as careful as normal people, if they know that they are colour-blind?

Pickford: This is a possibility, but it is extraordinary how many colour-blind people do not know that they are defective. You would not believe it until you tested a few hundred of them. Many others refuse to accept it, although they really know.

Ripps: You mentioned the recent cytogenetic work which indicates that there is an inactivation of one of the X-chromosomes during the formation of the somatic cells, which would explain the mosaicism or expression of the defective trait in a female carrier. We examined some of these female carriers and, to complicate matters further, we find that on occasion they do not express, even in a mild form, the defective trait which is expressed fully by their sons, the affected males. A. E. Krill and A. Schneiderman recently reported on protan carriers ([1964]. *Invest. Ophthalm.*, 3, 445), and an analysis of their results by my colleague Dr. Siegel indicated that these carriers were tritan-like, rather than protanopic or protanomalous. We have looked at some carriers in complete achromatopsia and found on one occasion a deuteranope. So the picture has become extremely complex; we just do not know at what level we are observing things when we speak of colour vision and just how it would be manifested in these two situations.

Pickford: It is not unlikely that more than one kind of defect is sometimes present in the same pedigree. This inactivation of the X-chromosomes apparently could affect both eyes, and the inactivation is itself

highly variable, and might or might not occur. If it affected both eyes, the person would exhibit some degree of the defect, and some people think that a heterozygote might be fully defective in some cases, but personally I am extremely sceptical.

Ripps: That is highly unlikely.

Pickford: Some people believe it. And you could also have cases like C. H. Graham's famous person in whom the inactivation is possibly complete in so far as it affects one eye rather than the other eye, and you have a mosaic (Graham, C. H., Sperling, H. G., Hsia, Y., and Coulson, A. H. [1961]. *J. Psychol.*, 51, 3). But on the whole my own data suggest that women who must be heterozygotes are affected to about 50 per cent; about half show a measurable defect on my tests. And this defect is indistinguishable from defects that might be shown by women who are not heterozygotes, so you can never tell. The data also suggest that the defect is, on the average, like the defect of the major defective relative, but not always. I have also tested the mothers and fathers of three achromatopes, and all showed a general colour weakness of a minor kind, suggesting that they were heterozygotes.

Cresitelli: Professor Pickford, does colour-blindness occur in the apes and monkeys?

Pickford: I am told that it does, but I have not seen any. I believe Walter Grether found a deuteranomalous cebus monkey.

Rushton: De Valois surely investigated this by behaviour experiments. There were panels illuminated by pure yellow light and others illuminated by a red-green mixture, with rewards if the monkey chose the right place. The macaques' Rayleigh match was exactly "normal". The only time that the macaques could not distinguish yellow from red-green was when the investigator was deceived too. But one species, I think the spider monkey, was found to be protanomalous, for the monkey was confused when yellow was matched by a reddish red-green mixture, but could distinguish the mixture that is judged identical by normal men.

Wolfbarsh: I think De Valois did not find any colour defects in macaques. But this is the one animal case where we know what the normal vision is.

Rushton: I have done some measurements on the pigments in the eyes of the red-green colour blind and there are two things that can be said

fairly definitely. First of all, the extreme forms of protanomaly and deuteranomaly closely resemble protanopes and deuteranopes. Each of them has only one visual pigment in the red-green range that can be measured by our technique, so that to a first approximation we can say that these people are obtaining their vision in the red-green range almost entirely from one pigment, the red-sensitive pigment erythrolabe in the case of the deuteranope, chlorolabe in the case of the protanope. We know from the anomaloscope readings that the anomalous trichromats must have another pigment to make the discrimination that they do; and because their readings are in general abnormal, that pigment must be an abnormal pigment. It becomes a matter of very great interest to know the nature of that abnormal pigment, and on this there is no reliable information. J. F. Schouten ([1937]. Thesis, University of Utrecht) made the interesting suggestion that there was just one extra pigment X in both protanomaly and deuteranomaly, so that these two conditions were erythrolabe plus X or chlorolabe plus X. It could be hoped that Stiles' method of two-colour increment thresholds might show what the extra sensitivity was, in the same way that he has shown π_3 and π_4 , but I have never succeeded in doing this. All the deuteranomalous or protanomalous subjects have only shown the major Stiles mechanism, just as they have only shown the major pigment. So we are left with a strong presumption that an abnormal pigment is present in very small amount.

Louvenstein: I wonder whether our knowledge of what is contained in the rods and cones is not complete enough now to ask the biochemical question of what, in a scheme of "one gene-one enzyme", is the connexion between these hereditary defects and the basic colour vision mechanism?

Pickford: There must be many more than even six genes because not only is the protan defect inherited as a protan defect, but an individual protan defect is inherited as such. I have tested families in which cousins have exactly the same defect, and consequently there must be quite a number of separate genes which have specific, quite narrowly definable effects. And as Dr. Rushton said, we have little idea of the mechanism underlying these variations of sensitivity.

Rushton: The evidence from Wald's laboratory is fairly clear that the *retinene* portion is likely to be the same for the red pigment and the

green pigment, so there must be something about the protein structure, or the charges that Dr. Dartnall spoke about, that define the resonance and hence the colour absorption for the particular proteins.

Dartnall: I would think there is a strong degree of probability that this is true. Presumably all the human visual pigments are based on retinene₁, for retinene₂ pigments are, so far, unknown in animals higher than the amphibia.

Rushton: And therefore the genetic feature would be something in the building-up of those specific proteins in the cones. I have a very different approach from Professor Pickford, because he is dealing with what the people *can* see and match, which is a complex thing to sort out, and as he quite properly said, many detailed features enter and seem to be inherited truly. The things that I see are at a cruder level—chiefly that defectives lack nearly all of one of the pigments. The colour problem of the protan, for example, arises chiefly from the fact that nearly all his red-green information is channelled through the single pigment chlorolabe. Colour discrimination is poor because very little information enters through the contrasting channel; but matching is also "wrong" because the extra quanta are caught not by erythrolabe but by an abnormal pigment.

AFTER-IMAGES AS A MEANS OF INVESTIGATING RODS AND CONES

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WHEN the retina is stimulated by flashes of light appearing at regular intervals of about $1/50$ th second, the individual flashes are not seen. The sensation of vision persists at a high value for a time sufficiently long to bridge the period during which the stimulus is absent. This can be termed a short-period after-image, although this phenomenon is usually grouped under the title of persistence of vision. The experimental work on flicker is well documented by le Grand (1957).

However, if the retina is stimulated by a single brief flash of light of high luminance, a faint sensation of light remains, and decays over periods of up to about one minute. If the stimulus is of small area, and then a large area surface of lower luminance is viewed, a darker region is seen in place of the original stimulus. This is termed a long-period negative after-image. If the primary stimulus is coloured, the positive after-image is approximately of the same colour (at least initially), whereas the negative after-image appears with the complementary colour.

Undoubtedly, positive after-images are the more important to investigate, since they are connected with some fundamental continued activity in the retinal neurones. On the other hand, negative after-images are produced by restimulation of the retina. They appear whenever the restimulated area is of sufficiently high luminance to swamp the positive after-image effect, which nevertheless is always present. They are related to adaptation and

simultaneous contrast phenomena, and thus do not lead so directly to an understanding of basic retinal functions.

PREVIOUS LITERATURE ON AFTER-IMAGES

There is a vast and, to a great extent, useless literature on the subject of after-images, since until recently most workers have described their experimental work in qualitative terms only, and without standardization or statement of their conditions (Duke-Elder, 1932). This has been due partly to the difficulty of evolving suitable measuring techniques. There was also the mistaken feeling that after-images were elusive and fleeting phenomena which were not amenable to quantitative investigation.

According to von Helmholtz (1962), positive and negative after-images of windows were described as early as 1634 by Peiresc, and they were discussed in a letter from Sir Isaac Newton in 1691 (Brewster, 1840). Even Newton thought that after-images were a product of the imagination, and does not seem to have considered the possibility that they might have had origins in the eye. A thorough historical survey of the work on after-images up to 1922 is given by Berry (1922). Great names such as Goethe, Fechner, Purkinje, and Thomas Young appear in the literature, but mostly they found little time to devote to the study of after-images. Berry's main conclusion was that there appeared to be little agreement in the various accounts of the phenomena.

It was not until 1923 that any serious attempt was made to study the time-course of decay of the positive after-image. Lasareff (1923) derived the relation for the apparent brightness J of the after-image from experimental work:

$$J = A + B e^{-\alpha_3 t},$$

where A , B and α_3 are constants, α_3 being about 0.01 when t is in seconds. He projected the after-image on to a luminous screen, and

adjusted its brightness to obtain a balance between a positive and negative after-image.

Little further work appears to have been done until that of Craik and Vernon (1941). They projected the after-image on to a black disk and modified the luminance of its surround to match the apparent brightness of the after-image. They found the after-image of a white light stimulus of diameter $3\frac{1}{2}^\circ$ decayed exponentially with a time-constant of about 30 seconds.

Pannevis (1947) measured the brightness of the positive after-image by equating it with a negative after-image formed by re-stimulation. He found his results could be represented by the equation

$$H = Kt^a,$$

where H is the apparent brightness of the after-image, t is time, and K and a are constants. His stimulus was 2° square and of retinal illumination about 2×10^4 trolands. He found an exposure of 1 second gave the after-image with maximum apparent brightness.

Brindley (1959) has applied a null method to determine the conditions which produce equivalent after-images. From experiments on negative after-images (Brindley, 1962) he has concluded that very long-period (e.g. 15 minutes) negative foveal after-images depend upon the diffusion of the photo-products into cones of the same spectral type, thus decreasing their sensitivity. Trezona (1960) has measured the threshold stimuli needed to evoke after-images of various kinds. Alpern and Barr (1962) timed the duration to disappearance of the after-image by means of a stop watch.

It is, however, clear that the measurements mentioned above are open to criticism. In the first place, if the after-image is projected on to a luminous surface in order to assess its brightness, the re-stimulation will affect the subsequent course of the decay. Even if the after-image is projected on to a dark area adjacent to a luminous area, simultaneous contrast effects will be present. Secondly, some of the other measurements are incomplete, and only the maximum

brightness or the total duration of the after-image has been measured, and not its instantaneous value of brightness as a function of time.

THE BINOCULAR MATCHING TECHNIQUE FOR THE MEASUREMENT OF THE APPARENT BRIGHTNESS OF POSITIVE AFTER-IMAGES

The author's method of binocular matching of after-images is free from the objections mentioned above (Padgham, 1953). The method is similar to that used by Wright (1946) in his researches on colour. In this technique, a brief light stimulus of high luminance produces an after-image in one eye. The resulting sensation of brightness is then balanced binocularly without fusion by a comparison stimulus in the other eye, which can be varied to follow the variation of brightness in the after-image.

The apparatus has been described previously (Padgham, 1953). A dental impression is used to fix the observer's head, and artificial pupils of 1 mm. diameter are used. The primary stimulus used in a large number of experiments is a rectangle subtending $2^{\circ} \times 1^{\circ}$, and the comparison stimulus in the other eye is of the same size, but displaced vertically to avoid fusion. The luminance of the comparison stimulus is continuously under the control of the observer by means of a rotating knob. This moves a logarithmic neutral density wedge, the instantaneous position of which is plotted on a rotating drum.

EXPERIMENTAL RESULTS

The results of the experiments with this apparatus are published (Padgham, 1953, 1957, 1963), and can be summarized as follows:

(i) Using a white light stimulus of colour temperature $2,900^{\circ}\text{K}$, of size $2^{\circ} \times 1^{\circ}$, and of two luminances (retinal illuminations of 2×10^6 and 1.2×10^6 trolands), and exposures from 0.02 to 6 seconds' duration, on a retina dark-adapted for 10 minutes, the

decay part of the after-image curves could be represented by the equation

$$R = Ct^{-x},$$

where R is the retinal illumination in the comparison eye needed to balance the after-image sensation in the other eye, t the time reckoned from the beginning of the stimulus, x is approximately 3, and C is a constant.

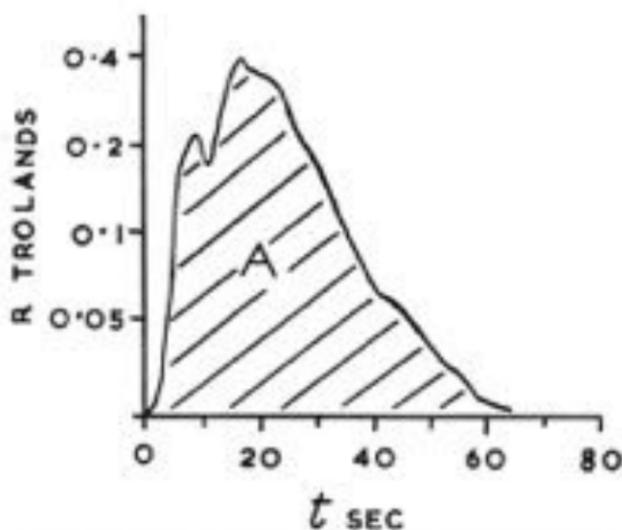


FIG. 1. A typical after-image curve of $\log(R)$ against t . White light stimulus of colour temperature $2,900^{\circ}\text{K}$, retinal illumination 2×10^6 trolands, and time of exposure, $t_e = 1$ sec.

(ii) Using stimuli of different angular size, of white light of colour temperature $2,900^{\circ}\text{K}$ and of retinal illumination 2×10^6 trolands for 1 second, it was found that with stimuli 5° square, $2^{\circ} \times 1^{\circ}$, and of 20 minutes of arc in diameter, the values of x were 3.5, 3.0 and 3.3 respectively. The respective time constants were 21, 12 and 6 seconds. Most after-image decay curves from stimuli of large area show a characteristic double maximum with peaks at about 10 and 20 seconds. However, the 20 minutes of arc in dia-

meter stimulus gives rise to after-images with a single peak only at about 8.5 seconds. It is thus probable that the 10-second peak is due to cone after-effects and the 20-second peak due to rod after-effects.

(iii) Using monochromatic stimuli at 510, 546.1 and 630 nm., of size $2^\circ \times 1^\circ$ and retinal illumination about 1×10^8 trolands for

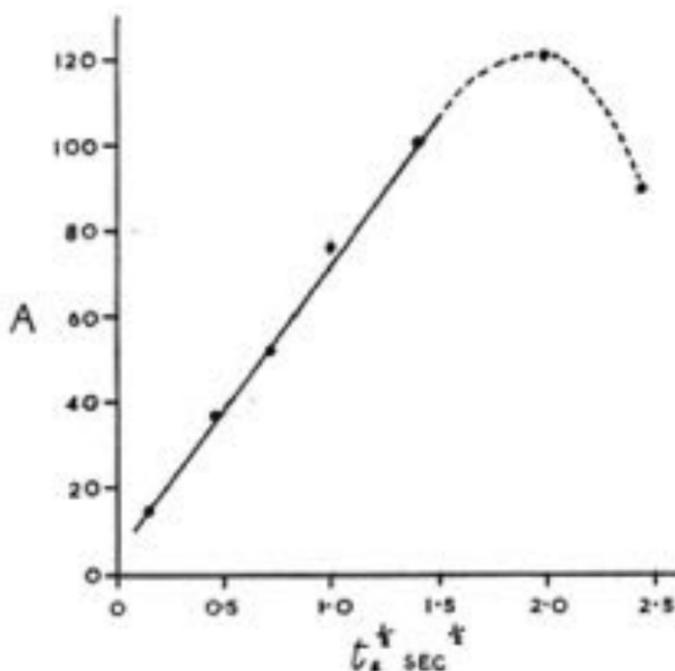


FIG. 2. A plot of A against $t_s^{1/2}$.

1 second, the after-image decay curves were exponential of the form

$$R = Ce^{-\mu t},$$

where μ and C are constants.

The values of μ for the three radiations 510, 546.1 and 630 nm. were 0.3, 0.1 and 0.4 respectively, giving time constants of 3.3, 10 and 2.5 seconds, respectively.

(iv) Positive after-images appear to follow a reciprocity law.

That is, the effect of the after-image is related to the product $R_s t_s$, where R_s is the retinal illumination provided by the stimulus, and t_s is the time for which the stimulus acts. This law has been verified for maximum apparent brightness of after-images, up to a value of $R_s t_s$ of 5×10^6 troland-seconds, and for the duration of the after-image up to values of about 1×10^6 troland-seconds, both with stimulus exposures of up to about 1 second. Beyond these values, a longer exposure produces a smaller effect for the same value of the product $R_s t_s$.

(v) A ripple with a period of about 6 seconds was observed on the decay curves of the after-images, although it is not yet known whether this is real, or in fact is observational "hunting" about the true balance position.

(vi) A plot of the area A under the after-image decay curves (of $\log R$ against t , Fig. 1) against the square root of the time (t_s) for which the stimulus acts, reveals a straight line up to values of t_s of about 2 seconds for a stimulus of 2×10^6 trolands, and up to values of t_s of 6 seconds for a lower luminance stimulus of 1.2×10^6 trolands (Fig. 2). These results give the relation

$$A \propto \sqrt{t_s}$$

DISCUSSION

After-images of high luminance stimuli appear to be of cone origin and the question of why the apparent brightness of the after-images of white light stimuli follows a power-law type of decay, whilst those from monochromatic stimuli decay exponentially, has been attributed to the fact that cone receptors with different colour sensitivities appear to have different time constants (Padgham, 1957). Each may exhibit an exponential decay of its after-images when acting alone, but when acting together after a white light stimulus, their combined effect could appear to follow a power law.

One cannot in fact investigate this problem fundamentally by using monochromatic stimuli of high luminance. As is known from colour mixture data, this is because different types of colour receptor have overlapping spectral response curves, and thus one cannot stimulate a single type of receptor without also stimulating others.

This work is however now being extended to enable the colour of after-images to be continuously measured during their decay. It might thus be possible to compute the individual decay curves of the different types of colour receptors from the colour mixture data thus derived.

The question we must now ask is: why does the sensation of light continue for about a minute after the cessation of a high-luminance stimulus? That is, why does the optic nerve continue to fire without a stimulus for this length of time? Since we do not know precisely how the discharge of the optic nerve fibres originates, it is perhaps premature to evoke elaborate theories concerning the origin of positive after-images. Nevertheless, perhaps a discussion of the results of experiments on after-images may open another avenue of attack on the problem of visual excitation.

Craik (1940) and Cibis and Nothdurft (1948) have described experiments in which the retina is rendered blind by pressure which prove conclusively that after-images have their origin in the retina. There thus seem to be four possible lines of thought. First we have the photochemical theory. This is based on the fact that the photochemical substances in the retina decay in a chain reaction, and on the assumption that the amount of each substance increases proportionally to the concentration of the substance from which it derives, whilst at the same time it is decaying into the substance which follows it. Furthermore, regeneration processes are taking place. If we also assume that the rate of firing of the optic nerve is proportional to the concentration of one of the substances, we arrive at an expression for the decay of brightness of the after-image in which an oscillatory term is superimposed upon an exponential

decay. This theory is not new, and was stated quite early by Peddie (1922).

The main argument against this theory seems to be the discrepancy between the time constants of the after-images for large and small fields (21 seconds and 6 seconds respectively), and those quoted by other workers for the regeneration of the rod and cone photo-products. Thus Rushton (1961a, 1958) quotes time constants of 360 seconds and 130 seconds for the regeneration of the rod and cone pigments respectively, and Weale (1962) quotes corresponding values of 260 seconds and 115 seconds. It seems difficult to reconcile these figures, unless the firing of the optic nerve during the after-image is not related to the concentration of the visual pigments in the receptors, but to some other substance with a smaller recovery time at perhaps the bipolar or more probably the ganglion level.

This leads to the second theory, which arises from the suggestion by Rushton (1961b) that in the eye of *Limulus* there is a transmitter chemical in the optic nerve ganglion, the presence of which changes the electrical resistance of the cell membrane, which in turn is related to the frequency of firing of the optic nerve. Could it be that this chemical during its exponential decay could give rise to the exponential decay in the positive after-image?

If the rate of production of the chemical (as suggested by Rushton) is proportional to $\log(R)$, where R is the retinal illumination provided by the stimulus, then in our experiments, the curves of $\log(R)$ against time (t) (R being the retinal illumination in the comparison eye needed to binocularly match the after-image sensation in the other eye) represent the rate of production of the chemical in the comparison eye as a function of time. Thus the integral

$$\int_0^t \log(R) \delta t = A,$$

(Fig. 1), represents the total quantity of transmitter chemical (Q) produced in the time t , t being the duration of the after-image.

In the after-image eye, however, we can assume that there is an initial quantity of this chemical Q' produced by the flash of light of retinal illumination R_1 for time t_1 . Furthermore, we can assume that this quantity of chemical begins to decay exponentially, and that the rate of firing of the optic nerve is proportional to its concentration at any instant of time. We thus see that Q and Q' are related by simple proportion. From our results, $A \propto \sqrt{t_1}$ (Fig. 2), and thus $Q' \propto \sqrt{t_1}$. In other words, the quantity of transmitter chemical liberated during a flash of time (t_1) is proportional to $\sqrt{t_1}$. This does not exactly fit with Rushton's suggestion that the rate of production is proportional to $\log(R_1)$ since in time t_1 the quantity produced would be $t_1 \log(R_1)$. One would expect also that a transmitter chemical, to be effective, would need to decay much more rapidly than the after-image appears to do.

The third suggestion is based on the fact that since the membrane potential V of the ganglion cell is directly related to the frequency of firing of the optic nerve, it is the exponential discharge of this potential within the cell which gives rise to the after-image. This does not fit the facts, however, because V is related to the logarithm of the light intensity, that is, $V \propto \log(R)$, and if V were to decay exponentially one would expect $\log(R)$ to decay exponentially. What we have found is that R decays exponentially, although perhaps it is worth recalling that in our experiments the retinal illumination R is evoking normal vision in one eye, whereas it is balancing binocularly an after-sensation of light in the other eye.

The fourth suggestion is that the centrifugal bipolar cells (or amacrine cells with axons) provide a closed circuit from the bodies of the ganglion cells to the fibres of the receptor cells, and then back through the bipolar cells to the ganglion cells. This could form oscillatory circuits which could give rise to decaying oscillatory potentials, which might be responsible for the after-images. The argument against this explanation is the fact that in oscillatory electrical circuits, the oscillatory potentials decay exponentially,

and we have the same lack of correspondence with experimental data as that stated in the previous paragraph.

CONCLUSION

Thus no theory of after-image formation seems to be very satisfactory at the moment, although it appears that the kinetics of the after-image decay might well be more nearly related to those of a transmitter chemical released during the passage of nerve impulses or to some other substance, than to the kinetics of the photochemical reactions in the visual receptors.

These experiments on after-images thus suggest that different types of visual receptors and their neural connexions give rise to after-images with different time properties. We cannot help speculating about the origin of after-images, and naturally such speculations are very much concerned with the question of how the optic nerve discharges are initiated by the action of light radiation on the visual receptors. On the other hand, theories as to the origin of the optic nerve discharges must necessarily include an explanation of the phenomenon of after-images.

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DISCUSSION

Lowenstein: What is the evidence that the after-image which you have observed arises in the retina?

Padgham: The evidence is from K. J. W. Craik ([1940]. *Nature [Lond.]*, **145**, 512). One applies pressure to the eye, which blinds the retina, and if one then produces an after-image and then presses the eye, the after-image disappears and reappears when the pressure is released. This is confirmed by other workers. My own evidence is that it is probably seated in the cone; but this is the main evidence.

Rushton: The evidence is a little stronger than that; if you are pressing on the eye properly at the time of the image-generating flash you do not see the light at all. But on removing the pressure you see its after-image, so it could not have been any kind of memory trace that was rekindled by resumption of nerve signals from the eye. I have repeated Brindley's artificial protanopia that way; if after blinding the eye by pressure you expose it to a very strong red light, and then release the pressure, you get a wonderful blue-green appearance, and when you look at the spectrum there is no red at all. Just as Brindley (Brindley, G. S. [1953]. *J. Physiol. [Lond.]*, **122**, 332) described, it is yellow as far as can be seen, and therefore red is invisible. That is not a psychological accommodation to the colour, because you never saw the red light which

produced that adaptation, so it must be in the eye and not in the brain.

Klüver: In studying the after-image sequence following momentary retinal illumination physiologists and psychologists, in the course of the last 75 years or so, have identified bright and dark, positive and negative, in fact seven or more after-image phases. One of these phases is the so-called Purkinje image. I recall that the physicist D. B. Judd sent me in 1927 a reprint of his doctoral thesis "A quantitative investigation of the Purkinje after-image" (Judd, D. B. [1927]. *Amer. J. Psychol.*, **38**, 507). To account for his findings he advanced an hypothesis rarely mentioned nowadays, the bioluminescence hypothesis. Has this been developed further by anybody? Or has it been demolished by now?

Padgham: I remember Judd's paper. I think I should state that I cannot reproduce many of these phases, the colour phases, dark phases and so on. Some of them are below the limit of my apparatus. I can only really follow the first decay, and then one does tend to get various small variations. But working with small fields, I have never seen much colour in after-images. I think some of this work must relate to re-stimulation.

Pickford: We have done many experiments on this with classes and have never had any difficulty in reproducing the colour effect, although it might be because we have a big field, of course.

Padgham: But have you done it in complete darkness?

Pickford: Yes.

Padgham: I want to extend my work on measurement of the colour of the after-images and I am building a colorimeter to do this; I shall have to use much larger fields. What size of field do you use?

Pickford: It is about a 20° field.

Rushton: I do not think the bioluminescence hypothesis is valid. Our equipment for measuring pigments has an arrangement for exposing the retina to a very bright light (a million trolands, for instance) for 0.03 second. During this time the light returning from the retina in the ophthalmoscopic arrangement is cut off by an electromagnetic shutter. These flashes are repeated at 10 per second and in the intervals between them the shutter is withdrawn so that the retina may be directly observed in the ophthalmoscope. It is easy to see the small patch that had been intensely illuminated 50 milliseconds earlier and to compare it with the unilluminated surround. No difference is seen; no phosphorescence;

only the complete and uniform spread of darkness. If any bioluminescence was excited by light it was finished in 40 milliseconds.

Klüver: It is interesting that the bioluminescence hypothesis crops up again and again. For instance, Christine Ladd-Franklin tried to explain the reddish-blue arcs and the reddish-blue glow of the retina on such a basis (Ladd-Franklin, C. [1926]. *Proc. nat. Acad. Sci. [Wash.]*, **12**, 413; [1927]. *Science*, **66**, 239).

Rushton: I know there are the blue arcs, and J. D. Moreland has shown them to us beautifully. But there is no bioluminescence that you can see in that experiment. I made my own observations because I came across an old booklet written by a photographer to acquaint the public with X-rays, which had only recently been discovered. He claimed that he could photograph the image on his retina, by looking first at a bright object and then at the photographic plate. He actually published the print from the photographic plate showing the piece of illuminated newspaper that he had been fixating a moment ago. But he was cleverer than I am, because I could not get anything, in what should be far more favourable conditions.

Klüver: It was pointed out that the after-image may disappear on applying pressure to the eye. Perhaps it is of some interest that after-images may also disappear when an insensible alternating current of low frequency is applied. It is generally known that within a certain frequency range this method of stimulation produces flicker phenomena which can be observed with open as well as closed eyes. I found that under certain conditions, when applying the stimulating current, negative after-images which happened to be under observation would almost entirely disappear or change radically in appearance. Cessation of stimulation and consequently of the electrically produced flicker would lead immediately to a reappearance of the after-image and to a restoration of its normal properties.

Padgham: This gives weight to the suggestion that the after-image is electrical in origin.

Monnier: Dr. Padgham, I am interested in your latencies in connexion with the "off effects" in humans. What is your latency from the end of the flash until the after-image appears? Do you have the same latency for the after-image as for the image itself?

Padgham: The first peak is at about 10 seconds. There is a rapid rise

here; it is so rapid that one cannot in fact follow it with the apparatus, which is why one picks up the after-image after perhaps four or five seconds.

Monnier: Our "off effect" starts about 30 to 50 milliseconds after the flash, so that subjectively yours come much later.

Padgham: Could I just say that my stimulus is of the order of 1 or 2×10^6 trolands, whereas the maximum illumination I use in my comparison eyes is of the order of 1 troland; so there is an initial period when the glare of light from the stimulus prevents one making a match.

Pirenne: May I raise a point which refers to a recent paper by H. B. Barlow and J. M. B. Sparrock ([1964]. *Science*, **144**, 1309). They made matches of the after-image with a comparison field, the image of which was stabilized on the retina. This I think is not the case in your new experiment, and it raises experimental and theoretical problems. It seems a good idea to try to stabilize the comparison field on the retina, because by definition the after-image must be stable on the retina, or so one would think. The outcome was that the matching luminance, under those conditions where the stability of the retinal image was as good as possible, was the same as that of the "equivalent veiling luminance" (the concept introduced by Stiles and Crawford). In brief, my point is that after-images, being stable on the retina, may not look as bright as one would expect, because of the Troxler effect, that is because of fading, probably due to neural factors.

Stiles: You remarked, Dr. Padgham, that the comparison eye was viewing the comparison stimulus below the photopic level. Did this mean that it was viewing the comparison stimulus in extrafoveal vision?

Padgham: I think it does, yes.

Stiles: You made a very interesting point about the difference between various spectral stimuli; I think they were 510 nm. and 630 nm. Did you have enough curves to draw a relative action spectrum, say, for a given level of intensity of the after-image, so as to get an after-image spectral sensitivity? From the results you gave this might be a very narrow curve, I would imagine?

Padgham: I am afraid I have not enough data for this. It is only recently I have come across an ultra-high pressure mercury arc, which is much brighter than the Siemens arc, and although I have ordered one of these I have not yet used it. But my difficulty was that my xenon arc, filtered

with a 630 nm. red interference filter, gave me a retinal illumination of only 0.6×10^6 trolands, while my 541 nm. green stimulus was 1×10^6 and my 510 nm. blue-green was 0.9×10^6 trolands. So they are not really the same, and the difficulty with working below a million trolands is that the after-images are relatively faint; their brightness only goes up to 0.2 or 0.3 trolands, and they are difficult to measure with any accuracy. Until I get much brighter stimuli I would not like to publish anything on the relative efficiency, but it is something I have in mind to do. The present evidence suggests that it would be a rather narrow curve. I think this ties up with your work, Dr. Lennox-Buchthal?

Lennox-Buchthal: Yes; it is the only psychophysical finding so far that does! It has been a great puzzle to me that within the cerebral cortex there are apparently mechanisms which narrow down the ability of single cells to respond over the whole spectrum. Single cells in the geniculate, if given enough intensity, no matter what their spectral sensitivity curves are, can be made to respond over the whole spectrum, but in the cortex one cannot do that. No matter how much one increases the intensity, a single unit still will not respond except to blue if it is blue-sensitive, or red if it is red-sensitive, or green if it is green-sensitive. If you find narrow curves, that is the only psychophysical result which corresponds to what we find physiologically.

GENERAL DISCUSSION ON THEORIES OF COLOUR VISION AND HUMAN COLOUR VISION

INTRODUCTION

Rushton: I would like to ask some questions of the speakers who have already presented their papers, as well as to make some comments of my own on the material that has already come up.

I would like first to raise some questions which Professor Pickford can perhaps answer. What puzzles me about these genetic factors is what happens to the vision of the people in whom more than one of these factors occur. I know almost nothing about the genetics except that deuteranopia and protanopia are not alternatives, but are located in different places on the sex chromosome, and so one might expect that there was a small chance, and not such a very small chance, that these two conditions might occur simultaneously. I should have thought that the chance of a male deuteranope also inheriting protanopia was precisely the chance of a protanope woman inheriting protanopia with the other chromosome also, and therefore being a manifest protanope. Now, I am sure that in your experience you have found manifest female protanopia, but I think it is unlikely that you have come across anyone with what I would have thought to be the natural result of having no gene for making erythrolabe and no gene for making chlorolabe, namely blue vision only. This has been recorded in one family only, that of three brothers in Michigan, recorded by H. R. and O. M. Blackwell ([1959]. *Archos. Soc. am. Oftal. Optom.*, 2, 73) and these subjects had just what one would expect—a blue cone sensitivity, of rather low luminosity, the shape of the blue spectral

sensitivity coinciding rather closely with Stiles' π_1 , and with rod vision near the fovea differing from normal scotopic vision, because of absorption by macula pigment. When the Blackwells' results are plotted and the π_1 curve is drawn through, they fit very closely together.

The other thing one might expect is that the tritanope and protanope conditions would come together in one individual and form another kind of cone monochromat. The best paper on the cone monochromat is that of Dr. Weale (Weale, R. A. [1953]. *J. Physiol. [Lond.]*, **121**, 548); he went into this in detail, considered the genetics in a way that most people working on colour vision don't, and came to the conclusion that this couldn't be the case on many grounds and that the cone monochromat was not just due to the genetic concurrence of a protanope and a tritanope, although frequency alone might be consistent with that idea. One of the strongest arguments was that if that were so, you would expect a high preponderance of cone monochromats among males because protanopia is sex-linked and tritanopia is not sex-linked, but he found about equal numbers of both sexes.

One further point that concerns genetics is that there is a considerable variation in the amount of red cones and green cones among normal people. The red and the green cones are quite normal ones, but the numbers can vary among people with normal colour vision over quite a big range. Baker and I (Rushton, W. A. H., and Baker, H. D. [1964]. *Vision Res.*, **4**, 75), took normal students from the class and measured red versus green sensitivity by flicker photometry in which red light alternates with green light and the subject varies the relative intensities until the flicker is at a minimum. We found that the log R/G sensitivity thus measured was distributed on a Gaussian curve of frequency in our population of 200. Moreover the 10 most red-sensitive had a high proportion of erythrolabe, and the 10 most green-sensitive had a high proportion of chlorolabe on the fovea. So it is reasonable to interpret the results as showing that in a normal population

there is a wide scatter in the ratio of red to green cones upon the fovea.

I want now to turn to Dr. Padgham's paper, but first I would like to say a word about Dr. Crawford's introduction. Most of the questions of particular interest that he raised have been dealt with in this session but there is one thing that he didn't raise at all, namely some of his own contributions on the subject. Over the years Dr. Crawford has made a number of surprising and important contributions, which have been the more trustworthy because it has always been perfectly clear that he never has an axe to grind. One of these contributions leads to my next point. It is his demonstration that the dark-adaptation curve and the increment threshold curve vary in the same way with the area of the test flash used. This means that we can get over the embarrassing question that was brought best into focus probably by Arden and Weale (Arden, G. B., and Weale, R. A. [1954]. *J. Physiol. [Lond.]*, **125**, 417), that the dark-adaptation curve has a different shape depending whether you use an object of rather small diameter or a test flash of large diameter, if we use Dr. Crawford's principle of equivalent background. If for example, after 100 seconds of dark adaptation, the threshold with one area of test flash corresponds to a log background field of say, -5.4 , then this correspondence also holds for all other areas of test flash, and the equivalent background field (-5.4) is the real thing which goes hand in hand with whatever is happening in dark adaptation (at 100 seconds).

Now what *is* happening in this process of dark adaptation? There has been bleaching of the pigment and it has been claimed for a long time and established for a rather short time that the regeneration of the visual pigment is what goes hand in hand with the equivalent luminance and causes the rise of threshold. It is easiest to measure the relation between dark adaptation and regeneration of pigments in the cones. If a red test flash is used, for example, dark adaptation and erythrolabe regeneration return to normal along the same curve. The relation is even better known

for rods where Dowling first showed it in the rat using the electroretinogram (Dowling, J. E. [1960]. *Nature [Lond.]*, **188**, 114).

Now we come to the situation in Dr. Padgham's experiments. H. B. Barlow and J. M. B. Sparrock worked with a stabilized comparison image ([1964]. *Science*, **144**, 1309). Since the after-image is stabilized on the retina, they compared it for brightness with a stabilized surround in the same eye. The subject wore a little sucker on the anaesthetized cornea which carried a small target which could be seen. A small lens brought the target into focus on the retina. The target was a white ring through the centre of which the flash of bleaching light was given. It was possible to shine light from the side to illuminate the white ring around the hole now filled with a positive after-image, and so to adjust the surround luminance that these two stabilized images appeared equally bright. The centre and surround were kept in balance throughout the course of dark adaptation. When the actual luminance of the ring is compared with the equivalent background luminance of Dr. Crawford, measured independently by Dr. Crawford's method, both measured in trolands, they fit absolutely. We therefore reach the conclusion (which I had never believed possible) that what is putting up the threshold in the early stages of dark adaptation is not what Barlow used to call "dark light", but is the real brightness of the after-image, and if you think, as Dr. Padgham certainly does, that the brightness of the after-image is nothing like adequate, the answer would be that with the *stabilized* image, as in this experiment, the appearance of the image gets dimmer and dimmer, and thus misleads us.

Now, what can be said about bleaching and the formation and brightness of the after-image and the raising of the threshold and so on?

The crude and speculative diagram of Fig. 1 (p. 269) represents an attempt to relate the adaptation to background and to bleaching and the brightness of fields and after-images. It starts with the Hubbard-Kropf (Hubbard, R., and Kropf, A. [1959]. *Ann. N.Y.*

Acad. Sci., **81**, 388) idea that the close fit of 11-*cis* retinene to protein hinders some enzymic activity that is freed when retinene is isomerized to the all-*trans* form.

In Fig. 1 the active enzyme site is represented in a cavity walled off from the substrate by the well-fitting 11-*cis* lid (a). The impact (b) of a quantum on the lid springs it open (all-*trans* isomer) and

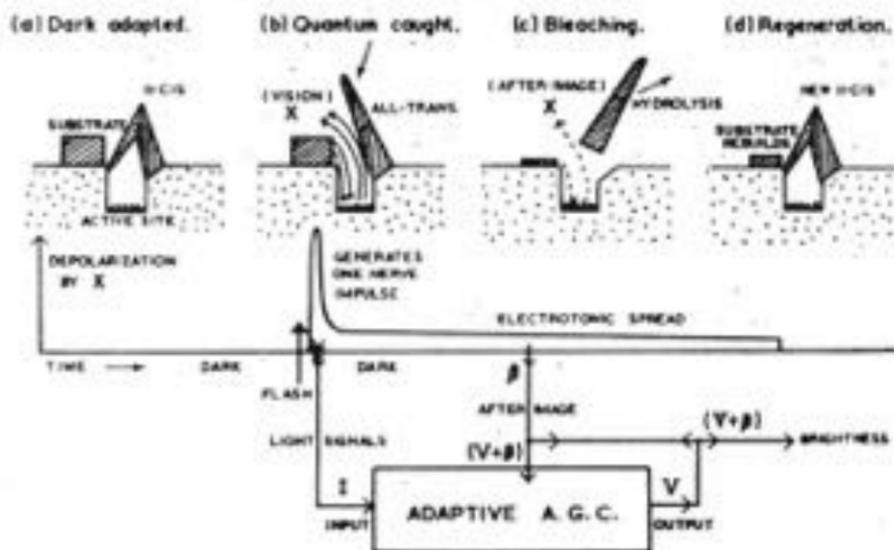


FIG. 1. (Rushon). Conjectured relation between pigments and vision (see text). Top line: molecular configuration. Second line: chemical transmitter X. Third line: input, feed-back and output of automatic gain control mechanism (A.G.C.).

the substrate reaches the enzyme and is turned into a pharmacological substance "X" with nerve-stimulating properties. Bleaching (c) is the hydrolysis which removes the retinene from attachment to the protein. Regeneration (d) is the union with a fresh 11-*cis* molecule. This occurs on average 5 minutes after bleaching with rods and four times as fast with cones.

The second line in Fig. 1 shows the level of X at various stages. Immediately upon light activation (b) the level is high, for there is accumulated substrate to be transformed; later the level is low, merely keeping pace with substrate replacement; at (d) X ceases

to be formed and substrate builds up. This describes the phenomenon relating to a single molecule of visual pigment. The lower part of Fig. 1 indicates the application to vision. Each quantum caught generates one nerve signal at the moment when X reaches its peak. The flux of signals enters an automatic gain control (A.G.C.) mechanism whose output constitutes a parametric negative feed-back, similar to the scheme proposed by M. G. F. Fuortes and A. L. Hodgkin ([1964]. *J. Physiol. [Lond.]*, **172**, 239). The output of this system also is the retinal basis for the sense of brightness. Now, the very small residual level of X , because it lasts so long, may add with all the other bleached residues to give an appreciable signal β . This does not enter the input of the A.G.C. box but it enters the feed-back, where it adds to the output V and produces a sense of brightness—the after-image.

This may strike you as a very odd and unlikely scheme; but we have some very odd phenomena to explain and this scheme will account quantitatively for some of them. If we assume that a flash is threshold when the output V is increased by a fixed amount ΔV , we may deduce (i) Fechner's increment threshold relation, that threshold varies linearly with background, (ii) the dark-adaptation relation, that log threshold is proportional to the amount of free opsin, and (iii) the Barlow-Sparrock relation that the equivalent luminance of the after-image is proportional to the amount of pigment still bleached. The analysis would take us too far to be developed here, but it may be found (Rushton, W. A. H. [1965]. *Proc. roy. Soc. B*, Ferrier Lecture, in press) in relation to normal thresholds and (Rushton, W. A. H. [1964]. *Photochem. Photobiol.*, **3**, in press) in relation to the physiological results of multiple quantum hits per molecule.

Finally, Fig. 2 (from Baker, H. D., and Rushton, W. A. H. [1965]. *J. Physiol. [Lond.]*, **176**, 56) shows the visual pigments in the normal subject. The black and white circles show the red and green pigments by transmissivity difference spectrum in conditions where

superficial stray light had been reduced to about 1 per cent of the light from the retina. The triangles show the transmissivity difference spectrum in the deuteranope and in the protanope; the continuous lines are Stiles' π_3 , and π_4 ; the dotted line is Hsia and Graham's threshold spectral sensitivity curve for the deuteranope

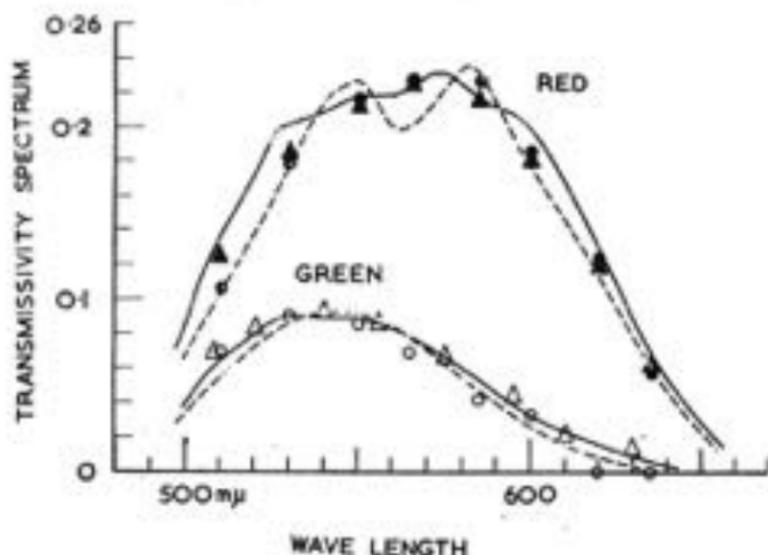


FIG. 2. (Rushton). Black circles, transmissivity difference spectrum of red pigment *erythrolabe* in normal eyes; black triangles, the same in the deuteranope. Continuous curve, Stiles' π_3 spectral sensitivity; dotted curve, Hsia and Graham's threshold for deuteranopes. White circles, triangles and corresponding curves show the analogous relations for the green pigment *chlorolabe* in normals and protanopes, Stiles' π_4 and the threshold for protanopes.

and protanope. When these points are put on to Dr. Marks' curves, they fit fairly well with the figures he found for the human eye (see Fig. 3 of Rushton, W. A. H. [1965]. *Nature* [Lond.], Newton Lecture, in press).

★ ★ ★

Finally I wish to present the evidence Dr. Naka has recently obtained that in the tench (*Tinca*) there is a visual pigment with

maximum action at 690 nm. in addition to three other pigments whose action spectra correspond to the pigments measured in single cones by Dr. Marks. It is always hard to argue from electrophysiology to pigment in any situation where more than one pigment enters, since we know so little about the nerve interaction between the signals from the two pigments that may contribute to the physiological change used as the criterion for the action spectrum. Dr. Naka has recorded the potential change of S-cells when lights of different wavelength and energy are flashed or maintained. In Fig. 3 the upper curve (black circles) shows for a red-green colour cell the amplitude of potential increase (depolarization) that followed a light flash of wavelength 720 nm. as the log intensity increased, as shown by the horizontal scale. The increase reaches a ceiling and further red light will not increase it. This is shown by white circles, where the same flashes were superimposed upon a steady bright background of light (750 nm.) of intensity sufficient to reach the ceiling (the same level as for the black circles but plotted displaced upward for clearness). The deep red flash now had no effect. But a flash of shorter wavelength did, as seen in the lower curves of Fig. 3. As G. Svaetichin and E. F. MacNichol have shown ([1958]. *Ann. N.Y. Acad. Sci.*, **74**, 385), long and medium wavelengths have opposite actions on this type of cell: red depolarizes, green hyperpolarizes. In Fig. 3 this antagonism is seen, for the white circles, at the deep red ceiling, are "pulled down" by flashes of shorter wavelength when these are strong enough. The black circles show us what is taking place. *Weak* flashes of 660 nm. light excite only the "red" cones and act exactly like flashes of 720 nm. light, the black circles rising on the same curve, the white circles remaining unaffected by the flash. But with an intensity of more than 2.5 log units of 660 nm. light a new feature arises. It is the excitation of the "green" cones that subtracts the "green potential" from the "red potential". It is seen to pull down equally the ceiling (white circles) and the rising red curve (black circles). Further experiments have shown that the

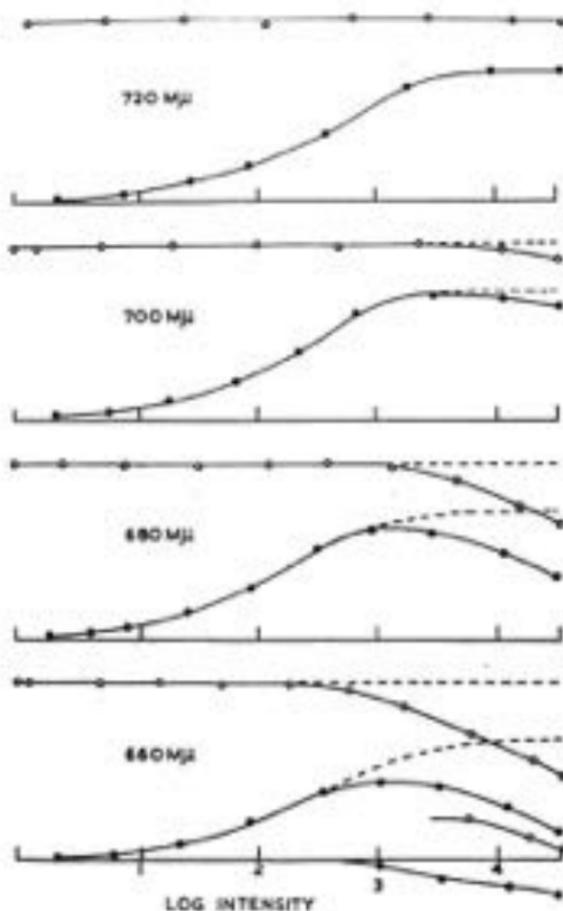


FIG. 3 (Rushton). Black points plot as ordinates the S-potential depolarization of a red-green cell when a flash of light of wavelength indicated in each row is delivered whose log intensity is plotted horizontally. White circles plot depolarization (displaced upwards, for clearness) when a strong light of 750 nm. is added to the light flashed. To the extent that the flash excited the red cones it has no effect when added to the 750 light. To the extent it affects the green cones it "pulls down" the white circles from their ceiling.

whole white circle curve with its downpull is unchanged when the intensity of red saturating light is altered.

If then we suppose that light of 660 nm. acts through two inputs, through signals from red cones and through signals from green cones, we have here a way of sorting them out. For the red-cone action is without effect in the saturated state (white circles), and

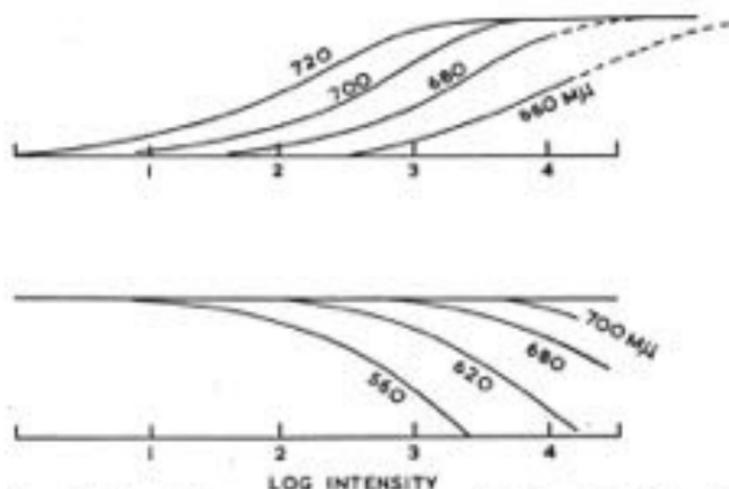


FIG. 4 (Rushton). *Lower curves*: the downpull of the white circles of Fig. 3; all lie on the same curve laterally displaced. The displacement gives the action spectrum of the green mechanism. *Upper curves*: the rise of the black points of Fig. 3 before the downpull sets in; all lie on the same curve laterally displaced. This gives the action spectrum of the red mechanism.

the green-cone action is the same no matter how supersaturated is the red. Hence the effect of 660 nm. (and other wavelengths) upon the green cones may be measured in isolation simply by comparing the down-pulling from the ceiling of the white circles. Fig. 4 shows some curves similar to those drawn through the black and white circles of Fig. 3. The lower set (from the white circles) shows, as would be expected, that the curves have all the same shape and are merely displaced along the horizontal scale; the upper set shows the same for the black circles. The lateral displacement of the lower curves, coupled with the energy output from

the spectrum we used, allows us to plot the action spectrum of the green pigment, isolated because at the red saturation obtaining, added red had no effect, hence the red component of the spectral

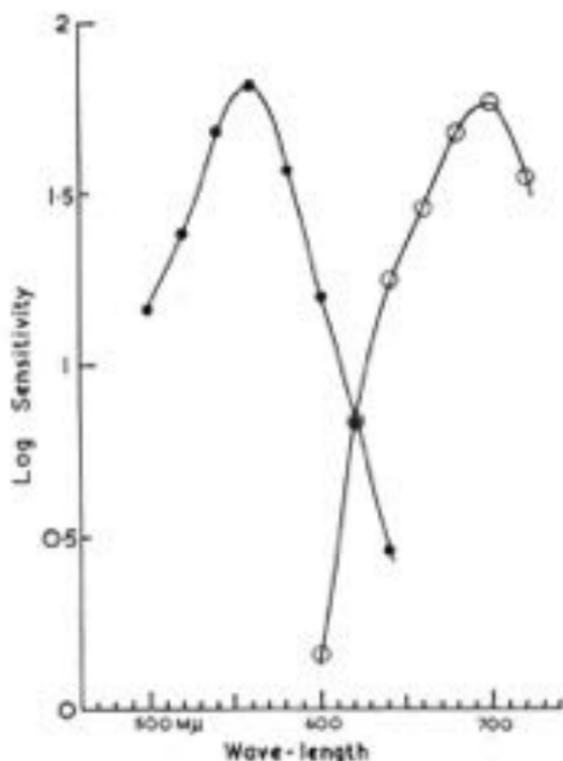


FIG. 5 (Rushton). Action spectra of red and green pigments in the tench as determined from Fig. 4. The maxima lie at 550 and 690 nm. Both curves appear narrower than expected from Dartnall's nomogram.

light contributed zero. A similar calculation with the upper curves of Fig. 4 allows us to plot the action spectrum of the red pigment, isolated because if green cones had been active, they would have pulled down the white points as much as the black points; but the white points are seen to lie at ceiling and are not pulled down at all, consequently the black points also are quite uninfluenced by

green cones in the range shown. The red and green action spectra thus isolated are shown in Fig. 5. The green lies at about 550 nm. near where Dr. Marks has found it, but the red lies at about 690 nm., where its presence surprised us.

GENETICS OF COLOUR VISION

Pickford: You raised the question of the combination of protan and deutan genes and asked how many times a protan gene and a deutan gene occur on the same X-chromosome. The result would be different in men and women because women generally have two normal genes on the other X-chromosome and the result would be normal, but in a man there would be no neutralizing normal gene, and the result would be some kind of double defective hemizygote. The colour vision of such men is a somewhat open question.

Rushton: But have you ever seen people like those Michigan boys, that is, people who have only blue cones?

Pickford: No, I haven't.

Rushton: Yet the frequency of protanopes and deuteranopes in the population is about 1 per cent so you would expect to see one in about 10,000; the question is, why haven't you?

Pickford: I have never found anybody who saw nothing but blue but I haven't tested as many as 10,000 people—not more than 1,500 with careful tests. Some kind of protan gene occurs with some kind of deutan gene in man about one in 1,000 times but there are 9 different types of combination and if you include women, I believe there are 81 different types more (cf. Pickford, R. W. [1951]. *Individual Differences in Colour Vision*, pp. 363-364. London: Routledge and Kegan Paul). You are giving me the problem of thinking of these in terms of pigments, but I don't think we know enough about the pigments to say. As you said yourself, the peculiar visual pigments of the anomalous trichromats are at present unknown.

With regard to the frequency of the slight variations you mentioned, in man there is an extraordinarily narrow curve of frequencies; about 15 per cent of men show a minor defect of red-green or yellow-blue vision (Pickford, R. W. [1951]. *Loc. cit.*, pp. 266-271), or both together, and these may be the ones you have got. The proportion depends on where you draw the line. Approximately 15 per cent of men show a minor defect without being failures on a standard test of colour blindness, because of these slight variations that occur. The standard deviation is extremely small if you exclude the major defectives, and these, in any case, form separate groups.

Lowenstein: Are both eyes always the same?

Pickford: I have been asked this by a number of people who say that their eyes are different but I have always found them exactly the same. In some women (the heterozygotes) it is said that they aren't the same because of the mosaic effect we mentioned, but I have never found anyone who had any difference.

DARK ADAPTATION AND AFTER-IMAGES

Padgham: You raised the question of the use of stabilized images, Dr. Rushton. I imagined from the work of R. W. Ditchburn, D. H. Fender and others that the sensation in a stabilized image disappeared after a matter of seconds.

Rushton: The sensation doesn't disappear. Unless you lost consciousness, I don't see how it could. Discrimination disappears, particularly fine discrimination. I have never been able to have fairly coarse bars of a grating disappear with a stabilized image myself; of course one may say that it wasn't properly stabilized. But there is no question of all nerve signals disappearing. The pupil will still respond in a perfectly normal way to the mean light.

Padgham: So it is colour and form?

Rushton: Yes, I think so.

Padgham: I wonder whether the difference in the technique

between Barlow and Sparrock's experiments and my own was enough to explain the difference between their decays of something like 45 minutes and mine of 1 minute.

Rushton: The luminance started at 1 millilambert. I expect the bleaching would be of the order of 10^7 troland-seconds.

Ripps: I believe it was delivered as a flash in the Barlow and Sparrock experiments, so that its effective bleaching may have been less than yours, which then certainly would not account for the difference.

Rushton: Why should it be less? It should be more, if anything, if it was a flash. A brief flash of the same energy bleaches more. It doesn't matter what the time of the delivery of the light is, provided that not much pigment is regenerated during that process. So anything up to about three-quarters of a minute for rhodopsin would be equivalent.

Ripps: I was under the impression from W. A. Hagin's work that flash photolysis can be less efficacious, so that this might not necessarily apply (Hagin, W. A. [1955]. *J. Physiol. [Lond.]*, **129**, 22P). I think Barlow and Sparrock also used a discharge lamp, a flash lamp, of the order of 24 milliseconds' duration. We are concerned here with the bleaching, since Dr. Padgham is trying to account for this longer recovery.

Rushton: What you said was perfectly correct, but I don't think it would account for this difference.

Wolbarsht: From experiments by Dr. Rushton and G. Westheimer ([1962]. *J. Physiol. [Lond.]*, **164**, 318), L. E. Lipetz ([1961]. *Science*, **133**, 639) and H. B. Barlow and J. M. B. Sparrock ([1964]. *Science*, **144**, 1309) it appears that there may be a large (possibly neural) component in dark adaptation, which causes a change in sensitivity unrelated to the reduction of pigment concentration from bleaching. The experiments by Lipetz on the frog retina seem to be the most direct. Two places within the receptive field of a single ganglion cell in the frog retina were stimulated and their respective sensitivities measured. Then one was light adapted,

and the sensitivities of both were measured again. Although the two points were some distance apart, it was found that the sensitivities of both points were raised by a large amount. It was presumed that the spatial separation of the two precluded any scatter of light from the light-adapted point into the point in the dark sufficient to account for the change in sensitivity at it. However, in the frog the receptive fields are often quite complex and in the type used by Lipetz in his experiment it was not excluded that the only sensitive point was in the centre of the field and that all other peripheral points have a response only insofar as they scatter light into the sensitive central point.

Dr. H. G. Wagner and I have repeated this experiment in the goldfish retina with some variations. The spatial distributions of the sensitivities of the "on and off" processes were ascertained with considerable precision. In the goldfish retina the "on and off" processes fall off in sensitivity at different rates as we move from the centre to the periphery of the field (Wagner, H. G., MacNichol, E. F., Jr., and Wolbarsht, M. L. [1963]. *J. opt. Soc. Amer.*, **53**, 66). Thus, we feel that these changes in sensitivity could not be explained by scattered light from the periphery falling on a central sensitive point, but represent to a large extent an actual change in density and distribution of the receptors connected with the ganglion cell. Here, we felt we could be certain that we had placed the stimuli and the adapting field over active responding retina.

Our latest experiments confirm the thesis that light adaptation is largely neuronal and that no matter where we place the patterns of light and dark within the receptive field, the sensitivity of all parts of the field appears to change in a similar fashion. That is, the action of light on any part of the field depresses the sensitivity of all parts of the field by approximately the same amount. It should be pointed out that our experiments have been on a cone system and that it is possible that rods may act somewhat differently. In any case, we intend to do some more experiments using both rods and cones to see if the relationship is still valid.

Rushton: First I would like to say what a very neat method this is, to take the difference in distribution of the two fields to control the question of the scattered light. The result is precisely what was to be anticipated—this is what I suggested the original experiment for—and the idea is simply that the increment threshold does not occur in the rods at all, but occurs somewhere down the line.

We know that many rods converge upon one optic nerve fibre; the change in increment threshold occurs somewhere at this convergence. With a luminous field, a great flux of impulses converges but its size is moderated through the automatic gain control box which I suppose is near the site of convergence; the gain is changed for all paths coming into the box, including those from receptors not exposed to the field, provided that they lie in the same receptive field. This description applies to increment thresholds, but as you say, it has also to explain dark adaptation which I don't think you were studying: yours was an increment threshold.

Wolbarsht: Our difficulty was that these were very probably cones, and the minute we turned the light on the threshold went up again.

Rushton: You don't have your preparation in a cup, as Dr. Naka does, so you can't expect to get good results! For the dark adaptation we have to assume something is coming down from the rods that is proportional to the bleaching, and that is what this term β in my model is doing; that is the thing that modifies the gain of the box so long as regeneration is not complete.

A NEW VISUAL PIGMENT AT 690 nm. IN THE TENCH

Crescitelli: Dr. Marks, is your instrument capable of detecting a pigment at 690 nm.? Have you found one?

Marks: The instrument is adequate but we found no pigment. However, the test that we ran to find an infrared receptor wasn't extensive enough. We examined only 25 cones and found no infrared receptors. We have been up to 730 nm.

Wolbarsht: Have you found these results in goldfish, Dr. Rushton?

Rushton: Goldfish and tench are both cyprinids. No, we have not examined goldfish.

Land: One point about chlorophyll reversal is that if a human being were sensitive at 690 nm., leaves would be light and dark at the same time, because that is where chlorophyll reflects heavily, and it may be that in this fish we have an example of what we were discussing earlier, the alternative meaning of adaptation. Where there are a lot of algae, and you are starting to lose light, the fish might make up for it by having sensitivity at that wavelength.

Wolbarsht: Dr. Rushton, have you examined your neutral density filters to make sure they are not becoming transparent at 690 nm.? The ordinary neutral density filter does become transparent at 690 nm., enough to account for the shift in the curves. We had a lot of trouble with this ourselves.

Naka: We tested this with the photometer and found that it was not so bad. There was *some* change in neutral density, but it was less than 0.1 log unit.

Wolbarsht: I would never say that one does not find a pigment: I have found a number myself. None of them agreed, by the way, with anything that Dr. Marks has found in the same retina. One question I have is: is this a rhodopsin-type pigment? I found it impossible to tell from your curves.

Rushton: It is narrower. Dr. Dartnall, when the porphyropsin wavelength is well out in the red, do you expect it to look narrower or broader on a wavelength plot?

Dartnall: Broader.

Rushton: So I feared. If then it looks narrower, it really is narrower.

Lowenstein: In your experiments, Dr. Naka, you have the visual elements in contact with the pigment cells. Could what you observe be due to a pigment other than a visual pigment? Could the screening pigment come in?

Naka: We considered this possibility very recently and we made the same preparation as Dr. Svaetichin usually does—that is, we isolated the retinal layer and removed the pigment epithelium—and we obtained the same result.

Dartnall: I have made digitonin extracts from tench retinae but have not succeeded in finding the 690 nm. pigment Dr. Rushton describes. I have not even found one at 620 nm., which according to Wald's experiments on the synthesis of cyanopsin is where one might expect the photopic pigment of a retinene₂ system to be. In fairness, however, I should state that my extraction procedures were carried out in deep red light—which is the best one can do, saving an infrared image convertor—and this light could very well bleach such long-wave pigments as we are talking about. I would like to ask Dr. Rushton if he has any evidence for a cyanopsin-like pigment, that is, one maximal at 620 nm., and whether his 690 nm. curve could possibly be a manifestation of such a pigment. He mentioned that his pigment has a narrow absorption curve, and this was rather a red signal to me, for I have come to think that there are no narrow-band pigments. When they seem to exist I believe this can be because a normal broad-band visual pigment has bleached, not to the retinene stage but to some intermediate condition, such as the meta-pigment. Then, because of overlapping between the two spectra, one gets a narrow difference spectrum (Dartnall, H. J. A. [1960]. *Nature [Lond.]*, **188**, 475; but see also Dartnall, H. J. A. [1956]. *J. Physiol. [Lond.]*, **134**, 327). Thus in 1962 (Dartnall, H. J. A. [1962]. *In The Eye*, vol. 2, ed. Davson, H. London: Academic Press) I ventured to interpret Dr. Rushton's narrow-banded erythrolabe, with λ_{\max} at 590 nm., as an iodopsin-like pigment ($\lambda_{\max} = 560$ nm.) bleaching to the meta-pigment stage ($\lambda_{\max} = 480$ nm.), and subsequent events, namely, the recent discoveries of a 560 nm. pigment in the human retina, have, perhaps, lent some substance to this prediction. Similarly, in the case of the narrow 690 nm. curve, could this be due to cyanopsin bleaching to meta-cyanopsin?

Rushton: This is an action spectrum, not a difference spectrum.

Dartnall: I know. But if these intermediate photoproducts, not normally encountered in solution, take part in the visual process in an antagonistic sense *in vivo*, you could still get an action spectrum that resembled the type of narrow-band difference spectrum we are talking about (see references quoted above).

Weale: There is a finding to support what Dr. Dartnall has said (although it is a phenomenon of very low temperatures); I refer to the new pigment pre-lumirhodopsin which has exhibited a maximum at 540 nm. in pure rhodopsin eyes (by pure rhodopsin I mean one which has a maximum at 500 nm.). If there is a cyanopsin peak at 620 nm., I don't think it would be stretching the wavelength scale unduly if a pre-lumirhodopsin based on cyanopsin were to be found in the neighbourhood of 690 nm.

Rushton: From other cells Dr. Naka has obtained evidence of pigments with maxima at about 620 nm. and in the blue region, and thus we confirm the three pigments that Dr. Marks has measured and are a little embarrassed to have to add so unexpected a fourth.

Svaetichin: Dr. Rushton, your experimental data and reasoning are very convincing indeed in support of a pigment at 690 nm. If I did not have at my disposal our cone pigment absorption data, I believe I would be ready to accept your interpretation. In our Fig. 10 (p. 197) the spectral response curve D displays a depolarizing, upwards, maximum far out in the long wavelengths and close to your 690 nm. pigment. Exposing the retina to a very strong short-wave spectral light selectively abolished the hyperpolarizing, downward, responses of the spectral response curve E, which was recorded after the strong bleaching. The point of interest is that the spectral maximum of the depolarizing response was now shifted to about 590 nm., which agrees with one of the three cone pigments of this Centropomidae fish retina, the pigment maxima being at 470, 542 and 590 nm. (Fig. 4, p. 189). For this reason I hesitate to accept the 690 nm. pigment proposed by you and Dr. Naka.

Marks: I feel like Dr. Dartnall about pigments that don't fit the nomogram; especially when they are narrower, I suspect the presence of some sort of filter. Perhaps the filter is the pigment epithelium itself. I mentioned the experiments in which we searched for infrared receptors with a very long-wave red light. I should add to these the experiments in which we searched for such cones with blue light, since that presumably wouldn't bleach away the pigment that you have found. That brings the number of cones examined up to 50 or 60, and that number together with the principle that all visual pigments are nomogram pigments, which has yet to be disproved with good data, makes me doubtful about your finding.

Lowenstein: Dr. Rushton, the point has been reached where one might either take a vote, or ask you whether you have been convinced by the negative observations made on the findings on the 690 nm. pigment!

Rushton: All the things that have been said against it are very reasonable, and Dr. Naka and I have been influenced by that. This is a surprising thing that falls out of pattern in just the manner that has occurred more than once before in electrophysiology of the retina. Many spectral sensitivity curves that have been derived from electrophysiological criteria have revealed *narrow* sensitivity curves; for example, Granit's modulators fall in that category, and nobody now believes that those are narrow-banded pigments; everybody believes that those are inhibitory interactions, just as Dr. Lennox-Buchthal has found for the spectral sensitivity of cells in the cortex. But such a misinterpretation of results does not seem applicable to our case. If you believe our observations, there is no chance, as far as I can see, of this kind of inhibitory interaction. We were dealing with a potential that *saturated* and became quite unresponsive to one colour while remaining sensitive to another. This allows the two to be separated analytically. And, if I may attack for a moment, how do you know, Dr. Marks, that in every one of your cones there is not, in addition to the pigment you have

found, an infrared-absorbing pigment, which you have always bleached away in making the preparation?

Marks: For the reason I have just said. The first light that strikes some of the cones is blue light, when we dissect in blue light and scan from the blue. Then when the wavelength reaches 620 nm, the absorption reaches a maximum, and at longer wavelengths the absorption falls again. If this pigment is like the 620 pigment, it wouldn't have been bleached away by then. So this merely reinforces my statement, and yours, that this is not an ordinary pigment, but is much more photosensitive and is bleached away in much stranger conditions.

Rushton: And might be the sort of thing Dr. Weale was suggesting?

Marks: Yes, possibly.

Land: No one has yet suggested what the structure of this pigment might be; just as some of the photographic sensitizing dyes are very unstable, this pigment might have corresponding lability and be able to exist only under your special conditions. But I am still concerned that if this pigment were found to exist in man one would need a whole new receptor system to handle it, because chlorophyll would look brilliantly light instead of dark.

Pirenne: In the sensitivity to long wavelengths there is a barrier at about 1μ , due to thermal radiation inside the human eye. This background of purely thermal radiation in the human eye shoots up tremendously just after 1μ , so that there should be a thermodynamic limit to the sensitivity of any visual system to long-wave radiation. It would be very interesting to know how close animals do come to this limit. Some exclusively diurnal animals might be more sensitive to the red than we are, if their absolute threshold is much higher than ours.

Weale: Then fish having a lower body temperature than man would conceivably benefit by using longer wavelengths?

Pirenne: Yes; one might find that arctic fishes are more sensitive to red light.

ANIMAL COLOUR VISION

THE PHYSIOLOGICAL BASIS OF COLOUR VISION IN HONEYBEES

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SINCE the investigations of von Frisch in 1914 it has been an acknowledged fact that bees can distinguish colours. Von Frisch and among his students especially Daumer (1956) showed this ability to be exceptionally fine. True, the bees' ability does not extend as far into the long-wave end of the spectrum as does man's. Red light beyond 650 nm. is simply invisible to them. On the other hand they can see ultraviolet light of 300 nm., and very likely all the way down to 250 nm.; whereas normal human vision ceases at wavelengths shorter than 380 nm. Just as with man, the bee's colour wheel is closed. If one mixes the colours which lie at both ends of the spectrum, one obtains a series of shades which move continuously from red into violet, the purple tones, which are themselves not contained within the spectrum. Similarly if one mixes the colours which lie at opposite ends of the bee's visible spectrum (yellow-orange and ultraviolet) one obtains colour qualities which bees do not confuse with any other colour of the spectrum and which in this respect correspond to the purple tones in human colour vision. One can produce all colours visible to bees by carefully combining three correctly chosen colours; for example, spectral yellow (588 nm.), blue-violet (440 nm.) and ultraviolet (360 nm.). Moreover, one can represent these three colours as the vertices of a colour triangle and show the proportions

in which every other colour is a mixture of these three. One can also understand the laws of colour vision in bees if one assumes three types of visual cells with different spectral sensitivities. The qualitative reactions of bees in his experiments led Daumer (1956) to postulate a yellow, a blue (or blue-violet) and an ultraviolet receptor.

As with all insects, the eyes of bees are composed of individual ommatidia, which in turn consist of visual cells surrounded by pigment cells. The visual cells are very long and slender, 250 by about 7μ . They adjoin at the centre as a rule, arranged somewhat like eight sections of an orange around a common axis. Towards the axis they form the rhabdome, a peculiar structure of four rather than eight sectors. Each pair of visual cells meets in a common structure which consists of microvilli, tiny tubules visible only under the electron microscope and running the entire length of the cell parallel to the axis (Goldsmith, 1962). Distal to the visual cells lies the dioptric apparatus, a crystal cone and a lens formed by the cornea. The proximal ends of the visual cells continue as nerve fibres connecting with the next ganglion cells.

In order to measure the spectral sensitivity of individual visual cells, we inserted glass capillary microelectrodes into the eyes. The diameter of the electrode tips was around 0.1μ . Monochromatic light was produced by interference filters, which spectroscopic measurements showed had only one maximum between 200 and 2,500 nm. (Autrum and von Zwehl, 1962, 1963, 1964).

By rotating the circular plate on which they were mounted, the filters could be brought one by one to intersect the beam of light. A xenon high-pressure lamp served as source. Its light was first cast on a semi-transparent cold light mirror in order to prevent heating of the lenses, interference filters and neutral grey filters. Heat radiation and a small part of the red end of the spectrum passed through the mirror; whereas the remainder of the visible spectrum and ultraviolet were reflected for use. Neutral grey filters of coated quartz glass (Balzer and Company, Liechtenstein) were employed

to equalize the intensity (quantum count) of the monochromatic lights within ± 2 per cent accuracy. A thermocouple connected with an amplifier and galvanometer (Kipp and Zonen) was used for calibration purposes. All the lenses in the optical system were made of quartz. A frosted Perspex disk 1 mm. thick was placed about 3 mm. in front of the eye of the bee. Perspex is transparent to the long-wave ultraviolet light used in these experiments. The absorption of the Perspex was included in the calibration.

The animals were fastened to a block with collophonium so that their heads could not move. With the head at the centre of a Cardan suspension system the faceted eye could be turned into the light beam in such a way that the axis of the ommatidium under investigation directly faced the oncoming light and yielded maximum illumination potentials. In one series of experiments the temperature of the surroundings was held at 30° C (measured by thermistor; cf. Thurm, 1963). Measurements at 20° C yielded no differences of significance for the results considered here.

The stimuli normally lasted 310 msec. In a few experiments stimuli of 50 or 100 msec. were separated by long pauses. The duration of the stimuli and the pauses between them (varied between 0.6 seconds and several minutes) had no influence on the spectral efficiency.

The preparation was subjected to constant control stimuli to guarantee that the sensitivity did not change during a series of measurements. Each efficiency curve was obtained by recording the responses from 318 nm. to 660 nm. and back at least once, or in the reverse order beginning from 660 nm. In many cases the sensitivity of the impaled cell remained constant for so long that recordings through the entire spectrum could be repeated several times in different directions. Wherever it was possible the dependence of the receptor potential height upon the stimulus intensity was also determined.

Receptor potentials were displayed on an oscillograph screen and recorded photographically.

The microelectrodes were inserted through a section of the eye from which the cornea had been removed. The exposed area was covered with vaseline. It was certain that recordings were made exclusively from intact ommatidia because of the great distance separating them from the opening in the cornea.

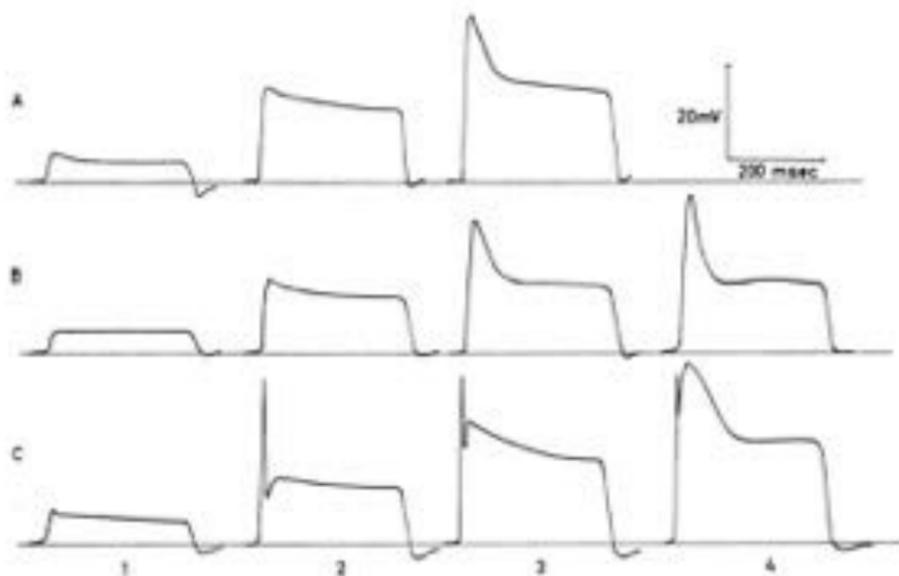


FIG. 1. Receptor potentials from single visual cells of worker bees and drones. Relative intensity of stimulus:

A (worker bee): (1) $J = 0.1\%$; (2) $J = 5.5\%$; (3) $J = 100\%$.

B (drone): (1) $J = 0.2\%$; (2) $J = 4\%$; (3) $J = 50\%$; (4) $J = 100\%$.

C (drone; an impulse appears in the rising phase of the receptor potential): (1) $J = 1.4\%$; (2) $J = 6.5\%$; (3) $J = 22\%$; (4) $J = 100\%$.

As soon as the electrode tip enters a visual cell, the resting potential appears. It lies between 45 and 80 millivolts.

The receptor potentials of the bees were always monophasic and depolarizing (Fig. 1). Within the first 20 msec. they reached a maximum which dropped off to a plateau. All measurements were based on the maximum depolarization.

Just after the onset of the receptor potentials in drones there often appeared a single or rarely 2 or 3 spikes just before the maximum

was reached (Fig. 1C). The spike disappeared when the stimulus intensity was lowered and only monophasic depolarization was observable. We made no investigations as to the source of the spikes described by Naka and Eguchi (1962).

Equal quanta stimuli evoke responses which are a function of the wavelength. The corresponding curve, the spectral efficiency

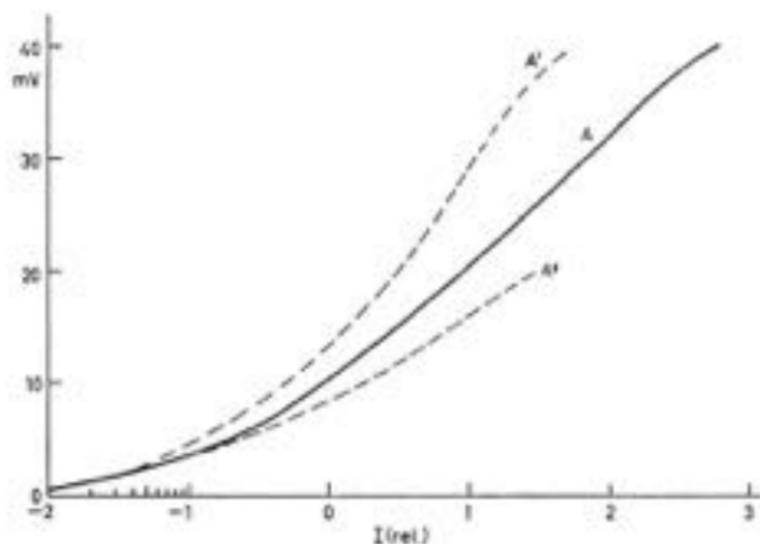


FIG. 2. Height of the receptor potential (in mV) in relation to the stimulus intensity (in relative log units). A: average values of all cells measured; A' and A*, the extreme curves of single cells.

curve, is however, not as significant physiologically as the spectral sensitivity curve. For whereas the efficiency curve is based on the constant physical magnitude of the stimulus, the sensitivity curve is based on the constant physiological magnitude of the response.

Sensitivity curves can be calculated from efficiency curves if the relationship between depolarization and stimulus intensity is also known. This relationship was determined on a large number of individual sensory cells (Fig. 2) and was found to be independent of their spectral characteristics.

The first recordings were made on drones. Their visual cells of

$9\ \mu$ in diameter are somewhat larger than those of worker bees, and it is much easier to insert a microelectrode into them.

In the dorsal area of drone eyes visual cells were found with a maximum sensitivity at 340 nm, together with others with a maximum at 450 nm.

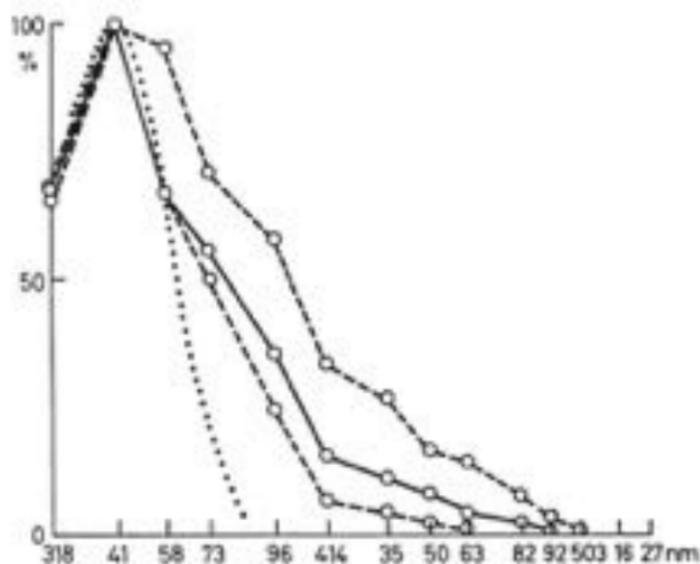


Fig. 3. Sensitivity curves from drone visual cells with λ_{\max} of 340 nm.

Sensitivity curves: —, average of 15 cells; - - -, the narrowest and broadest curves of different single cells. Dartnall's nomogram curve for λ_{\max} of 340 nm,

Figure 3 shows the average values from recordings on fifteen visual cells of drones, with a maximum sensitivity at 340 nm. In addition the two extreme curves, the narrowest and the broadest, are also shown. In each case the sensitivity falls off in the violet range to a very small percentage of the maximum.

The maximum sensitivity of the second type of visual cell found in drones lay at 450 nm. Fig. 4 shows average values from measurements on sixteen cells. The two extreme sensitivity curves, the narrowest and the widest, diverge but very slightly.

By way of comparison, the absorption curve for retinal pigment with an absorption maximum at 450 nm. is also given (Fig. 4). This curve was obtained from the nomogram of Dartnall (1953) for the corresponding retinal pigment. The agreement between the sensitivity curve and the absorption curve (for the retinal pigment) is astonishingly good.

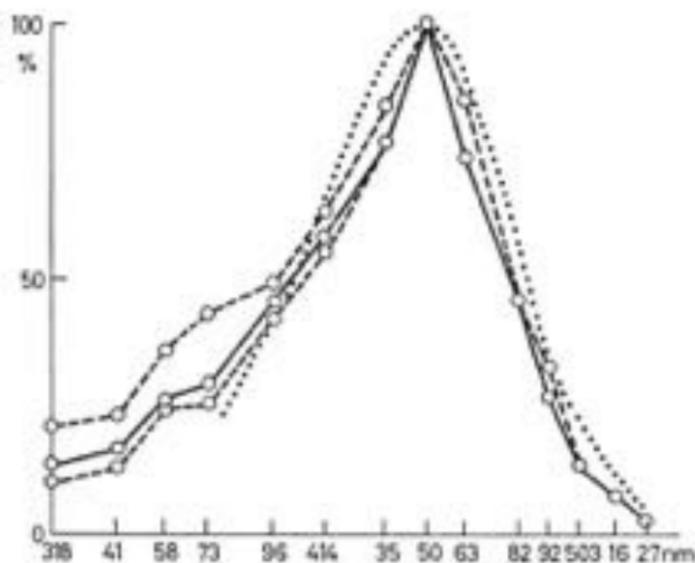


FIG. 4. Sensitivity curves from drone visual cells with λ_{\max} of 450 nm.

Sensitivity curves: —, average of 16 cells; ----, narrowest and broadest curves of different single cells. Dartnall's nomogram curve for λ_{\max} of 450 nm,

In the extreme ventral area of the drone eye—and so far only there—visual cells have been found with a maximum sensitivity at 530 nm. (Fig. 5).

Figure 5 shows the corresponding efficiency curve, the calculated sensitivity curve, and the curve resulting from Dartnall's nomogram for retinal pigment with an absorption maximum at 530 nm. Here too the correlation between the nomogram curve and the sensitivity curve is high.

It is harder to insert microelectrodes successfully into the visual cells of worker bees.

Cells with a maximum sensitivity at 530 nm. can be found throughout the entire compound eye of the female workers. Fig. 6 gives the sensitivity curve plotted from the average values of measurements made on 28 cells. The nomogram curve for a retinal pigment with the same absorption maximum is also given for comparison.

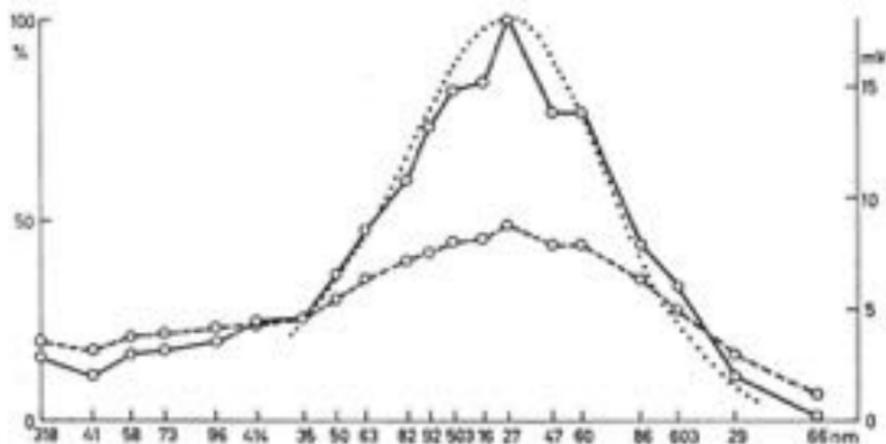


FIG. 5. Efficiency and sensitivity curves for drone visual cells with λ_{max} of 530 nm.

Efficiency curve (mV on right-hand side), - - - - -; sensitivity curve (relative values on left-hand side), —, Darmall's nomogram curve for λ_{max} of 530 nm.,

The visual cells of both drones and workers which we have investigated show the same spectral characteristics. The sensitivity of the 530 nm. receptor extends into the long-wave side of the spectrum to about 650 nm., that is, into the orange. In that region of the spectrum which our eyes see as red, the receptors of the bees' eyes fail to react. On the short-wave side the sensitivity extends into the ultraviolet. However, there is no second maximum as in the case of rhodopsin.

We did not find a receptor in the workers with a maximum at 450 nm. as we did in drones. Instead we found two significantly

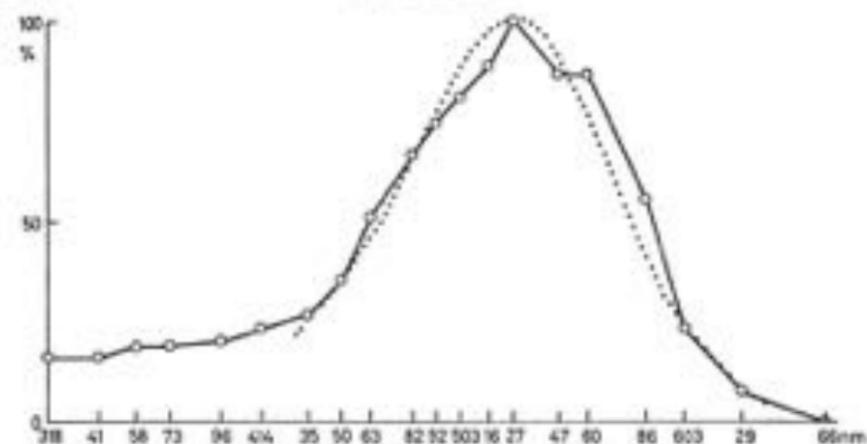


FIG. 6. Sensitivity curve for worker bee visual cells with λ_{max} of 530 nm. Sensitivity curve, average of 28 cells, —, Dartnall's nomogram curve,

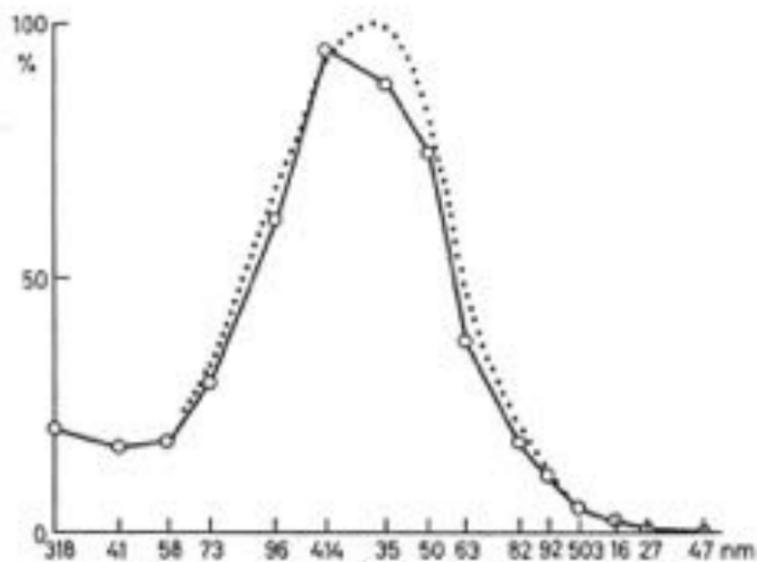


FIG. 7. Sensitivity curve for worker bee visual cells with λ_{max} of 430 nm.

Sensitivity curve, average of 3 cells, —, Dartnall's nomogram curve,

different receptors with maxima at 460 and 430 nm. The 460 maximum appeared only in two cells. Figs. 7 and 8 show the sensitivity curves for these two receptors and the corresponding nomograms.

Sensitivity curves with a maximum in the ultraviolet at 340 nm. were also found in worker bees (Fig. 9), but these curves always had a more or less distinct second maximum at 530 nm. At times the 530 nm. maximum was even higher than the ultraviolet maximum.

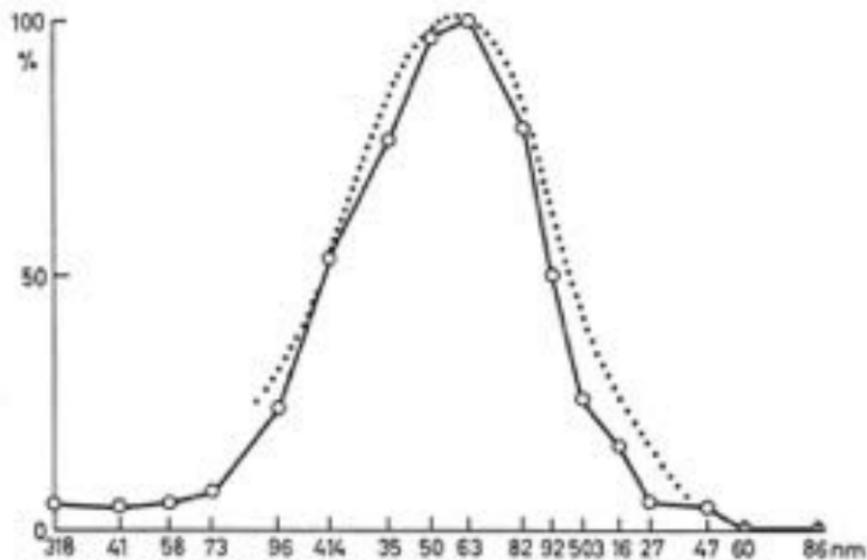


FIG. 8. Sensitivity curve for worker bee visual cells with λ_{max} of 460 nm.
Sensitivity curve of one cell, —, Dartnall's nomogram curve,

One might assume that the curves with the double maximum represent the summation of the receptor potentials of two visual cells. This assumption is given strong support by the fact that the relative height of the two maxima was subject to great variation and was not reproducible in different cells. The ratio of the 530 nm. potential to the 340 nm. potential varied between 0.65 and 1.8. The exact opposite to these curves is the regular pattern and slight variation in curves of only one maximum.

In a few cases both in drones as well as in workers, curves were obtained with other double maxima—for example, at 350 and 450 nm. These curves also failed to show a reproducible connexion between the maxima. The relative heights of the maxima were different in every case.

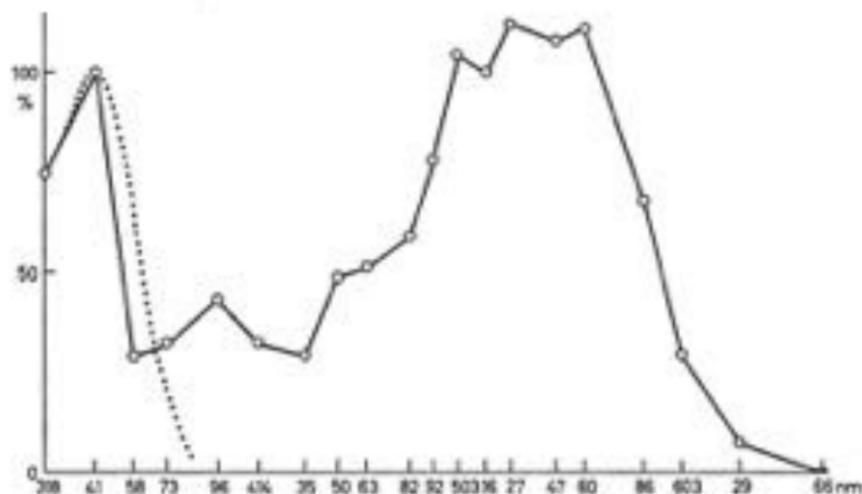


FIG. 9. Sensitivity curve of worker bee visual cell.

Sensitivity curve of one cell, —, Dartnall's nomogram curve for λ_{\max} of 340 nm,

One could consider two hypotheses to explain the two maxima in these sensitivity curves:

- (1) Several visual pigments occur in a single visual cell.
- (2) The microelectrode picks up potentials from two neighbouring visual cells.

The great variability militates against the first assumption. It is much more likely—and also for anatomical reasons—that the potentials of two neighbouring cells are added together at the electrode. According to the electron micrographs of Goldsmith (1962), the visual cells of the bee are contiguous. Their membranes are separated by a third membrane which is only 50 to 100 Å thick. It could happen that the tip of the advancing microelectrode pushes against the wall of cell A (Fig. 10), causing it to bulge into cell B, and

at that point pierces the two walls together. In such a case the depolarization in one cell would spread electrotonically to the other at the point of penetration and the potentials of both cells would be effective at the electrode tip. The same explanation would account for the variation in the composite curves. The extent to which the potentials of one cell would spread to the next would depend on the final position of the tip of the electrode and on the electrical impedance through the wound.

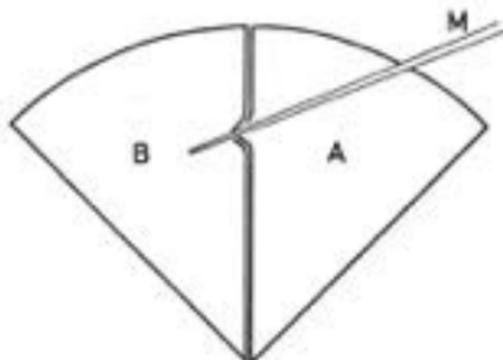


FIG. 10. Microelectrode (M) piercing two visual cells A and B (see text).

This assumption allows one to interpret the curves with two maxima as the sum of two sensitivity curves, the components of which can be separated by subtracting known curves with a single maximum from the composite curve. Thus the sensitivity curve in Fig. 9 arises through summation of one cell with a maximum at 530 nm. and another cell with a maximum at 340 nm. The right side of the curve between 435 and 650 nm. corresponds quite exactly to the sensitivity curve with a maximum at 530. The maximum is flat and the differences between 530 and 560 lie within the limits of error in measurement. Added to the 530 nm. receptor is an ultraviolet receptor with a maximum at 340 nm. The maximum at 396 nm. comes from adding the potentials of the two cells together.

Von Frisch (1914), Kühn and Pohl (1921), Kühn (1927), and Daumer (1956) studied the ability of female worker bees to distinguish spectral colours by analysing their behaviour. Their visible spectrum extended from 300 nm. (or below) to about 650 nm. Compared with human vision, the long-wave end of the spectrum is shortened whereas the short-wave end is considerably lengthened. Thus there is complete agreement between the results of our investigation of single visual receptors and the behaviour experiments. The receptor with the maximum at 530 nm. has no significant sensitivity beyond 650 nm. On the other hand, bees have a special receptor for ultraviolet with a maximum sensitivity at 340 nm. which is still at 70 per cent of maximum at 318 nm. A visual pigment with these properties has yet to be identified. The ultraviolet sensitivity of the human eye results from a secondary maximum of rhodopsin, which also lies at 340 nm.

Bees have a hard time distinguishing colours in the "yellow region" between 650 nm. and about 530 nm. Yellow and orange look very much alike to them, if not fully the same (Daumer, 1956). This conclusion corresponds very closely to the sensitivity curves (Fig. 11). Down to about 610 nm. only one receptor responds. Then a second receptor starts to come in weakly. Between 530 and 510 nm. bees begin to perceive a change in colour and one notices a similarity to the neighbouring region of shorter wavelength. According to Kühn (1927) and Daumer (1956) the "yellow region" connects with a transitional region which extends from 520 or 530 nm. to about 480 nm. This is the "blue-green region" in which bees can distinguish very slight changes in wavelength. Now, the ability to distinguish spectral colours is going to be best developed where the slope of sensitivity in different receptors is of opposite sign. In the "blue-green region" at 530 nm. the sensitivity of the green receptor begins to fall off. Simultaneously the sensitivity of the 460 and also the 430 nm. receptor starts to rise. One is again confronted with the remarkable agreement between the course of the sensitivity curve and the results of behaviour experiments.

In the adjoining "blue region" from about 480 nm. to around 410 nm., behaviour experiments show some ability to distinguish colours, though not so great as in the transition area between 530 and 480 nm. The characteristics of the visual cells indicate that the ability to distinguish colours should grow considerably smaller at the short-wave end of the "blue region", for the active receptors here drop off together almost in parallel.

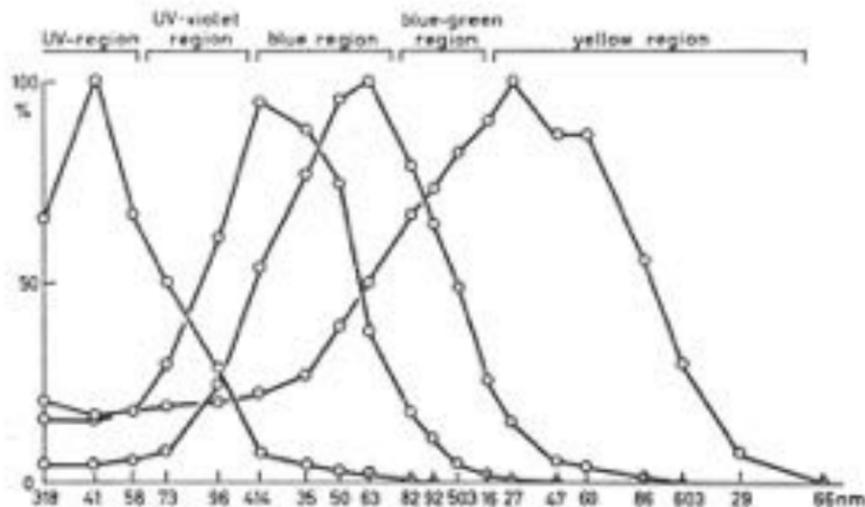


FIG. 11. Sensitivity curves of the receptor cells of the worker bee eye with maximal responses at 340, 430, 460 and 530 nm. Regions of colour differentiation after the training experiments of Kùhn (1917).

Behaviour experiments show a second transition area between 410 and 360 nm., where slight differences in colour are discernible. Here the sensitivity of the 340 nm. receptor begins and rises sharply. Daumer (1956) found that the addition of only a small percentage of ultraviolet to spectral blue-violet (440 nm.) was enough to make the resulting mixed colour look different from blue-violet to bees.

Accordingly, the results of the behaviour experiments agree exceptionally well with the spectral characteristics of the individual visual cells which give rise to colour perception. The behaviour of

bees can therefore be correlated with the characteristics of individual visual cells and can even be deduced from them. The data on the bee's ability to distinguish colour is, however, not sufficiently extensive to permit an exact comparison.

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[Professor Autrum was unfortunately prevented by the illness of Mrs. Autrum from attending the symposium, and his paper was not received in time for it to be read and discussed by the other participants.]

THE SPECTRAL SENSITIVITY AND VISUAL PIGMENT CONTENT OF THE RETINA OF *GEKKO GEKKO*

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THE visual cells of nocturnal geckos, though usually referred to as rods, have a number of morphological features which suggest some relationship to cones. The transmutation theory of Walls (1934) attempts to rationalize these morphological indications of intermediacy. Gecko visual cells are not all of one type, however, since morphological examinations (Underwood, 1951; Tansley, 1964) indicate the presence of single cells, two types of double cells and occasionally triple cells. Moreover, more than one photopigment has been detected in retinal extracts of several species of geckos (Crescitelli, 1963a). Morphologically and biochemically, the gecko retina is not a homogeneous structure and several types of visual functions may be associated with such a retina. Perhaps colour vision is present, either fully developed or as a residuum of some phylogenetic character. My research in this field aims to define more specifically the types of receptor elements of the gecko retina and then to discover the biological significance and functional rôle of these. I have undertaken such a study with the retina of *Gekko gekko*, a large nocturnal lizard which has many advantages for a study of this type. It is the current status of this programme which I am presenting here.

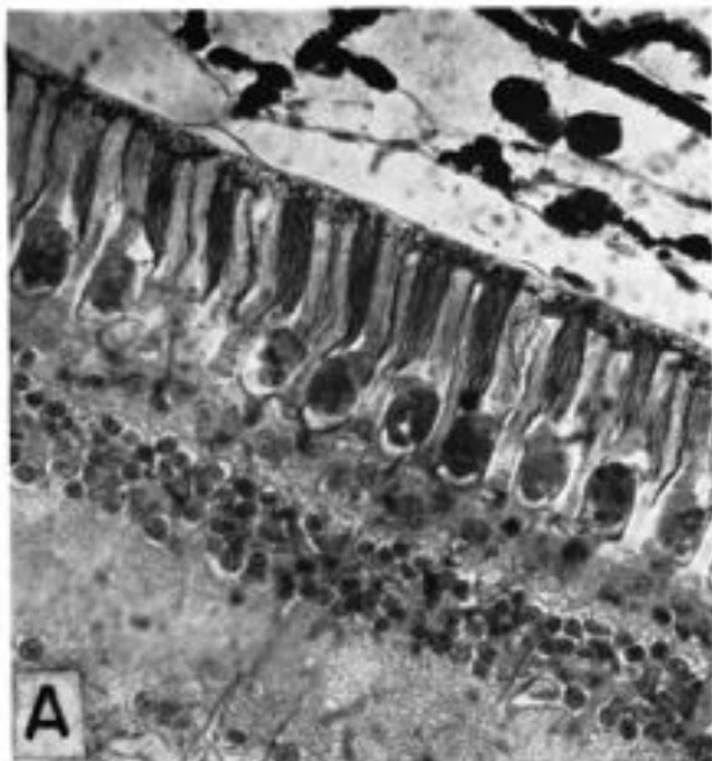
THE RETINA OF *Gekko gekko*

Gekko gekko has a typical gecko retina (Fig. 1) with the visual cells beautifully organized in rows which run at right angles to the

vertical pupil. Two types of double cells, types B and C of Underwood (1951), alternate with each other in these rows. One row consists almost exclusively of type B cells, in which the accessory member has a large ellipsoid, with prominent mitochondria, and a very large paraboloid. In such a row the chief and accessory members lie alternately above and below each other (Fig. 1). The row which alternates with the type B cells consists not only of type C doubles but also of type A singles and of occasional triple cells, either in linear or triangular array (Fig. 1). The outer segments are large and cylindrical and no oil droplets can be seen in any of the cells. Though the visual cell composition is not identical in all regions of the retina, there is no obvious fovea, but there is a typical gecko *conus papillaris* (Fig. 1).

Two photopigments have been extracted from the retina of this gecko (Crescitelli, 1963*b*). One of these absorbs maximally close to 521 nm. while the second pigment, less precisely located at 478 nm., is present in extracts as about 8 per cent of the total photopigment concentration. Both pigments have been shown to be retinal₁ (retinene₁) chromoproteins and the evidence is good that both, rather than being products of *in vitro* reactions, are present in the retina. No evidence has been found for the presence of a classical rhodopsin, so widespread in the rods of terrestrial vertebrates. It is expected, therefore, that the spectral sensitivity of this animal should not be in the usual spectral location for rhodopsin systems. This expectation has been realized, for Denton (1956) discovered the sensitivity curve, based on the pupil response to light, to be located, not in the region of 500 nm., the usual location for rhodopsin systems, but some 20 nm. further toward the red. There are two aspects of this comparison of spectral sensitivity with visual pigment data which require further clarification. One is the rôle in spectral sensitivity of the second pigment (at 478 nm.), whose existence was not known at the time of Denton's work. Can the spectral sensitivity curve be shown to include this pigment system as a participant? The second point is concerned with the possibility

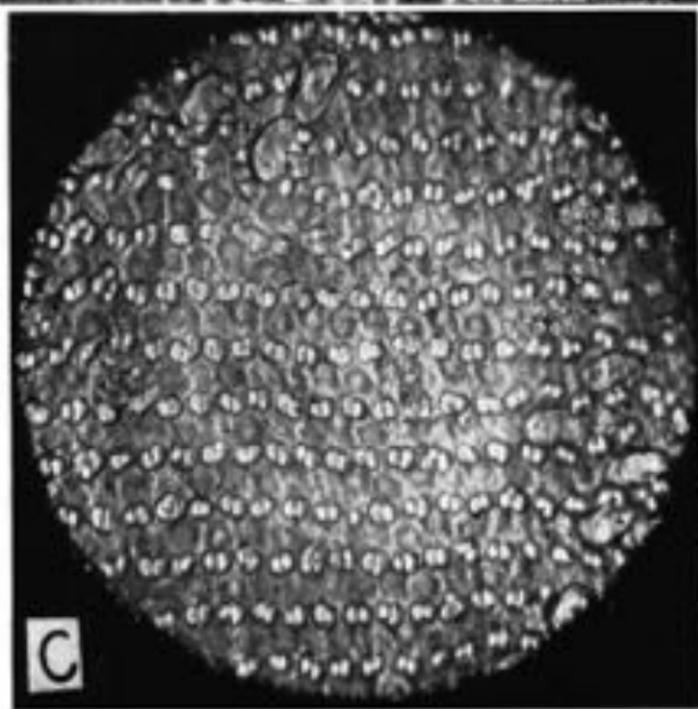
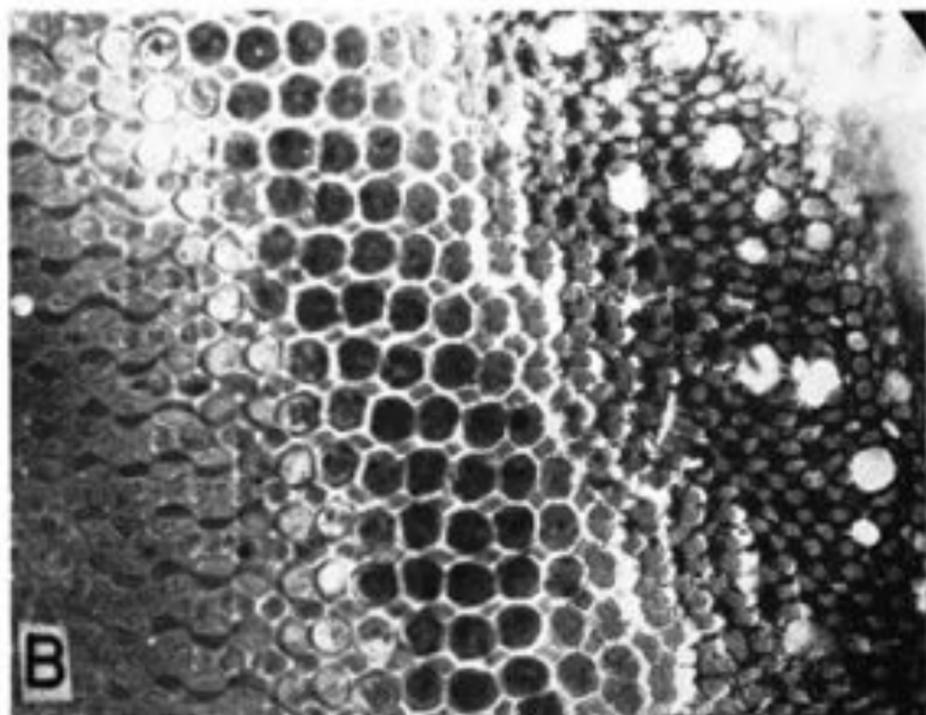
FIG. 1. The retina of *Gobio gobio*.



1a. Section showing visual cells. Note cells with large, heavily stained ellipsoids containing mitochondria. These are the accessory cells of the type B doubles. Alternating with these are some type A singles and type C doubles. Kolmer's fixative, Harris haematoxylin and Mallory Azan, sections cut at $8\ \mu$.

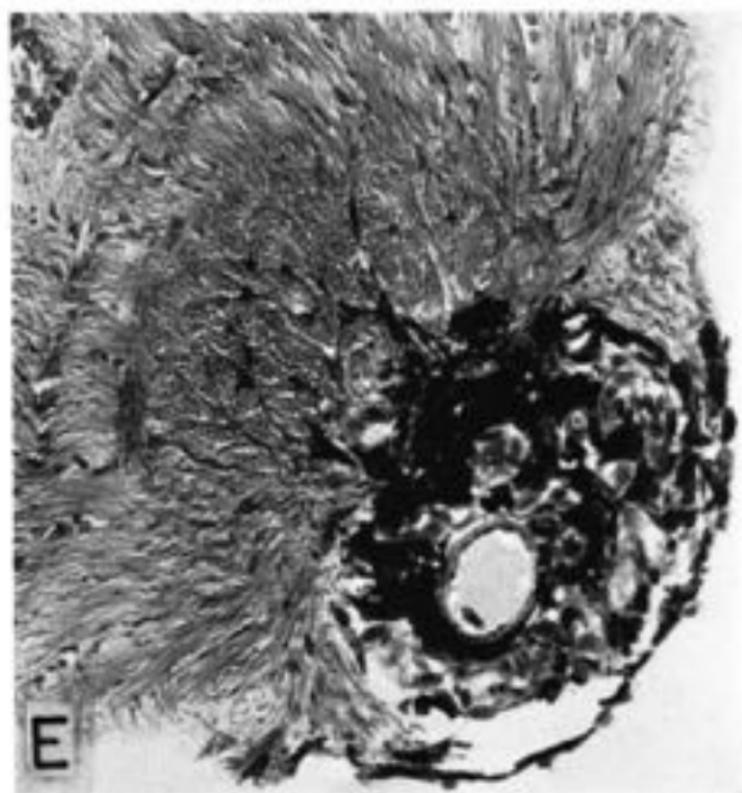
1b. Tangential section of fixed stained retina showing (from right to left) outer segments embedded in pigment epithelium, ellipsoids, and parabolooids. Note that at the level of the parabolooids one sees the alternation, in position in each row, of chief and accessory cells of type B doubles. Note also the alternation of rows of type B doubles with rows containing type A singles and type C doubles.

1c. Photomicrograph of fresh, isolated retina placed on slide with outer segments up. Note precise alignment of double cells in rows with a few triple cells. These double cells are probably type C doubles, the type B outer segments having been pulled off, but their large ellipsoids may be seen out of focus in between the rows of type C outer segments.





1d. Tangential section of a fixed eye to show optic disc with comus papillaris near centre of section.



1e. The comus papillaris enlarged to show blood vessels with blood.

of the occurrence in the gecko retina of a third photopigment system in the orange-red region of the spectrum. At present, there is no biochemical evidence for the existence of such a system but in two species of geckos (*Tarentola mauritanica* and *Hemidactylus turcicus*) the spectral sensitivity curves, in comparison with the density spectra for the photopigments, are shifted toward longer wavelengths and have elevated sensitivities in the range from 530 nm. to 620 nm. (Crescitelli, 1963a). Denton's results showed no such discrepancy, but his method of substituting test flashes in place of an orange-red adapting light is possibly not favourable for a demonstration of such red-sensitive mechanisms. This paper is specifically concerned with a clarification of both these points in connexion with the spectral response function of *Gekko gekko*.

THE ELECTRORETINOGRAM OF *Gekko gekko*

After some attempts had demonstrated that the pupil reaction to light is too uncertain for precise measurements of retinal sensitivity, the electroretinogram (ERG) was adopted for use as an index of response. Though the origin of the ERG is uncertain, there is little doubt that, properly used, it can serve as an adequate criterion for determining spectral sensitivity. An example of this is the excellent agreement which has been found between the spectral sensitivity curve of the aphakic human eye, determined by means of the ERG, and the density spectrum of cattle rhodopsin, which is spectroscopically similar to human rhodopsin (Dartnall, 1962). The ERG recorded from the unanaesthetized *Gekko gekko* in the dark-adapted state has a characteristic pattern. At stimulus intensities high enough to yield the maximum potential, this pattern consists of a cornea-negative *a*-wave, a rapidly rising positive deflection on which are seen several small perturbations, and a slowly declining, relatively smooth decay of the positivity (Fig. 2). As the stimulus intensity is lowered, the *a*-wave is reduced in magnitude and is then lost, the perturbations are diminished and the positive deflection is

decreased in magnitude and in rate of rise (Fig. 2). Light adaptation, produced by steadily illuminating the eye while testing the responses to brief test flashes, caused a major change in this pattern. The adapting coloured field was produced by use of a grating (Bausch and Lomb) monochromator, while the coloured test stimuli were brief (33 msec.) flashes given by an optical channel which employed a regulated tungsten source, interference filters, neutral filters, and a motor-driven, sectored-disc shutter to deliver flashes. Adaptation with light restricted to either end of the spectrum, that is, 400 nm. and 690 nm., caused the ERG pattern to be altered only slightly, as is shown in Fig. 2 (column 2). This consisted of a sharpening of the positive peak, a more rapid decay of the positivity, and an increase in prominence of the perturbations. The maximum potential which was obtainable under these conditions was about the same as for the dark-adapted eye. Increasing the effectiveness of adaptation by changing the wavelength to 670-650 nm. caused the ERG pattern to be further altered in the same direction (Fig. 2, columns 3 and 4). The rounded positive peak was abolished and a structured pattern with a pointed, prominent component was produced. The maximum potential was still not greatly reduced from that of the dark-adapted eye, which means, since the sub-maximal potentials were greatly reduced from the corresponding values of the dark-adapted eye (Fig. 2), that the rate of increase of potential with increase in stimulus intensity was greater for the light-adapted eye. By further changing the adapting field to the range of 650-600 nm., the structured pattern was retained but the amplitude of the potential was so reduced that it was not possible, with the light intensities available, to elicit maximum responses (Fig. 2, column 5). The surprising feature of these results is the speed with which these changes occurred. Less than 10 minutes was required to complete the transformation of ERG pattern, going in either direction. By use of this technique of adaptation, similar in principle to the partial bleaching method employed in analysis of photopigments in retinal extracts, the excitability of the eye to

DARK ADAPTED LIGHT ADAPTED 690 m μ & 2030 LIGHT ADAPTED 650 m μ & 2030 LIGHT ADAPTED 650 m μ LIGHT ADAPTED 600 m μ

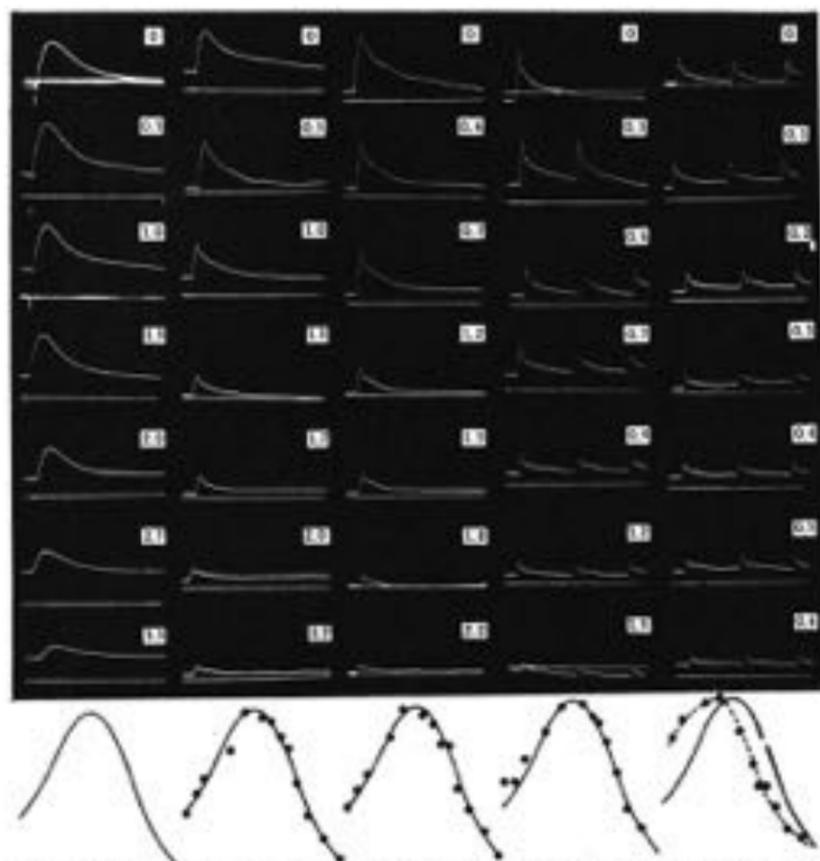


FIG. 2. Electroretinograms recorded from the dark-adapted eye and from the eye in four states of light adaptation, as indicated at tops of columns.

Coloured adapting illumination (690, 650, 600 nm.) (690 nm = 690 m μ) was delivered by use of a grating monochromator. The Corning 2030 filter served to cut out unwanted radiations and to limit the adapting field to the red end of the spectrum. Numbers with each record give the densities of the neutral filters used to vary intensity of test flash. In each case the test flash was a 33 msec. flash of light at 526 nm. The gekko, set up in a plaster of Paris mould of his body, was unanaesthetized but given 2-3 mg. of gallamine triethiodide (Flaxedil), subcutaneously, at the start of the measurements. The pupil of the eye was dilated and immobilized by means of a drop of Flaxedil introduced behind the spectacle through a small opening made in the spectacle. Recording of the potentials was accomplished through use of silver-silver chloride wick electrodes, one touching the cornea through the opening in the spectacle, the other in contact with the cranium, which was exposed by scraping away the skin. The recording system included a 20-second time-constant amplifier and the 502 Tektronix oscilloscope. For stimulating the retina the single ribbon filament of the tungsten lamp was brought to focus at the plane of the cornea, as in Maxwellian view, the visual angle being 35°, large enough to fill the retina with light.

Miniature graphs at bottom illustrate the spectral sensitivity curves obtained from the dark-adapted eye (full line, which is repeated for each column) and from the eye adapted as indicated at tops of the columns. The points added to each graph are the results of the adaptation. Explanation in text.

light was reduced in progressive stages and the spectral sensitivity determined at each of these stages by means of the test flashes. It was hoped thereby that separate spectral mechanisms, if they exist in the retina of *Gekko gekko*, would be revealed.

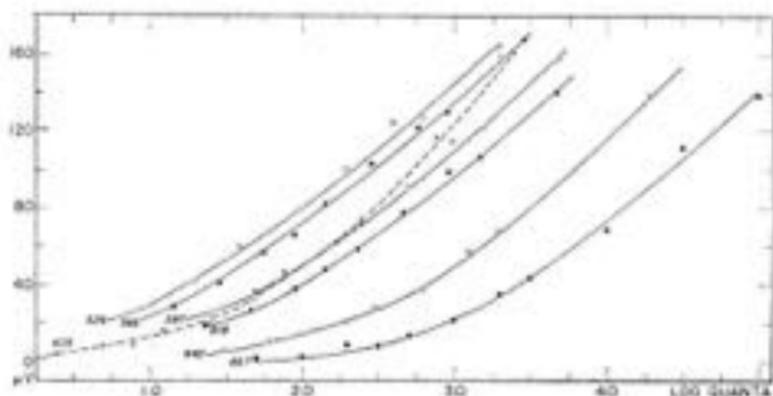


FIG. 3. Amplitudes of ERG (microvolts), from beginning to peak of positive swing, are plotted as functions of the logarithm of the relative number of quanta in the test flashes.

These are selected curves representing the responses at six wavelengths (indicated next to each curve) but they are typical of the results with all 15 wavelengths which were tested in each experiment. The relative number of quanta were determined by first measuring the relative energy, at the position of the eye, with a thermopile-amplifier-recording system, and then converting the energy to relative quanta. The value for the 667 nm. filter was assigned the 100 per cent level and all other test wavelengths were expressed as fractions of this maximum. The test light was shown to be restricted to the visible region of the spectrum and this was accomplished by removing heat radiations through use of a water cell and heat-absorbing filter. Note that the curve for 405 nm. (dashed line) departs considerably from the course followed at other wavelengths.

For the determinations of spectral sensitivity, the height of each electrical response from the start to the peak of the positive deflection was measured, and these values (in microvolts) were plotted for each of the fifteen test wavelengths as functions of the logarithms of the relative number of quanta. The results, illustrated by the data of Fig. 3, are represented by a systematic family of similar curves, the only differences being the distribution of the individual curves along the horizontal axis according to spectral sensitivity.

The only definite exceptions to this statement are the curves for the two shortest wavelengths (405 and 422 nm.), which, at higher levels of stimulus intensity, become steeper than the remaining curves, as if an additional factor were contributing to the responses. This feature of the results, well illustrated by the data of Fig. 3, was obtained consistently. It is a point mentioned by Dodt and Walther (1958a) in connexion with the spectral sensitivity of the human eye in the ultraviolet region of the spectrum. Dodt and Walther considered that lens fluorescence could account for the elevation in the ultraviolet. I have not yet measured the spectral absorption curve for the *Gekko gekko* lens but it appears to the eye to be optically clear with no trace of a yellow pigment.

MOST PROBABLE DENSITY SPECTRUM FOR THE PIGMENTS IN *Gekko gekko*

A comparison of spectral sensitivity with visual pigment absorption requires knowledge of the density spectra of the pigments. Digitonin extracts of the retina of this gecko, though relatively pure, contain, in addition to the two photopigments, photostable impurities, some of which absorb at the blue-violet end of the spectrum. The density spectrum of such a solution is not suitable for comparison with the spectral sensitivity. To obtain a suitable density spectrum the procedure outlined below was used. The mean density spectrum, as obtained directly from optical density measurements on ten solutions, shows very little dispersion of the data from 500 nm. to 700 nm. (curve 1, Fig. 4). At wavelengths shorter than 500 nm. these data have an increasing amount of dispersion attributable to varying amounts of impurities in the several extracts. The individual spectrum having the smallest amount of coloured impurity, as judged by the lowest value of the ratio of density at the minimum to density at the maximum, is an approximate indicator of the upper limit of the density spectrum of the photopigments. This individual spectrum is given by filled

points (Fig. 4). To obtain an indication of the lower limit I have selected the difference spectrum which was obtained by totally bleaching an extract to which had been added hydroxylamine.

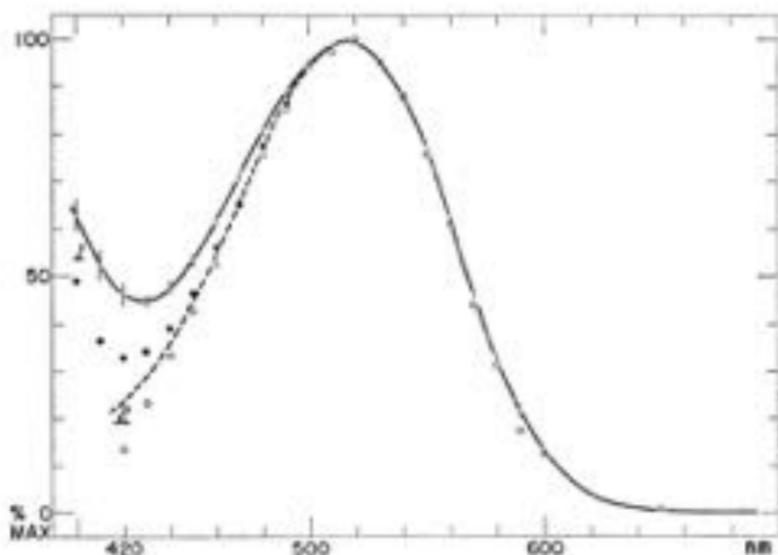


FIG. 4. Method of computing the most probable density spectrum.

Curve 1: measured optical density, average curve for 20 solutions. The vertical lines indicate the standard errors associated with these measurements. Filled points: optical density curve for the purest of the 20 solutions, only values below 500 nm. being given. Circles: hydroxylamine difference spectrum obtained in an experiment in which the solution was bleached to remove both photopigments; this was carried out after adding one drop of 0.1 M neutralized hydroxylamine to 0.6 ml. of the solution. The dashed line (curve 2) is the curve midway between the filled points and the circles. This is taken as the most probable density spectrum. At wavelengths longer than 500 nm. all the data are in accord and no corrections are needed. Further explanation in text.

This difference spectrum (circles, Fig. 4) is in excellent agreement with the mean density spectrum at wavelengths above 500 nm., indicating that the latter, in this wavelength region, represents the true density spectrum of the photopigments. At wavelengths shorter than 500 nm. the hydroxylamine difference spectrum is located below the density spectrum of even the purest solution. Since such a difference spectrum, even in the presence of hydroxyl-

amine, is the result not only of the bleaching of the photopigments but also of the formation of coloured products of bleaching (the retinal₁ oxime in this case), the difference spectrum, at shorter wavelengths, is expected to be lower than the density spectrum of the visual pigments. It is reasonable, therefore, to assume that a curve midway between the density spectrum of the purest extract and the hydroxylamine difference spectrum would represent the closest approximation which is possible to the true density spectrum. This curve (curve 2, Fig. 4) is therefore used for comparisons with the spectral sensitivity functions. It is referred to as the most probable density spectrum.

SPECTRAL SENSITIVITY FUNCTIONS OF *Gekko gekko*

The dark-adapted eye

Two separate studies were made on the dark-adapted eye. The first, carried out during the period from November, 1962 to February, 1963, used thirteen lizards and employed Farrand interference filters for producing coloured test flashes. These filters have half-band widths of 11 to 20 nm. A second separate study with six geckos was made about a year later, this time using a new set of interference filters (Optics Technology) with half-band widths of 10 to 20 nm. The results of both these studies are summarized here (Figs. 5 and 6), and it is clear that some of the erratic distribution of the points obtained in the first study is missing in the data of the second series, and that the results of the latter study probably represent a closer approximation to the truth. Both sets of data indicate the maximum of the sensitivity curve to be not at the position of rhodopsin systems, but shifted toward longer wavelengths by about 17 to 20 nm. In this respect the results agree with those of Denton (1956) obtained with a different physiological index of activity, namely, the pupil response. Compared with the most probable density spectrum, the better sensitivity curve of the second study is broader, with somewhat elevated sensitivities at the

short- and long-wavelength regions of the spectrum (Fig. 6). The disparity at longer wavelengths is significant, for the standard errors of the means for the two sets of data indicate the two curves to be

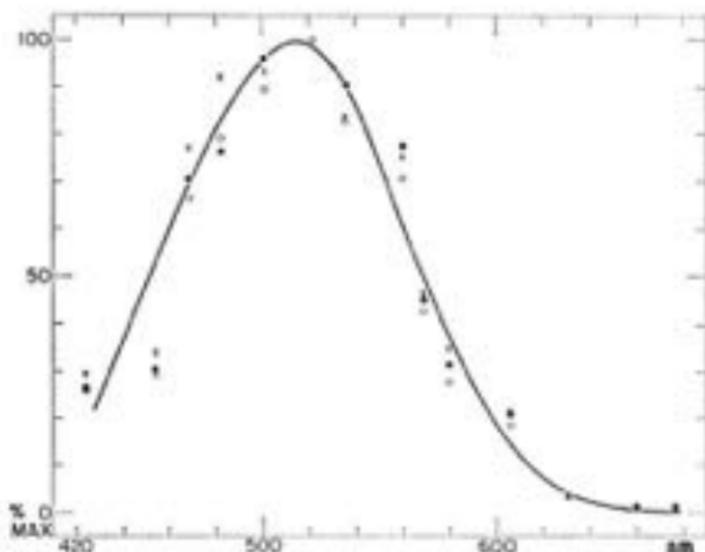


FIG. 5. First investigation. Spectral sensitivities for the dark-adapted eye (filled circles), for the eye adapted to 600 nm. (crosses) and for the eye adapted to 400 nm. (open circles).

The data for the dark-adapted eye are the means of 13 experiments with 11 animals. The data for the light-adapted eye are the means based on 6 experiments for the 690 nm. condition and 7 experiments for the 400 nm. condition. The standard errors were computed and they overlap all means. Therefore, one curve of best fit (filled line) is drawn to represent the sensitivity curve for all three states of the eye. The adapting illumination was restricted to the extremes of the visible spectrum. This was done by using, in addition to the monochromator, a Corning cut-off filter with the 690 nm. light and an interference filter with maximum transmission at 400 nm. for the 400 nm. light. This sensitivity curve is based on low criterion levels of the ERG, as described in the text.

two to three standard errors apart. *Gekko gekko*, like *Tarentola* and *Hemidactylus*, appears to have an elevated sensitivity at longer wavelengths which is not accounted for by the known photopigments. The interpretation of this elevated sensitivity will be discussed later after some other results have been presented.

The data in Figs. 5 and 6 are based on relatively low levels of electroretinographic responses, that is, values of 20 to 80 microvolts, which represent amplitudes at 15 to 30 per cent of the maximum potentials. If the response criteria are set higher, at 50

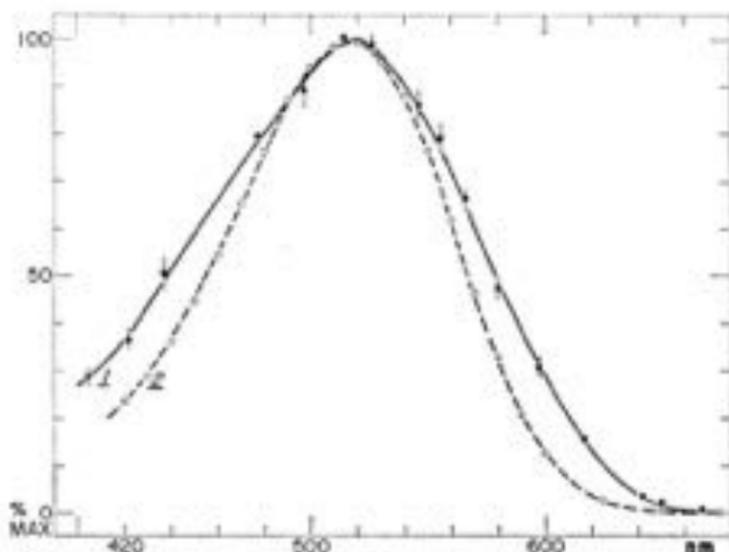


FIG. 6. Second investigation. The mean spectral sensitivity curve for the dark-adapted eye (curve 1) compared with the most probable density spectrum (curve 2).

The vertical lines indicate the standard errors of the data, while the diameters of the circles give the standard errors above 480 nm. for the density spectrum. The most probable density spectrum was derived as indicated in Fig. 4. The sensitivity curve is based on low criterion responses obtained in 6 experiments.

or 70 per cent of the maxima, the sensitivity curves are higher in the violet region. The point at 405 nm. is especially increased, going from 25-30 per cent sensitivity at the lower criteria to 40-50 per cent at the higher levels. The sensitivity curve at these very short wavelengths is subject to a number of uncertainties which at present cannot be adequately evaluated. Absorption by the lens, lens fluorescence and other factors may contribute in this region of the spectrum. In addition, the uncertainty about the exact location of

the density spectrum makes it unprofitable to speculate on the significance of the disparity at shorter wavelengths between the visual pigment data and the sensitivity curve.

The eye adapted to light at 690 nm. and 400 nm.

One interpretation of the elevated sensitivity at longer wavelengths (Fig. 6) is that the retina of *Gekko gekko* has receptors with maximum absorption in the orange-red region. Such a system might be revealed by adaptation of the eye to light restricted to the red end of the spectrum. A series of experiments was carried out in which the spectral sensitivity was determined for the condition when the eye was illuminated with light at 690 nm. In order to be certain that the adapting field was limited to the longer wavelengths, a Corning 2030 cut-off filter was interposed in the output beam of the monochromator. Calibration of this filter showed that it transmits maximally above 700 nm. and that transmission falls off rapidly below 700 nm. reaching a value of 0.14 per cent of maximum at 625 nm., and remaining at this low value down to 400 nm. This level of adaptation altered the ERG, as shown in Fig. 2, but the spectral sensitivity remained essentially unchanged (Figs. 2 and 5). Moreover, adaptation with light at 400 nm. led to the same result, namely, a spectral sensitivity curve similar to that of the dark-adapted eye (Fig. 5). In other words, adaptation with light restricted to either end of the visible spectrum produced no evidence of retinal mechanisms located either at the short or the long wavelengths which could be separated out under these conditions of light adaptation.

The eye adapted to light at 670 nm. to 650 nm.

Light adaptation effective enough to produce the drastic change in ERG pattern from the smooth to the peaked form (Fig. 2) still failed to alter the spectral sensitivity function (Fig. 2). In other words, this function was stable under conditions when the threshold (for low criterion responses) was raised by 1.5 to 2.0 log units.

From these results no evidence at all can be adduced for the existence of a separate mechanism functioning in the orange-red portion of the spectrum. What, then, is the explanation for the elevated sensitivity at longer wavelengths which is not accounted for by the visual pigment composition? One interpretation relates to the

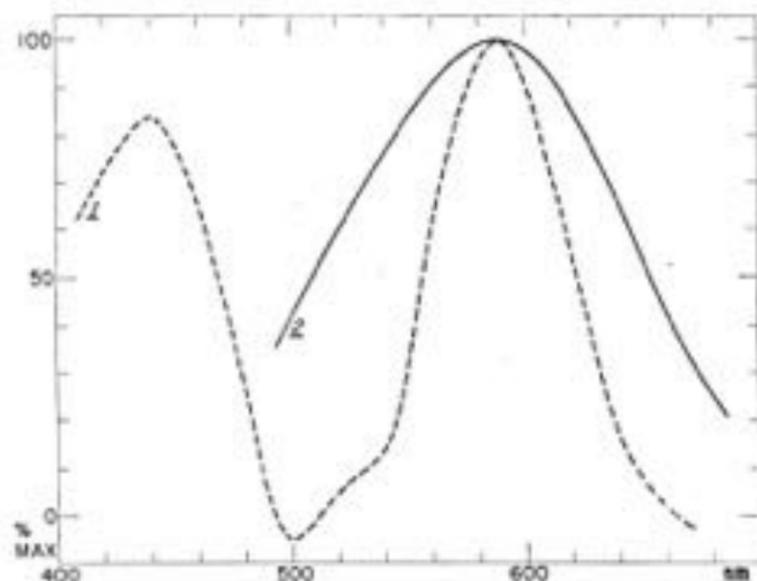


FIG. 7. Curve 1: a plot of the differences, as a function of wavelength, between the sensitivity function (curve 1, Fig. 6) and the most probable density spectrum (curve 2, Fig. 6). Curve 2: a hypothetical visual pigment with wavelength for maximum at 588 nm., as deduced from the Dartnall nomogram.

selective reflectivity of the gecko fundus. Johnson (1927) examined the eyes of three nocturnal species of geckos and stated that "they all have a brick-red fundus". His examination did not include *Gekko gekko* but I have clearly observed the orange fundus of this gecko. There are no intraretinal blood vessels in geckos but, like other lizards, these animals have a conus papillaris which projects into the vitreous from the optic disc (Fig. 1). The conus appears to be well supplied with blood vessels (Fig. 1).

A curve of the differences, as a function of wavelength, between the most probable density spectrum and the spectral sensitivity function has a double-humped form (Fig. 7) with one peak at about 440 nm. and the second at 588 nm. The form of the difference curve at longer wavelengths has no resemblance to the curve for a visual pigment in this spectral location as deduced from the Dartnall nomogram (Dartnall, 1953). The entire difference curve resembles the graphical representation, given by Dodt and Walther (1958*b*), of the difference between the spectral sensitivities of the albino and pigmented rabbits, a difference which was interpreted as an effect of reflectivity by blood in the albino animal. The peaks of the difference curve for *Gekko gekko* (Fig. 7) are located at somewhat shorter wavelengths in comparison with Dodt and Walther's result but at present it is not possible to state what other factors in the *Gekko gekko* fundus are determining the reflectivity. All that can be said is that selective reflectivity is seen to occur from the *Gekko gekko* fundus, that blood vessels are present in the conus, and that adaptation by either red or violet light does not produce any alteration of the spectral sensitivity curve. Fundus reflectivity could account for the elevated sensitivity in the orange-red region of the spectrum and might account for some of the increased sensitivity at the shorter wavelengths.†

The eye adapted to light at 650 nm. to 600 nm.

So far no evidence has been produced which indicates that separate spectral mechanisms are demonstrable by the technique of selective adaptation. The presence of two photopigments in extracts of this retina was a constant reminder that such separate mechanisms might exist and might be demonstrated by appropriate adaptation. Such a demonstration was eventually achieved

† An additional factor which may explain some of the discrepancy between the sensitivity curve and the most probable density spectrum (Fig. 6) is the *in situ* density of the visual pigments, which may be high enough to make the absorption spectrum broader than the density spectrum. This factor is now under study and will be evaluated in a later publication.

by use of adapting fields in the range of 650 nm. to 600 nm. Such adaptation reduced the amplitude of the ERG to test flashes while the pattern retained its peaked form (Fig. 2). The results of an individual experiment on one animal are summarized by the miniature graphs at the bottom of Fig. 2. The sensitivity function for the eye in the dark-adapted state is given by the full line at the bottom of column 1. This curve is repeated for the remaining four columns so that appropriate comparisons can be made. As already explained, adaptation to light at 690 nm. and 650 nm. led to no significant alteration in form of the sensitivity curve, and these facts are summarized in columns 2, 3 and 4 (Fig. 2). A significant change did occur when light at 600 nm. was used for the adapting field. In this case the sensitivity curve was shifted to shorter wavelengths by about 15 nm. (column 5). Three experiments of this type were successfully completed, the mean results being shown in Fig. 8. The curve for the dark-adapted eye (curve 1) is the mean of six experiments using six lizards. The vertical lines indicate the standard errors for these data. The curve is based on low criterion responses of the eyes. In comparison, curve 2, also a mean result, was constructed from data obtained from three animals in which the responses were recorded while the animals were adapted: in one case, to 600 nm.; in the second, to 625 nm.; and in the third, to 640 nm. The dispersion of the data is in the same range as in curve 1, for the dark-adapted eye. Adaptation under these conditions resulted in a definite shift, to about 505 nm., of the sensitivity curve but the form of this curve remained essentially unchanged (Fig. 8). The effect was as if the adaptation had selectively reduced a portion of the contribution to the ERG from a mechanism at longer wavelengths, presumably the 521-mechanism, thus revealing the participation of a mechanism at shorter wavelengths. This may well be the mechanism based on pigment 478.

The result just discussed (Fig. 8) involves a comparison of retinograms from the dark-adapted eye with responses from the light-adapted eye, which are smaller in magnitude and different in wave

form. It would be more convincing to achieve the same result as that seen in Fig. 8 by using more nearly comparable records. After some preliminary trials, this was finally accomplished by determining the spectral sensitivities of the same eye during adaptation with light at 630 nm. and at 430 nm. These two conditions of adaptation

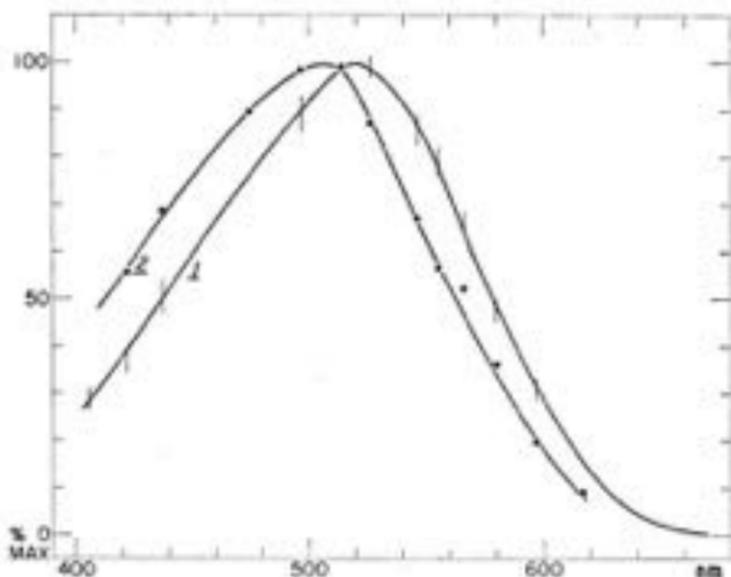


FIG. 8. The effect of adaptation with light at wavelengths below 650 nm.

Curve 1: the mean sensitivity curve for the dark-adapted eye of the second investigation, with standard errors again indicated. Curve 2: mean sensitivity curve based on 3 experiments with 3 lizards, in which adaptation to 600 nm., 625 nm. and 640 nm. was employed. Standard errors were about the same as for curve 1.

reduced the excitability of the eye to about the same level and led to electrical responses of about the same amplitude. Three experiments with three geckos were included in this 630-430 procedure. The result was the same for all three experiments and the mean curves for the three determinations (Fig. 9) adequately illustrate the outcome. Adaptation to light at 630 nm. resulted in a sensitivity curve (curve 1) which, in comparison with the result for adaptation

with light at 430 nm. (curve 2), is much higher at shorter wavelengths and lower at longer wavelengths. In other words, adaptation with blue light appeared to suppress a mechanism which was active in the eye during adaptation with red light. This result lends strong support to the previous statement (Fig. 8) concerning the presence of a retinal mechanism function at shorter wavelengths.

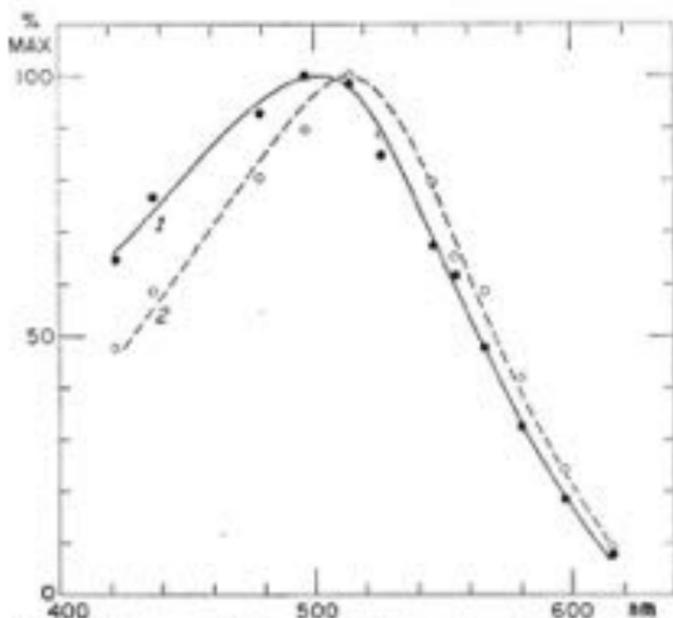


FIG. 9. Mean sensitivity curves (3 experiments) showing the result obtained with light adaptation at 630 nm. (curve 1, filled circles) and at 430 nm. (curve 2, open circles).

For each of the single experiments the curves were displaced as shown in the mean curves here. Note that curve 2 is narrower than curve 1. Explanation in text.

Indications of spectral specificity in the ERG pattern

The ERG of the dark-adapted eye (Fig. 2) is relatively free of fine structure which might be examined for indications of spectrally specific components. The ERG from the light-adapted eye is usually so complex that analysis of the many and varied components offers a formidable task. The conditions of the 630-430

experiments appear to offer some promise of discovering whether or not there are separate components in the ERG associated with separate spectral mechanisms. The electrical responses obtained in the 630-430 experiments were not only reduced in magnitude, but were simplified in pattern. Two main components—an initial fast wave and a second, slower elevation—were constant and prominent features of these responses. A cursory examination revealed that the records, in response to the different wavelengths of test flashes, were not equivalent, and that, in fact, a match in height of the fast wave did not result in a match of the slow component. This information is summarized in Fig. 10, in which the responses to four different wavelengths (437, 479, 597, 616 nm.) are listed in four columns and arranged within these columns according to intensity of test stimulus. The responses to 437 nm. are characterized by a relatively prominent slow wave and an initial, fast deflection, which, when it is discernible, is no larger, and is usually smaller, than the slow wave. In comparison, the fast wave is more prominent in the responses to 479 nm. and, in fact, is larger than the slow wave at the higher stimulus intensities. Even at 479 nm., however, the slow wave becomes relatively larger as the stimulus intensity is lowered. The responses to shorter wavelengths are thus characterized by a relative prominence of the slow wave. A different result is seen in the records at longer wavelengths (Fig. 10). To flashes at 597 nm., for example, the fast wave was very obvious, even for low stimulus levels, and one has some difficulty in being certain that a distinct and separate slow wave exists. This result is confirmed by the retinogram taken with test stimuli at 616 nm., in which a clearly dominating fast wave is observed to occur for the full range of stimulus intensities. The records of Fig. 10 make clear the point, at least for the range of stimulus values given here, that no equality of match with respect to magnitude will yield an equality of match in regard to total wave form. This is especially convincing when the adjacent records of columns 2 and 3 (Fig. 10) are compared with each other, since such records are approximately

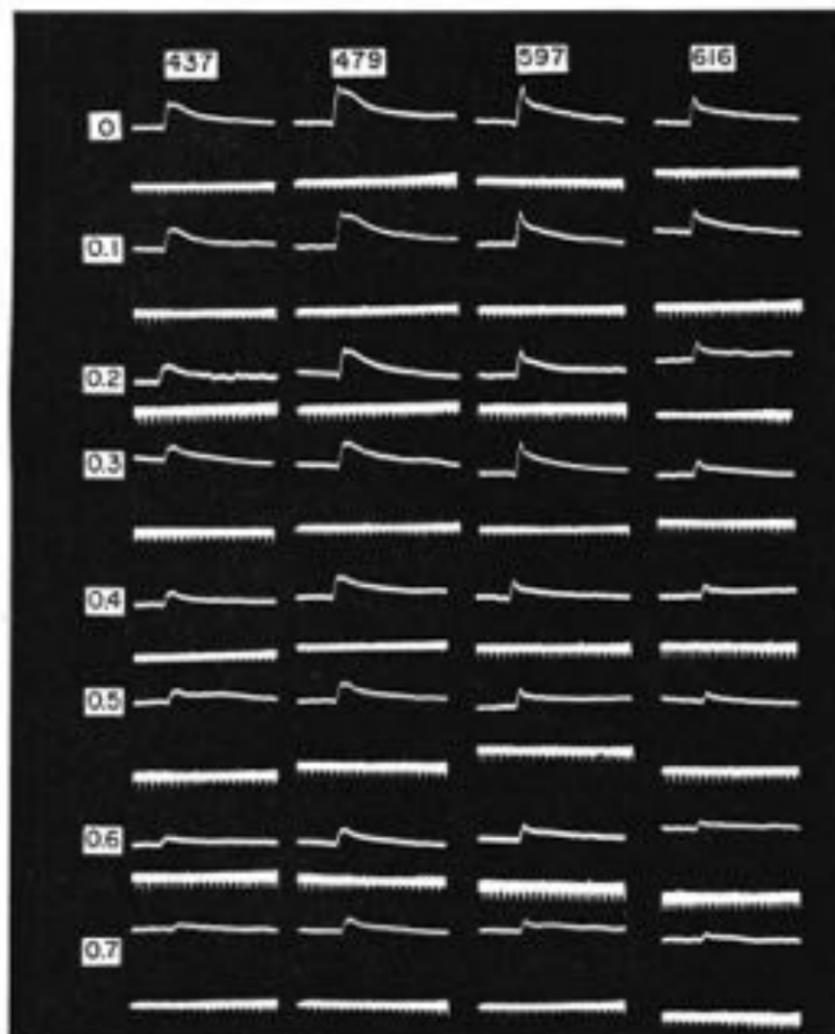


FIG. 10. Spectral specificity in the ERG pattern.

This is the result of an experiment in which the ERG was recorded while the eye was adapted to light at 630 nm. Shown here are the results obtained with stimuli at 4 different wavelengths (437, 479, 597 and 616 nm.) and at 8 different stimulus intensities (0 to 0.7 are values of neutral density filters). Note the presence of an initial faster wave and a second slower component. The latter is more prominent in records taken at 437 and 479 nm., while the former is the prominent feature of records taken at 597 and 616 nm.

Further explanation in text.

equal in height with respect to the fast wave. This account of the spectral specificity of the *Gekko gekko* ERG is of the nature of a preliminary statement since at the time of writing I have not completed the study of the records. I hope to be able to determine for the same animal the spectral sensitivity based on the fast wave and on the slow wave. At the moment, however, the results appear to indicate that the ERG does contain signs of a spectral mechanism at longer wavelengths, possibly the 521-system, and of a mechanism at shorter wavelengths, possibly the system utilizing the 478 pigment.

DISCUSSION: DUALITY OF THE RETINA OF *Gekko gekko*

The results presented here, most of which are appearing in print for the first time, give a fairly coherent picture of the retina of *Gekko gekko* as a structure in which duality is a feature. Two photopigments, two spectral sensitivity functions, two components in the ERG have been suggested and these appear to be correlated phenomena. Yet these results were obtained from a retina which any competent histologist would recognize as a pure rod retina. But these rods may not all be identical. Differences have been reported on the basis of morphology (Underwood, 1951; Tansley, 1964). The shift in spectral sensitivity to shorter wavelengths produced by appropriate adaptation suggests that the two pigments are present in separate visual cells. I am hopeful of eventually being able to detect each of these two pigments in the visual cells of the isolated retina, in order to determine the organization of these photopigments in relation to the visual cell types.

When I first compared the data on visual pigments of *Tarentola* and *Hemidactylus* with the spectral sensitivity functions of these two geckos (Crescitelli, 1963a), the hypothesis of a third photopigment system was suggested. This hypothesis must be abandoned in regard to *Gekko gekko*, as there is no justification for it in interpreting the elevation in the sensitivity curve at longer wavelengths.

The failure to abolish this elevation by adapting the eye to red light, the presence of a vascularized conus, and the observed selective reflectivity of the fundus all lead to a rejection of the hypothesis of an "orange-red" mechanism. Instead, an explanation in terms of the reflectivity of the fundus is substituted. A similar explanation may apply to the results obtained with *Hemidactylus*, since Johnson (1927) reported seeing a red fundus in this gecko. This is probably not the explanation for the elevated sensitivity at 550-570 nm. which Arden and Tansley (1962) reported for the diurnal gecko, *Phelsuma inunguis*. In addition to the fact that this elevated sensitivity was depressed by adaptation with red light, is the observation by Johnson (1927) that these diurnal geckos have, not a red, but a grey fundus. Selective reflectivity by blood in the intraretinal blood vessels of the albino rabbit also serves to explain the increased sensitivity in the red region which has been observed to occur in this animal (Dodt and Walther, 1958b).

The duality of the ERG pattern (Fig. 10), with the clear suggestion that the fast and slow components possess different spectral properties, again emphasizes the concept of a dualistic differentiation in this retina. The ERG duplicity is of special interest here because of its apparent resemblance to the results obtained from the retinae of several vertebrates. The classic researches of Adrian (1945, 1946) come to mind in this respect. Adrian reported the presence in the ERG of man and other vertebrates of dual waves which behaved, in many respects, like the fast and slow components of the *Gekko gekko* retina. He presented good evidence in favour of the interpretation that the two waves are associated with photopic and scotopic activity. Yet here in the *Gekko gekko* retina—a so-called pure rod retina—we find responses so strikingly similar to those reported for the classical duplex retinae. It helps little in this case to seek refuge in an explanation which involves neuronal interactions. The presence of two visual pigments and the suggestion that the fast and slow waves are separately associated with each of these pigment mechanisms implicates the visual cells.

The transmutation theory (Walls, 1934) may eventually take us even further than Dr. Walls suspected.

Acknowledgement

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DISCUSSION

Pedler: It is of interest that these particular receptors in this species all have the cone type of pedicle. There are minor ultrastructural differences in the pedicle but in terms of the number of channels they appear to have and the number of bipolars which are available, they are identical.

Land: What are the habits of the gecko? You called him nocturnal: does he see at night?

Crescitelli: He is active at night and feeds at night, and is quiescent during the daytime.

Land: How does the level of illumination at which he is active compare with the level of illumination at which you study him?

Crescitelli: The level of illumination in the experiments is much higher.

Klüver: Has the gecko very extensive eye movements?

Crescitelli: This animal is very suitable as an experimental subject in this respect also. The eye movements are small, the eye muscles are poorly developed, and there are no eyelids.

Klüver: Could you use the optokinetic nystagmus as a response indicator in colour vision studies?

Crescitelli: Some behavioural response such as striking at a moving object might be used. The pattern of the retinal cells may be correlated with what Dr. Svaetichin has already suggested, movement and pattern detection.

Rushon: You showed us an illustration, Dr. Crescitelli (Fig. 1c), in which you speculated that the outer segments might be lost, in rows; if this can be achieved regularly by stripping off the retina in a particular way, you have a very good means of sorting out some of the properties, first of all with extraction, because you will know that that particular set of outer segments is lost; if you could (I don't know whether this is possible) extract the eye cup for pigments, you would have the outer segments already separated. Dr. Svaetichin and Dr. Wolbarsht have studied the electrophysiology of excised retinæ and very often, Dr. Svaetichin, I think you remove the rods by blotting paper and are left with a pure cone retina; is that right?

Svaetichin: Yes, most of the rods can be removed and also the pigment by means of the blotting paper technique, which was originally invented by W. Kühne.

Crescitelli: When I took that photograph it concerned me considerably, because of the possibility that my visual pigment analysis was not typical, so I repeated the visual pigment analysis extracting the whole eye cup. It turned out that there was nothing from the whole eye cup which was different from the results obtained with isolated retinæ.

Rushon: So you have tried the discrimination on the basis of what I suggested, and it is no good, as far as pigments are concerned? So probably they have no outer segments.

Marks: Mr. William Dobelle and I have made some microspectrophotometric curves for the receptors of the gecko and we have not found any 478 nm. pigment yet, but we were bedevilled by the separation of those receptors which stick to the retina and those which fall off.

We tried one partial bleaching experiment and found only one pigment, at 520 nm.

Dartnall: Dr. Crescitelli, you compared *density* spectra with spectral sensitivities; what about *absorption* spectra? Did you allow for the concentration of pigment in the photoreceptors? It might reduce the discrepancy a little.

Crescitelli: Yes, that is true. I have not done that yet.

Donner: Dr. Crescitelli, you plotted the magnitude of response against log quantum intensity and you had a different course for the blue light; would that not indicate a gradual change in spectral sensitivity when you go from low to high intensities?

Crescitelli: Yes, I think so. I have not measured the absorption by the lens yet but I don't think there is much absorption in that region, as it looks optically clear. I think you are right and there is a change in spectral sensitivity.

Stiles: In your discussion of the shape of the electroretinogram, I gathered that you felt that the initial rounded part would be associated with receptors containing one pigment, the 480 pigment, and the sharper part with the other, 521, pigment; if so, what are your ideas on whether an electroretinogram pattern could be regarded as a summation of two patterns of these different shapes?

Crescitelli: The remarks I made about those two waves only applied to the extreme light-adapted condition, where most of the electroretinogram was obliterated. I do not know what relation those two waves have to any of the waves that appear from the dark-adapted eye, so at present I dare not speculate on that. There are many waves, of course, in the dark-adapted retina and we have to see what their spectral sensitivities are and how they summate. I suspect that there won't be just one blue-sensitive wave but a number of them and that they in some way sum, but that is a formidable task and so I have tried to simplify it first.

COLOUR DISCRIMINATION IN CATS

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SOME ten years ago, Meyer, Miles and Ratoosh (1954) reported an experiment in which they tried to train a group of cats to choose between two lights that differed in their spectral compositions. The fact that they were unsuccessful led them to conclude that the domestic cat is colour blind, and that their data thus confirmed the early, careful study that De Voss and Ganson (1915) had performed.

What they did not mention was that this result had been a disappointing outcome for the group; the plan had been to use the habits, once they were established, in some visual cortical research. The picture then was somewhat simpler than it is today (Thompson, Johnson and Hoopes, 1963), and it seemed a reasonable guess that Visual I and Visual II bear some relation to the duplex nature of the retina. The cat was chosen for this work because these regions of its brain are surgically accessible, and because the works of Clark (1942), Granit (1947), Chang (1952) and Chacko (1949) all suggested that this species should be capable of forming certain hue discriminations.

Figure 1 recalls the training situation that was used in this experiment; in retrospect, it still appears to be a proper format for the kind of study that was done. It was a Wisconsin General Test Apparatus (WGTA) which, though built initially for monkeys (Harlow, 1949), has been shown by J. M. Warren to be useful as a training instrument for cats as well. One advantage of this somewhat cumbersome device is that it minimizes both the temporal and spatial separations of the cue, the reinforcement and the response.

These are factors that, in monkeys, are of great importance in the efficiency of apparatus (Meyer, Treichler and Meyer, 1965), and it is entirely probable that they would be of great importance for the cat as well. Here a pair of coloured lights project upon the upper surfaces of two small wooden blocks; the animal, to make a choice, pushes one aside, and either finds or does not find some food. At that time, its paw, the food or food-well, and a screen around the

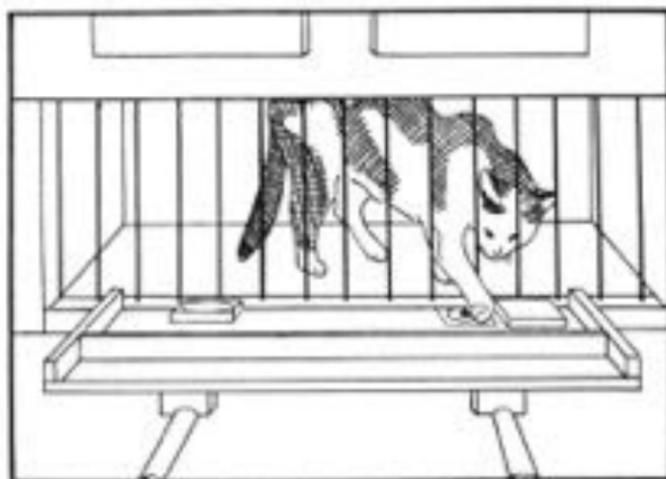


FIG. 1. The apparatus used by Meyer, Miles and Ratoosh.

food-well are illuminated by the same discriminative cue that had appeared upon the surface of the block that it displaced.

Figure 2 reiterates the outcome of the study, in which cats were given reinforcements for responding to green (Wratten 61) which was paired first with red (Wratten 23A) and then with blue (Wratten 47) for 1,000 training trials per pair. The animals did not show signs of learning when these cues were varied randomly in brightnesses, and yet, as soon as brightness was converted to a cue, the animals at once began to learn.

Disappointing though it was, this result appeared to be the same as that obtained by Gunter (1954), who had independently considered that the problem merited a reinvestigation. It is most

remarkable how similar these studies, which appeared within months of each other, were in terms of rationales, methods of approach, criteria, and overall conclusions. The outstanding differences were type of apparatus (Gunter used a Y alley box) and the fact that Gunter had determined, for some subjects, how much coloured light would match a white light. Thus it seemed that, in so far as negative results can ever settle issues of this sort, the answer to the question as to whether cats see hue was that it is unlikely that they do.

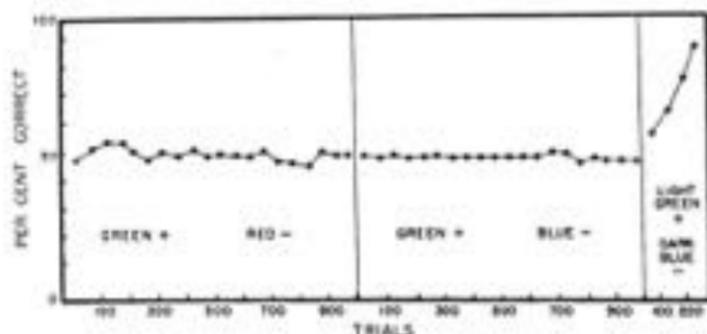


FIG. 2. The results obtained by Meyer, Miles and Ratoosh.

It was nonetheless a fact that, of eleven studies carried out within this century, the works that have suggested colour blindness in the cat are in a very slight minority. In the past, the argument has been that these four studies (De Voss and Ganson, 1915; Gregg *et al.*, 1929; Meyer, Miles and Ratoosh, 1954; Gunter, 1954) were the ones to be relied upon; all the rest had been subjected to what seemed to be a very telling set of criticisms. But there are some recent works with positive results that, on the surface, are impeccable, and hence we are no longer quite so sure that cats cannot, in fact, discriminate between two hues.

The first is some unpublished work that Clayton (1963) has reported to a recent national convention. Earlier, while working with the form of apparatus that was used by Meyer, Miles and

Ratoosh (1954), he had trained a group of cats to make discriminations between red and blue papers that had been inserted into two transparent food-well covers. Before running this experiment, Clayton had given all his subjects training with a problem series that involved positional reversals. This was calculated to reduce their tendencies, which are known to be quite strong in cats, to stay with a position if the problems posed to them in WGTA's are difficult. Clayton (1963) was unsatisfied with these results because the stimuli could not be specified, and also noted that some several thousand trials had been required to teach the cats the red-blue habit.

His second study, therefore, was performed with Wratten filters, two of which—described as red and green—were two of the filters that had previously been used in the research of Meyer, Miles and Ratoosh (1954). A third, a blue, was similar, but not identical to that employed by Meyer, Miles and Ratoosh (1954); maximal transmissions were described as being near the maxima for Granit's (1947) modulators. In addition, Clayton (1963) used a fourth, yellow filter that cut off at 520 nm., peaked at 589 nm., and had just a minimal absorption in the region of the red and infrared.

Clayton (1963) worked with two young female cats that had not had prior training in the WGTA. He began by pairing red with grey, with grey defined in terms of absence of a spectral filter, and, in phase I of this problem, he confounded colour with position for nine trials before reversing left-right cue relations. When this phase was mastered, the positions of the cues were governed by a Gellermann (1933) series, and this same procedure was maintained when other pairs of colours were presented to the cats. With these methods, Clayton seemed to show that both his cats discriminated grey from all colours, taking grey to be the colour of the light that he used before the Wratten filters were inserted.

Both cats also were successful when blue was paired with either green or yellow colour cues, but they both failed yellow versus either red or green when trained for 1,350 trials. One cat learned

the red-green problem fairly rapidly, but the other found it difficult; the latter cat was doing poorly after having had some 1,800 trials of practice with it.

A second programme that concerns us is the Sechzer-Brown (1964) investigation of the red-green problem. In this work, four cats were trained to make a heterochromatic brightness match choice between a pair of red and green, opal, plastic panels that were placed at one end of a runway apparatus. First, each of these coloured panels was randomly made darker than the other, and each cat was trained to choose that panel; then, when a criterion of learning had been reached, the brighter coloured panel's luminance was varied. The responses of the cats were used to find the points at which these two coloured panels could be said to be of equal brightness in the sense that subjects chose each panel just as often as the other. This information was used to control brightness cues in the actual colour discrimination study. The cats were then trained to choose red (Wratten 25A) or green (Wratten 58) when these were paired and brightnesses were varied. All four cats succeeded when presented with this task, but notably it took the best of them no less than 1,350 training trials to reach a very high criterion of learning.

The third and last experiment within this general group, the Mello-Peterson (1964) investigation, differs from the others in that operant techniques were used instead of two-choice situations. Basically, the method was the one that Miles, Ratoosh and Meyer (1956) had used in work with guinea pigs which, it may be recalled, had led them to conclude that guinea pigs are also colour blind. The essential features of the training situation were, as can be seen in Fig. 3, a panel for the stimulus, a lever for the cat, and a food-delivery mechanism. The measure of discrimination was a change in rate associated with a change in outcome that was signalled by an alteration in the nature of the cue presented on the panel.

The pair of outcomes that were used in this investigation are

described, in operant behaviouristic terms, as variable-interval (VI) and extinction schedules. In the VI schedule, the experimenter arms the lever to deliver reinforcement after some brief, variable interval of time has gone by since the last such reinforcement. This produces very stable, malleable rates, and these provide a background of responding that is higher than when, in extinction, reinforcements cannot be produced by lever-pressing. Operants are said to be discriminated if a given schedule's pattern of responding is

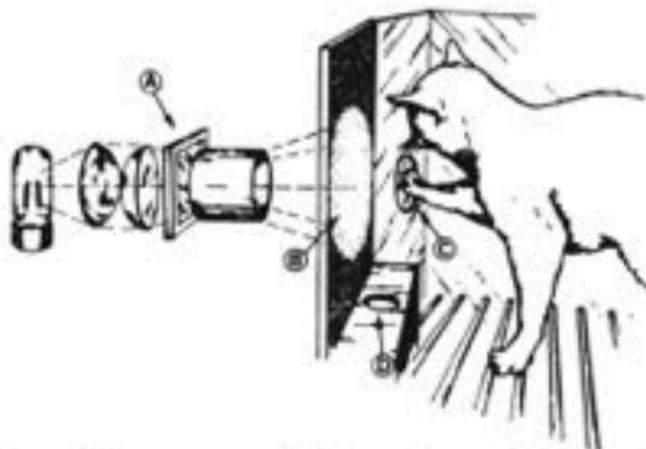


FIG. 3. The operant conditioning equipment of Mello and Peterson.

evoked as soon as some external stimulus, for example, colour, is presented. Thus, if cats respond according to a VI schedule when a red light is presented to them, and then cease responding, or at least reduce their rates when green light signals the onset of extinction, one has established that the cats are capable of telling these two stimuli apart. Naturally, the same precautions must be taken here to exclude factors such as brightness learning as in situations where the two cues are presented at one time instead of in succession.

In the Mello-Peterson (1964) experiment, three cats were given training on a VI schedule; colours were then introduced, and this

contingency was maintained when a red (Wratten 24, 29) light was presented. When, however, yellow (Wratten 3, 8, 16), green (Wratten 57, 58, 61, 91) or blue (Wratten 45, 47B, 98) light was

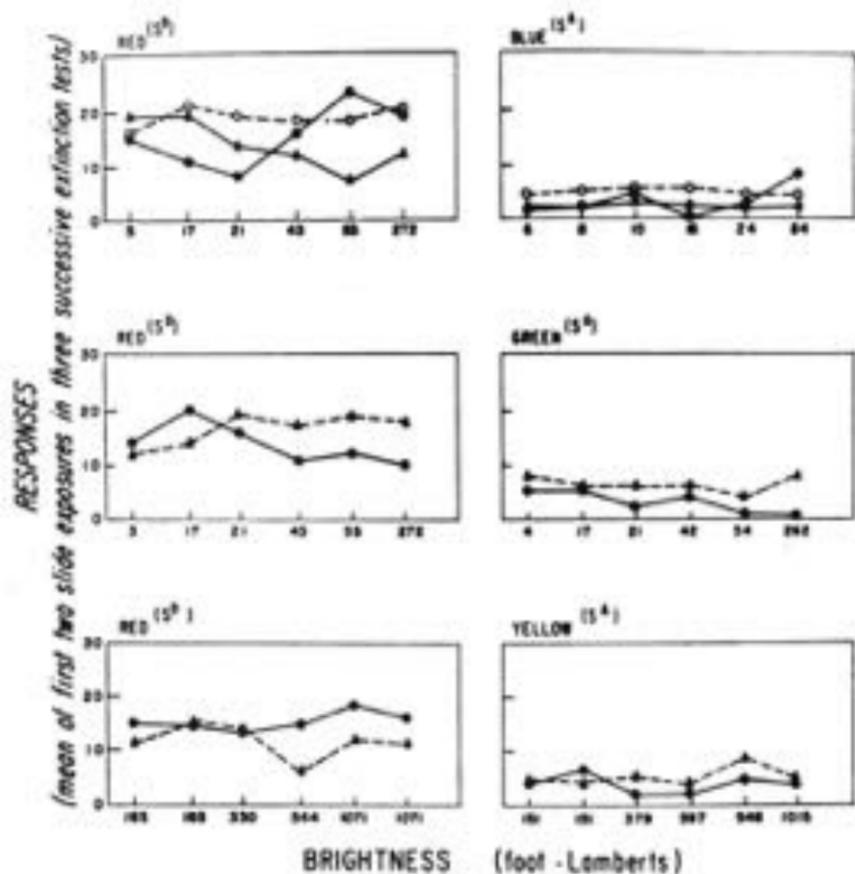


FIG. 4. Mello and Peterson's responses-to-extinction measures.

presented, lever-pressing never yielded food, and the first of several questions that were asked was whether cats can form a rate discrimination based upon the differences between these colours. The answer was that all the cats learned all the several tasks, and then, when reinforcement was withheld, made more responses to extinction in the presence of a red light than to any other colour.

This last finding, which we reproduce in Fig. 4, was independent of the brightness levels; here, again, we have convincing evidence that cats are capable of hue discrimination.

As we thought about these data, it appeared to us that the results had posed two different problems. One, of course, was whether we ourselves could duplicate the sorts of outcome that had been described; the second, and the more important from our point of view, was why the early methods had not worked. That is, our presumption was that these experiments had shown that cats discriminate some colours, and that the instructive thing for us to do would be to ask why this is difficult to show. If, indeed, the list of cat capacities includes a modicum of hue discrimination, cats are most reluctant, it would seem, to make this known to the comparative behaviourist. Thus the nearly 1,500 trials that were required in the Sechzer-Brown (1964) experiment, and the more than one year's training that had gone into the Mello-Peterson (1964) investigation, make one wonder why it takes the cat so long to learn a problem that is simple for a primate.

Some have argued that these difficulties have their source in the incentives that have been employed. Thus the cat has been described as so cantankerous and unaccommodating as a subject that, unless one uses shocks or other punishments, a failure to perform is meaningless. Yet there is no proof at all that one can get, from cats, a higher level of discrimination through the use of shock-avoidance methods than one can with methods based on positive incentives. It has also been proposed that cats will not work well for food rewards unless they have been starved, but this, once again, is an opinion that has not been fully validated by the facts.

The problem that such arguments inevitably raise is why it is that cats have failed to learn in situations that were shown to be appropriate for setting up another kind of habit. Although there are bases for believing in a drive-incentive-difficulty interaction, that is, in relationships of which the Yerkes-Dodson law would be, if correct, a good example, it is not so easy to accept the point of



FIG. 5. The apparatus used by Meyer and Anderson.

view that interactions of this sort could be so strong that they could be appealed to in this situation.

It has therefore seemed to us more likely that the cat's disinclinations with respect to colour have their bases in some factors that are unrelated to the motive-and-incentive system. For example, it is known that training situations in which several cues are relevant may generate a differential transfer to one class despite the fact that all the cues presented can be shown, by other means, to be discriminable. After Lashley, this is termed perceptual dominance, and possibly the best examples of it can be found in certain studies carried out with monkeys by J. M. Warren (1954).

A review of studies in this area (Meyer, Treichler and Meyer, 1965) suggests that such perceptual dominance occurs most strikingly when one of the dimensions one employs is more discriminable than the other. Yet, it must be emphasized again that all these cues can be discriminated by the subject; it is simply that one class may not control a habit if some other potent class is present. These things have been touched upon by Clayton (1963) with respect to problems posed by strong position habits, and that cats prefer to go to brightness has been stressed in the report of Sechzer and Brown (1964).

All of the above considerations had a place in the design of our experiment; we propose to look at it in some detail because we plan no other publication for it. The study was made possible because the invitation to attend this conference in London almost coincided with an offer of a cat that J. M. Warren held in high regard. This cat, which was known familiarly as Hugh, was so good at discrimination learning that it seemed to Warren that, if any cats see hue, this cat, Hugh, was surely in the group. Hugh and three more able cats, one supplied by Warren and two from our own laboratory, constituted the experimental group from which the results here reported were obtained.

Figure 5 presents the apparatus that we built; in form, it was a WGTA. The stimulus lights were projected on a pair of white,

diffusing surfaces attached to 3-inch squares of plastic that were covers for two food-wells set into a black, plastic tray. The food-wells were themselves surrounded by white surfaces, and hence, when subjects pushed aside the covers, the food-wells and the bits of liver that they might contain appeared within the centres of the cues. Pins centred upon the covers and some low metal ridges were employed to predispose the cats to move the covers by some other means than touching them upon the edges nearest to the cage. The operator's body was concealed by low partitions, and during his arrangements for a trial, he lowered an opaque screen that was fitted just in front of bars that kept the cat within its cage.

The projection system was placed above the cage, and terminated in a pair of Leitz projection lenses which were orientated up-and-down. Therefore it was possible, by shuttering these lenses, to select materials that were placed within the upper or lower halves of 2-inch slides. Exit beams were taken to a first-surface mirror and reflected downward to the tray, but were passed through masks that were arranged above the tray and held a pair of simple filter slots. Filters slipped into these slots were neutral densities, and hence their functions were to vary brightness; colour filters were contained in filter sandwiches, and these were placed in the projector proper. Each of the projection slides was so constructed that it held a left-hand and a right-hand pair; differential shuttering of lenses thus was used to alter the positions of the cues.

We will limit our discussion to results with just two colours, namely red and green. We used Wratten filters 24 and 61, whose dominant wavelengths were 612.5 and 534 nm. These were mounted in the slide frames in conjunction with transparencies containing black and white striations which, when projected on the food-well covers, were each 7 mm. wide. These striations were included so that we could start the cats on problems in which colour cues were correlated with a pair of patterns that we knew that cats could readily discriminate. These were patterns in which the striations were oblique, but perpendicular within a pair; that

is, the striations were at 45 degrees, or 135 degrees as measured from the edges of the two food-well covers.

Basically, our notion was that, after cats had learned the combination colour-pattern problem, we would very gradually reduce the latter cues by varying the slants of the striations. Thus, when cats had mastered problems in which colour cues were paired with perpendicular striations, we proposed to alter each striated pattern by a step of five degrees toward vertical. Hence, when nine such steps had been completed, pattern cues would be eliminated, but they would have been removed in very gradual steps instead of being suddenly reduced.

After one such introductory series, during which the cats did very well, the patterns were eliminated and the cats were trained to make discriminations that could have been based either on colour or on brightness differences. The data were then analysed in terms of the presumption that the cats would try to follow brightness, and from the results that we present in Fig. 6, it seemed that we could get a reasonable brightness match through the addition of a 1.2 log unit neutral density filter to the green. This was checked by transfer tests in which either the non-reinforced red or the reinforced green was replaced with white light. It was calculated that if brightness was, in fact, controlling the behaviours of the cats, the red-white and the green-white comparisons presented should yield red- and green-going probabilities of 95 and 42 per cent. On the basis of 200 trials per animal, two of which were under each condition, the obtained performances were 91 per cent and 37.2 per cent. Further, when we plotted the responses of the cats as a function of the log differences between the values of the white filter and the different filters used with either green or red filters, we obtained indifference points, as shown in Fig. 7, that were separated by approximately 1.2 log units.

Next, the matching data were employed in the design of a set of filter combinations that reduced the payoff for a brightness strategy to something close to 60 per cent. The cats were trained with these

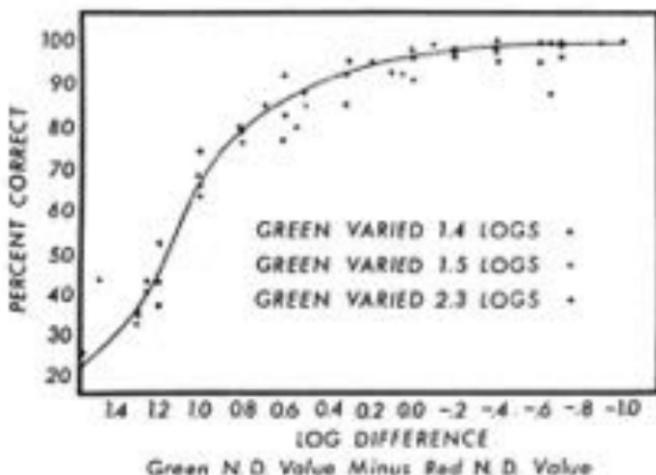


FIG. 6. Performance as a function of the log difference between the neutral density filters that were used in combination with the coloured filters. The 1.4 and 1.5 series were obtained with a 3290°K bulb; the 2.3 log series was run when the source was a 3350°K bulb.

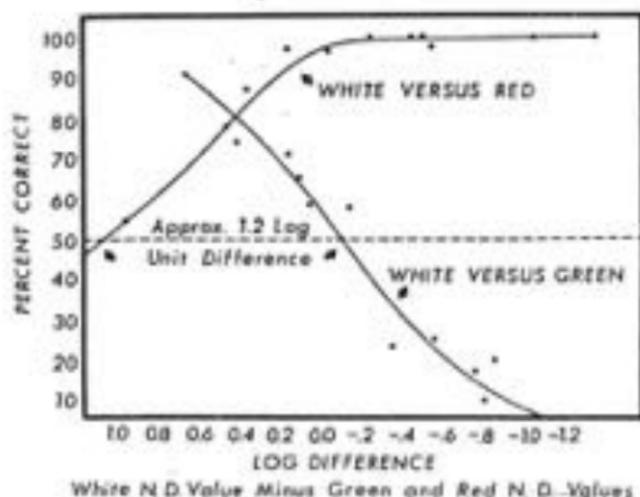


FIG. 7. Performance in the white-red and white-green situations as a function of the log difference between the white neutral density filter and the neutral density filters used with red and green filters. The curves are visual fits to the data.

new combinations for 12 days, or 600 total trials per cat, and mean performance in this series proved to be approximately 70 per cent. Hugh, the best cat of the group, made 78.5 correct responses to these stimuli, and we had a first suggestion that the animals were capable of hue discrimination.

At this point, we modified the system to include a lamp of somewhat higher lumen output. When these changes had been made, the cats were trained again with reds and greens of varied brightnesses. These were so selected that, if brightness differences had not been altered inadvertently, the payoff ratio for a brightness strategy would now be something close to 73 per cent. The cats were trained in this way for a 10-day period, or 550 trials per cat; the mean performance of the group was 76 per cent, and day-to-day performances were stable. This suggested that the cats would comfortably accept this relatively modest rate of payoff, even though a cue that they presumably could see was always there to mark the food's location.

In the final series, then, filter combinations were selected that would be expected to reduce the payoff for a brightness strategy to a point quite close to the 50-50 minimum. The patterns that have been described before were introduced, and colour-pattern cue combinations were presented until, at each stage in the reduction, each cat reached a criterion of 45 correct responses to the 50 daily trials.

The initial problem, or the case in which the slants were 45 and 135 degrees, took the cats from 13 to 17 sessions (650 to 850 trials) to master. The first eight steps in the reduction of the pattern cue required 1 to 3 days (50 and 130), 1 to 2 days (55 and 125), 1 day (60 and 120), 1 day (65 and 115), 1 day (70 and 110), 1 to 2 days (75 and 105), 1 to 3 days (80 and 100), and 1 to 3 days (85 and 95) respectively. Two cats, who took 3 and 8 more days respectively, passed the ninth and final cue reduction; at this writing, the remaining cats have not succeeded after 5 and 10 days' further training. Yet, all cats within the group are doing very well, so well that we

think it probable that the laggards, like the others, will eventually achieve the levels that we have prescribed. Their last two performances were 82 per cent and 84 per cent respectively, or 4 and 3 responses, daily, short of where we planned to terminate the study in the first place.

On this basis, we agree that cats are capable of learning to discriminate some colours. A better statement is that it is possible, but painful, to extract the evidences of it. Cats do not, as we have seen, so readily give up a brightness-based discrimination habit; we suspect that, when they are responding in this mode, they do not pay attention to the hues. Colour, of the various hypotheses that cats can possibly adopt when being trained, seems in view of our results and others in this field to be by far the least preferred of all.

Acknowledgement

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DISCUSSION

Willmer: When you were training your cats you began by combining colour cues with patterns which you knew cats could distinguish, but when the two sets of lines became vertical, could you remove the grating altogether?

Meyer: Yes, you can remove them, and you can defocus the grating. You can also change over the slides that bear the lines, and you can adjust their position; by these means you can get rid of whatever cues there might be in terms of small irregularities in gratings. We have also used several different sets of 90° figures. All these things do not prevent the maintenance of a learned discrimination.

Dartnall: Can one assume from the difficulty of establishing a sense of colour in the cat that colour is not important to a cat, or is it a matter of intelligence? I often wonder what would happen if we took a group of human beings, did not tell them what we were going to do with them, slightly frightened them perhaps, and then put them in a maze and let them get on with it. This is what we do with animals.

Meyer: In virtually any animal training experiment you cannot inform the animal of the nature of the solution to the problem, and yet you can train cats with discriminable cues to do some most remarkable things. Furthermore, in brightness discrimination training, you cannot tell the cat that it is a brightness problem that he is supposed to solve, and yet he solves it very quickly. You can only tell him to do a certain thing by the reinforcement contingencies with which you provide him. I think the cat is well equipped in terms of what we can call cat intelligence to learn a simple hue-discrimination problem.

Dartnall: This difficulty they have might suggest, then, that colour vision is not very important to the cat.

Wolbarsht: I have talked to Dr. D. H. Hubel and Dr. T. N. Weisel about this problem, in an effort to convince them that the cat has colour vision and they should therefore use monochromatic stimulation, and

they always reply that they do not find any units which are sensitive to colour. That does not mean that the units are not there, because Granit's reports certainly indicate that (Granit, R. [1948]. *Brit. J. Ophthalm.*, **32**, 550; [1951]. *Ann. psychol.*, **50**, 129), but it means that they are probably fairly rare, so that at least with certain sampling techniques you do not see them at all.

Land: I have forgotten the exact details of Granit's modulator curves, but we characterize filters by where the peak happens to be, and of course it is the integrated area that counts; I wonder if these filters might be rather broad, compared to the location of his modulator, so that if you took the integrated area of these filters, they might spread over several modulators?

Meyer: This is a problem whenever one must use light that is not monochromatic.

Land: One could use the sodium line and the mercury line and so on.

Donner: In the cat Granit derived his modulators by calculating the change in the shape of the spectral sensitivity curve after various kinds of colour adaptation (Granit, R. [1945]. *J. Neurophysiol.*, **8**, 195). He never found fibres or cells that showed an isolated spectral sensitivity of the modulator type. A consequence of this is that the sensitivity curves obtained have been changed by nervous interaction processes in the retina.

Meyer: The fact that Granit could not record cat modulators without differential preadaptation was the principal physiological comfort that we had in 1954.

Wolbarsht: In the last experimental paper that Granit published on vision ([1948]. *Brit. J. Ophthalm.*, **32**, 550) he showed that there was a shift with wavelength in certain cells from an on-response to an off-response. Those were the last experiments he did on vision, and why he started using electrical polarization—to find out why there was a shift from "on" to "off".

Sjöstrand: Professor Meyer, you said that the guinea pig does not show colour vision. Do you intend to do the same kind of study with the guinea pig as you have done with the cat?

Meyer: I am not prepared to tackle the guinea pig again. I think the Miles, Ratoosh and Meyer ([1956]. *J. Neurophysiol.*, **19**, 254) experiment provides the reason why I'm not enthusiastic. In this study we projected

signal lights at intervals of two minutes each. These lights differed with respect to brightness and in spectral composition, but the guinea pig was only reinforced for bar presses falling at the end of an interval in which the light presented had been green. We found that they pressed as often when the light was either red or blue as they did when it was green, even though they quickly learned to stop responding when a systematic brightness difference was employed to mark the times when

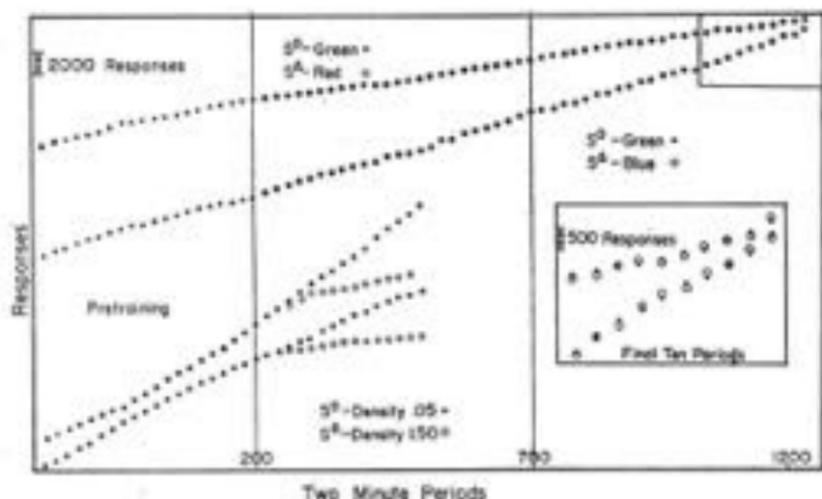


FIG. 1 (Meyer). Cumulative response record with an arbitrary zero for each subject. When discrimination training began, after 200 periods, both S^D and S^A records are recorded together; therefore, the continuous number of periods recorded along the axis of abscissae are doubled.

pressing would not lead to food. As we see in Fig. 1 from this paper, the animals did not respond to differences in hue within a thousand cue presentations. Hence, although it may be that the guinea pig sees colour, this does not seem very likely now.

Rushton: The first year I worked on vision was in Professor Granit's laboratory (where I was deflected from nerves to eyes) and I thought then that I had quite definite evidence of a red mechanism in the cat's eye by microelectrode recording from a few, but not all, of the ganglion cells we tried. I projected upon the retina a red-green light mixture in which red was polarized vertically and green horizontally, so that by

turning a polaroid in the beam one could change from red to green and back. If only one receptor served the photopic dominator the change would only be detected as a brightness difference and could be eliminated by adjustment of the red/green energy ratio. With many ganglions this condition could be reached; with some it was impossible. With these there was enhanced red sensitivity and no red/green ratio permitted an uneventful change. It was as if in addition to photopic dominator receptors there were red receptors that easily responded to the red light when so adjusted that the dominator receptors did not detect it. I have never published this and it probably needs confirmation, but it has left me with a strong impression that there are red receptors in the cat's eye.

There is one other point: if you were to hear me sing you might get the impression that I don't know one note from another, and if you were a psychologist you might set up an experiment in which you would play C on the piano one day and E on the next and reward me for successful discrimination between the two notes. Now, though I can distinguish a major third well enough, I have no absolute pitch. I need the recent memory of the first note in order to judge its relation to the second. If you leave a day or an hour in between I am afraid you will confirm your impression that I can't tell one note from another. Many of the experiments by which animals are tested for discrimination between red and green are for the *memory* of the different kinds of sensation, and to compare a mosaic of green and red squares on a chessboard with identical green and green squares, which would not depend so much upon the memory but simply upon the colour discrimination, might be an easier way to detect such discrimination in a cat, but of course it wouldn't be detecting the same thing as you are investigating.

Meyer: Yes; but this is not so much a problem when one uses simultaneous colour presentation as it is when one uses successive presentations in conditioned operant procedures. In the simultaneous procedure, memory time is surely very short.

Svaenichin: Why is it so easy to train a fish to discriminate colour? Are fishes so much more intelligent than cats?

Meyer: They might be better equipped with the colour mechanism.

Pickford: Surely it is not a question of intelligence, because this is rote learning which you are testing anyway.

Pirrense: How do you view the question of intensity, Professor Meyer?

It may be that the relative importance of the rod and the cone systems in the cat is such that in most of these experiments the cats see colours as we see them in moonlight—and in moonlight it is not easy to be guided by colour. Using high intensities with light-adapted cats one might be able to detect better colour vision in them. But it is always a difficult technical problem to obtain coloured lights of high intensities.

Klüver: When I studied night monkeys some thirty years ago, histologists generally insisted that the retina of *Aotes* is a pure-rod retina. And Woollard, here in London, had reached the conclusion that it has no macula or fovea (Woollard, H. H. [1927]. *Proc. zool. Soc. Lond.*, p. 1). In view of these findings I thought it challenging to test for pattern discrimination and colour vision. I found that pattern discrimination was, under comparable conditions, about as good as my own and consequently I was not surprised when many years later a deep foveal pit was discovered in the retina of *Aotes trivirgatus* (for a brief report on some of my behavioural findings cf. Chacko, L. W. [1954]. *J. anat. Soc. India*, 3, 11). When, in a long series of experiments, I tested for colour vision I obtained results which were difficult to interpret but at that time led finally to the conclusion that colour discrimination was possible at certain levels of illumination but not at others. It would seem to be of special interest that *Aotes*—being the only monkey that is truly nocturnal in its behaviour—may have colour vision under certain conditions.

Pirenne: It is also possible that the cat pupil might contract more than that of the monkey; this may be a further difficulty in studying the cat. In sunlight the area of the cat's pupil, contracted to a narrow slit, seems to be much smaller than that of our pupil.

Meyer: There is a curious comment by Ingvar on the intensity of the monochromatic light that he used in his experiment on cerebral visual centres. He said that he used the monochromator that Granit used in his experiments, but that a human observer with his eye in the full beam path could not discriminate the hue.

Pickford: Would it be possible to use saturation as a variable?

Meyer: Yes, that would be very interesting; in fact it was part of the Sechzer and Brown experiment. They desaturated the reds and greens after they had established their discrimination and found that the discrimination could be maintained to a substantial desaturation. I haven't yet tried that myself.

Lennox-Buchthal: I would just like to add that it is clear that cats do have units which respond differently to different colours but they are not nearly as responsive to different colours as they are to brightness, and the observer is rewarded by seeing changes with brightness and studies those in the cat, whereas in monkeys there is a very obvious and clear selection with regard to colour and the observer is rewarded with that and studies it. So the animal really trains the observer!

GENERAL DISCUSSION

PSYCHOLOGICAL ASPECTS OF COLOUR VISION

Klüver: In considering the psychological aspects of colour vision I am reminded, first of all, that the author of one of the most important books on general sensory physiology, J. von Kries ([1923]. *Allgemeine Sinnesphysiologie*. Leipzig: Vogel), held that sensory physiology is different from all other fields of physiology in that its problems are closely related to, or even identical with, certain problems of psychology, epistemology and logic. In fact, he held that sensory physiology and sensory psychology are practically indistinguishable. Such views, I should point out, were held by a man who was rather "hard-boiled" in his own scientific researches. When Selig Hecht made energy measurements to determine the minimum energy required for vision he discovered that von Kries long ago had arrived at practically the same values without even making any energy measurements. No wonder, then, that Hecht expressed his admiration for von Kries's great skill and care in evaluating reflections, absorptions, lens factors and the like. In any event, the dictum that sensory physiology and sensory psychology are practically indistinguishable should be seriously considered.

When it comes to studying visual functions the psychologist is not likely to forget that there are not only receptors but that there is a brain, more specifically, a cerebral cortex, behind the eyes. In 1923, the same year in which von Kries published his book on sensory physiology, one of the great Swedish brain researchers, S. E. Henschen, published his famous paper on the "40-year battle" of the visual centre. He insisted that the long battle had finally demonstrated that there is such a thing as a "cortical retina" and that it is the area striata which represents the cortical visual

centre (Henschen, S. E. [1923]. *Z. ges. Neurol. Psychiat.*, **87**, 505). A psychologist interested in visual functions, including colour vision, will certainly want to know how such functions are affected by the destruction of the striate area. For a long time it was generally assumed that such a destruction, in man, leads to complete blindness, although it is worth mentioning that only at the end of World War I did Saenger report on what he thought to be the first case of permanent cortical blindness following occipital injury (Saenger, A. [1919]. *Neurol. Zbl.*, **38**, 210; Klüver, H. [1927]. *Psychol. Bull.*, **24**, 316). Unfortunately, the patient was not carefully studied and visual functions were not even tested under conditions of dark adaptation.

In monkeys, removal of the striate cortex leads to a degeneration of the lateral geniculate bodies and therefore to an elimination of the geniculo-striate system. I have studied visually induced behaviour in such "bilateral occipital monkeys" in long-term experiments (Klüver, H. [1941]. *J. Psychol.*, **11**, 23; [1942]. *Biol. Symp.*, **7**, 253; [1951]. *In Cerebral Mechanisms in Behavior*, pp. 147-199, ed. Jeffress, L. A. New York: Wiley). Animals pre-operatively trained to respond differentially to visual stimuli of various kinds were retested after the operations or examined in new stimulus situations. In some instances, as many as 30 to 45 trials a day were given for more than 2,000 days after the occipital lobectomy. Such animals are not blind, since under conditions of dark adaptation absolute brightness thresholds of between 4 and 20 micromillilamberts can be obtained. Such values do not appear significantly different from those found in normal monkeys and man. However, if we examine the responses of the dark-adapted bilateral occipital monkey to luminous stimuli differing in brightness, area, shape, colour and/or distance, including responses to various kinds of intermittent light stimuli, it turns out that practically all its differential reactions can be understood by assuming the effectiveness of one factor only, namely, the effectiveness of differences in the quantity of light entering the eyes. The fact that the

elimination of the geniculo-striate system leaves us with density of luminous flux at the eye as the chief determinant of visually induced behaviour has important implications.

In studying the behaviour of a normal monkey, that is, of a monkey with an intact geniculo-striate system, we discover that its visual environment is structured in terms of "objects" with properties which stay put, as it were—properties such as size, brightness, colour, shape and position. We find experimentally that responses, for instance, to "size" are, within wide limits, not affected by changes in distance, brightness, colour, shape and position. Similarly we find that responses to "colour" are not affected by marked changes in brightness, size, shape, position and distance. Furthermore, we can show experimentally that size, colour, brightness, shape and many other properties of objects are not markedly influenced by changes in illumination or in the character of the illuminant. In other words, the geniculo-striate system guarantees an approximate constancy of the objects and their interrelations in the visual environment. This system therefore provides the normal monkey with a visual world that has a remarkable degree of stability. The elimination of this system leads to an elimination of constancies—even of constancies of a primitive order, such as "brightness constancy"—thereby putting the organism at the mercy of the never-ending energy fluctuations that continuously affect the retina. Without this system, the animal tends to behave like a photocell, responding merely to fluctuations in intensity. Such a formulation of the experimental results implies, of course, that visuo-spatial properties are no longer effective determinants of visually induced behaviour. The experimental findings strongly suggest that an elimination of the geniculo-striate system leads to an elimination of visual space with its dimensions.

To return to "colour vision". The fact that the bilateral occipital monkey no longer responds to visuo-spatial differences means that it no longer responds to "surface colours". Things with

surfaces reflecting light, that is, "objects", apparently no longer exist for such an animal. When I studied responses to "colour" in bilateral occipital monkeys, under conditions of light as well as dark adaptation, I found, on the one hand, that spectral effectiveness curves similar to the scotopic luminosity curve of the human eye could still be obtained and on the other hand, that wavelength itself was no longer behaviourally effective. In other words, the approximate constancy of "hue" in the presence of intensity changes and a wide range of other changes had disappeared in such animals. The fact that a permanent loss of colour vision is one of the consequences of eliminating the geniculostriate system raises the question of the rôle the receptors can possibly play in effecting colour vision. It seems paradoxical that the eye or, perhaps more accurately expressed, the "visual sector" of the central nervous system, minus the geniculostriate system, should furnish absorption curves of photopigments and relative spectral effectiveness curves and yet furnish us no reasons for speaking of "colour vision" at all, at least not in the subhuman primates which I studied. Just what has the eye to do with colour vision?

In dealing with the relations between peripheral receptors and centres it is in this connexion perhaps not surprising that the mechanisms involved in central regulation should have become of special interest to psychologists. To be sure, anatomists such as Cajal were interested in this problem and greatly contributed to an understanding of centrifugal systems. It is a hopeful sign nevertheless that more recently physiologists, and especially electrophysiologists, have turned their attention to problems of central regulation in different sensory systems (Rossi, G. F., and Zanchetti, A. [1957]. *Arch. ital. Biol.*, **95**, 199). Our own experiments concerned with determining the effects of destroying the cortical "visual centre" have shown the necessity of specifying "constancies", that is, visual "iso-functions", in physiological terms. It is possible that further studies of central regulation may provide us with a physiological basis for what was called 30 years ago the "counter-reaction

of the centres". In fact, a psychologist could conceive of no more important job than the analysis of cortico- or centrifugal systems in every sensory modality.

Pickford: I was extremely interested to hear Dr. Land put forward his ideas; it seems to me that he has got hold of something very important in his formulation and I don't doubt that this is a possible way of looking at things. But I wonder how far this can be generalized and how far it will prove to be an explanation or theoretical framework which can be extended quite considerably in psychology. The phenomenon of constancy in colour is only one aspect of constancy; constancy is extremely general. The fact that you can turn your head and the room doesn't go round is a phenomenon of constancy; it affects every form and aspect of perception. Therefore the retinex system could be a partial aspect of or a point of approach to a concept or series of concepts of the psychological mechanisms bearing on size, shape, distance and stability of the environment.

One point in relation to this is the great variability of constancies. Here again I have great difficulty with a group of this kind which talks in absolutes. (This is itself a constancy phenomenon, of thought rather than perception.) The reluctance to think of individual differences is characteristic of physicists and physiologists and people of that kind, who appear to think that if they test one subject they have finished. There are tremendous variations between individuals, including the matter of constancies; constancies are affected by the size, area, and the condition of perception, by the temperament of the person, and by all kinds of other factors, and you cannot assume that two individuals have the same constancy. You can never assume in fact, that constancies are absolute; they are extremely variable. The result of this is that the application of a theory like Dr. Land's is going to be very complex. But I would stress that I found it one of the most interesting things put forward here.

Lowenstein: It is very interesting how silent physiologists become

when talked to by psychologists. This is so in part for a historical reason which I couldn't go into in detail, and is in part due to fundamentally different approaches, which are both very useful but which haven't yet met on a common level.

SPECTRAL SENSITIVITY OF THE RABBIT RETINA

Monnier: In this part of the symposium on comparative colour vision it may be interesting to specify the position of the rabbit, as concerns the spectral sensitivity of its retina.

It is usually admitted that rabbits are more or less colour-blind. Various results suggest, however, that their rod retina may be sensitive to short wavelength stimuli. Thus J. B. Watson and M. J. Watson observed a spectral sensitivity of the rodents to blue light in behavioural experiments ([1913]. *J. anim. Behav.*, **3**, 1). E. Dodt and V. Elenius ([1956]. *Pflügers Arch. ges. Physiol.*, **262**, 301), recording the retinal responses of the rabbit's retina with microelectrodes, found that the sensitivity maximum shifts from 470 nm. to 460 nm. after light adaptation. A Purkinje shift was never observed, but there was also in some elements an increased sensitivity in green (520 nm.) during the photopic state.

With Schwartz and Jordan (Monnier, M., Schwartz, A., and Jordan, P. [1962]. *Vision Res.*, **2**, 189) we determined the spectral sensitivity of the rabbit's retina for a constant amplitude of the electroretinogram (b-potential). The spectral sensitivity curves thus obtained showed higher values after dark than after light-adaptation, the b-potentials of the electroretinogram being higher in the dark (Fig. 1). However, the form of the dark-adaptation curve was not very different from that of the light-adaptation curve. There was always a maximum in the blue-green (498 nm.). This distribution corresponds to the absorption curve of rhodopsin, according to R. J. Lythgoe ([1937]. *J. Physiol. [Lond.]*, **89**, 331) and H. J. A. Dartnall ([1953]. *Brit. med. Bull.*, **9**, 24). Here also a Purkinje shift was not detectable; however, there was a slight

hump-like increase at 452 nm. after light adaptation, suggesting sensitivity to indigo. A new series of experiments with Vatter and Koller (Vatter, O., Koller, T., and Monnier, M. [1964]. *Vision Res.*, 4, 329), confirmed this slight hump-like increase of the spectral sensitivity in the indigo band after light adaptation and showed in addition a smaller increase in green-yellow (521-543 nm.).

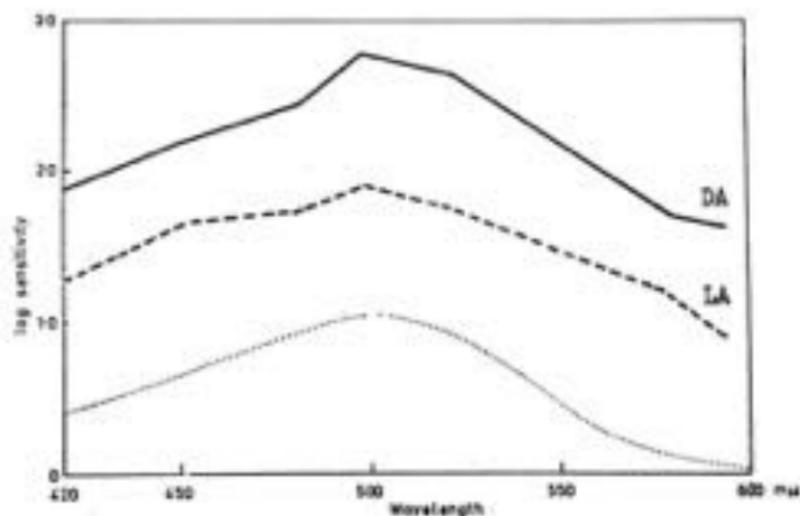


FIG. 1 (Monnier). Average curves of retinal sensitivity based on constant amplitude electroretinogram responses to variable stimulus energies and wavelengths. (Modified from Monnier, Schwarz and Jordan, 1962.)

— = dark adaptation (4 rabbits).
 ---- = light adaptation (5 rabbits).

With L. Hoesli and V. Golovine we controlled these previous results with the Mnemotron computer CAT, in a greater series of waking rabbits, applying equal energy colour stimuli with interference filters of Schott (Fig. 2). (All colour stimuli had the same energy of 33.8×10^{-6} watt/cm.²) The stimulation frequency was 1 in 2 seconds for light adaptation and 1 in 30 seconds for dark adaptation. The flash duration did not exceed 20 msec. For light adaptation, we had to compute 50 responses and for dark adapta-

GENERAL DISCUSSION

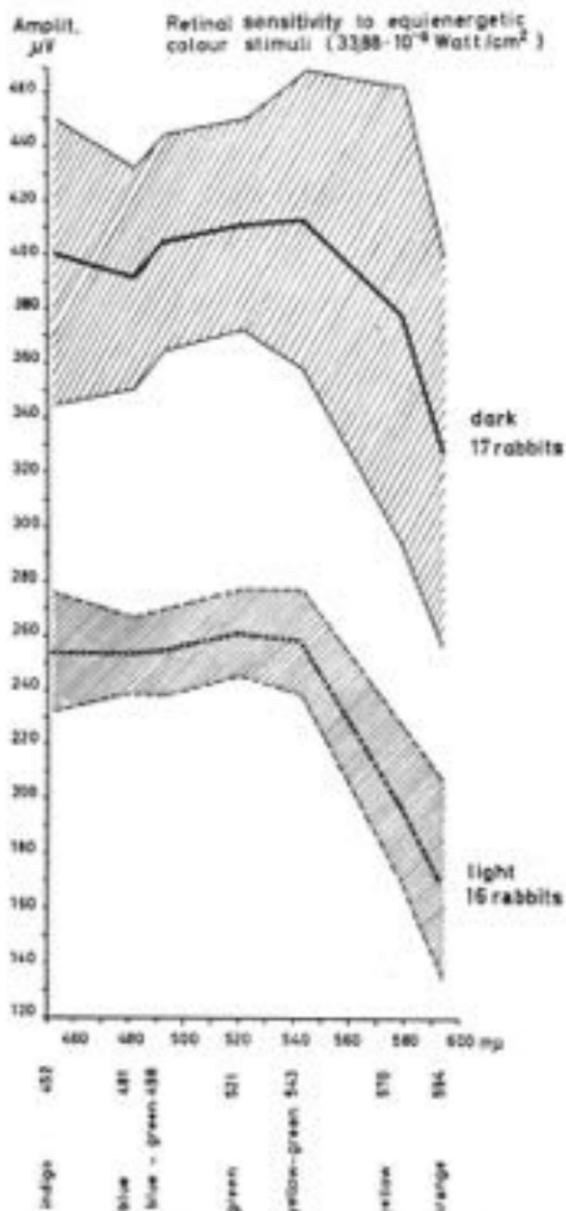


FIG. 2 (Monnier). Average curves of retinal sensitivity based on variable electroretinogram responses to colour stimuli of equal energy.

— = dark adaptation (17 rabbits).
 - - - = light adaptation (16 rabbits).

tion 30 responses, using an analysis time of 250 minutes in both cases.

We plotted the variations in amplitude of the b-potential on the ordinate and the wavelengths on the abscissa. Since the various rabbits provided electroretinograms of different basic values, the average curve shows a great dispersion, especially in dark adaptation. However, in spite of this dispersion, there is a significant difference from the rhodopsin absorption curve, since the curves do not fall towards the short wavelengths as they do towards the yellow and orange bands. After light adaptation especially, the electrical responses of the retina to green-blue, blue and indigo stimuli keep a constant height. This suggests that the rabbit's retina has good sensitivity to short wavelengths, especially after light adaptation. By contrast, the steep fall of the curve towards orange (594 nm.) suggests that the rabbit has bad sensitivity to long wavelengths such as red (625 nm.), as already suggested by M. F. Washburn and E. Abbott ([1912]. *J. anim. Behav.*, **2**, 145). This animal probably does not see the scarlet coats of the English hunters!

Our findings are in agreement with Granit's results in rodents such as the rat (Granit, R. [1941]. *Acta physiol. scand.*, **2**, 93) and the guinea pig (Granit, R. [1942]. *Acta physiol. scand.*, **3**, 318). Recording the spikes from isolated elements of the retina in the guinea pig, he found in the photopic state some retinal elements responding with a maximum in blue (450-460 nm.) and others in green (530 nm.). Finally, R. A. Weale ([1955]. *J. Physiol. [Lond.]*, **127**, 572), measuring the reflected light from the retina in guinea pigs, found a shift of the absorption maximum from 470 nm. to 460 nm. after light adaptation, as in the rabbit.

From all these data we may conclude that the rabbit and rodent retinae have good spectral sensitivity for blue; this sensitivity seems to be more pronounced in the rabbit than in the guinea pig.

Lowenstein: Is the rabbit a nocturnal animal?

Pedler: It has a nocturnal retina, structurally.

Pickford: It seems that the cat and the rabbit are like extreme protanomalous subjects, with a shortened red end of the spectrum and very little red/green discrimination.

Lowenstein: As Dr. Dartnall remarked earlier, moonlight is redder than daylight.

LINES FOR FUTURE RESEARCH

Weale: I want to cast an eye into the future, especially in connexion with the work on pigments. Like everyone else I am thrilled by the results obtained by Dr. Marks on individual cones, and I am probably reflecting his own feelings in saying that ultimately it would be desirable to examine the pigments *in vivo* rather than *in situ* even though *in mortuo*, to quote Dr. Crawford. The method of reflectometry has provided one such possibility, but it is rather ancient; it goes back to 1897. It is clinical, since it is really an extension of the ophthalmoscope, and I think Dr. Rushton will agree that it has had its day.

Rushton: I should have thought that there are some useful things still to be done.

Weale: The question is whether they cannot be done by transmission densitometry rather than by reflection densitometry. This problem is relatively easy to tackle in animals; by an extension of Dr. Svaetichin's technique one can drill holes in the sclera and shine a light through them. I expect several people here have done this, but it has not yet been mentioned, and it should be brought up as a possibility for the future.

We have tried photographing rods through the eye. It is more complicated in man, but even here one can try new techniques. One can shine light in through the mouth and watch it coming out through the eyes; the spectrum is limited because of blood absorption, but it can be done. Optical colleagues can develop long working distance objectives, and it is feasible that we shall be able to isolate smaller and smaller areas in the living eye.

There are difficulties which the agreement between Dr. Marks'

data and other results should not be allowed to obscure. I think I am right in saying that Dr. Stiles' π_5 mechanism is maximal at 590 nm. and not at shorter wavelengths. I also think that the Stiles-Crawford effect of the second kind, the hue shift effect, will be extremely difficult to account for if the red-sensitive pigment is ultimately located at 570 nm. From that point of view I would be happier to see it at the longer wavelengths. This doesn't invalidate the data we have so far; it is possible that when the retina is removed one does not have to contend only with red light. Oxygen tension is altered, for example. But this is sheer speculation because we are talking about the future. It is also possible that the 570 pigment is a metastable product.

I don't want to mention the term "transient orange" because one is dealing with another part of the spectrum, but we know that even visual purple gives rise to light-sensitive secondary substances and it is possible that if you remove the cones from the retina you are not really dealing with a primary pigment.

Stiles: The interpretation of the hue shift in directional sensitivity depends at the moment on making certain assumptions as to how the primary intensity effect operates, and although, in common with J. M. Enoch and the Dutch workers, I have made the same kind of theory, I would think that the theoretical interpretation is so uncertain that this would not be a very cogent argument in considering the position of the maximum in the red curve. However, if I may turn from the directional effects to the question of the pigment in relation to psychophysical measurements, I would like to indicate a piece of computational work which might be worth doing.

It arises from a question put to me by Dr. Marks, who asked whether one could represent the data of colour matching if all the pigments in the human eye were of rhodopsin type. This question has not been attacked directly. What one has done is to select sets of sensitivity curves derived from colour matching. To justify such selections you have to make assumptions which you cannot

verify by ordinary colour matching; you have to introduce other ideas, such as colour adaptation studies, and then to select the particular set of three linear combinations of your colour-matching functions, to get what you might call your fundamentals, and which are conceivably pigment absorption curves. But it is an interesting speculation to investigate what would happen if the only assumption is that there are three pigments all absorbing by rhodopsin-type processes, that is, with that shape of spectral sensitivity curve. I don't think three of this kind will work but I haven't done the essential calculation, which is this.

We know that by expressing the colour-matching functions obtained by ordinary colour-matching methods in a particular co-ordinate system called the W. D. Wright system you can eliminate the effect of any pre-retinal absorption. You jettison part of your information in doing this but you leave some of it behind. If you have three rhodopsin-type curves and allow the wavelengths of the maximum to vary in various ways, you can calculate the colour-matching functions for any positions of these three maximal wavelengths. When you have reduced the actual colour-matching data to the form which eliminates the effect of any pre-retinal pigmentation you can compare what you have with the predictions, similarly reduced, of any set of three rhodopsin-type pigments. Using a computer you can determine what particular set of three wavelengths of the maxima will give the best fit to your experimental colour-matching data. In order to do this at all rationally you must know the probable error associated with your colour-matching data. We have some information of this kind, at least for 10° colour-matching values (Stiles, W. S., and Burch, J. M. [1959]. *Optica Acta*, 6, 1). It should be possible to make the calculations, either allowing λ_{\max} for all three rhodopsin-type curves to vary, or perhaps fitting one or two of the λ_{\max} values from other considerations.

For any derived optimum set, you could then determine the corresponding absorption of the pre-retinal (inert) pigment

which would have to be combined with the rhodopsin-type curves to get the actual data. I don't think that any set of three rhodopsin-type curves will give results within the actual known errors in the colour-matching data, but even so, the analysis could be instructive.

Svaetichin: May we consider the problem of just where and how colour matching occurs in the retina and in which histologically-known structures one may imagine that this occurs; and which are the laws we know from psychophysics on which we have to base this?

Land: I can make one contribution about where it does not occur, starting from the other end. If using one set of conditions you generate a colour in a scene and give it to one eye, and give the same colour to the other eye, but under an entirely different set of conditions—different wavelength composition, different energy—if the colours are the same they will fuse cortically; if the colours are different you will have retinal rivalry independent of what the composition is at the retina. Cortical fusion depends on the fusion of the sensation and is independent of the structure of the stimuli. So that is the other end of what you are talking about.

Weale: I can throw some light on the very beginning. We did an experiment in which we produced trichromatic matches with monochromatic lights and then bleached the human fovea with both the monochromatic lights and the trichromatic matches, and the bleaching produced was the same with fairly different stimuli; you can test this in various parts of the chromaticity diagram. So it seems that if you produce the mixing at the retinal level, the pigment level, it will remain mixed.

Stiles: How intense was your adapting light?

Weale: It was of the order of millions of trolands.

Stiles: I am surprised you didn't upset your matching, because such high intensities are certainly likely to do this.

Weale: The actual matches were done at a lower level and then both the monochromatic light and the matches were increased by

the same amount. Whether or not they were still matched at those high intensities is impossible to tell, as the lights are just white, or very nearly so.

Pirenne: With regard to this problem of where colour matching occurs, the simplest logical approach, in the case of uniocular matching, is to say that two lights A and B having different spectral compositions will match when light A and light B act in the same way upon all the different retinal receptors involved. Thus the stimulation produced in cone X by light A is the same as that produced by light B. The stimulation caused in cone Y is also the same whether light A or light B is used; and so on for all the other cones or receptors involved. Since none of the receptors is able to detect a difference between lights A and B, no subsequent nervous mechanism will be able to do so, and there will be a match between A and B. On this basis colour matching occurs at the most peripheral level, that of the retinal photoreceptors (cf. Pirenne, M. H., and Marriott, F. H. C. [1962]. *In The Eye*, vol. 2, ed. Davson, H. New York and London: Academic Press).

Svaetichin: I think a problem remains as far as the physiology of colour matching is concerned; I agree there is no point in discussing this purely on a psychophysical level.

Rushton: I agree with what Dr. Pirenne has said; metameric matches are matches which have exactly the same bleaching power in all the pigments involved, so the inputs to the physiological mechanism must be identical and then everything that subsequently happens must also be identical; there is virtually no evidence to the contrary. As Dr. Stiles says, the matches break down at very high values, probably because, as Brindley analysed it, there is self-screening which is removed, but otherwise I don't see that there is any problem unless you are going to go away from the fundamental notion of three cone pigments, which as far as I know satisfies everything that we know on the subject of colour-matching. If you have no objection to that, I think that is the complete answer.

Dr. Land spoke earlier about photographic sensitizers. Would it be possible to have a red sensitizer in connexion with a pigment, to help us to see in the red, and would that be expected to change the shape of the Dartnall curve?

Land: This raises the question of what the mechanism of energy transfer from these pigments is, because to me one of the more attractive ways of transferring the energy would be the way it is done in photography. A very small amount of dye spread through the receiving element would transfer the energy directly to some nervous mechanism—in which case dark adaptation becomes a very simple mechanism. If you remove part of the dye by bleaching, it is not there to do the absorbing; the rates are about right; the dye could go up and down in effective concentration as the light changed slowly.

You have raised the further question, Dr. Rushton, of whether one could use the sensitizer with another pigment; the answer would be "Yes, easily", provided that in the system all energy transfer is by the solid state crystal mechanism. However, rabbits living on green leaves, or human beings, who want to see grass, don't want too much sensitivity in the far red, because that will give a negative image that will destroy the contrast of the positive image. The rabbit falls off in sensitivity there for a very good reason, because he is a grass-cater. So you could use the sensitizer to give you a deformation of the curve but in my opinion you do not want too much of it.

Stiles: Surely the answer is definitely "Yes", because if you take a red sensitizer in a photographic system you get a hump on the descending part of the original absorption curve, and the resultant is certainly not the same shape as the original. I would think the answer to this suggestion, which has intrigued me for some time, is that this is the kind of thing that might be wanted.

Dartnall: There is at least one case where you might have a photosensitizing system, and that is in the green rod of the frog. There is unfortunately a contradiction in the literature here.

W. Kühne ([1878]. On the Photochemistry of the Retina and on Visual Purple. Ed. Foster, M. London: Macmillan) says that the green rods are bleached by red light, but E. J. Denton and J. H. Wyllie ([1955]. *J. Physiol. [Lond.]*, **127**, 81) say they are not. However, they drew their red lights from different parts of the spectrum.

Rushton: Donner and I measured the electrosensitivity and we confirmed Denton and Wyllie. That is to say, there was blue sensitivity and red light didn't do anything.

Donner: We later used quite strong red fields but did not get any depression of the green rod sensitivity in the blue part of the spectrum (Donner, K. O., and Denton, E. J. [1962]. *Vision Res.*, **2**, 352).

Dartnall: How then does one account for this statement of Kühne's?

Svaetichin: We expected to encounter the so-called green rods in the suspensions of rod outer segments obtained from toad and frog retinæ. However, our microspectrophotometric measurements carried out on more than 200 individual rods showed only one absorption maximum at 500 nm.

Donner: Did you ever inspect a freshly-dissected retina?

Svaetichin: I agree with you that the green rods are nicely seen in the light transmitted through the receptor layer of the isolated retina, but I wonder if it could be some kind of "optical artifact".

Dartnall: How does one account for the fact that this optical artifact apparently contains a visual pigment with λ_{max} at 430 nm.?

Svaetichin: I really don't know, but we should have encountered at least 20 green rods among the 200 frog and toad outer segments that we measured. Possibly there is a selection in the procedure of making the suspension of the receptors.

Rushton: This "artifact" was photographed by Denton and Wyllie ([1955]. *Loc. cit.*).

Crescitelli: In the crab-eating frog there is quite a large amount of the blue-absorbing pigment, much larger than Dr. Dartnall and Dr. Donner found in *Rana esculenta* and *R. temporaria* respectively.

Dartnall: Why are the green rods of the frog green? Why do

they have a photostable red-absorbing pigment, as well as a photosensitive blue-absorbing pigment, do you suppose? If this red-absorbing pigment does not act as a photosensitizer, can anyone think of a function it might have?

Pedler: Could I suggest that in the future, since we are discussing future research needs, we might concentrate a little less on pigments and more on retinal function. The problem here is to find out how the transduction takes place, as Dr. Land was saying. The pigments may do what we say they do; but how does the information they generate get transferred to the cortex? What form of information is this? For this we need not only electron microscopy and photochemistry, but we need to break down the barriers more thoroughly than we have done before and to enthuse mathematicians who specialize in logic networks and this sort of thing. I think this is a promising trend for the future, in conjunction with electron microscopy and all the other techniques. We can no longer be pure morphologists.

FUTURE INTENTIONS OF PARTICIPANTS

Dartnall: Our future intentions in this part of the field lead on from the work I presented in my paper. You will recall that as the result of our survey of the visual pigments of British and Mediterranean fishes, Dr. Lythgoe and I think we have evidence for a clustering of λ_{\max} in five parts of the spectrum. This seems to us a finding of some importance that is almost bound to have a structural implication, and in my talk I put forward my suggestion of what I thought this might mean in structural terms. What interests us particularly is to what extent this clustering phenomenon is spread throughout the rest of the spectrum. To discover this we have to look for another batch of visual pigments from another environment, a batch with λ_{\max} outside the range we have so far studied. As Dr. Crescitelli has said, the visual pigments of terrestrial animals form a fairly invariant group. They mostly have the 500 nm.

pigment; his geckos are an interesting exception. We think a collection of 30 or 40 fishes from the yellow waters of the Nile (for example) might provide some more cluster positions (assuming the pigments are adapted to this yellow environment). If we could add to the five we have already found, we should be happy.

Land: Have you persuaded a good theoretical chemist to examine the series of resonance systems?

Dartnall: Well, I even hope to try this myself. I thought I would work on some fairly simple polypeptides, whose structure is known, and try to couple them with retinene₁ and retinene₂. It would be interesting to determine the λ_{max} values.

Land: One wants to find out whether it is an interruption of conjugation or a loading that is equivalent to more conjugation.

Munz: The transition between retinene₁ and retinene₂ pigments in the same species is a very interesting matter and one that has not been investigated fully as yet. It seems from our evidence that control of the proportions of retinene₁ and retinene₂ is rather different in the salmonids from the control that seems to be present in the rudd. My intention is to look into the influence of photo-period as opposed to total amount of light, and I hope also of wavelength, that is, the action spectrum of the retinene₁-retinene₂ control mechanisms.

Pedler: We are working now on the structure of the outer plexiform layer next to the receptors, using a combination of serial reconstruction, silver impregnation and information theory. I think here lies the clue to the function of receptors, and so we are going to try to work out how the receptors are connected to the elements of the second neurone, to make some hair-raising assumptions about them, and to put it into the form of electronic hardware. Then we want to construct models of what we have found out in the living system.

Sjöstrand: We have roughly the same ambitions as Dr. Pedler. I also want to see whether we can get additional information on the molecular structure of the outer segments, and with the informa-

tion we already have and the improvement of resolving power, which has improved to 15λ from 30λ a couple of years ago, to see whether there is any structural difference in connexion with dark and light adaptation; I don't think it is very hopeful, but it is something that should be looked at. I also hope to collaborate with physiologists to study the embryonic development of structure in the retina, and correlate that with the development of function.

Louvenstein: One thing which I hope you will do is to check up again on membrane continuity, because to us physiologists this is very important, and I had a great shock to hear you say that you had given up the idea that there is continuity.

Sjöstrand: That is one of the first problems that I want to look into.

Willmer: I would like to raise the question of whether it is legitimate to take the colour-blind subject as an example of what is occurring in the normal, because if it is, the work we did some years ago on the foveal centre of colour-blind people throws considerable light on the mechanism of colour vision. If you restrict the visual field of a colour-blind person so that he is only using the foveal centre (by using a field something of the order of 30 minutes across and with central fixation), he becomes totally colour blind and has no sensation of colour at all. I interpret that to mean that he has only one type of receptor, and therefore if you plot the sensitivity curve of that individual under those conditions you obtain the sensitivity curve of the one type of receptor that he happens to possess. This applies both to protanopes and deuteranopes, and the curves produced are almost superimposable on the chlorolabe-erythrolabe curves. If you assume that these are the two curves that you get in the normal fovea, it is legitimate to say that the curves will probably cross somewhere, and the simplest assumption is that they cross at the point in the spectrum which the normal person, when his vision is confined to a small field, matches with white. In other words, there is a wavelength, 574 nm. , which the

normal person matches with white when he is restricted to this small field and central fixation. The differences between the two spectral absorption curves (plotted logarithmically) also measure the saturation of hues which the normal person sees as he observes wavelengths on either side of 574 nm. That being so, it would seem that the mechanism of colour must depend on a differentiating machine and that if there is no mechanism for distinguishing the outputs from the two types of receptors, then a colourless sensation is evoked. As soon as the receptors are responding differently, colour is evoked, and the intensity of the colour is related simply to the difference between the ordinates of the two spectral log sensitivity curves. In other words, if you have a cell which is detecting the difference between the responses of the two primary receptors, and it is fairly easy to visualize mechanisms which would do this, as soon as that cell responds it can send a message up to the brain, and tell you that this is (say) greenness, and the extent of the response of that cell will tell you the extent or saturation of the greenness; and *vice versa* for redness. The whole approach is sensible but it may break down on two grounds: one is that you are restricting yourself to this absurdly small field and so errors in measurement are rather large; secondly, you are assuming that the protanope and the deuteranope have the same sorts of cones as the normal person has. I would like this problem reinvestigated with more modern methods.

Rushton: This particular wavelength number you mention, 574, is of interest with regard to C. H. Graham and Y. Hsia's one-eyed deuteranope ([1958]. *J. opt. Soc. Amer.*, **48**, 614). They put lights at various wavelengths into the bad eye and asked her to match a normal spectral output from the other eye until the colour was the same. She matched the whole of the red-green range of the spectrum with 575 or something like that, so this is a match on a large field and not on a small field. Secondly, it may be of interest in the theory of the Bezold-Brücke effect, the change of hue with intensity of light. The invariant hue, where the output of the two

might be expected to be the same, is also just about this same wavelength, 575 nm. And this also is the wavelength where Stiles' π_4 and π_5 cross. It would be very nice if the rate of bleaching of the red and green pigments were here equal but unfortunately, by my measurements the red pigment, erythrolabe, seems to bleach much faster than chlorolabe.

Weale: I cannot confirm that but I can confirm that the Bezold-Brücke invariant hues don't exist; according to J. D. Moreland they disappear when precise methods of measurement are used ([1955], Ph.D. Thesis, University of London).

Land: Dr. Willmer's feeling about this is a good feeling. We have to be careful to think of the two curves as important as far as shape goes but independent of each other as far as the vertical axis goes; that is, they may not be interchangeable in terms of energy, and it is only under special conditions that you can treat them as if they were drawn on the same graph paper, as it were, although their shapes become continuously important.

Willmer: There is a big step between the primary receptors and what the cell which is differentiating is going to pick up. The green receptor is giving a certain number of impulses per second and the red receptor is giving a certain number of impulses per second, or a certain change, anyway, and this third cell is being acted upon by those two in opposite senses, so that if the green receptor, for example, is more active than the red receptor, green will be perceived. One has first to find how the energy of the absorption event is converted to the event which is to be subtracted.

Crawford: I am working on colour matching, and it is curious that whatever experiment one does, something new always turns up unexpectedly. We have hit on several effects which seem to be interesting. There is an effect of adaptation, for instance, an account of which will be published very shortly; this is the effect of adaptation at quite ordinary levels of illumination, not of vigorous bleaching-out of most of some pigment or other. What happens is that, according to the conditions of adaptation and the colour of

the field that one is looking at, the eye produces different colour-matching functions. We don't know why, but they seem very reproducible and they occur for the fovea, for the parafovea, for large fields and for small fields, so it is obviously something fairly fundamental. At the same time we have discovered what appears to be a lability of colour-matching functions; they drift about.

I might put in a slightly frivolous word here for Professor Pickford's benefit: when a physicist wishes to discover the average performance in colour matching he takes a hundred observers; on the other hand, if he were to take but the one observer and find out how his colour-matching processes work I feel he would have achieved a great deal; one observer is sometimes justifiable and may produce results of great significance even though every other observer in the world is more or less different. After all, we communicate with each other because we assume that we are approximately identical.

We are also trying to investigate the occurrence or non-occurrence of additivity, that is, linear-additivity, in the colour-matching processes of the eye. This is rather tiresome to investigate with accuracy, because the effects show at their greatest in the parafoveal regions of the retina where precise psychophysical observation is most difficult, but nevertheless it seems to be producing results which are unexpected, and of course, as in pure physics, where some famous man said "Small corrections are the fun of physics", these divergencies from the laws of the past provide not only interest, but progress in our knowledge.

Pickford: I am sorry if my remarks should be taken amiss; no one could be a greater admirer than I am of the work of people here, and it is quite true that some psychologists have almost gone too far in their insistence on statistics, and will not agree that anything is important unless it has statistical significance. This means that very often they simply don't realize that they may have found something out. I have tried to show this in discussing my frequency tables.

Crawford: Nothing is significant unless it is statistically significant.

Pickford: And nothing is statistically significant unless it is related to credibility: that is the real criterion. We have to give the statistical probability, and then it is a question of how much confidence to place on the result of our experiment. Many results which do not reach generally accepted levels of confidence are most important and interesting. I have the impression, in any case, that the psychologist is interested in differences between individuals to a greater extent than the physicist or physiologist, but this depends on one's outlook.

Marks: My future interests were expressed by Dr. Pedler, who said that he intended to use the electron microscope to examine many cells simultaneously, which is no problem with the electron microscope, and to apply a mathematical analysis to the interrelations. But it is a problem with microelectrodes, and my interest is to try to study the electrical activity of many cells simultaneously and apply a mathematical analysis to the interrelationships.

Pickford: One of my aims is to follow up the colour vision of women who are heterozygotes, if we can get enough of them, and see how far Mary Lyon's theory of the inactivation of the X-chromosome is relevant here (Lyon, M. F. [1962]. *Amer. J. hum. Genet.*, 14, 135). Dr. Rushton mentioned the colour vision of hemizygotes; if we could identify them, which is quite a problem, we should find out something about their colour vision. Another interesting problem is that yellow-blue variations of a small and even larger kind are really quite frequent in the population; I would like to test several hundred people and to follow up those who showed any yellow-blue abnormality by testing their relatives, and so to work out something about the genetics of small yellow-blue variations. The greatest thing of all would be to test human groups with something better than the Ishihara test and to see whether one could identify the frequencies of the different types of colour blindness in different human groups. This is a world-wide problem.

Padgham: There are four things my colleagues and I are trying to do. I am hoping to measure continuously the colour of after-images as they decay, and by analysis of the colour-mixing data perhaps to find three colour receptors which have different decay curves. We are also trying to carry out the suggestion which Dr. Stiles made after Dr. Land's talk; we are trying to measure binocularly the apparent colour of areas. To take a simple example, if you take a large green disk and have a small white spot in the middle, the spot looks pink or red. I would add three stimuli to try to match that red sensation and relate the luminance to the colour in the other eye. We are also carrying out the direct estimation technique of S. S. Stephens to try to obtain a scale of apparent brightness. The final thing we are trying to do is to measure the photopic luminous efficiency curve of the human eye at high luminance. The standard CIE photopic luminous efficiency radiation curve was based on measurements all of which were below the level of luminance necessary for certain photopic vision.

Rushton: My chief interest for a long time has been adaptation. I really started investigating the pigments to see how much they controlled it, and now I have an idea of how much they do. But the subject of adaptation will still be a major problem in vision when I am dead and gone. The pattern seems to be that adaptation occurs not in the receptors but somewhere further down the line. I hoped that the S-potentials would give a clear indication of the mechanism that represented negative feed-back; we haven't been able to get any evidence whatever to support that, although it is a plausible idea and I still believe something of the sort must be true.

The question I am getting more and more interested in is the effect of the spread of the surround on adaptation, the sort of thing that Dr. Land has been describing and the sort of thing that Dr. Stiles and Dr. Crawford were analysing at the end of the twenties: how much is due to scattered light and how much to nerve interaction; how much is integrated at the retinal level; how much depends upon integrations at much higher levels. These are

difficult problems but they infiltrate so many aspects of vision that there is a prodigious variety of approaches that may yield some information. I think adaptation is even more fascinating than colour and the study, Sir, less bedevilled by lunatics!

Svaetichin: I entirely agree with you, Dr. Rushton, in your views on retinal adaptation. In this connexion I would like to remind you of some of our experiments which clearly show that the receptors are linear over a considerable range of intensities of illumination (Fatehchand, R., Svaetichin, G., Mitarai, G., and Villegas, J. [1961]. *Nature [Lond.]*, **189**, 463; Fatehchand, R., Laufer, M., and Svaetichin, G. [1962]. *Science*, **137**, 666). This means, of course, that some mechanisms subsequent to the receptors must be responsible for the Weber-Fechner non-linearity.

Pedler: Some recent work of ours might be of interest to Dr. Rushton. I did experiments on 23 lizards in conditions of dark adaptation and found that there are many ultrastructural changes, and only one of these is in the neurones; the rest are in the radial fibres of Müller.

Weale: If adaptation has nothing to do with the receptors, why did you show us a slide of the correlation between pigment regeneration and adaptation, Dr. Rushton?

Rushton: That all the light and everything that follows from it is due to the receptors is not to be doubted. The ultimate cause of anything that happens in light is that the quanta are caught, and of course they are caught in the receptors; but adaptation is the result of what happens after the receptors have caught their quanta and transmitted their signal; after that they are no less able to catch a quantum than they were before except by the trivial amount by which they have lost visual pigment.

Marks: In the diagram you showed us of that correlation, Dr. Rushton (Fig. 1, p. 269), there was a quantity β which I recall represents the amount of bleached pigment. Is that a fortuitous connexion down into the neural loop or does the neural loop know the value of β somehow?

Rushton: β is an influence, probably a chemical one, and depends upon the amount of pigment bleached, but I think that the rods themselves respond to the catching of any quantum at any time by a single invariant signal. What that signal does depends upon the organization of the retina.

Land: As I indicated, we feel that the really remarkable phenomenon is the persistence in the steps of the lightness scale at random variability of illumination, and so one of the first things we want to understand is how that can be; how does the eye know what the lightness scale is when a physicist couldn't possibly tell you what it is?

Secondly, since the opponent phenomena on the unitary level seem to be real, we need some way of getting from those to the concept of the lightness scales associated with the separate pigments. Finally, we are interested in the treatment of the spot, and the spot in the surround, as a mathematically degenerate example of the generalized lightness scale.

Cresitelli: One of the things that I am very much interested in now is the part of the visual pigment that few have paid much attention to, the protein, and so I shall continue looking at the visual pigment from the point of view of the conformation of the retinene to the protein. In addition to the visible region I plan to examine the circular dichroism at different pH values, and in the ultraviolet region, to see how bleaching alters the circular dichroism. In addition, I am going to investigate the bullfrog tadpole, where we know that a change from porphyropsin to rhodopsin occurs during metamorphosis. I want to take the opsin at different stages of metamorphosis and combine it with the specific retinene₁ and specific retinene₂. This is an experiment I have long been wanting to do but the pure compounds were not available; we now have available chemically pure retinene₂.

Meyer: I am interested in colour vision in cats now for exactly the same reason as I was interested in it ten years ago. I am also interested in space perception, contour perception and the way they

relate to the cerebral cortex. In general, I am interested in doing with the cat what Professor Klüver did with monkeys.

Lennox-Buchthal: We are more interested in the monkey cortex, now, with reference to colour vision, and it has puzzled us that one finds opponent colour responses in the retina and in the geniculate which cannot be found in the area of foveal representation in the cortex. An opponent colour response has been found in the area of peripheral retinal representation in monkey cortex by K. Motokawa and his co-workers, but we find a trichromatic code in the cortex. How is this trichromatic code derived from the opponent colour code in the geniculate? Since our original work we have been able to use long colour flashes and have not found clear "off" units among these tightly colour-coded units. It is our working hypothesis therefore that the "on" characteristics of each geniculate cell are passed on to a single cortical cell in the case of the red and blue-sensitive units; only in the case of the green-sensitive units are there occasionally traces of opponent colour responses. To test our hypothesis, we have been applying a direct current across the cell through the recording electrode and have found, interestingly enough, that a negative direct current, the electrode as cathode, either excites the cell directly or increases its responsiveness to flash, quite opposite to the effect of direct current in cats. We are in the process of exploring this effect and its relation to colour coding in the cortex in more detail.

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Lowenstein: It is obvious that to sum up this most instructive symposium and our weighty discussions would be a practical impossibility. May we therefore accept the programmatic statements which we have just heard from the participants as a most fitting and at the same time forward-looking summary. I think you will all agree that the hope I expressed in my opening remarks has been amply fulfilled. We have together accomplished a good piece of work.

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