Ciba Foundation 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

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Under the guidance of its distinguished Trustees, the Foundation offers accommodation to scientists from all over the world at its home in Portland Place. Foremost in its activities is the organization of small conferences, the proceedings of which are published in book form in the manner of the present volume. The Foundation convenes many other informal discussions between research workers of different disciplines and different nationalities and each year invites an outstanding authority to deliver a special lecture. An exchange programme between French and British postgraduates is conducted and a library service is available. Furthermore, the Ciba Foundation attempts in every other way possible to aid scientists, whether they be Nobel Laureates or young graduates making their first original contribution to research.

The purpose of the Ciba Foundation, which is to promote international co-operation in medical and chemical research, is symbolized in the armorial bearings by five interlaced rings representing the continents, a black sacrificial cock (emblem of Aesculapius) holding a medical caduceus, and three regular hexagons for chemistry. Its domicile in London is indicated by the red sword of St. Paul and the British lion; the wyvern and the crozier, symbols associated with Basle, refer to the sponsoring firm located in this ancient Swiss town.

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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 (mμ) 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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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†

H. J. A. DARTNALL AND J. N. LYTHGOE

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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 retinene$_1$ or on retinene$_2$. 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 retinene$_1$ series the known pigments have λ_{max} ranging from about 430 nm. to 562 nm.; in the retinene$_2$ 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 retinene$_1$ series and 110 nm. in the retinene$_2$ series. The retinene$_2$ pigments in

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† This paper was presented to the symposium under the title "Relations between retinene$_1$ and retinene$_2$ visual pigments."

general have absorption bands situated at longer wavelengths than those of the retinene$_1$ pigments, but there is a considerable region of overlap. Another obvious fact is that the retinene$_1$ pigments are much more numerous than those based on retinene$_2$. Future discoveries 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 retinene$_2$ pigment at 543 nm.

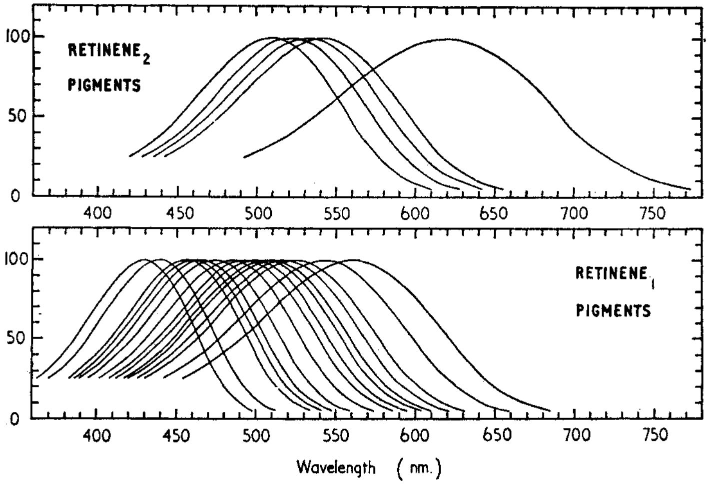


FIG. 1. The main absorption bands of the visual pigments in the two series. The λ_{max} of retinene$_2$ pigments range from 510 nm. in the wrasse (*Labrus merula*, Brown and Brown, cited by Wald, 1960) to 620 nm. (cyanopsin, Wald, Brown and Smith, 1953), and those of the retinene$_1$ pigments from 430 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.)

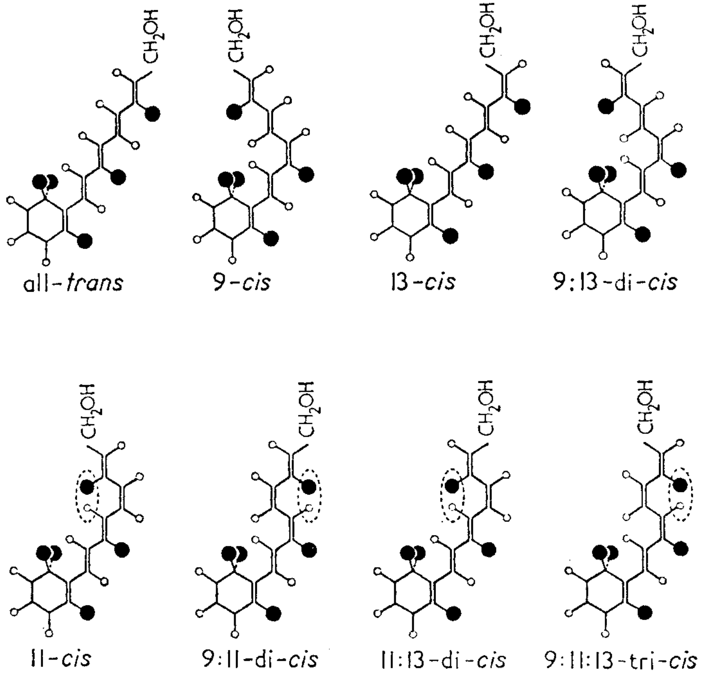
How are we to account for the remarkable variation in spectral properties of the visual pigments, a variation that enables species to evolve pigments suitable for their photic environment, and, in some species where more than one pigment is possessed, possibly to enjoy colour vision? It is instructive 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 retinene$_1$ or on retinene$_2$. 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 retinene$_1$ and of vitamin A$_1$ should be possible. The structural formulae of these are shown in Fig. 2. Since retinene$_2$ and vitamin A$_2$ differ from their "A$_1$" 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 A$_1$ or A$_2$, 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 retinene$_1$. 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 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,



all-*trans* 9-*cis* 13-*cis* 9:13-di-*cis*

11-*cis* 9:11-di-*cis* 11:13-di-*cis* 9:11:13-tri-*cis*

FIG. 2. Structural formulae for vitamin A_1 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 ($—CH_3$) groups. Similar formulae apply to the vitamin A_2 isomers (additional conjugated double bond at carbon 3 in the ring structure), and to the aldehydes (retinenes) of the vitamins. (From Dartnall, 1962*b*.)

1953, 1962*a*) 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" ($\lambda_{\text{max}} = 500 \pm 2$ nm.) and the classical cone pigment iodopsin ($\lambda_{\text{max}} = 562$ nm.) in the retinene$_1$ series and between the analogous "porphyropsin" ($\lambda_{\text{max}} = 522 \pm 2$ nm.) and cyanopsin ($\lambda_{\text{max}} = 620$ nm.) in the retinene$_2$ 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, Dobelle 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 $retinene_1$ and $retinene_2$ series.

RELATION BETWEEN λ_{max} OF $RETINENE_1$ AND $RETINENE_2$ PIGMENTS IN IDENTICAL-OPSIN PAIRS

Wald, Brown and Smith (cited by Wald, 1953) found that the opsin of a $retinene_1$ pigment could be induced to combine with a suitable isomer of $retinene_2$ to form a synthetic analogue. The opsin used was derived from the 498 nm. visual pigment of cattle. The synthetic $retinene_2$ pigment so formed had $\lambda_{max} = 517$ nm., that is, 19 nm. higher than the natural $retinene_1$ pigment. Wald, Brown and Smith (1953) also performed a similar experiment using the opsin of chicken iodopsin ($\lambda_{max} = 562$ nm.). The $retinene_2$ analogue ("cyanopsin") formed in this case had $\lambda_{max} = 620$ nm., namely 58 nm. higher than the natural $retinene_1$ pigment.

These two examples are the only direct substitution experiments that have been carried out. There are several instances, however, where a $retinene_1$/$retinene_2$ 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 $retinene_1$ pigment of $\lambda_{max} = 510$ nm. and a $retinene_2$ pigment of $\lambda_{max} = 543$ nm. Under natural conditions the proportions of these two pigments vary according to season, the 543_2 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 proportion of the retinene$_2$ 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 retinene$_1$/retinene$_2$ pair of pigments have been found to behave similarly (Dartnall, 1962*a*, p. 411; Bridges, 1964*a*).

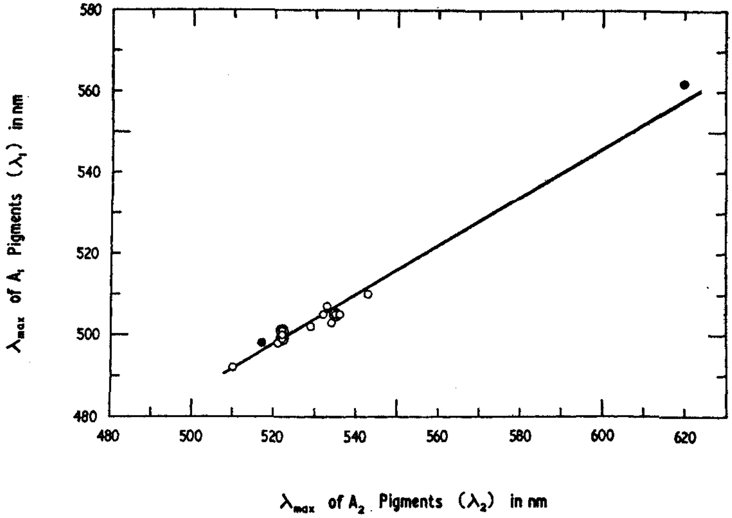


FIG. 3. The relationship between the λ_{max} of retinene$_1$ and retinene$_2$ pigments in identical-opsin pairs. Empty circles represent the sixteen naturally occurring pairs and filled circles, the data obtained in Wald, Brown and Smith's direct substitution experiments (see text). Equation to the line is $\lambda_1 = 0 \cdot 60\,\lambda_2 + 186$.

In general whenever a species contains *only* a retinene$_1$/retinene$_2$ 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 retinene$_1$/retinene$_2$ pair. In Fig. 3 the λ_{max} of the retinene$_1$ pigment is plotted against that of its retinene$_2$ counterpart for sixteen of these pairs (empty circles). The two species omitted from this plot are the tench (467_1 and 533_2) and the scarid, *Sparisoma cretense* (486_1 and 520_2). The tench pair are omitted because it is not absolutely certain that the 467 pigment is based on retinene_1 (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 retinene_1 and retinene_2 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 Marseilles, 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 retinene_1, although retinene_2 pigments were also present in a few instances (see Table I). The λ_{max} of the retinene_1 pigments were found to extend from 468 nm. to 528 nm., a result that significantly widens the known range of retinene_1 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

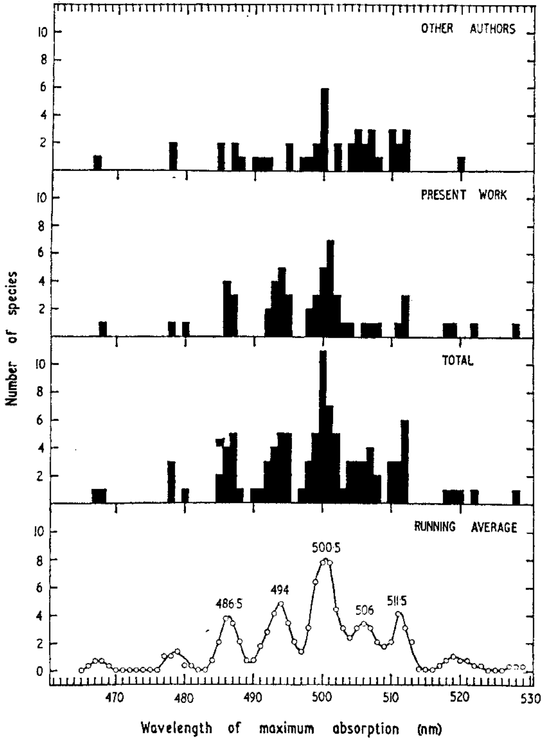


FIG. 4. The distribution of retinene$_1$ visual pigments in teleost fishes. The histograms give the plot of pigment λ_{max} against frequency of occurrence (number of species). Previously published data, present work, and the total data are separately shown. The "running average" curve was obtained from 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 retinene$_1$ pigments from 41 species; the work of previous authors gives 45 retinene$_1$ pigments from an additional 42 species. Thus the total survey includes 102 retinene$_1$ 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 retinene$_1$ 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 retinene$_1$ and retinene$_2$ series (Fig. 3) it can be calculated that the *retinene*$_2$ analogues of our retinene$_1$ 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 retinene$_2$ 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 retinene$_2$ analogues of our retinene$_1$ 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 retinene$_1$/retinene$_2$ 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 retinene$_1$ and retinene$_2$ pigments (Fig. 3). To answer this objection we need to consider the distribution of pigment λ_{max} in fishes that possess *only* retinene$_1$ pigments in the one case, and *only* retinene$_2$ pigments in the other.

Twenty of the species listed in Table I possess retinene$_2$ as well as retinene$_1$ pigments. Removal of these still leaves 63 species having retinene$_1$ 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 retinene$_1$ pigments.

The retinene$_2$ list (33 species) is more seriously depleted by removal of "paired pigment" species. Thus Bridges' 18 new species (Bridges, 1964*c*) include 9 such members, while the 15 retinene$_2$-containing species in the literature harbour another 7, including the only two 543_2 examples (rudd and chub with 543_2 and 510_1). The 17 species possessing *only* retinene$_2$ 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* retinene$_1$

pigments on the one hand, and *only* retinene$_2$ 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.

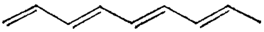
STRUCTURAL IMPLICATIONS OF CLUSTERING

Although this paper deals exclusively with fishes, a study of the lists of visual pigments of other animals (Dartnall, 1960*c*) 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 retinene$_1$ 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* retinene$_2$ positions, viz. 501, 513, 524, 534 and 543 nm.—four of which, in fact, are found in *natural* retinene$_2$ 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



(1)

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