NATIONAL PHYSICAL LABORATORY

SYMPOSIUM No. 8

Visual Problems of Colour

VOLUME I

LONDON: HER MAJESTY'S STATIONERY OFFICE
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SELIG HECHT
1892-1947

NATIONAL PHYSICAL LABORATORY

SYMPOSIUM No. 8

Visual Problems of Colour

A Symposium held at the
National Physical Laboratory
on 23rd, 24th, 25th September
1957

VOLUME I

LONDON: HER MAJESTY'S STATIONERY OFFICE
1958

PREFACE

This book contains the Proceedings of a Symposium on the Visual Problems of Colour which was held at the National Physical Laboratory from 23rd to 25th September, 1957. The first session of the Symposium was devoted to a Selig Hecht Commemorative Lecture by Professor George Wald, which is here reproduced. The forty papers which followed are grouped in these Proceedings under Sessions.

As far as possible, revisions suggested by authors to their papers as presented have been included; it is thought that no revision of scientific significance has been omitted. In some instances, new material has been added. Several contributors to the discussion supplied written copies of their remarks. The rest of the discussion has been compiled from the record taken of the proceedings.

The Laboratory wishes to express its thanks to Professor George Wald, to the authors of papers and to the gentlemen who acted as Chairmen of sessions.

One or two symposia are held at the Laboratory each year. Some are on subjects of direct industrial interest and others are on more academic themes. The printed proceedings of these symposia are published by H.M.S.O., the present volume being the eighth in the series.

NATIONAL PHYSICAL LABORATORY,
TEDDINGTON,
MIDDLESEX.

December, 1957.

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Chairman:
Professor Y. Le Grand
(Muséum National d'Histoire Naturelle, Paris)

* Unfortunately, the manuscript of this paper was not available at the time of printing.

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SESSION I

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THE SELIG HECHT COMMEMORATIVE LECTURE

VISUAL PROBLEMS OF COLOUR

Opening Address

by

G. B. B. M. SUTHERLAND

MY predecessor, Sir Edward Bullard, started symposia at the National Physical Laboratory about 1953 and we hold at least one each year, generally on a topic in which one of the Divisions has a special interest and has made significant contributions. This year it is the turn of the Light Division, which is under the guidance of your Chairman, Dr. Sayce.

The particular topic chosen is one which is not only of great practical importance, but also intensely interesting to scientists in four different fields, i.e., physicists, physiologists, psychologists and biochemists. We therefore invited representatives from each of these sciences to take part in this Symposium, and are most gratified by the responses we have had. On behalf of my colleagues I now welcome you to the National Physical Laboratory and hope that the time you spend here will be interesting, profitable and enjoyable.

In a very real sense this may be regarded as the first N.P.L. Symposium in Biophysics, a field which promises to be as exciting as that of nuclear physics. There is a growing realisation among physicists that, having dealt fairly successfully with the physical explanation of the properties of inanimate matter, the time has now come when they should turn their attention to the much more remarkable properties of living matter, hitherto the preserve of biologists and physiologists. Dirac once remarked that now that quantum mechanics had provided the equations governing the behaviour of electrons, the whole of chemistry was really just a branch of mathematics. In the same sense, it can be said that before too long physiology may become a branch of physics.

It is significant that one of the largest of the Research Institutes in Moscow is that devoted to Biophysics and that we have already planned a Symposium for November, 1958, on "The Mechanisation of Thought Processes".

You already know that this morning is being devoted, very properly, to the commemoration of a great pioneer in the field of vision - the late Professor Selig Hecht. What some of you may not know is that we have the privilege of Mrs. Hecht's company to-day, and on behalf of all of you I wish to thank her for making this long journey and to say how very pleased we are that she can be with us on this occasion.

(Dr. G. B. B. M. Sutherland, F.R.S., is Director, National Physical Laboratory, Teddington).

THE SELIG HECHT COMMEMORATIVE LECTURE

Introductory Remarks

by

The Chairman

EXACTLY ten years ago, a very successful Conference on Colour Vision was held in Cambridge, England.

At that Conference a leading part was taken by the pioneer American worker in the subject, Professor Selig Hecht.

Professor Hecht's sudden death immediately following his return to the United States deprived the world of a man whose ideas and investigations on vision had been a main line of advance for nearly thirty years.

Had he lived, it is certain that he would have been here with us today and we are greatly honoured by the presence in his place of Mrs. Hecht, his widow.

In tribute to his great achievements, this Symposium is associated with his name and this, the opening Session will be devoted to a Selig Hecht Commemorative Lecture given by his former student and collaborator, Professor George Wald of Harvard University.

THE SELIG HECHT COMMEMORATIVE LECTURE

RETINAL CHEMISTRY AND THE PHYSIOLOGY OF VISION

By GEORGE WALD

George Wald, born **18th November**, 1906, obtained his B.Sc. degree at New York University in 1927, majoring in zoology. In the same year, he began graduate work in zoology at Columbia University under Professor Selig Hecht. On receiving the degree of Ph.D. there in 1932, he was awarded a National Research Council Fellowship in Biology, which took him to the laboratory of Otto Warburg in Berlin-Dahlem. There Dr. Wald first identified vitamin A in the retina, completing the identification at Professor Karrer's laboratory in Zurich. Subsequently, he was with Otto Meyerhof at the Kaiser Wilhelm Institute in Heidelberg and then the Physiology Department of the University of Chicago.

In 1934, Dr. Wald came to Harvard University, originally as a tutor in Biochemical Sciences, becoming Professor of Biology in 1948. Among the many distinctions he has received may be mentioned the Eli Lilly Award for "Fundamental Research in Biochemistry" in 1939; the Lasker Award of the American Public Health Association in 1953, and the Proctor Medal of the Association for Research in Ophthalmology in 1955. Dr. Wald is also a Fellow of the American Academy of Arts and Sciences in Boston, and a member of the National Academy of Sciences.

THE SELIG HECHT COMMEMORATIVE LECTURE

RETINAL CHEMISTRY AND THE PHYSIOLOGY OF VISION

By GEORGE WALD

IT is a great privilege to offer this lecture to the memory of my former teacher, Selig Hecht. I have always been proud and glad to work, not in his shadow, but in his light; for he cast a wide and penetrating light in which at all times one could see just what was there, for good or ill; usually, I am glad to say, for good.

Hecht and I attended together the last such meeting as this, held in Cambridge ten years ago. After the meeting we spent several days together in Cambridge, in part with Dr. Stiles; and then several days in London. It was more than I had seen of Selig Hecht in all the years since leaving his laboratory in 1932; and I am grateful for it, for when I said good-bye to him in London to return home, it was the last time I ever saw him.

One of the great themes of Hecht's work was that much of visual physiology originates in the substances and processes of retinal chemistry. He went at this both ways, at times attempting to infer the chemistry from the physiology, a risky and difficult enterprise that led sometimes to happy consequences, sometimes disappointing, but which he pursued always with great boldness and skill. When opportunity offered, he also worked the other way, studying the chemistry directly and relating it as he could to the physiology. Indeed, he made signal contributions to all the areas I shall touch upon here.

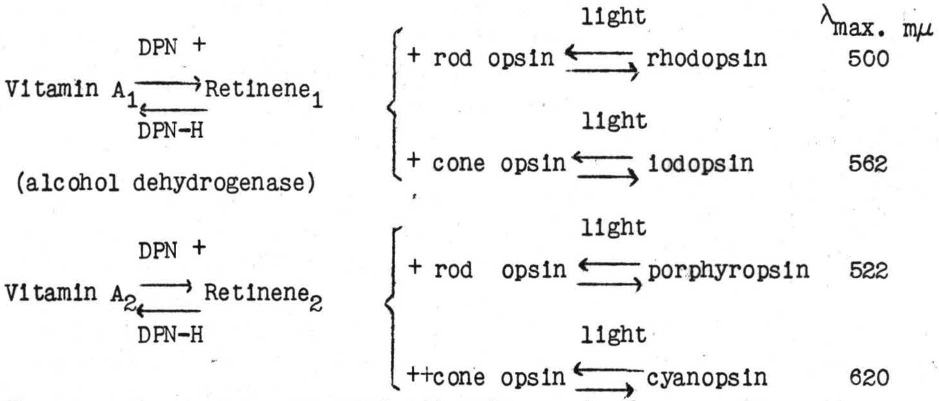
Not all of visual physiology can be approached in this way. That part of it which derives from the retinal elements involves two kinds of properties: individual and collective. The collective properties involve the interaction of large numbers of retinal elements, and are not to be sought in any one of them; so, for example, visual acuity, most of intensity discrimination, the flicker function, and, to a degree, colour vision. The individual properties come to reasonably full expression in each retinal element. Among them I would reckon spectral sensitivity, the slower components of light and dark adaptation, and the failure of vision in vitamin A deficiency known as night-blindness. It is specifically the individual functions, which come directly out of retinal chemistry, that are the subject of the present paper.

I. VISUAL CHEMISTRY

LET me begin by reviewing briefly what we know of the chemistry of the excitatory processes in the rods and cones. This has now reached a point of great simplicity. Most of it can be summarized in a few sentences and such a diagram as lies below.

To say it briefly - there are two vitamins A, A_1 and A_2, differing only in the fact that A_2 possesses an extra double bond in the ring. There are the two corresponding aldehydes, retinene_1 and retinene_2 *(refs. 1,2)*. There are also two opsins, specific proteins of the outer segments of the rods and cones; rod opsin and cone opsin. The combinations of the two retinenes with the two opsins yield the four major photosensitive pigments of vertebrate vision.

The retinenes and vitamins A form oxidation-reduction couples with reduced and oxidized Coenzyme 1 (DPN), catalyzed by the enzyme, alcohol dehydrogenase. The equilibrium point of this system lies far over toward the vitamins A, so that the retinenes tend to be reduced almost as rapidly as they are formed. In the dark, however, the opsins remove whatever retinenes are present to form the visual pigments. The system has then no recourse but to keep forming more and more retinene by the oxidation of vitamin A. To say this another way, opsin "traps" retinene in the form of visual pigments, so forcing the oxidation of more vitamin A to retinene, so that both these reactions proceed essentially as one. The entire array of systems can be summarized as follows *(refs. 3,4)*:



$$\text{Vitamin } A_1 \underset{\text{DPN-H}}{\overset{\text{DPN }+}{\rightleftarrows}} \text{Retinene}_1 \left\{ \begin{array}{lr} & \lambda_{\text{max.}}\ m\mu \\ + \text{ rod opsin} \overset{\text{light}}{\leftrightarrows} \text{rhodopsin} & 500 \\ + \text{ cone opsin} \overset{\text{light}}{\leftrightarrows} \text{iodopsin} & 562 \end{array} \right.$$

(alcohol dehydrogenase)

$$\text{Vitamin } A_2 \underset{\text{DPN-H}}{\overset{\text{DPN }+}{\rightleftarrows}} \text{Retinene}_2 \left\{ \begin{array}{lr} + \text{ rod opsin} \overset{\text{light}}{\leftrightarrows} \text{porphyropsin} & 522 \\ ++\text{cone opsin} \overset{\text{light}}{\leftrightarrows} \text{cyanopsin} & 620 \end{array} \right.$$

For present purposes one need add only one further consideration. This involves the *shape* of vitamin A and its corresponding retinene. These molecules exist in a variety of different shapes, geometric or *cis-trans* isomers of one another. In the most stable, all-*trans* configuration, the side chain of the molecule stands out relatively straight *(fig. 1)*. A *cis* linkage involves ordinarily a rotation of 180^0 at the double bond, resulting in a bend in the molecule. Such *cis* linkages are readily formed

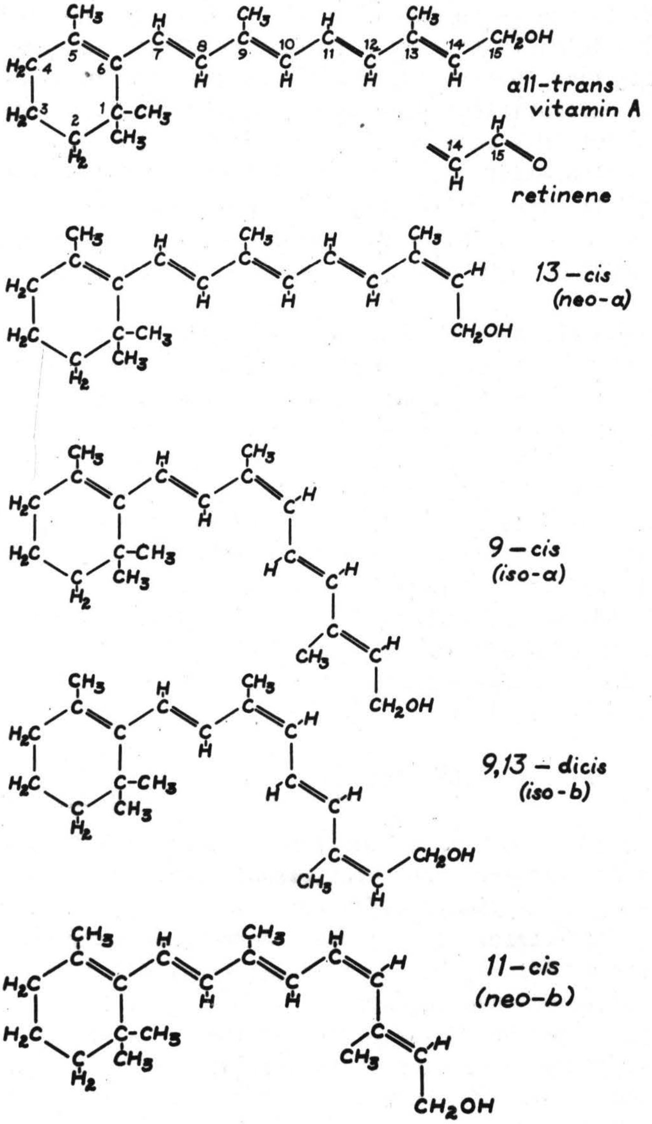


Fig. 1. Geometric isomers of vitamin A and retinene. A similar series of structures, differing from these only by possessing an extra double bond in the 3,4 position, represents vitamin A_2 and $retinene_2$. All the known visual pigments have the hindered *cis* configuration of $retinene_1$ or $retinene_2$, neo.*b* (11-*cis*), as prosthetic group. The 9-*cis* isomer, iso-*a*, forms with the same opsins a closely related series of photosensitive artefacts, the iso-pigments.

at double bonds 9 and 13. At double bonds 7 and 11, however, rotations of 180^0 cannot occur, because of steric hindrance between side groups, either $-CH_3$ and $-CH_3$ in the 7- position, or $-CH_3$ and -H in the 11-position. Hence one expected originally to find only four geometric isomers of vitamin A or retinene: all-*trans*, 9-*cis* (iso-*a*), 13-*cis* (neo-*a*), and 9,13 *dicis* (iso-*b*).

All these configurations have been isolated and identified *(ref. 5)*; but it has turned out that none of them forms a visual pigment. That job is done by a fifth configuration called neo-*b*, which contains a hindered *cis* linkage at double bond 11 *(fig. 1) (refs. 6,7)*. Such a hindered linkage represents not only a bend but a twist in the molecule; and the departure from planarity, since it interferes with resonance, had been expected to lead to a lowered stability.

In fact, however, the neo-*b* isomer is surprisingly stable, once formed; though apparently it requires special conditions, notably a special access of energy, for its formation. All the methods we have yet found for converting other geometric isomers of retinene to neo-*b* involve the use of light *(refs. 6,8)*. The light that is effective in this process includes of course only those wavelengths which retinene can absorb - blue, violet, and near ultraviolet light. Any geometric isomer of retinene in solution, irradiated with such light, is rapidly isomerized into a steady state mixture of all possible isomers, including neo-*b*. When such irradiation is performed in alcohol solution, a surprisingly high fraction of the product, about 25%, is in this hindered *cis* configuration. Using this method, we have crystallized a large amount of the neo-*b* isomer *(ref. 9)*. It has also been prepared by total synthesis *(ref. 7)*.

II. ONE RETINENE, MANY OPSINS

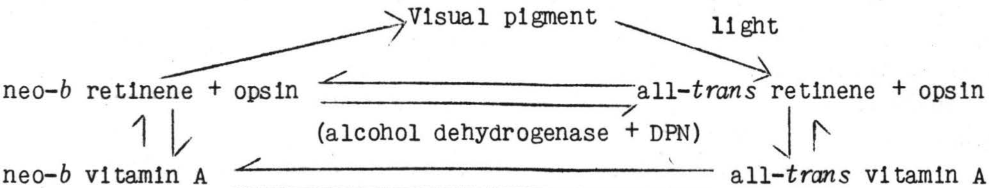
I think we can now be permitted two important generalizations: *the geometric configuration of retinene from which visual pigments are made is always the same; the opsin is always different.*

The retinene precursor of a visual pigment is always neo-*b*, whether one is dealing with vitamin A_1 or A_2, or the corresponding retinenes; and whether vertebrate or invertebrate visual pigments. It has recently been shown that the chromophore of squid rhodopsin is neo-*b* retinene *(ref. 10)*; and we may by inference conclude this also of the visual pigments of crustacea, since their eyes have so far been found to contain only neo-*b* vitamin A *(refs. 11,12)*.

This generalization may at once be coupled with another. In every instance the retinene which emerges when a visual pigment is bleached with light is in the all-*trans* configuration. Indeed, in her contribution to this Symposium, Hubbard proposes that the primary effect of light on a visual pigment is to isomerize its neo-*b* chromophore to all-*trans*. Since the all-*trans* molecule does not fit the surface of opsin as does the neo-*b* configuration, this transformation always results in some degree of instability. In vertebrate rhodopsins, at very low temperatures or in the dry condition *(ref. 13)*; and in some invertebrate rhodopsins even at 20°C and with access to water (squid: *refs. 14,10;* lobster: *ref. 15)*, the combination of all-*trans* retinene and opsin is relatively stable; this is metarhodopsin. Released from these special conditions, all-*trans* retinene and opsin part company; and this is bleaching.

A cycle of *cis-trans* isomerization is therefore an intrinsic component of all visual systems. In all of them retinene enters the visual pigment in the hindered *cis* form, neo-*b*, and leaves in the all-*trans* configuration. It must then somehow be bent and twisted back into the neo-*b* configuration before it can take part again in the regeneration of visual pigment.

All the types of visual system now known can therefore be characterized with some such diagram as follows:



In sharp contrast with this universality in the configuration of vitamin A and retinene, every animal species possesses a different opsin. For those who are not biochemists, I should like at once to re-state this generalization in the context of a wider one.

It is a truism in biochemistry that every species makes specific proteins, different from those of every other living species. So, for example, no two species of animal are known to possess the same haemoglobin. Haemoglobin consists of a ferroporphyrin chromophore called haem, bound to a type of protein called globin. The haem is always the same, but the globin is different in every species so far examined. Indeed, some species, as for example man, possess at least two different haemoglobins in each individual. Furthermore, the globins of vertebrates differ characteristically from those of invertebrates.

Despite all these differences, it has proved useful to call all these substances haemoglobin, designating each by the name of the animal from which it comes, and recognizing that some animals possess more than one haemoglobin, that vertebrate and invertebrate haemoglobins are characteristically different, and indeed all the complications that enter this situation.

This is also the state in which we find the visual pigments. The opsins of rods are different from those of cones; and every species possesses a specific rod or cone opsin. Indeed a few species (see below) appear to possess more than one rod opsin. Thus far, the opsins of invertebrates have proved to be characteristically different from those of vertebrates; we have already mentioned the relative stability of metarhodopsin in invertebrates as compared with vertebrates, which derives from this source. I think nevertheless that as with the haemoglobins we should be well advised to call all these pigments by a few great class names, recognizing that each name designates not a substance but a family of substances which differ and consequently have somewhat different properties in every species.

A signal instance of this situation lies in the recent discovery by Denton and Warren *(ref. 16)* that deep-sea fishes possess rhodopsins displaced in the spectrum relative to those of surface fishes. This has since been confirmed by Munz *(ref. 17)*. The λ_{max} of the deep-sea forms lies at 478-485 mμ; whereas surface forms have rhodopsins with λ_{max} near 500 mμ. Indeed, Munz has reported finding one deep-sea fish that has two rhodopsins, one with λ_{max} about 487 mμ, the other with λ_{max} about 500 mμ, both derived from retinene$_1$.

These observations raise two obvious questions: At what depth does this change occur? And what is its mechanism?

It hardly seems likely that the change from the surface to the deep-sea type of rhodopsin occurs abruptly. One should rather expect to find some more or less gradual transition with depth. We have recently examined a number of fishes from middle depths, and seem to find such a transition *(ref. 18)*. Little more is to be expected here than some degree of statistical regularity, for fishes wander widely in depth, and at every depth display a wide range of rhodopsins.

Nevertheless, some correlation between λ_{max} and depth has begun to emerge *(fig. 2)*. Rhodopsins obtained near the surface have λ_{max} about 500 mμ; the λ_{max} slips toward shorter wavelengths with increasing depth until at about 200-400 fathoms λ_{max} falls within the range 475-485 mμ. This is probably near the limit of the change, for it is about the limit to which surface light penetrates, even in the clearest ocean waters.

Of what type of retinene are deep sea rhodopsins made? Apparently of neo-*b* retinene, as are other rhodopsins. So for example, the visual pigment of the deep-sea lancet fish, with λ_{max} 480 mμ, bleaches to a mixture of opsin and all-*trans* retinene. When this mixture is incubated with neo-*b* retinene, it regenerates rhodopsin with λ_{max} 480 mμ, indistinguishable from that extracted from the lancet fish. The same is true of a fish from middle depth, the cusk, which possesses rhodopsin with λ_{max} about 488 mμ, and after bleaching regenerates with neo-*b* retinene the same pigment *(ref. 18)*. The peculiar characteristics of the rhodopsins of

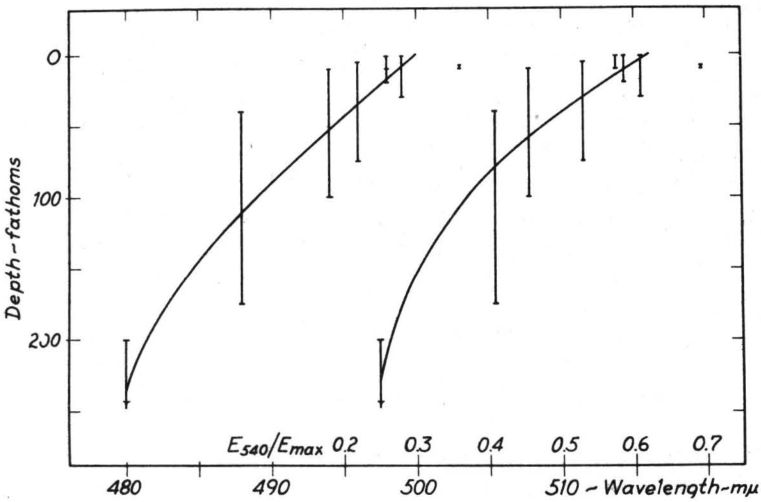


Fig.2. Variation of the absorption spectra of rhodopsins of marine fishes with summer range of depth. Left, λ_{max}; right, E_{540}/E_{max}, the ratio of extinction at 540 mμ to that at the maximum. This ratio offers a convenient and accurate index of the position of a rhodopsin absorption band in the spectrum. (Modified from *ref.18*).

deep-sea fishes are derived, not from any unusual retinene, but from distinctive *opsins*. The variation of λ_{max} with depth in the rhodopsins of marine fishes presents us with a particular instance of the general condition that every species possesses different opsins.

Lately we have encountered another peculiar instance of this generalization. The first absorption spectrum of rhodopsin to be measured was that of human rhodopsin. König had asked his colleagues Köttgen and Abelsdorff to prepare to measure the absorption spectrum of frog rhodopsin, hoping that with the skill so obtained they might eventually go on to human rhodopsin; but before that could be done, König was unexpectedly presented with a single human eye, from which König and Köttgen *(ref. 19)* prepared a solution of rhodopsin. Its difference spectrum had λ_{max} near 500 mμ.

About sixty years passed before human rhodopsin was again prepared, this time again from a single eye, and its difference spectrum was measured *(ref. 20)*. The λ_{max} was found near 500 mμ, and the authors concluded that the absorption maximum probably lay at 497 ± 2 mμ. They proposed that this rhodopsin be called "visual pigment 497."

Recently we have prepared reasonably pure human rhodopsin and find that its λ_{max} lies at 493 mμ, at a shorter wavelength therefore than that of any other terrestrial vertebrate so far encountered (*ref.21*). Indeed, it resembles the rhodopsin of a marine fish living at a mean depth of about 60 fathoms.

The absorption spectra of human rhodopsin and of the product of its bleaching are shown in *fig.3*. The pigment bleaches to the usual mixture of all-*trans* retinene and opsin. When this mixture is incubated with neo-*b* retinene, it regenerates rhodopsin with λ_{max} 493 mμ, identical with that extracted from the human retina. Again, therefore, the peculiar characteristics of human rhodopsin go, not with an unusual chromophore, but with a specific human opsin.

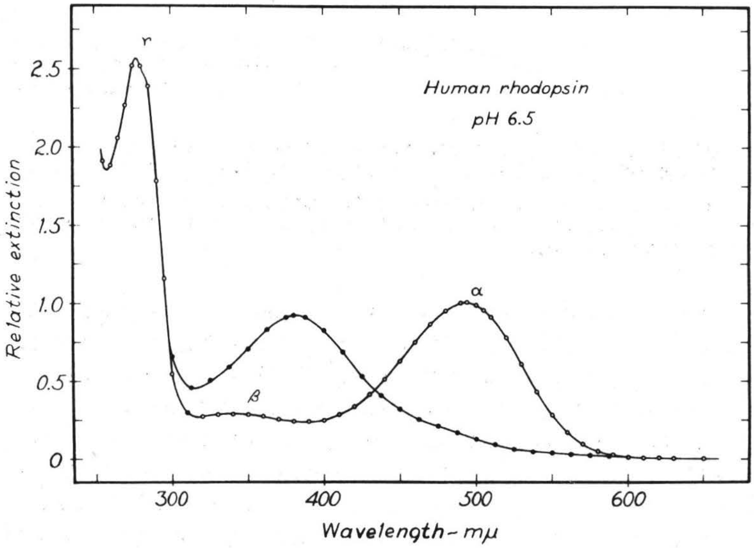


Fig.3. Absorption spectra of human rhodopsin and the product of its bleaching in 2% aqueous digitonin solution. The E_{max} of the α-band of rhodopsin has been set arbitrarily at 1.0. On bleaching in orange (non-isomerizing) light, the α- and β-bands of rhodopsin are replaced by the absorption band of all-*trans* retinene (λ_{max} 380 mμ). The γ- or opsin band remains unchanged. (From *ref.21*).

We should like, at least for the present, to call all visual pigments derived from rods and involving neo-*b* retinene$_1$ as prosthetic group *rhodopsins*, designating each one by the name of the animal of origin. Since the visual pigments of the invertebrates so far examined - squid, lobster, euphausiid crustacea - apparently involve the same prosthetic group, and possess very closely related properties, we should like for the present to call them also rhodopsins.

Since Munz has found *Bathylagus wesethi* to possess two rhodopsins with λ_{max} 480 and 500 mμ, I see nothing better to do than to state that. Similar ranges of variation in λ_{max} will of course eventually be found in all the other visual pigments. Indeed, specific variations must be expected in *all* the measured properties of all the visual pigments. We have already found wide differences in the rates at which rhodopsins from different species are synthesized, so that though in the chicken iodopsin is synthesized with enormously greater speed than rhodopsin, cusk rhodopsin is synthesized as rapidly as chicken iodopsin *(ref.22)*. So it is that everything we measure, in every type of visual pigment, will vary more or less with species and at times even within a single species. This is the way proteins are. We shall have to live with them here, as one does in all the other realms of biochemistry; and a simplicity of nomenclature should do no harm and delude no one, so long as we know what we are talking about.

III. SPECTRAL SENSITIVITY

THE first physiological property I should like to discuss is the spectral sensitivity of vision, the so-called luminosity function. In a sense this is the most basic of visual properties, since it defines for us and other animals what we mean by light. It has been recognized since first indicated by König *(ref.19)* that the spectral sensitivity is closely related to the absorption spectrum of the relevant visual pigment.

The conditions which govern such a comparison have for the most part been adequately defined: (a) The absorption spectrum of the visual pigment should be stated in terms of percentage absorption; and since such a curve varies in shape with the value of the percentage absorption, one should know the percentage absorption of the visual pigment *in situ* in the retina. Fortunately, this is not an important consideration unless the maximum percentage absorption rises above 10%. Below this value, the percentage absorption is proportional to the extinction; and the absorption spectrum plotted either as percentage absorption or extinction is virtually invariant in shape *(ref.23)*. (b) The spectral sensitivity function should be quantized; that is, it should state the reciprocals of the numbers of incident quanta at each wavelength needed to evoke a constant visual effect *(ref.24)*. (c) Any absorption by ocular structures that intervene between the incident light and the receptor organelles acts as a filter, distorting the intrinsic spectral sensitivity of the receptors themselves. Therefore, the spectral sensitivity measured in terms of quanta incident on the eye should be corrected for ocular transmission, so as to yield a function as though measured at the receptor surface *(ref.19)*. A fourth condition will emerge later in this discussion.

That the spectral sensitivity of human rod vision resembles closely the absorption spectrum of rhodopsin has been understood in a general way since König and Köttgen *(ref.19)*. Now that we have absorption spectra for other visual pigments, one can pursue this relationship further. *Fig.4* shows the absorption spectra of chicken rhodopsin and iodopsin in

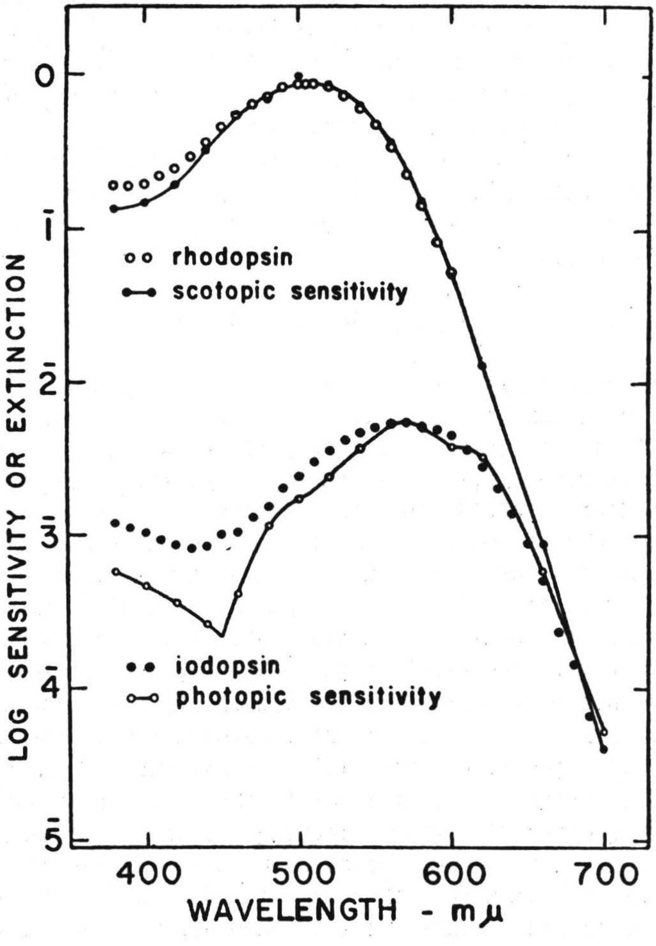


Fig.4. Scotopic and photopic sensitivities of the pigeon, measured by a behavioural procedure *(ref.25)*, compared with the absorption spectra of chicken rhodopsin and iodopsin *(ref.68)*. The spectral sensitivity data represent the means of measurements on three birds. (From *ref.25*).

solution, compared with recent measurements by Blough *(ref.25)* of the spectral sensitivities of dark- and light-adapted pigeons. We do not yet have comparable data on the chicken, though the electroretinographic measurements of Armington and Thiede *(ref.26)* on dark- and light-adapted chickens compare very well with the absorption spectra of the visual pigments shown here. It should be noted also that Mrs. Brown in our laboratory has recently extracted iodopsin from the pigeon retina, and finds it to have virtually the same spectrum as chicken iodopsin.

It is clear from *fig.4* that the scotopic luminosity function - like that measured earlier by Donner *(ref.27)* - is in good agreement with the absorption spectrum of rhodopsin. There is some discrepancy, however, between the photopic sensitivity and the absorption spectrum of iodopsin, particularly in the violet and blue regions of the spectrum. This is readily explained by the presence of coloured oil globules in the cones of both pigeons and chickens, which act as individual colour filters through which light must pass before entering the photosensitive outer segments. The oil globules are of four kinds: red, orange, yellow, and colourless. In the pigeon the dorso-temporal quadrant of the retina is much deeper red in colour than the other three quadrants, owing to a special concentration of red globules in this area (the "red field"). It is on this area that the images of objects fall at which a pigeon pecks. Blough's experimental procedure depends upon pecking, and his measurements particularly involve the red field of the retina.

Some years ago we extracted and crystallized the pigments of the oil globules of chicken and turtle retinas *(ref.29)*. They are all carotenoids: astaxanthin in the red globules, a mixture of xanthophylls such as is found in chicken egg yolk in the orange globules, and a carotenoid hydrocarbon, i.e., a carotene, in the yellow globules. The spectra of these pigments are shown in *fig.5*. All of them absorb light principally in the violet to blue-green. It is because these pigments lie as a screen over the cones that one observes a decreased photopic sensitivity in this region of the spectrum.

A comparison of spectral sensitivity with the absorption of visual pigments when such obstructions are not in the way is shown in *fig.6*. Here the lines are the absorption spectra of chicken rhodopsin and iodopsin; the points are Granit's micro-electrode measurements on light- and dark-adapted frogs, snakes, cats, and guinea pigs. The fit is obviously good. The shift in visual sensitivity toward the red as one goes from dim to bright light, from rod to cone vision, is of course the Purkinje phenomenon *(ref.30)*. It consists fundamentally of nothing more complex than the transfer of vision from dependence upon the absorption spectrum of rhodopsin in dim light to dependence on the absorption spectrum of iodopsin in bright light.

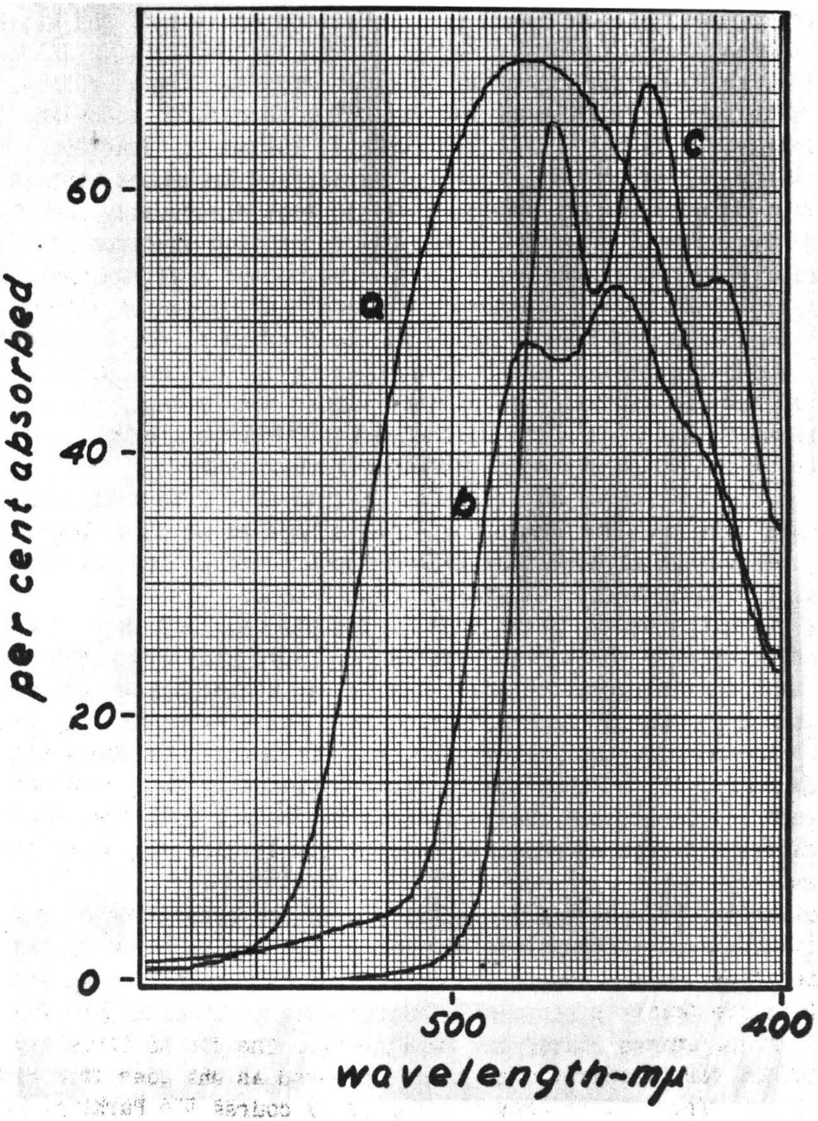


Fig. 5. Absorption spectra of pigments of the cone oil droplets of the chicken retina. The pigments, which are all carotenoids, had been crystallized, and their spectra measured in hexane solution. (From *ref.29*).

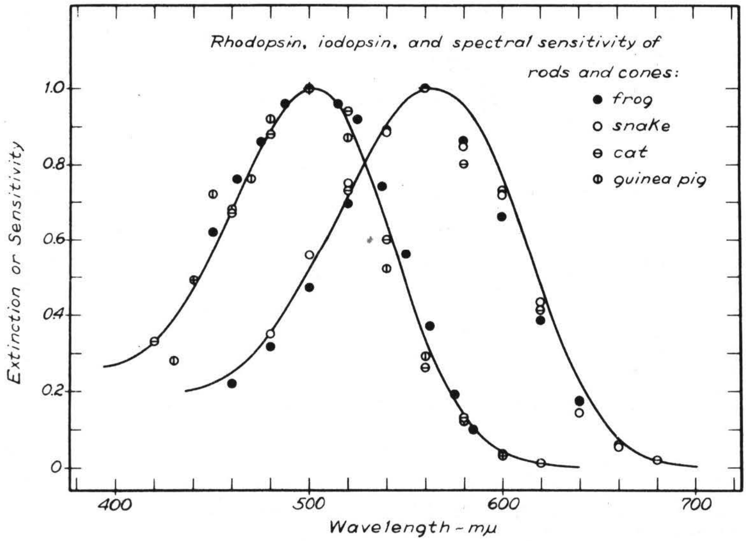


Fig.6. The absorption spectra of chicken rhodopsin and iodopsin (solid lines), c pared with the scotopic and photopic sensitivities of various animals (points), measured electrophysiologically by Granit *et al.* The spectral sensitivities have been quantized. Scotopic data: frog *(ref.97,* p.292), c *(ref.98)*, and guinea pig *(ref.99)*. Photopic data: frog *(ref.100)*, snake *(ref.101)*, and cat *(ref.102)*. (From *ref.68*).

This figure implies also that iodopsin, which we have extracted as yet only from the chicken, the pigeon, and a marine turtle, may in fact have a much wider distribution in animals. We have already emphasized that one can expect to find iodopsins covering a wide range of λ_{max}. Indeed, we have already found in marine fishes red-sensitive pigments with λ_{max} 525-530 mμ, which we think of as iodopsins *(ref.31)*. Nevertheless, just as 500 mμ represents a most usual position for rhodopsins, so about 560 mμ seems to represent a most usual position for iodopsins.

Fig.7 shows the same comparison for a visual system based upon vitamin A_2. Here the absorption spectra of porphyropsin and cyanopsin are compared with Granit's electrophysiological measurements *(ref.32)* on dark- and light-adapted tench. The photopic sensitivity function fits very well the absorption spectrum of cyanopsin. The scotopic sensitivity function, however, is maximal at about 533 mμ, whereas the porphyropsin spectrum - obtained from the yellow perch - is maximal at about 522 mμ. This is the most usual λ_{max} for porphyropsins. Dartnall however, *(ref.33)*

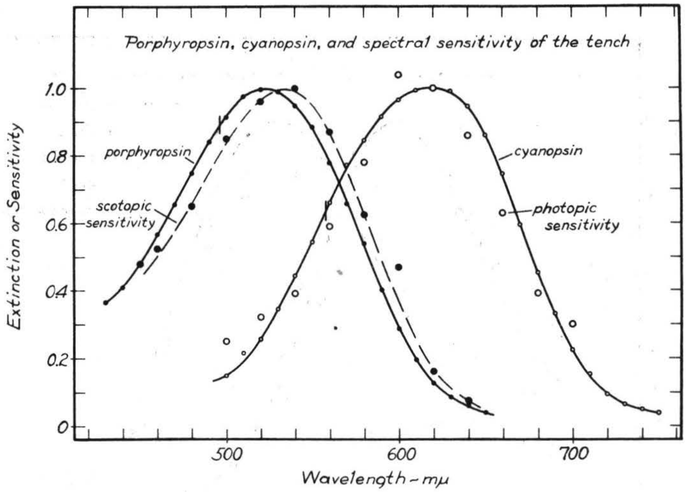


Fig.7. Absorption spectra of porphyropsin and cyanopsin (lines, small circles) compared with the spectral sensitivities of rod and cone vision in a fresh-water fish, the tench (broken line, large circles). The spectral sensitivities were measured electrophysiologically by Granit *(ref.32)* in opened eyes from which cornea and lens had been removed. The photopic sensitivity agrees well with the absorption spectrum of cyanopsin *(ref.103)*, but the scotopic sensitivity is displaced about 10 mμ toward the red from the porphyropsin of the yellow perch *(Wald, Brown and Brown, unpublished observations)*. On the other hand this scotopic sensitivity agrees well with the absorption spectrum of porphyropsin from the tench, with λ_{max} 533 mμ, as reported by Dartnall *(ref.33)*. (From *ref.3*).

has reported that tench porphyropsin has in fact λ_{max} 533 mμ, so that had the comparison shown in *fig.7* been made with the tench pigment, it might have displayed an almost exact correspondence.

It may be concluded from these and comparable data that in both rods and cones, and in systems derived from both vitamins A_1 and A_2, the spectral sensitivity of vision is based intrinsically upon the absorption spectra of the visual pigments.

In this regard human vision - specifically human cone vision - raises special problems. It has been plain for some time that the human photopic sensitivity function does not agree in λ_{max} with the absorption spectrum of chicken iodopsin; nor of course should it. The uncorrected spectral sensitivity measured in the human fovea is maximal, as is the absorption of chicken iodopsin, at about 560 mμ; but when the foveal luminosity function is corrected for the transmission of the yellow human lens and the yellow macular pigmentation, or better still, if one avoids both pigments by measuring the photopic sensitivity in a peripheral region of the lensless human eye, one finds it to be maximal at about 545 mμ *(refs.34,35,36)*.

The photopic luminosity of the human eye is of course a composite function, involving probably the spectral sensitivities of three types of cone. Stiles's analysis several years ago yielded the first reliable estimates of the positions in the spectrum of these sensitivity functions: a blue mechanism at about 440 mμ (corrected for ocular transmission), a green mechanism at about 540 mμ, and a red mechanism at about 590 mμ *(ref.37)*. Experiments in our own laboratory involving a somewhat different method confirm the position of the peak of the violet receptor at about 450 mμ (not corrected for ocular transmission) *(ref.38,* and *Auerbach and Wald, unpublished observations)*. We find this receptor in normal subjects, protanopes and deuteranopes, though it was absent in a tritanope. We find it also in fields as narrow as $1/8^0$ in the central fovea. As reported in the present symposium, Rushton is now succeeding in measuring the absorption spectra of the red and green components *in situ*, and finding them close to Stiles's estimates.

We may look forward therefore to the eventual characterization of at least three pigments in human cones, with λ_{max} probably near 440, 540 and 590 mμ. From all that we know of visual pigments, and from the behaviour of human cone vision in vitamin A deficiency, I would suppose that in these substances one is dealing with a single prosthetic group (neo-*b* retinene) united with different cone opsins. The 440 mμ pigment presents no difficulty in this regard, for we have repeatedly obtained photosensitive pigments - artefacts - with λ_{max} 440-450 mμ, formed by the combination of retinene with denatured opsin or even with such wholly unrelated proteins as egg albumen *(ref.39)*. Recently also Goldsmith *(ref.40)* has extracted a retinene_1 visual pigment from the bee with λ_{max} about 440 mμ.

Nor does the formation of a 590 mμ pigment from retinene_1 present insuperable obstacles; one can take some encouragement from the analogy to visual pigment formation offered by the antimony chloride test with retinene, which yields a blue, photosensitive product with λ_{max} 664 mμ (cf.*ref.6*, discussion on p.309). It is not absurd, therefore, to suppose that union with an opsin could raise the retinene λ_{max} as high as 590 mμ or higher.

A peak at 590 mμ is only a little more distant from iodopsin (λ_{max} 562 mμ) than one at 540 mμ. If we are reconciled to finding rhodopsins from λ_{max} 478 to 524 mμ (where Crescitelli *(ref.41)* has recently found that of the gecko), there should be no inconsistency in calling cone pigments in the range 540-590 mμ iodopsins. That would leave out the 440 mμ pigment, however; and my own choice would be to pay more heed to the uniquely human association of these pigments with colour sensations, and to their physiological and historical status by calling them chromopsins *V* (or *B*), *G* and *R*.

We may now return to the instance that introduced the association of absorption spectra of visual pigments with spectral sensitivity functions, König's classic comparison of the difference spectrum of human rhodopsin with the human scotopic luminosity function *(ref. 19)*. It is strange to realize now that we have lived with this comparison for sixty years, never doubting its essential validity, yet constantly repairing the data upon which it is based. König himself used a difference spectrum of rhodopsin, believing it to represent the absorption spectrum. Hecht and Williams *(ref. 42)* restated this comparison on the basis of their new measurements of the human scotopic luminosity. This they compared with Köttgen and Abelsdorff's measurements *(ref. 43)* of difference spectra of rhodopsin, still believing that the difference spectra were absorption spectra. Hecht and Williams also neglected to correct for ocular transmission, though König had introduced such corrections. Hecht and Williams found the scotopic sensitivity to be maximal at about 511 mμ, whereas the rhodopsin spectra with which they compared it were maximal at about 503 mμ. That is, they found the luminosity curve to be shifted some 8 mμ toward the red from the absorption spectra. They suggested that since in the outer segments of the rods rhodopsin is dissolved in a medium of considerably higher refractive index than in aqueous solution, its spectrum in the rods is shifted toward the red in accordance with Kundt's rule *(ref. 44)*.

In recent years this comparison has developed the further refinements already reviewed. We now have reasonably reliable absorption spectra, we understand that they should be plotted as percentage absorption, and that the luminosity function should be both quantized and corrected for ocular transmission. When all these things had been done, the spectral sensitivity function agreed very well with the absorption spectrum of rhodopsin. No discrepancy in λ_{max} remained; Kundt's rule was no longer needed.

This comparison in recent years, however, has usually involved the rhodopsins of other animals than man - frog rhodopsin initially, then cattle rhodopsin. Very recently Crescitelli and Dartnall re-introduced an estimated spectrum of human rhodopsin, with λ_{max} 497 mμ, which agreed very well with Crawford's recent measurements of scotopic sensitivity.

It is now plain, however, that the λ_{max} of human rhodopsin is at 493 mμ, and the entire absorption spectrum in aqueous solution lies at shorter wavelengths than the human scotopic sensitivity, however corrected. We are forced, therefore, to reconsider Hecht's suggestion that in the rod outer segments the absorption spectrum of rhodopsin is shifted toward the red, compared with the spectrum in aqueous solution.

To examine this possibility, we have measured the absorption spectrum of rhodopsin in suspensions of human rod particles; rather, we have measured its difference spectrum in such suspensions in the presence of hydroxylamine, which makes the difference spectrum identical with the absorption spectrum at wavelengths longer than 450 mμ *(fig. 8)*. Under these circumstances we find λ_{max} at 500 mμ, shifted 7 mμ toward the red, as compared with solution. The absorption band is also slightly *narrower* than in solution *(ref. 21)*. Indeed, the absorption spectrum in rod particles displays much the same characteristics as the spectrum of rhodopsin at very low temperatures (-40° to -60° C.) in a rigid medium *(ref. 13)*.

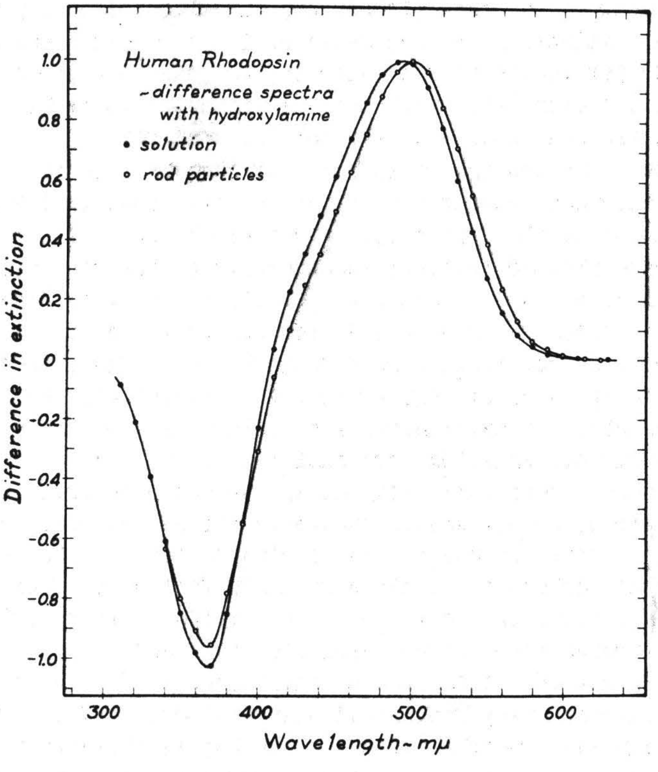


Fig. 8. Difference spectra of human rhodopsin in aqueous solution and in rod particles, measured in the presence of hydroxylamine. The λ_{max} in solution lies at 493 mμ; in a suspension of rod particles oriented at random it is at 500 mμ. The spectrum in the particles is also narrower than in solution. At wavelengths longer than 450 mμ these difference spectra are identical with absorption spectra. (From *ref. 21*).

We have also measured directly the difference spectrum of rhodopsin in the human retina. A portion of human retina laid flat upon a microscope slide and backed with finely ground Vycor glass to diffuse the light, was measured against an identical microscope slide and ground glass as blank. Then the retina was bleached in position and its spectrum was remeasured. The difference spectrum had λ_{max} 502.5 mμ, displaced slightly from the difference spectrum measured in rod particles, owing to the fact that in the absence of hydroxylamine the retinene produced by bleaching absorbs light in the region of the rhodopsin maximum, and so displaces the difference spectrum slightly toward the red. The change in extinction at 502.5 mμ was 0.067. This must be taken as minimal, since the retina was not dark-adapted, and since it makes no allowance for the passage of light through spaces between the rods. It corresponds almost exactly, however, with the highest extinction we have measured in unoriented human rod particles (0.042 per length of rod outer segment, taken as 25 micra) multiplied by the factor 1.5, applicable if rhodopsin is highly oriented relative to the transverse axes of the rod (cf. *ref. 45*).

It is therefore the absorption spectrum of rhodopsin in the rod structure that should be compared with the spectral sensitivity of human rod vision. The latter also, however, raises problems.

In *fig. 9* I have plotted together measurements of the scotopic sensitivity from five sources: Hecht and Williams *(ref. 42)*, Weaver *(ref. 46)*, Wald *(ref. 34)*, Flamant and Stiles *(ref. 47)*, and Crawford *(ref. 48)*. Weaver's measurements were made in a 2.6^{o} central area and appear to have involved considerable macular pigmentation; for this reason, only the data for wavelengths longer than 510 mμ, which are not affected by macular pigmentation, are included.

If these were not human luminosity data, one would congratulate all concerned on their close agreement, and rest content. Not entirely content, to be sure, for we still do not possess a single complete and detailed set of measurements throughout the visible range. Nevertheless, what data we have are in good agreement, and there is no difficulty in throwing such a freehand curve through them as the solid line in *fig. 9*.

Unfortunately, however, this is a human luminosity curve, and as such subject to the extraordinary process of standardization. The Commission Internationale de l'Éclairage (the C.I.E., or in English translation I.C.I.) has recently announced a standard scotopic luminosity function for young human eyes, measured in the peripheral region of the retina, based on the consideration of all the data shown, with particular weight attached to Crawford's recent measurements and my own *(ref. 49)*. The C.I.E. standard curve is shown as a broken line in *fig. 9*. It does several extraordinary things. In the neighbourhood of 425-475 mμ it introduces a double inflexion, a reverse curve. It tends also to be narrower at the maximum

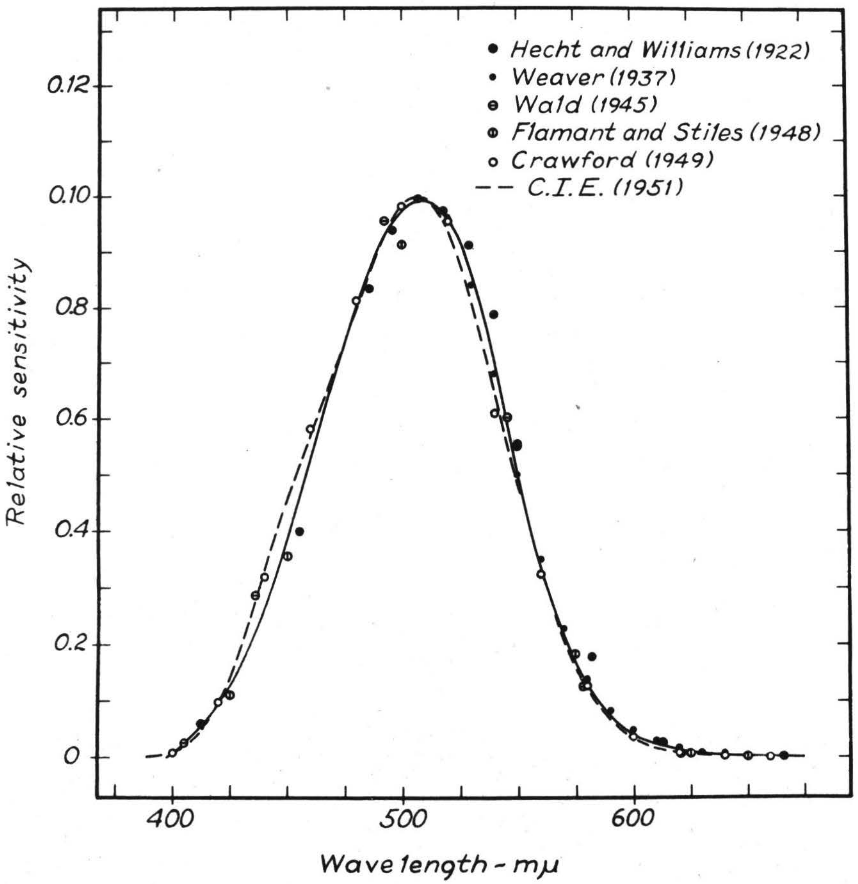


Fig. 9. Measurements of human scotopic sensitivity from five sources. Weaver's measurements, made in a 2.6^{0} central area, were used only at wavelengths longer than 510 mμ, to avoid apparent effects of macular pigmentation. The solid line is a freehand curve thrown through the points. The broken line shows the recently adopted C.I.E. (or I.C.I.) 1951 Scotopic Luminosity Function for Young Eyes *(ref.49)*.

than the data seem to indicate; and this mainly because, between about 500 and 540 mμ, it avoids the data entirely.

Standardization is a strange business. It is enough for a standard curve to be accepted; one should not ask in addition that it be right. I understand that the inch was originally defined as the length of three barley grains taken from the centre of an ear, laid end-to-end. The only point I should like to make here is that for comparison with the absorption spectrum of rhodopsin, the standard luminosity curve does not best represent the data, and I think we should be better served by the data.

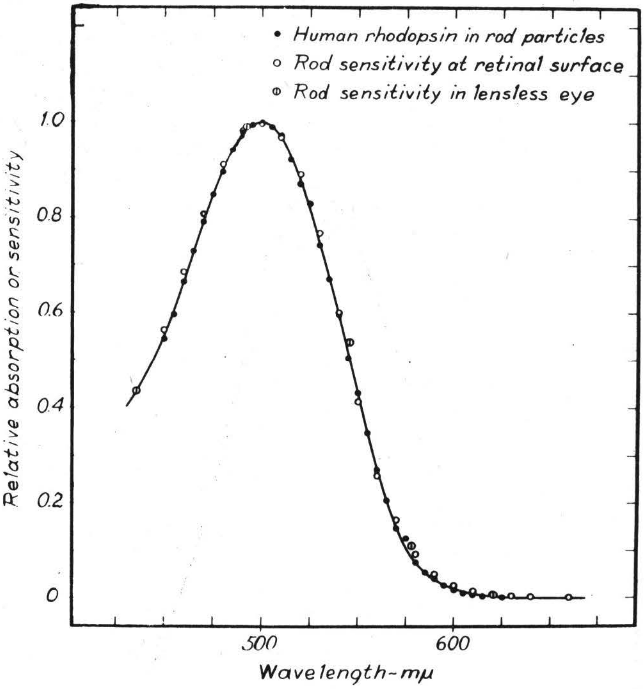


Fig. 10. **Human rhodopsin and the spectral sensitivity of human rod vision. The absorption spectrum of rhodopsin, measured in human rod particles, compared with the scotopic (rod) luminosity function, quantized and corrected for ocular transmission, so as to represent the sensitivity at the retinal surface; and with the scotopic luminosity (quantized) of the lensless human eye. In the latter, the yellow lens having been removed in the operation for cataract, and the macular pigmentation having been avoided by using a peripherally fixated field, one comes as close to the intrinsic sensitivity of the rods as is possible *in vivo*. (From *ref.21*).**

In *fig.10*, the absorption spectrum of human rhodopsin as measured in rod particles is plotted on the basis of percentage absorption, made up to a maximum absorption of 29%, corresponding to an extinction of 0.15 as estimated by Rushton *(ref. 50)* in living human eyes. For comparison with this curve I have used the freehand curve shown in *fig. 9*, now however recomputed in terms of the numbers of quanta incident at each wavelength, and corrected for human ocular transmission with the data of Ludvigh and McCarthy *(ref. 51)*. In effect, this final correction renders the measurements as though made at the retinal surface.

It is clear that the luminosity function so treated fits almost exactly the absorption spectrum of rhodopsin in rod particles. In *fig.10* I have plotted also the scotopic luminosity measured in an extra-macular region of the retina, in subjects who had lost their lenses in the operation for cataract *(refs. 34,52)*. Such measurements avoid the distortions introduced by the lens and macular pigmentations, and so represent as close an approach to the intrinsic sensitivity of human rod vision as is possible *in vivo*. It is clear that they too agree very well with the absorption spectrum of rhodopsin in rod particles.

I think we can conclude that when adequately measured the absorption spectrum of human rhodopsin and the spectral sensitivity of human rod vision correspond exactly. It is now clear, however, that this correspondence hangs upon a special phenomenon - the displacement of the absorption spectrum of rhodopsin toward the red in the outer segments of the rods as compared with aqueous solution.

We have known for a long time, yet have been reluctant to draw its consequences, that in the outer segments of the rods and cones, and indeed in all the photoreceptors we know, the visual pigments are not in free solution, but rather in a condition which closely approaches the solid state. Sjöstrand's electron micrographs and many made since have shown us that the outer segments of vertebrate rods and cones are highly organized structures consisting of one to several thousand layers, each no thicker than moderately sized protein molecules *(ref. 53)*. A comparable degree of organization has been revealed by the electron microscope in the rhabdomeres of insect eyes *(refs. 54,55)*, crustacean eyes, and in the so-called rods of the squid retina *(Wald and Philpott, unpublished observations)*. Long before electron microscopes were available, we knew that rhodopsin is highly oriented in the rod structure, through the observations of W. J. Schmidt of Giessen *(ref. 56)*, with the polarizing microscope. All these receptor structures are in fact little crystals, in the sense that most of their molecules, including the visual pigments, are fixed in position and highly oriented with regard to one another *(cf. ref. 57)*. They are not perfect crystals, but rather two-dimensional crystals. In the vertebrate rods at least, rhodopsin displays no special orientation with regard to light passing down the long axis of the rod, the normal direction of incidence *in vivo*; but for light passing through the rod transversely, rhodopsin exhibits a high degree of orientation, displayed in part by a striking dichroism *(refs. 56,58,59)*.

It is to this condition that we may ascribe the displacement of the absorption spectrum of rhodopsin in the rods toward the red, and its narrowing. The semi-crystallinity of rhodopsin in visual receptors seems to be a general condition; and to this degree we may expect generally such changes in spectrum as here observed. Entirely independently of our experiments, Denton, in the present symposium, reports finding such changes in a variety of rods. As a further consequence of their orientation, the visual pigments may be expected also to be denser in the receptors than in free solution, by a factor which, depending upon the degree of orientation, approaches the limit 1.5 *(cf. ref. 45)*.

IV. LIGHT AND DARK ADAPTATION

WE owe mainly to the work of Selig Hecht the prevalence of the idea that light and dark adaptation reflect physiologically the bleaching and resynthesis of visual pigments in the rods and cones. When the eye is exposed to light, the visual pigments are bleached; but since they are constantly being resynthesized, their concentration falls to some steady state level, maintained as long as the light is on. Concomitantly the visual sensitivity declines to a steady state level; and this is light adaptation. On returning to the dark, the visual pigments are regenerated to their maximal concentrations. Simultaneously the visual sensitivity rises to its maximal value; and this is dark adaptation.

It was Hecht who first tried to give these ideas exact expression by assuming a specific relationship between visual pigment concentration and visual threshold. The notion has somehow arisen that what Hecht assumed in this instance was a direct proportionality between visual pigment concentration and visual sensitivity. This assumption is spoken of sometimes as the "classical" view, meaning that it is wrong, but ancient enough to be forgiven. Hecht of course never made such an assumption. At one time he assumed that the concentration of visual pigments in rods and cones is proportional to 1/log threshold, and on this basis decided that the curves of dark adaptation followed the course of a second-order chemical reaction. This assumption, however, never could be rationalized convincingly. It is significant that after 1934 *(ref. 60)*, Hecht never attempted to fit a theoretical curve to the data of dark adaptation; and in 1942 *(ref. 61)* he stated the position as follows:

> "In general, human visual dark adaptation runs roughly parallel with the accumulation of visual purple in the dark-adapting animal retina. Efforts to study this parallelism experimentally have not been successful . . . In fact, even the sensitivity data of human dark adaptation, though very precise, are still without adequate theoretical treatment in terms of visual purple concentration changes."

Now that we can synthesize the visual pigments in solution, it is interesting to inquire whether the course of such syntheses resembles the course of dark adaptation. *Fig. 11* shows the dark adaptation of the human eye, measured in a peripheral area which includes rods and cones. Such data are usually plotted in the form, log threshold versus time in the dark. In the present instance they have been plotted as log 1/threshold or log sensitivity against time, for it is the sensitivity that we expect to display some parallelism with visual pigment concentration. The dark adaptation function is divided into the familiar two segments: a first rapid rise in log sensitivity to a constant level, attained in 4-5 minutes, marking the relatively rapid dark adaptation of the cones; followed by a

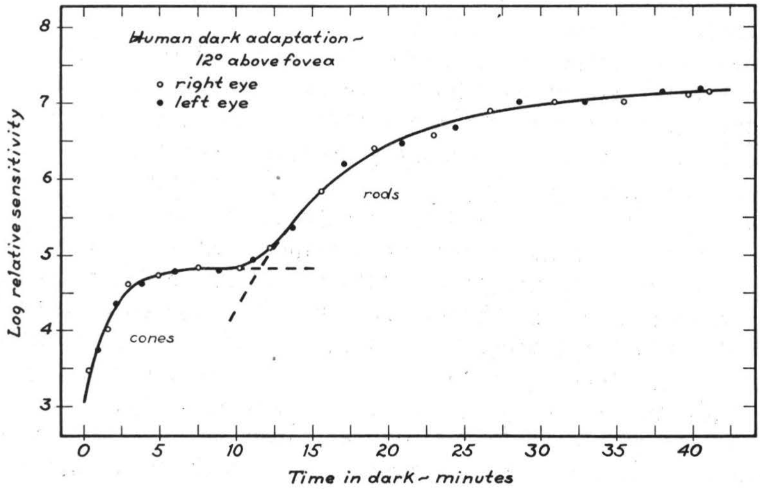


Fig. 11. Dark adaptation of the human eye, measured in a peripheral retinal area which contains both rods and cones. The dark adaptation of the cones is completed in about 7 minutes, that of the rods in about an hour. (From *ref.68*).

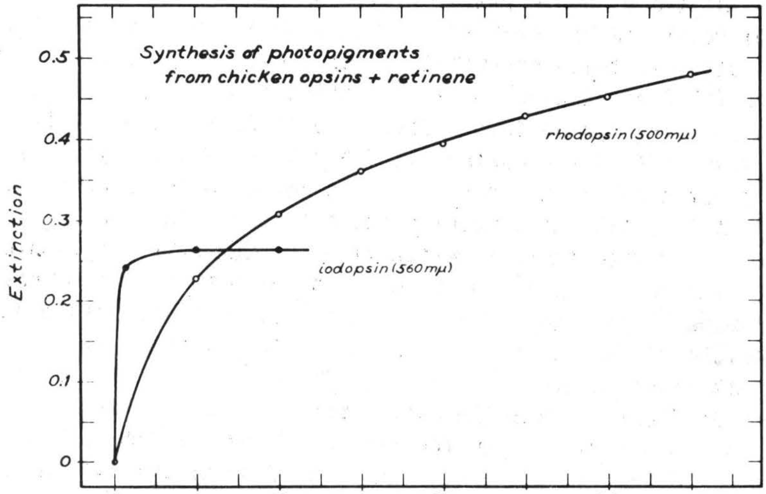


Fig. 12. Synthesis of iodopsin and rhodopsin in solution, from a mixture of chicken cone and rod opsins with neo-*b* retinene. At 23° C., the temperature of these measurements. iodopsin synthesis is complete within about 3 minutes, whereas rhodopsin synthesis continues beyond 40 minutes. (From *ref.68*).

second, much slower, rise of log sensitivity, the adaptation of the rods, virtually completed in about 45 minutes.

Fig. 12 shows the synthesis of chicken iodopsin and rhodopsin in solution. In earlier experiments we had learned that though both pigments are synthesized by incubating the respective opsins with neo-*b* retinene, iodopsin forms in solution at enormously greater speed than rhodopsin. For the experiment shown in *fig. 12*, we began by extracting a mixture of iodopsin and rhodopsin from dark-adapted chicken retinas. On bleaching this with orange (non-isomerizing) light, we obtained a mixture of rod and cone opsins and all-*trans* retinene. By preliminary trials we determined how much of this mixture was cone opsin, i.e., how much iodopsin could be regenerated with neo-*b* retinene. We then added exactly this amount of neo-*b* retinene, and since iodopsin forms so much more rapidly than rhodopsin, could follow the synthesis of iodopsin virtually alone. At this temperature it was completed in 3-4 minutes. Then we added more neo-*b* retinene, and could now measure the synthesis of rhodopsin. This takes about an hour to approach completion.

It is plain that the synthesis of iodopsin and rhodopsin under these conditions is extraordinarily similar qualitatively to the course of human cone and rod dark adaptation. The opsins are specifically different, the temperature is different, and indeed the essential biochemistry is different, for the combination of neo-*b* retinene with opsin is only the last link in the chain of reactions by which the visual pigments are regenerated *in vivo*. Despite all these essential differences, two points of correspondence emerge: (1) Just as human dark adaptation is much more rapid in cones than in rods, so the synthesis of chicken iodopsin in solution is much more rapid than that of rhodopsin. (2) The course of both types of function is qualitatively similar, provided that one compares the *logarithm* of the visual sensitivity with the concentration of visual pigment.

Owing to the extraordinary investigations of Campbell and Rushton *(ref. 62)* and Rushton *(ref. 63)* one need no longer pursue this comparison at such a distance. These workers have given us direct measurements of the rise and fall of visual pigment concentration in the human retina during light and dark adaptation. The bleaching of rhodopsin in the living human eye is shown in *fig. 13*. When the narrow pencil of light used to make the measurement is made to fall upon the central fovea - which of course contains no rods - exposure to bright light causes no measurable change. Similarly, when the testing light is thrown upon the head of the optic nerve, the blind spot, the bleaching light has no measurable effect. But at 5^0 from the fixation point, where there are both rods and cones, the bleaching light causes a rapid fall in rhodopsin concentration. At 10^0 from the fixation point, where the rod population is denser, the fall is

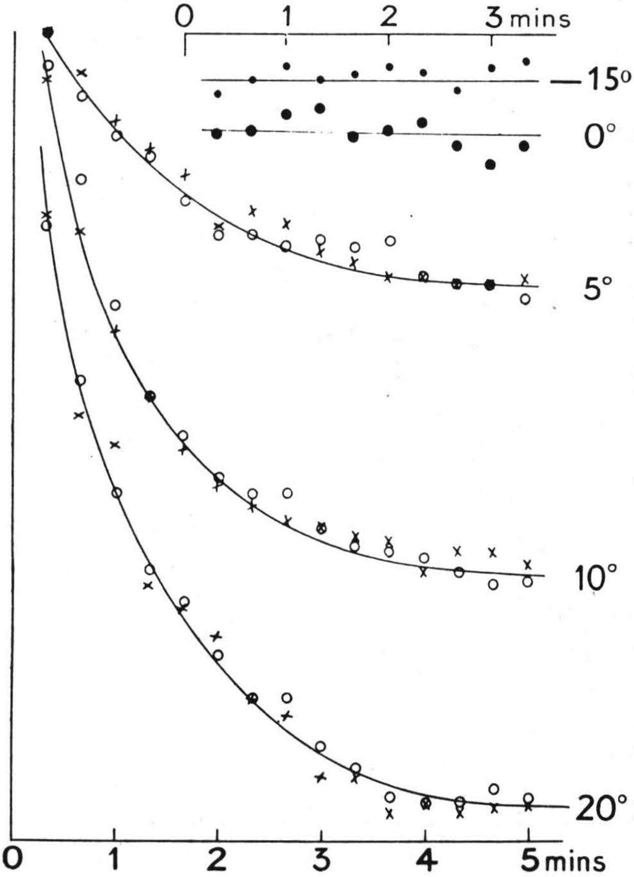


Fig. 13. The bleaching of rhodopsin in the living human eye, measured at 5 different locations in the horizontal meridian; e.g., "20°" here means 20° temporal to the fovea (0°), whereas -15° is on the head of the optic nerve, the "blind spot". Ordinates represent an arbitrary measure of rhodopsin absorption; abscissae are minutes of exposure to an intense light. Circles and crosses represent measurements made 3 days apart. On exposure of the fovea or blind spot, both of which lack rods and rhodopsin, no bleaching is observed; but at 5, 10 and 15° from the fovea, where increasingly large densities of rods occur, correspondingly larger amplitudes of bleaching are observed. In every case the steady state is attained in about 5 minutes. (From *ref.104)*.

correspondingly greater; and at 20° out, where the rod population is most dense, the fall in rhodopsin concentration is maximal. In all cases the steady state level is attained within about 5 minutes. We have known for many years that this is about the time it takes to light-adapt the human rods.

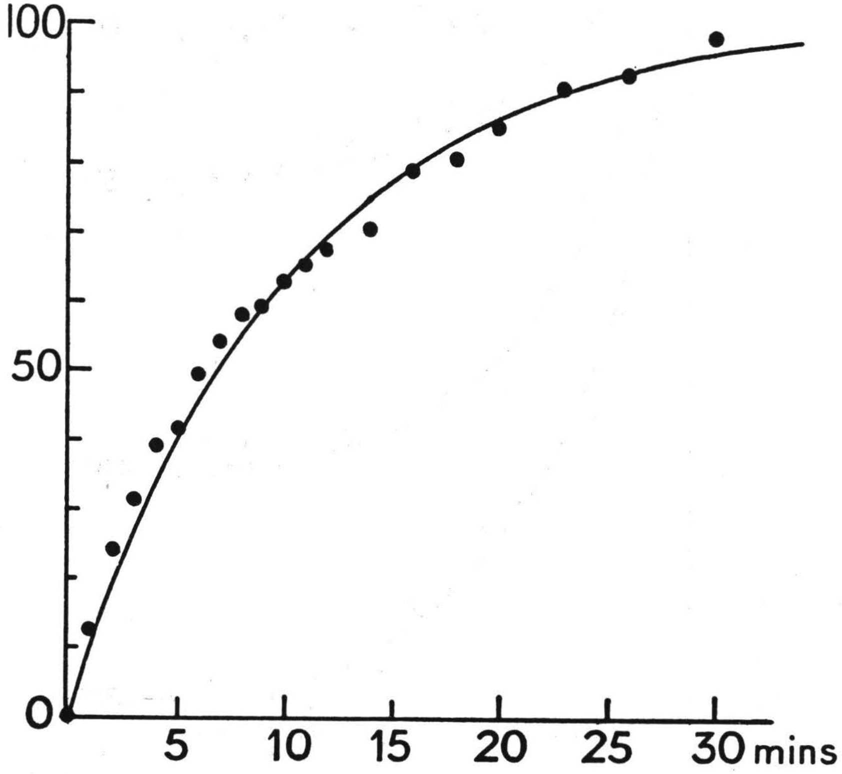


Fig. 14. Regeneration of rhodopsin in the living human eye, following exposure to bright light. Ordinates: percentage regeneration; abscissae: minutes in the dark. (From *ref. 104*).

Fig. 14 shows the resynthesis of rhodopsin in the living human eye following a strong light adaptation. At the end of 30 minutes the curve is still rising. It displays much the same course as the dark adaptation of human rods, as shown for example in *fig. 11*.

Rushton's paper in the present symposium shows comparable measurements of the rise of concentration of the pigments of human cones, measured during dark adaptation in the central fovea (cf. also *ref. 63*). The regeneration of the cone pigments is complete in 5-6 minutes, just as is human cone dark adaptation.

These measurements, therefore, demonstrate a striking parallelism between the course of human light and dark adaptation, rod and cone, and the course of bleaching and resynthesis of the visual pigments. Once again it should be emphasized that this similarity is evident only when the *logarithm* of the visual sensitivity is compared with the concentration of visual pigment.

I should like now to return to the much less direct comparison of visual adaptation with the synthesis of visual pigments in solution. We have already emphasized the species specificity of the opsins, and their striking effects upon the absorption spectra of the visual pigments. The various opsins also exhibit wide differences in their rates of combination with neo-*b* retinene. We have for example observed that the rod opsins of fishes tend to combine with neo-*b* $retinene_1$ or $retinene_2$ very much more rapidly than do the rod opsins of frogs or cattle. Indeed, as already noted, the rhodopsin of a marine fish, the cusk, forms in solution as rapidly as does chicken iodopsin.

The alligator possesses such a rapidly regenerating rhodopsin. Whereas the rhodopsins of frogs, cattle, or chickens regenerate from mixtures of neo-*b* retinene and opsin at 25^0 C. with half-times of 4-6 minutes, alligator rhodopsin is regenerated in a half-time of 10-20 seconds. This great disparity in the speeds of synthesis of rhodopsin in two cold-blooded animals, frogs and alligators, offers the opportunity to compare the reactions in solution with the dark adaptation of the animals, all at the same temperature.

This comparison is shown in *fig.15*. Dark adaptation was measured by following the height of the b-wave in the electroretinogram, in response to a constant stimulus. This is not the best way to measure it, but was the only way available at the time. It is clear that just as frog rhodopsin is synthesized slowly in solution, the course of rod dark adaptation in the frog is correspondingly slow; and just as alligator rhodopsin is synthesized rapidly in solution, so dark adaptation in the living alligator is correspondingly rapid.

We seem to have arrived at a reasonably clear position. It is that light and dark adaptation reflect the bleaching and resynthesis of visual pigments in the rods and cones; and that a measure of quantitative parallelism is observed if one compares the changes in the *logarithm* of the visual sensitivity with the rise and fall of pigment concentration.

V. ALARUMS AND EXCURSIONS

I characterized the position achieved in the last section as reasonably satisfactory. Yet the thought that light and dark adaptation have a primary connexion with chemical processes in the rods and cones has recently been seriously questioned by some workers. Indeed it has been repudiated in the strongest terms; it is described as "classical."

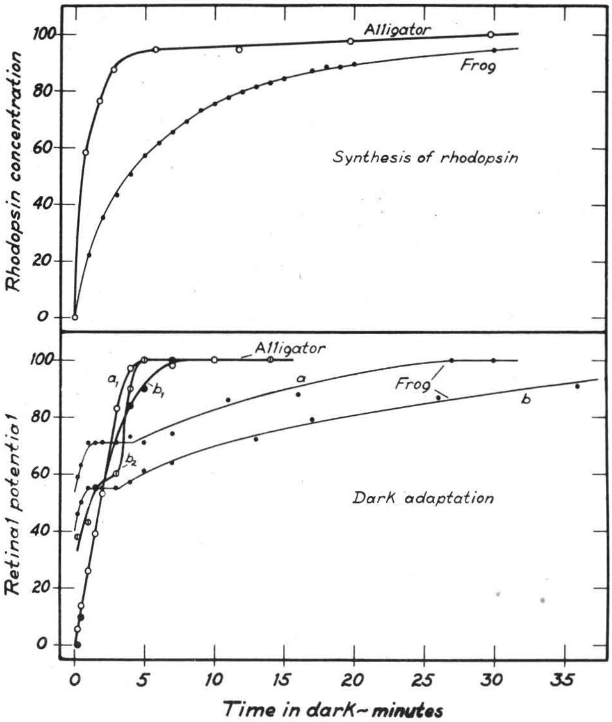


Fig. 15. Synthesis of alligator and frog rhodopsins in solution, compared with ERG measurements of dark adaptation in the living animals at the same temperature (25° C.). For the dark adaptation measurements, the height of the b-wave was measured for a standard test flash in the dark adapted eye. Then the eye was exposed to 2000 millilamberts for 15 seconds (a) or 30 seconds (b). Next the eye was stimulated periodically in the dark with the constant test flash, and the height of the b-wave recorded as it returned to the dark adapted condition. This is plotted in the lower part of the figure. Data are shown from one frog, and two alligators (subscripts 1 and 2). (From *ref.22*).

This rejection rests mainly upon four propositions: (1) that cones are "intrinsically" about as sensitive as rods, and therefore probably contain about as much photosensitive pigment; (2) that dark adaptation curves vary in shape with the size of the field, as a chemical reaction obviously would not do; (3) that so much of light and dark adaptation can be achieved with very small changes in concentration of photosensitive pigment that the latter must be of little account; and (4) that for all these reasons and more, light and dark adaptation are "neural" rather than "photochemical" in origin.

These propositions are so exciting that I could almost wish they were true, or if true, pertinent. I think that (1) is mistaken, (2) takes too much for granted, (3) is irrelevant, and (4) in need of further definition.

1. *"Intrinsic" sensitivities of rods and cones.*

The first proposition involves the following argument: The much higher sensitivities observed in rod vision as compared with cone are caused primarily by "summation" in the rods. When this factor is removed by measuring thresholds in very small fields, rods and cones are found to be almost equally sensitive.

Baumgardt *(ref. 64)* has compared the thresholds of peripheral and foveal fields in the dark-adapted eye. He found that in fields 45' in diameter the foveal threshold is 130 times as high as the peripheral; but as the fields are made smaller the thresholds approach each other, so that in fields 5' in diameter the foveal threshold is only 8 times the peripheral. Arden and Weale *(ref. 65)* have reported similar observations. In a field 2.7' in diameter, they found the threshold in the periphery and the fovea to be equal after 20 minutes of dark adaptation.

I should like first to note that in both cases the authors have tended to overstate their observations. Baumgardt, for example, says that the extrapolation of his observations to still smaller fields than he measured should have made the foveal and peripheral thresholds virtually equal; but in fact in fields smaller than 5', Riccò's law has begun to take effect even in the fovea - the intensity times the field area is nearly constant - and consequently the ratio of foveal and peripheral thresholds should hardly decline further. Similarly, Arden and Weale observed that the peripheral threshold is equal to the foveal after twenty minutes of dark adaptation; but at this time the foveal threshold is constant, whereas the peripheral threshold is still declining rapidly. Twenty minutes of dark adaptation merely represents the crossing point of both functions; it is clear that the peripheral thresholds will end considerably below the foveal thresholds. The correct conclusion to be drawn from both sets of observations, therefore, is that the foveal and peripheral thresholds in the dark-adapted eye approach each other as the size of field is decreased, but that the foveal threshold is appreciably higher than the peripheral threshold, even in the smallest fields.

Fig. 16 shows measurements made during the last war by Wald, Griffin and Hubbard *(ref. 66)*, showing the average thresholds of five observers for fields of various sizes, viewed binocularly in the fovea and 10^{0} outside the fovea in the dark-adapted eye. The test field was at a distance of 18.75 feet, since it had earlier been reported that the area-threshold relationship varies with the accommodation of the eye for distance. These data are in good agreement with earlier measurements, and show that the reciprocity of area and threshold (Riccò's law) holds approximately in peripheral (rod) fields smaller than about 1^{0} in diameter, and in foveal fields smaller than 1'-2' in diameter.

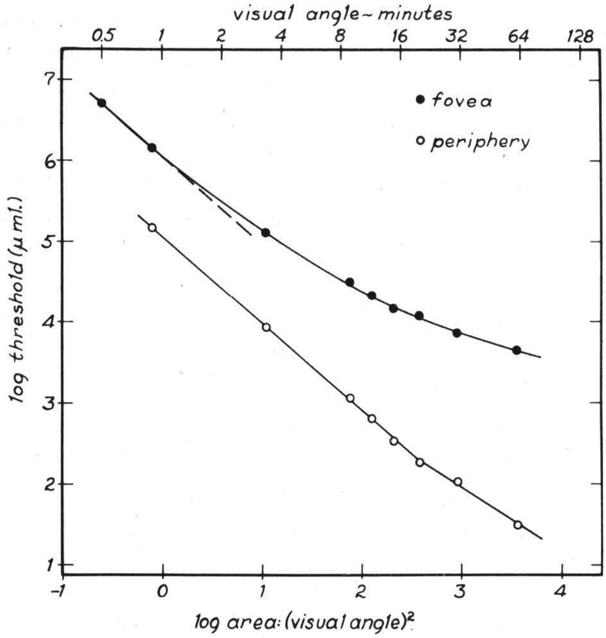


Fig. 16. The relation between visual threshold and area of field in the human eye. Averaged data from 5 subjects, measured in the completely dark adapted eye, with fields of various sizes fixated within the fovea or 10^o below the fovea. Circular fields, 19.75 feet from the eye, viewed binocularly, and exposed for 1/50 second. White light. Riccò's law, the reciprocity of threshold and field area, holds in the fovea only for fields which subtend visual angles smaller than 1 - 2 minutes. For rod thresholds in the peripheral retina it holds approximately for fields up to about 60 minutes in diameter. (From *ref.66*).

In fields 1^o in diameter, the foveal threshold lies 2.16 log units above the peripheral. As the field is made smaller, the foveal and peripheral thresholds approach each other, achieving a minimum separation at a field size of 1'-2'. Below this size of field, both functions are parallel, since both obey Riccò's law. The separation in this region is about 0.8 of a log unit. This is in good agreement with Baumgardt's observations made in 5' fields, and is not in serious disagreement with the measurements of Arden and Weale.

Of course whatever is said of human foveal cones should not be taken to involve cones in general, or even extrafoveal *human* cones. The cones of the human fovea are a peculiar species. In a sense, one might say of their outer segments that they are rods in all but their chemistry. They are thread-shaped organelles, with nothing cone-like in their appearance, and quite unusually long. The outer segment of a human cone is on the average 40 microns long in the central fovea, compared with 20μ at the foveal border, and 6-10μ in the peripheral retina; the average length of the outer segment of a human rod is 28μ *(cf. ref. 67*, pp. 346-348*)*. When Rushton *(ref. 63)* tells us that by direct measurement he finds evidence that the density of absorption by visual pigments in the human fovea is perhaps 1/3-1/2 as great as the density of rhodopsin in human rods, this is surprisingly high; but must be taken in conjunction with the extraordinary length of human foveal cones, as well as other factors (e.g., "funnelling" of light) which Rushton has discussed. We have as yet no evidence of such a condition in human peripheral cones. Indeed, the experiments in which Arden and Weale show that in a 2.7' field after 20 minutes of dark adaptation, the foveal cone threshold is equal to the peripheral rod threshold, also show that in the same field the peripheral *cone* threshold lies at least 2 log units higher. The statement, therefore, that cone thresholds lie very much higher than rod thresholds seems to hold even in very small fields in the human eye, provided they lie outside the fovea.

With the thought that the "intrinsic" thresholds of cones and rods are nearly equal, the curious notion has arisen that cones in general probably contain about as much visual pigment as rods. This has not only the appeal of iconoclasm, but offers a tempting relief from all the difficulties that have been experienced in pursuing cone pigments. I can only invite anyone who believes this to try to find them; that is, outside the human fovea, where Rushton seems indeed to be finding them. I do not seriously doubt Rushton's observation that the density of visual pigment in human foveal cones is surprisingly high; though even here Rushton has explained that this may not imply the presence of a large *amount* of pigment. The only direct experience we have had with the *amounts* of visual pigment to be obtained from rods and cones involves the chicken, in which we estimated that the rods contain about 13 times the extinction of visual pigment found in cones *(ref. 68)*.

More important, however, than all these things, the argument we are discussing involves, I believe, a fundamental misconception. This concerns the meaning attached to thresholds measured in very small fields.

It has been said that the threshold of rod vision rises rapidly as the size of field is decreased because of loss of "summation." Summation could have a great effect in large fields, but must decline in small fields, and could not occur at all in a field restricted to a single element. Hence thresholds measured in very small fields might inform us of the "intrinsic" sensitivities of the retinal elements, uninfluenced by summation.

Summation of what? If rods or cones are active, one can understand that they might summate their activities; but what can they summate before they have become active? What is it that a large number of receptors, each exposed to a luminance below its individual threshold, can summate so that the group as a whole can respond?

I think it must be clear that unless an intensity is sufficiently high to stimulate individual receptors, no summation can occur. But if individual elements are stimulated at the very low luminances that suffice to stimulate rods in large retinal fields, what is left of the argument that the "intrinsic" thresholds of individual rods are in fact very high?

I think that at this point it would be well to recall that the threshold of rod vision corresponds to the stimulation, not of one rod, but of several, virtually simultaneously. To stimulate rod vision, a number of rods must almost simultaneously each absorb at least one quantum of light *(refs.69,70,71,72)*. The smaller the visual field, the higher the luminance needed to achieve this result. The primary reason for the rapid increase in rod thresholds as the field is decreased in size is not that one is losing "summation," or approaching the "intrinsic" sensitivity of individual rods, but that one is decreasing the probability of several rods simultaneously absorbing quanta effectively.

Suppose that it were possible to restrict the visual field in the limit to a single rod; and suppose it were found that with a single rod one could never see, because presumably (as is possible) a single rod cannot fulfil the condition of "knocking at the door" the requisite number of times to arouse a visual sensation? What would this tell us of the intrinsic sensitivity of rods? - or of their density of visual pigment? Obviously, nothing whatever. I think this is the proper conclusion to be drawn from the experiments cited above: they tell us nothing of the intrinsic sensitivities of individual rods, and nothing of their content of visual pigment.

It seems to me that what comes out of this discussion is that the intrinsic sensitivities of individual rods and cones may be approached most closely, not in the smallest but in the largest visual fields. For a large visual field to respond, at least one individual element must respond. The lowest visual threshold one can measure, however large the field and however one arranges the experiment, may still be far *above* the thresholds of individual elements; it cannot possibly lie *below* them.

2. *Dark adaptation and size of field*

It has been observed that dark adaptation proceeds somewhat faster and further when measured in fields of moderate size (2^0 or larger) than when measured in exceedingly small fields (about 3' in diameter) *(refs.73,65)*. On the plea that a chemical reaction should proceed in the same way irrespective of the size of field in which one measures it, this observation has been taken to mean that dark adaptation cannot reflect the course of a chemical reaction, or of a chemical reaction "alone."

In fact, however, it is not at all clear that this observation makes difficulties for a chemical theory of adaptation. The synthesis of visual pigments in the retina proceeds, not in one vessel, but - in fields of moderate size - in hundreds or thousands of vessels, the rods and cones. What is more serious, in a dark adaptation experiment we sample this process, not in one vessel, but in whatever vessel is out ahead - from moment to moment always in the most sensitive rods and cones. Ordinarily we do not measure the same elements at the end of dark adaptation as we measured at the beginning. A dark adaptation curve represents the *envelope* - the low-threshold boundary - of the dark adaptation curves of the individual elements. The larger the population of retinal elements, the further this envelope may be expected to depart from the adaptation curve of a single rod or cone; and depart in the direction observed, yielding a more rapid and extensive adaptation the larger the field. The small fields employed in the experiments cited above were only 2.7'-3' in diameter, and included therefore less than a dozen rods. It is not surprising that they revealed very restricted ranges of dark adaptation. This means, however, not that dark adaptation involves necessarily something other than the synthesis of visual pigments, but only that in the retina this synthesis is followed under the peculiar circumstances we know.

3. *Disproportionality between visual adaptation and photochemical changes*

Beginning with observations of Granit and his co-workers *(refs. 74,75)* and pursued further by Rose, Baumgardt, Pirenne and Denton, and others (cf. citations in *ref. 76)*, it came to be realized that relatively little bleaching of visual pigment might occasion a disproportionately large rise of visual threshold. Rushton and Cohen *(ref. 73)* calculated that the bleaching of less than 2% of the rhodopsin in the human eye raises the threshold one hundred times; and later we found evidence that the bleaching of 0.6% of human rhodopsin raised the threshold 3300 times *(ref. 76)*. I think it is now clear that the bulk of light and dark adaptation go with the bleaching of the first small fraction and the resynthesis of the last small fraction of the visual pigment. I have offered a possible explanation of how this happens *(ref. 76)*, and have derived the mathematical relation between pigment concentration and visual threshold that follows from this view *(ref. 68)*. Indeed, this formulation supports the observation stressed above, that the *logarithm* of the visual sensitivity rises and falls in parallel with the *concentration* of visual pigment.

It would be absurd to suppose that because the changes in concentration of visual pigment that accomplish much of visual adaptation are small, they are for that reason any less significant. It should be obvious that the bleaching and resynthesis of the visual pigments is of the same nature, and must follow much the same course, whether we regard the entire quantity of visual pigment in the eye, or 1 % of it. So long as we are dealing with a sufficient number of pigment molecules to satisfy the demands of statistical mechanics, we can expect to observe regular kinetics, and indeed the same kinetics as one observes with 100 or 1000 times this quantity of pigment.

4. "Neural" v. "photochemical" components in visual adaptation

This brings us to the companion statement, that light and dark adaptation are essentially "neural" rather than "photochemical". I think that in fact light and dark adaptation do involve neural components; and that the essential problem is not to choose between neural and photochemical components, but to attempt to evaluate both.

In this regard I have been much interested in re-examining the extraordinary measurements of Julian Blanchard *(ref. 77)*. Blanchard measured dark adaptation following light adaptation to four luminances: 0.1, 1, 10, and 100 millilamberts. He did this in the conventional way, pursuing the rise of sensitivity for an hour. However, he also measured the instantaneous thresholds at the moment of extinguishing the adapting light; and measured in detail the rise of sensitivity during the first minute. He gives us, therefore, the data of human dark adaptation both on a second basis and on a minute basis.

The first observation I should like to make is that these data disclose a discontinuity. Some time ago I noticed the simple empirical relationship, that in human rod dark adaptation, the reciprocal of log threshold plotted against time frequently yields a straight line *(ref. 76)*. Actually one plots, not quite this, but the reciprocal of the difference between log threshold at time t ($\log I_t$) and at the end of dark adaptation ($\log I_f$); i.e., one plots $1/(\log I_t - \log I_f)$ against time *(fig. 17, right)*. Such a straight line yields a relatively unequivocal extrapolation to zero time, and so a value of $\log I_0$.

In *fig. 17* one series of Blanchard's measurements have been analyzed in this way. At the left are the measurements themselves, plotted as Blanchard plotted them, in terms of -log threshold or log sensitivity. On the right the minute-to-minute measurements have been re-plotted in the form of a straight line, so permitting the extrapolation of the sensitivity to zero time. This indicates that $-\log I_0 = 4.06$, i.e., 2.46 log units above the instantaneous log sensitivity actually measured. Blanchard's measurements show that most of the first minute of dark adaptation is taken with making up this discrepancy.

I would be prepared to agree that the rapid initial change of sensitivity, most of it completed during the first ten seconds of dark adaptation, represents something other than the chemical reactions we have

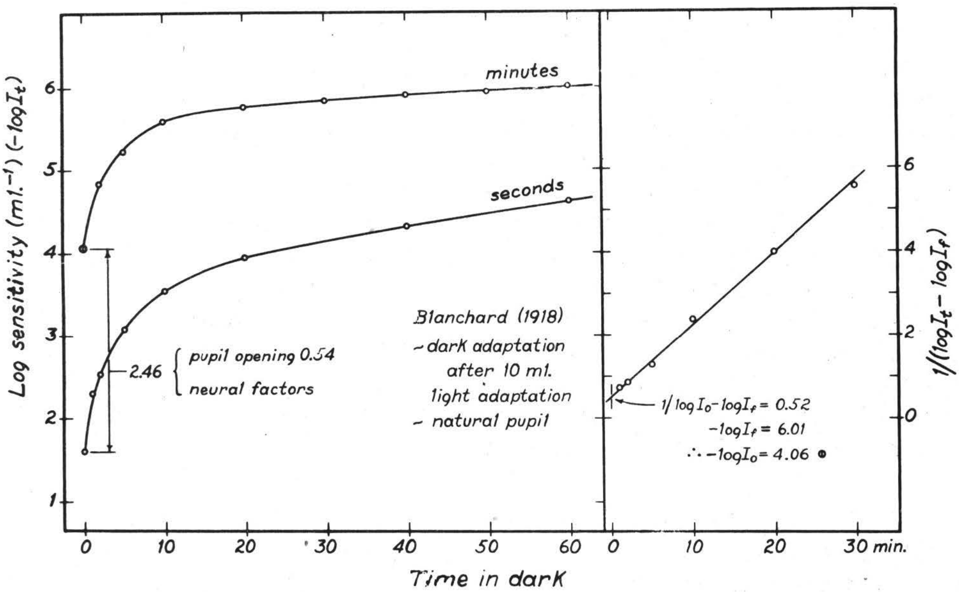


Fig. 17. Blanchard's measurements *(ref. 77)* of the dark adaptation of the human eye after light adaptation to a luminance of 10 millilamberts. White light, binocular view, natural pupils, test field diameter 4.9^0. The measurements are plotted in terms of seconds for the first minute, beginning with a special determination of the instantaneous threshold; and in terms of minutes thereafter for the first hour. At the right is shown a replotting of the minute-to-minute data, to yield an extrapolated value of the log sensitivity at zero time, $-\log I_0$. This value is used as the first point on the "minute" curve at the left. This calculated $-\log I_0$ lies 2.46 log units above the measured instantaneous sensitivity; most of the first minute of dark adaptation is occupied with closing this gap. Clearly the instantaneous sensitivity and most of the "second" function do not form part of the "minute" function. It is suggested that the latter is mainly concerned with the regeneration of rhodopsin, the "second" function mainly with other and more rapid types of change.

been considering. Since these measurements were made with the natural pupil, part of this change is probably caused by the opening of the pupil. According to Blanchard's measurements of pupillary size, this factor, following adaptation to 10 millilamberts, might account for a rise of log sensitivity of 0.54. The remainder of the rapid change, 2.46 - 0.54 = 1.92 log units, must be ascribed to other causes. It does not correspond to any known chemical reaction, and might in this sense be described as "neural". Some of it might be concerned with rapid restorative processes in the response mechanisms of the rods themselves ("Abklingen"), that have nothing to do with rhodopsin regeneration; some of it may involve similar reactions in the neurones and synapses of the visual pathway.

On the other hand, I think we have increasingly good evidence, in part reviewed in the previous section of this paper, that the relatively slow component of dark adaptation reflects the synthesis of visual pigments. This is the component that begins with the extrapolated value of log I_0, and takes more than an hour to complete *(fig. 17)*. Ordinarily it is all that we measure in a dark adaptation experiment.

I have put all of Blanchard's data through the type of analysis shown in *fig. 17*. All of it yields comparable results. These are summarized in *Table I*. It comes out that the slow "photochemical" component of dark adaptation is much smaller than the rapid "neural" component following adaptation to 0.1 and 1.0 millilambert. After adaptation to 10 millilamberts, both components are nearly equal; and after 100 millilamberts adaptation, the "photochemical" component is much the greater. These relationships would merit careful re-investigation.

TABLE I

Analysis of dark adaptation measurements of Julian Blanchard *(ref. 77)*. For 4 luminances of light adaptation, Blanchard measured the instantaneous thresholds, and dark adaptation through the first minute, and for the subsequent hour. He measured also the pupillary opening at various levels of light adaptation. These measurements were made with white light, binocularly, with natural pupils, using an unfixated square field that subtended an angle of 4.9^o with the eye. They are expressed as -log threshold or log sensitivity (-log I).

Column I: luminance of light adaptation. II: final log sensitivity (-log I_f), the asymptote of the dark adaptation curve. III: theoretical initial sensitivity (-log I_0) determined by extrapolating to zero time the straight line obtained by plotting $1/(\log I_t - \log I_f)$ against time in the dark. IV: log I_0 - log I_f, the range of the slow component of dark adaptation, here ascribed primarily to "photochemical" factors. VI: difference between the theoretical -log I_0 and the measured instantaneous sensitivity (V). Part of this difference is caused by the opening of the pupils (VII). The remainder (VIII) may be ascribed to "neural" factors, still undefined.

I Adapting luminance (milli-lamberts)	II Final log sensitivity (-log I_f)	III Theoretical initial log sensitivity (-log I_0)	IV "Photochem-ical" component (II - III)	V Instantaneous sensitivity (measured)	VI Pupillary + "neural" changes (III - V)	VII Log change in pupil area	VIII "Neural" component (VI - VII)
0.1	6.10	5.40	0.70	2.79	2.61	0.17	2.44
1.0	6.20	5.09	1.11	2.20	2.89	0.32	2.57
10.0	6.01	4.06	1.95	1.60	2.46	0.54	1.92
100.0	6.20	3.34	2.86	0.90	2.44	0.86	1.58

VI. VITAMIN A DEFICIENCY AND NIGHT-BLINDNESS

THE participation of vitamin A in the biochemistry of visual excitation introduces a special series of relationships. It connects the otherwise isolated visual processes with the general metabolism of vitamin A throughout the body, and with an external factor, its nutritional supply. One consequence of this connexion is that probably the earliest symptom of vitamin A deficiency in man and other animals is the rise of visual threshold known as night-blindness.

Because we are used to associating night vision with rods, it was once thought that dietary night-blindness is a failure specifically of rod vision. The first experimental studies of human night-blindness, however, showed at once that in vitamin A deficiency cone vision deteriorates with rod vision, and both recover together on administration of vitamin A *(refs. 78,79,80)*.

The realization that both iodopsin and rhodopsin are synthesized from the identical forms of vitamin A appears to offer a substantial basis for this relationship. It is true that iodopsin has not been demonstrated in the human cones; if present, it is presumably accompanied by at least two other cone pigments needed to account for normal human colour vision. Yet the observation that on administration of vitamin A, night-blindness is repaired as quickly and completely in the cones as in the rods implies that the human cone pigments as a group are probably synthesized from the same vitamin A.

It was hoped at one time that the experimental study of human night-blindness might provide an exemplary instance of the rise and cure of a human deficiency disease. Unfortunately, however, with the earliest of such experiments, in Hecht's laboratory and our own, stubborn discrepancies appeared which we were unable to clarify. They were of two kinds: (1) When placed on a vitamin A-deficient diet, certain human subjects begin immediately to go night-blind, whereas others show no effects, visual or otherwise, for many months. (2) Of the human subjects who did develop various degrees of night-blindness on vitamin-A deficient diets, some could be completely cured within a few hours by administration of a large dose of vitamin A, whereas others, though they showed some immediate improvement, required months of high vitamin A feeding to return to normal *(cf. refs. 81,82,83)*.

It should be said at once that of these two discrepancies, it is possible to adopt a reasonably satisfactory attitude with regard to the first. Human subjects have been shown to vary greatly in their liver stores of vitamin A *(refs. 84,85)*. Some human livers contain enough stored vitamin A so that, if used economically, it should tide a person over a period of deficiency lasting several years. It is altogether possible that the great variability in the times required for the development of night-blindness in human subjects derives directly from the very different amounts of vitamin A stored in the liver.

The intermediary between the vitamin A of the liver and the eye is the blood circulation. Much evidence has accumulated that as long as the blood vitamin A remains above a certain value (about 50 IU per 100 millilitres of serum) there is no night-blindness; and that when the blood vitamin A level falls below about 40 IU per cent, night-blindness regularly ensues. It seems, therefore, that so long as stores of vitamin A remain in the liver, the blood level is maintained, and vision remains normal. When the liver stores are exhausted, the level of vitamin A in the blood falls, and night-blindness is imminent *(refs.86,87,83)*.

The principal discrepancy that we face in this field, therefore, involves the slow *versus* fast cures of night-blindness. Some time ago I began to think that in this regard we may have tended too much to think of night-blindness and its cure in terms of vitamin A, and too little in terms of the other component of the visual pigments, the opsins *(cf. refs.57,3)*.

As soon as one begins to think of the opsins, this raises further thoughts. It is well known that in addition to producing night-blindness, vitamin A deficiency leads to a deterioration of tissues throughout the body, particularly epithelial tissues. Indeed, tissue degeneration is prominent in the eye itself. Shortly after night-blindness has appeared in rats, for example, one can observe the deterioration of the outer segments of the rods. Later, the inner segments of the rods also deteriorate, and then in order the external limiting membrane, the outer nuclear layer, the pigment epithelium, the outer molecular layer, and the inner nuclear layer *(refs.88,89,90)*.

It should be noted in this connexion that rhodopsin is an important *structural* component of the rod outer segment. It constitutes about 40% of the dry weight of a frog rod and about 14% of that of a cattle rod - respectively, about 60 and 22% of the rod proteins *(ref.76)*. If opsin were to begin to leave a rod, this should shortly appear as an anatomical degeneration of its structure; and whatever aspect of vitamin A deficiency caused a loss of opsin might equally involve the depletion of other retinal proteins, with the consequent deterioration of cellular anatomy in the other retinal tissues. In the etiology of night-blindness, therefore, we have to consider not only a depletion in the supply of vitamin A, but losses in opsin, and related with this, a progressive series of anatomical retinal lesions. The lack of vitamin A can be repaired very quickly by feeding it; but repairing the loss of proteins and particularly the degeneration of retinal tissues, might take much longer.

With a view to clarifying these relationships we have recently re-examined the course of vitamin A deficiency in the rat. The experiments were performed by a young Harvard undergraduate, John Dowling, during the summer months before and after his Senior year at college, and in whatever time he could spare from his studies during his Senior year. I think they constitute a remarkable achievement, for in the short time at his disposal Dowling managed single-handedly to explore the biochemistry of night-blindness, its physiology, and to a degree its histology *(ref. 91)*.

Not all these things were measured for the first time. I have already mentioned Tansley's and Johnson's histological studies. Special note should be taken also of Tansley's remarkably early investigation of rhodopsin synthesis in the normal and vitamin A-deficient rat *(ref. 92)*; and the excellent study by Lewis, Bodansky, Falk and McGuire *(ref. 93)* of the relative concentrations of vitamin A in the liver, plasma and retinas of rats on various levels of vitamin A intake and in vitamin A deficiency. There is also the provocative recent paper by Lowe, Morton and Harrison *(ref. 94)*, which opens new directions in the investigation of vitamin A deficiency.

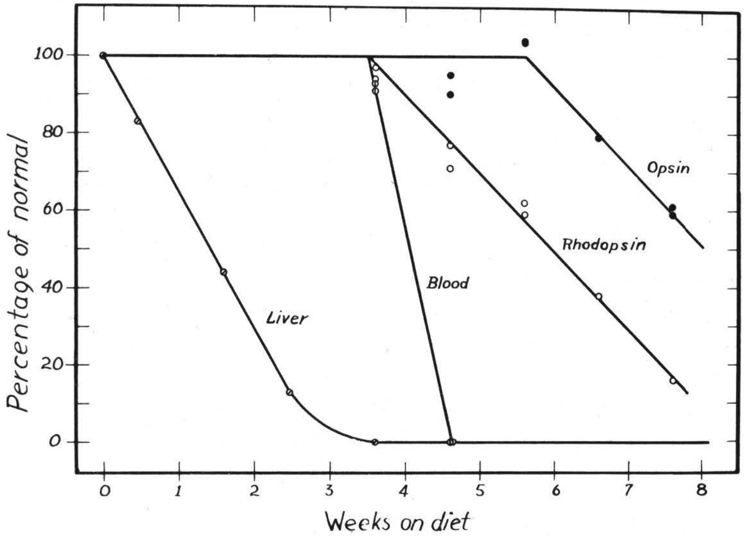


Fig. 18. Biochemical changes which accompany vitamin A deficiency in the white rat. Measurements of vitamin A content of the liver, vitamin A concentration of the blood plasma, and rhodopsin and opsin of the retina, all expressed as percentages of the normal values. As soon as the animals start on the deficient diet, the liver content of vitamin A falls. The plasma vitamin A remains normal until the liver is almost wholly depleted; and the rhodopsin level remains normal until the blood is depleted. The decline of rhodopsin marks the incidence of night-blindness. the decline in opsin the beginning of structural degeneration in the retinal tissues. (From *ref. 91*).

Fig.18 shows the results of a typical experiment, in which the vitamin A content of the liver, the vitamin A of the blood plasma, and the concentrations of rhodopsin and of opsin in the retina were followed simultaneously in a single group of rats on a vitamin A-deficient diet. The results are expressed throughout as percentages of the normal values.

The general plan of such an experiment was as follows. Male albino rats, 22-24 days old, weighing 33-36 grams, were divided into two groups, one of which received the standard USP Vitamin A-deficient test diet, the other a complete diet.

The deprived animals continued to gain weight, though more slowly than the controls, into the fifth week. In the fifth to seventh week their weight became constant, and thereafter it declined rapidly.

The liver vitamin A represents the content of the whole liver, so better to express the total vitamin A available to the animal; and this is rendered as percentages of the amounts of vitamin A stored in the livers of control animals at the time the deficient diet was started. The blood vitamin A, rhodopsin, and opsin are all expressed as percentages of the concentrations and amounts found in control animals of the same age.

Rhodopsin was measured by direct extraction from the retina, and mainly by the change of extinction at 500 mμ on bleaching. Opsin was measured by the capacity to regenerate rhodopsin on incubation of whole retinas with neo-*b* retinene. We had found that bleached rat rhodopsin or opsin, dissolved in aqueous digitonin solutions, exhibits only a negligible capacity to regenerate rhodopsin. For effective regeneration it was necessary to incubate the retinas themselves, after bleaching, for 4-6 hours at room temperature with neo-*b* retinene. The ratio of rhodopsin regenerated by the retinas of deficient animals to that regenerated by normal retinas represented the relative opsin content.

As soon as the rats were placed on the deficient diet, the stores of vitamin A in the liver began to fall, and declined almost linearly until in the third to fourth week the liver was wholly depleted. The blood maintained its normal level of vitamin A until the liver had been completely drained. Then the blood vitamin A fell precipitately, so that within a few days it went from normal values to zero. At this point the rhodopsin concentration in the retina began to decline, and in the succeeding weeks this fell regularly. This marked the onset of night-blindness.

There is a curious interval of several weeks in which, though the rhodopsin concentration is falling, the opsin level remains normal. During this period the rat possesses more opsin in the outer segments of its rods than it can find vitamin A to occupy. Then the opsin concentration also begins to fall; and this marks the point at which the first signs of structural degeneration appear in the retina.

In this period, however, following the seventh week of the diet, many other signs of deficiency become evident. The rats lose weight rapidly, and develop the classic ocular signs of vitamin A deficiency: xerophthalmia, xerosis, and keratomalacia. All these symptoms were observed in the last weeks of the experiment. The eyes were surrounded by a reddened area, and tended to become encrusted with dried exudate. The animal's coat was often rough and disarranged, and the gait unsteady. Several of the animals breathed with difficulty, and by the end of the eighth week all the animals not sacrificed in earlier experiments had died.

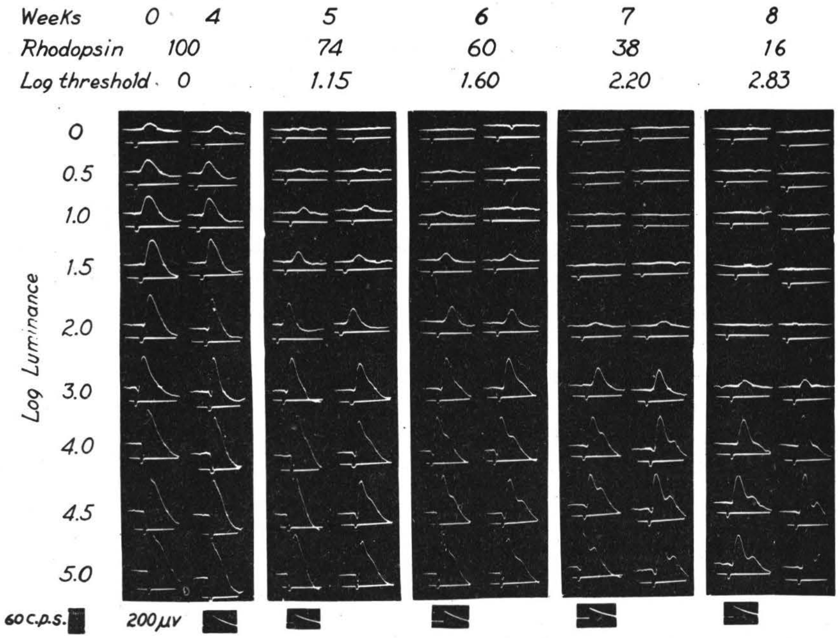


Fig. 19. The effects of vitamin A deficiency on the ERG. For the first 4 weeks on the deficient diet, both the rhodopsin level and the ERG remain normal; sets of ERG data from weeks 0 and 4 are shown. Thereafter, the rhodopsin level falls, the log threshold rises, and the ERG deteriorates (records each week from 2 animals): the b-wave grows smaller, the a-wave virtually disappears, and a second component in the b-wave is progressively delayed, so that by 7-8 weeks it appears as an independent wave. (From *ref.91*).

Fig. 19 shows electroretinograms measured in these animals. For this the animals were dark-adapted over-night, and anaesthetized lightly with nembutal (0.1 cc. per 100 grms.). The ERG was led off from the eye with two cotton wick electrodes moistened with Ringer solution, one placed on the side of the cornea, the other on a shaved area of the cheek. The intensity of the test light was controlled with neutral filters and a circular wedge, and it was delivered in flashes of 1/50 second. The ERG was recorded through a capacity-coupled Grass P4 preamplifier, used together with a Dumont oscilloscope.

The top of *fig.19* shows the weeks on the vitamin A-deficient diet, and under this the rhodopsin obtained from these animals, expressed as percentages of the amounts found in controls. Under this are measurements of the logarithm of the visual threshold; the normal threshold is given arbitrarily the value 1, hence the normal log threshold is 0. To determine the threshold, the dark-adapted animal was stimulated with sub-threshold flashes, the brightness of which was raised until the response could be just detected on the oscilloscope. This procedure was repeated several times until constant values were obtained.

After the threshold had been determined in this way, the ERG was measured for a series of luminances that involved a total range of 5 log units. The photographs of these responses are shown in the figure. For the first four weeks of the diet, no appreciable change occurred in such measurements; the figure begins, therefore, by showing in parallel the ERGs obtained with an animal just before going on the deficient diet, and another animal that had been four weeks on the diet. For each week thereafter the figure shows the ERGs measured on pairs of animals. These animals were afterward sacrificed and their retinas extracted for rhodopsin.

Fig.19 shows that the ERG remains normal as long as the rhodopsin level remains at its maximal value. As the rhodopsin level begins to fall, the visual threshold rises. This is the beginning of night-blindness.

In the succeeding weeks one can follow the development of three further changes: (a) As the threshold continues to rise, and the rhodopsin concentration to fall, the b-wave of the ERG in response to a given luminance of test flash becomes progressively smaller. Thus, for example, at log luminance 3.0, after four weeks of the deficient diet the ERG was relatively normal, but after eight weeks on the diet the b-wave had fallen to the just perceptible threshold level. (b) Even more rapidly than the b-wave, the a-wave declines in height, and finally hardly appears even at the highest luminance. Thus at log luminance 4.0, the normal animals exhibit a large a-wave along with a large b-wave; after eight weeks on the deficient diet, there is still a large b-wave, but the a-wave has almost vanished. (c) The b-wave is composed of two components, resulting, in normal animals, in a small inflexion at the top of the b-wave, evident particularly at the highest luminances. As the animals become deficient, this deflection moves down the descending limb of the b-wave, and finally appears as an independent maximum. This effect is associated with a progressively longer delay in the appearance of the second component of the b-wave - i.e., a relative increase in its latency - as the vitamin A deficiency progresses.

Figs. 20 and *21* show the numerical results of such experiments. *Fig.20* shows that the logarithm of the threshold rises linearly with the time on the diet, after a somewhat more rapid initial rise. Extrapolation of the straight line in *fig.20* to the normal threshold suggests that the threshold might have begun to rise after two and a half weeks on the diet; whereas in fact it began to rise one week later. A similarly linear rise of log threshold with time on a vitamin A-deficient diet has been observed also in human subjects *(refs. 79,95)*.

Fig.21 shows the relationship between the retinal content of rhodopsin and the logarithm of the threshold of the dark-adapted eye. Over most of the range the rise of log threshold - that is, the fall of log sensitivity - is linear with the decline in rhodopsin concentration. The initial rise of log threshold is a little more precipitate, yet does not depart greatly from the linear relationship. Here again, as in visual adaptation

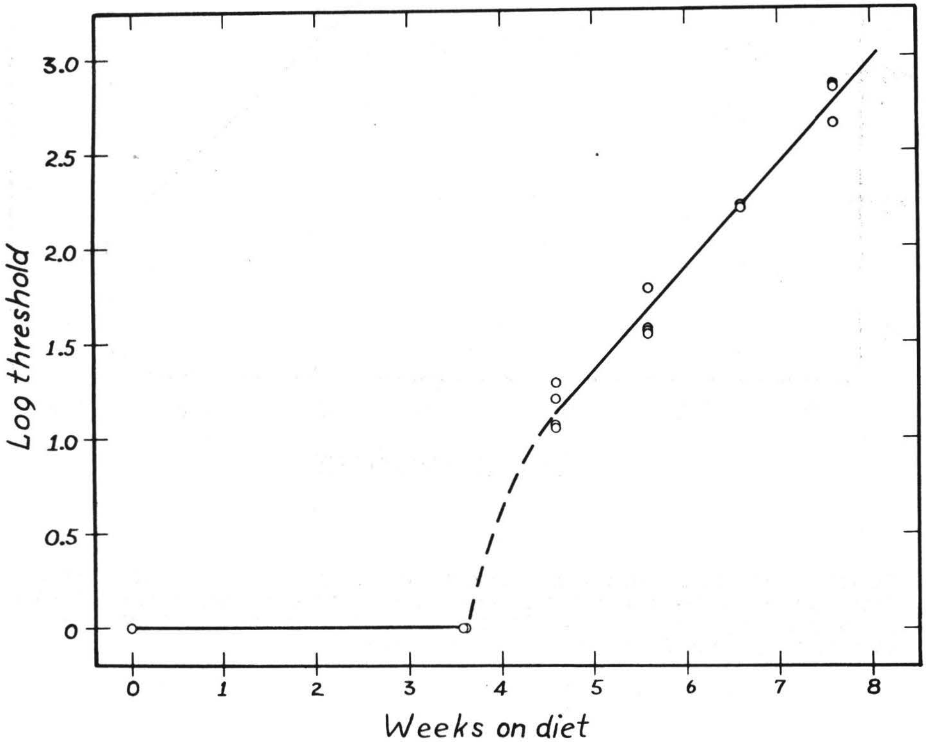


Fig. 20. Rise of log threshold with time on a vitamin A-deficient diet in the rat. (From *ref.91*).

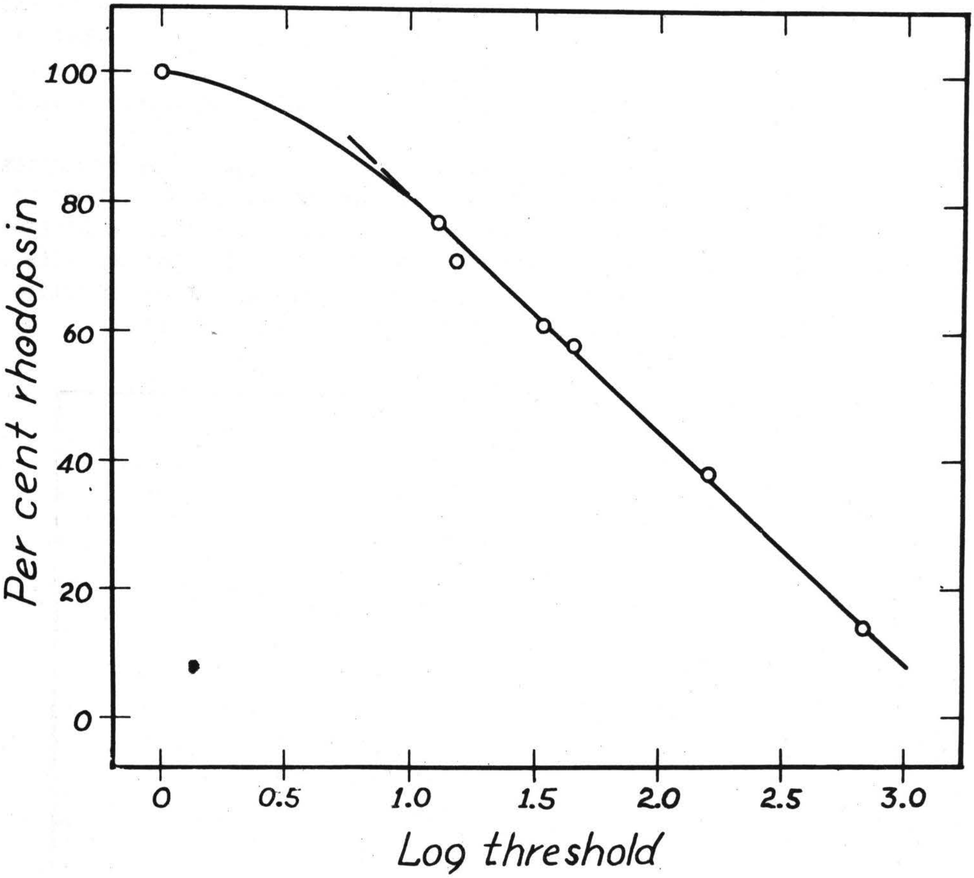


Fig. 21. Variation of log threshold with rhodopsin content of retinas of vitamin A-deficient rats. The threshold is expressed on an arbitrary scale, in which the normal log threshold = 0. The rhodopsin is expressed as percentage of that found in normal retinas. (From *ref.91*).

(cf. *Section IV* above), the log sensitivity appears to vary in parallel with the concentration of visual pigment.

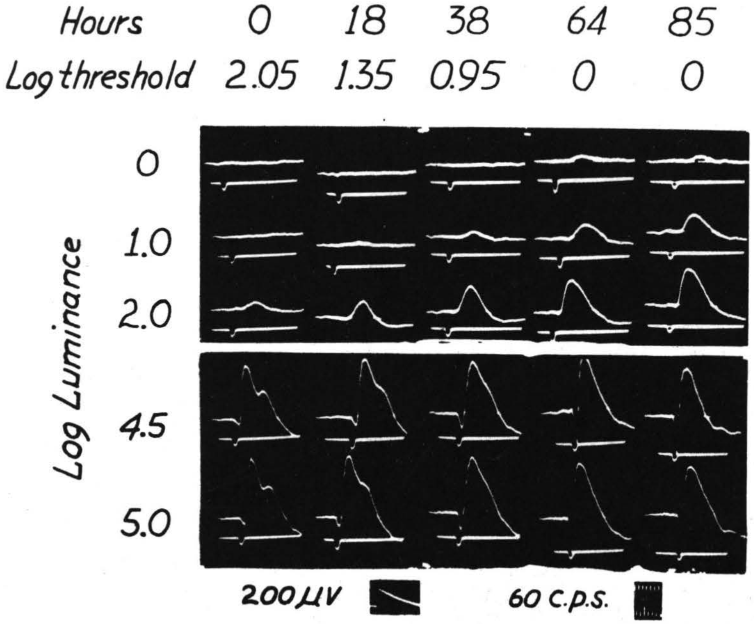


Fig.22. Recovery from night-blindness in the rat, on administration of vitamin A. Top: hours after intraperitoneal injection of all-*trans* vitamin A in oil; and below this, measurements of log threshold (0 = normal). ERG measurements at various luminances, made on this one animal. Within 64 hours the threshold has fallen to normal, and the ERG has retraced in reverse all the changes which had accompanied the development of night-blindness. (From *ref.91*).

Fig.22 shows the course of a recovery from night-blindness. At the beginning of the experiment this animal exhibited much the same ERG responses and log threshold as did the animals of *fig.19* in the seventh week of deficiency. Its visual threshold was a little more than 100 times normal.

The animal was given a large dose of all-*trans* vitamin A in oil, injected intraperitoneally. The threshold slowly fell, and reached the normal level within 64 hours. During this period the ERG retraced in reverse all the changes that had accompanied the development of night-blindness: (a) the responses at all brightnesses of test flash increased in size; (b) the a-wave returned to its former prominence; and (c) the delayed component of the b-wave once again ascended to its former position as a small inflexion at the top of the b-wave.

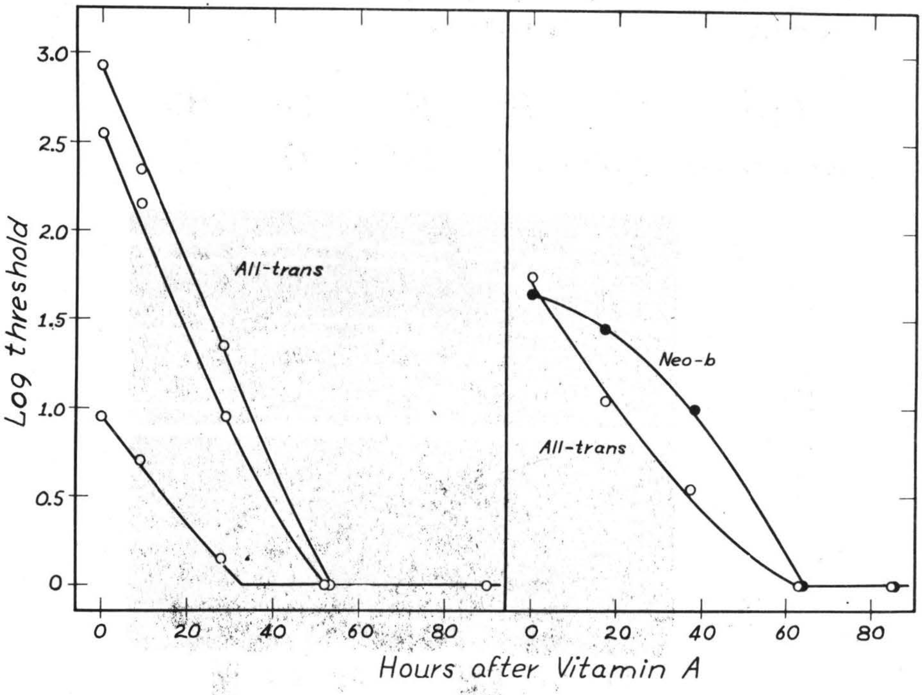


Fig.23. The cure of night-blindness by administration of vitamin A. The log threshold measured at various times after intraperitoneal injection of all-*trans* (open circles) or neo-*b* vitamin A (closed circles). Dosages: at left, 320 - 920 μgm. in 1 - 3 injections; at right 1 mgm. in 3 injections. (From *ref.91*).

Fig.23 shows a series of such recoveries from night-blindness after injection of all-*trans* vitamin A, and on one occasion neo-*b* vitamin A, in terms of the return of log threshold to normal. With all-*trans* vitamin A, the log threshold falls linearly in all cases, and has reached the normal level within 30-60 hours.

On the only occasion in which we injected neo-*b* vitamin A, the recovery displayed an initial lag. We have examined the vitamin A of the liver after injections of neo-*b* vitamin A, and have never found a trace of the neo-*b* isomer. Our interpretation of the delayed course of recovery following injection of neo-*b* vitamin A is that this isomer must first be isomerized to the all-*trans* configuration, and is conveyed in that form to the eye. In experiments with cattle we have looked for the neo-*b* isomer of vitamin A in the liver, kidney, and blood plasma, and in no case found any trace of it (*P. S. Brown, unpublished observations*). This isomer has so far only been found in eye tissues; and it seems probable that the isomerisation of all-*trans* to neo-*b* vitamin A occurs in the eye itself.

The degeneration of the retinal tissues that results from vitamin A deficiency in the rat has been described by Tansley (*refs. 88,89*) and by Johnson (*ref. 90*). For our purposes it is most important to orient the histological symptoms in the pattern of biochemical and physiological changes that we have described. We find that after six weeks of the vitamin A-deficient diet, when the rhodopsin has already fallen to perhaps half its normal value but the opsin is still intact, the retinal histology appears to be altogether normal. After eight weeks on the diet, however, when the opsin has fallen to perhaps half its normal value, and many overt signs of the deficiency have appeared, the retinal histology has become very much disturbed. The outer segments of the rods are attenuated, have a gnawed appearance, and have lost their former regularity of spacing. Only vestiges of the pigment epithelium remain. The clean line of separation that marked the external limiting membrane also is gone; and in the choroid the blood vessels appear to be occluded. Our initial supposition that when opsin goes, the outer segments of the rods should show structural deterioration, has proved to be correct; but by this time degenerative changes are evident also in many other tissues.

In the light of these data the etiology of night-blindness and the recovery from this disease seem to present a clear and simple series of changes. There is no suggestion in these measurements of any uncontrolled variable or the participation of any factor other than vitamin A itself. After the stores of vitamin A have left the liver, the level of vitamin A in the blood can no longer be maintained. After vitamin A has left the blood, the retina is no longer able to obtain sufficient vitamin A to occupy all its opsin. Lower than normal amounts of rhodopsin are formed, and this is the cause of night-blindness. At this point, at which the retina contains all its opsin, but insufficient supplies of vitamin A, night-blindness can be cured very quickly. On administration of vitamin A, as rapidly as the retina can acquire it, rhodopsin is built back to its normal level, and night-blindness is at an end. As the deficiency progresses further, however, opsin itself begins to decline in concentration; and at this time other tissues in the eye and apparently elsewhere in the body begin to deteriorate anatomically. It is not enough any longer to supply vitamin A to achieve recovery. Time must be allowed for the structural repair of the eye tissues before the visual threshold can return to normal. Recovery from night-blindness at this stage of deficiency is correspondingly slow.

It seems to us probable that human night-blindness involves similar relationships. The great variability in the times required for the incidence of night-blindness in human subjects deprived of vitamin A seems explained with Moore's demonstration of the great variability in the amounts of vitamin A stored in human livers. It seems to be true in man as in the rat that as long as the liver retains vitamin A, the blood level remains high (cf. *refs. 82,83)*. When the blood level sinks below a critical value (40-50 I.U. per cent; *refs. 86,96,83*), night-blindness appears.

The length of time required to cure human night-blindness on administration of vitamin A probably depends in man, as in the rat, on the degree to which the deficiency has induced changes more deep-seated than simple lack of vitamin A in the eye tissues. If the retinal tissues are normal and the opsin concentration complete, administration of vitamin A should accomplish recovery from night-blindness within a matter of hours, as has frequently been observed. When, however, the deficiency has progressed to the point at which the concentration of opsin has declined and other aspects of tissue deterioration have appeared, correspondingly long times must elapse after administration of vitamin A for the structural lesions to be repaired, and the visual threshold to return to normal.

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TRIBUTES TO SELIG HECHT

PROFESSOR H. HARTRIDGE (University of London) said:

I know that you will by now have given a great deal of thought to the matter of Hecht as a scientist. I wonder therefore if you would like me to speak about Hecht in his capacity of a friend, and for me to describe how we first met and what followed that meeting?

It must have been in June 1925 that Langley F.R.S., who was Professor of Physiology in Cambridge and Editor of the Journal of Physiology, received a letter stating that Hecht was on his way to this country, and would be staying with his wife and daughter in Cambridge for the long vacation.

At that time, Banister, Lythgoe and myself were studying the relationship between visual acuity and illumination. We were very excited to hear of Hecht's visit. A week or two later he came to the laboratory for the first time, and I immediately noticed two things about him, his cordial and friendly manner and the breadth across his shoulders. In the days which followed I was to learn that breadth was one of Hecht's characteristic features, and that it applied not only physically but also mentally. He had a wide knowledge of science and a generous disposition both to his family and to his acquaintances. We quickly became friends. He often visited us at the laboratory to see how our research was progressing, and to discuss the possible causes of the changes of acuity with illumination; and of course his family met mine, and at once a lasting friendship was established. In the evenings we would go to one anothers' houses, and at weekends we would take our combined families out, for trips on the river, for games in the woods, for visits to neighbouring beauty spots or places of historic interest. From time to time we would set out together to sketch from nature, he in crayons and oils; I in pen and ink and water-colours. Then in the evenings we would show our wives what we had done. Hecht always amused us by putting a "surround" around his sketches. He used to say that a picture could not be properly seen, unless it had a frame, which in this case consisted of a piece of white cardboard with an oblong hole in it. This he held in front of each sketch as he showed it to us. So time passed! Happy carefree days which went by all too quickly, and soon Hecht had to take his family back to America. From time to time he wrote suggesting that I and my family should pay him a return visit. But this never proved to be possible. If London University had had Sabbatical years set aside for study, travel or research, as Cambridge University has, it could have been managed, but unfortunately this was not the case. So it came about that we did not meet again for many years.

In 1948 my wife and I attended the International Physiological Congress at Oxford, and while there we met friends two of whom we invited to stay with us as soon as the Congress was over. We had no premonition of what was in store. It was planned that we should go home first and get everything ready, and that our friends should then follow, arriving later that same evening. This plan was carried out, but when we arrived we found three cars in our drive, one belonged to our doctor, the two others being unknown to us. On entering the house we learned that our second daughter Felicity, at that time a medical student studying "fevers" had returned home with a high temperature, had been put to bed and our doctor sent for. He in his turn had sent for a specialist in fevers, and the medical officer of health. Either it was a case of small-pox, or the worst case of chicken-pox that any of them had seen. The rash now covered the skin entirely, it also covered the mouth, the tongue, and the interior of the nose, but also other surfaces, probably including the intestinal tract. The case, the doctors said, must be treated as one of small-pox. The patient is far too ill to move to a fever hospital and so must be nursed at home, taking every precaution against cross infection, and spread of infection. Soon our visitors arrived and we explained the position to them. One decided to leave, the other to stay and help us. Quickly the first floor of our house was turned in to a ward, nurses arrived, a large steriliser with its drums and other accessories were sent in by the M.O.H., and the anxious work began. A week later the patient's condition began to improve and two day's later it was clear that the case was one of chicken-pox. But why do I record all this? What has it got to do with Hecht? It has everything to do with him, because he was the guest who decided to stay. Who else, but this kindly man, would have sacrificed valuable days on a visit to another country, in order to help to nurse someone else's sick daughter?

You may like to know what the outcome was. Felicity made a complete recovery and the fever left leaving no trace whatever. She went back to King's College Hospital, got qualified, got engaged, got married, and now has a plump and energetic baby daughter. We like to think, my wife and I, that Hecht played a decisive role in that recovery, and that it was owing to his help that there were no complications. We will always think of him with gratitude in our hearts.

DR. M. H. PIRENNE (University of Oxford) said:

SELIG HECHT'S great endeavour was the quantitative study of the biological processes of vision and photoreception, with particular reference to the photochemical reactions occurring in the peripheral receptors, which, as he thought, are bound to determine to a large extent the characteristics of the visual response. The contents of the very papers presented to this Conference do, I think, prove that such an endeavour is now generally regarded as scientifically legitimate and fruitful. Yet it must be remembered that in his day he had to meet opposition, notably from those who, as he put it, believed that precise investigation in this field was impossible because the biological reactions involved must be inherently erratic in their outcome or too complicated ever to admit of a simple explanation, particularly an explanation in terms of physics and chemistry.

Such an attitude of mind also carried a possible implication regarding his work. For Hecht did obtain accurate and significant results in experiments performed, not only on human subjects, but also on a wide range of animals - from the clam to the honey-bee. The implication was that these beautiful experimental results perhaps were "too good to be true." Those who collaborated with him know that this was not so. The records of preliminary trials Hecht invariably threw into the waste-paper basket; then, having perfected his method of experiment; he set to work. Now it is true that the results of the first trials often showed puzzling irregularities. But if such irregularities had been due to intrinsic properties of the system studied, no amount of experiment planning could have removed them - the quantum fluctuations of the stimulus acting upon the eye are in fact an intrinsic property of the system, and these indeed were never eliminated! Hecht got reproducible and consistent results from organisms which some would have considered very unpromising material. In the case of subjective visual experiments, he had a lifetime's experience of the complexities which, from an introspective standpoint, face the human observer. It seems that he took for granted that all his readers knew as much about this as he did, and in his publications he gave only his final results. Thus, it may sometimes be regretted that he did not publish a more detailed description of some of his work. The fact that he was a master of experimentation is, however, beyond doubt.

Hecht was something of a pioneer in a field which at the time attracted fewer first-class scientific minds than it does today. As he was a forceful personality, the relative isolation in which he worked explains that at times he may have appeared dogmatic. Yet dogmatism was not the impression he gave to those who really knew him. He was a scientist searching for truth. He was fearless in stating what he believed to be the truth. But he was also prepared and eager to accept new truths from others. In his dealings with collaborators he was a most human and sympathetic person, and won the affection of all those around him.

He was a painter and a man of discriminating taste, but first of all he was an artist in his work. A well-done piece of scientific research would fill him with delight, whether it happened to be just published, or was a classical work like Schultze's paper on rods and cones, with its beautiful drawings of the retina. His care in the preparation of his publications, particularly with regard to the bibliography, matched the care he gave to his experiments. His aim, he said, was not to add to, but rather to subtract from, the literature on vision.

No doubt some of Selig Hecht's theories - as is the fate, or the purpose, of theories - will be superseded, but many of his leading ideas will not. Others are gathering what he has sown.

DR. S. S. BALLARD (Scripps Institution of Oceanography, California) said:

I am presenting this tribute on behalf of the Armed Forces - National Research Council Committee on Vision, of the National Academy of Sciences of the U.S.A. If it is permissible, I should like to speak also on my own behalf, since I came to know Professor Hecht during the recent war, and profited greatly from his advice and counsel on practical matters involving vision.

Others have spoken of Professor Hecht's accomplishments in pure research. I wish to refer to his contributions made by applying his broad knowledge of physiological optics to problems of the greatest practical value. Early in World War II we found ourselves in a position where many decisions had to be made regarding such items as specifications for new optical instruments, lighting practices in the fleet, and night vision selection and training, to name just a few. The experimental data needed for making these decisions were simply not available. Therefore, those of us in the service - I was a reserve lieutenant in the Navy's Bureau of Ordnance - had to go to the best sources we could find for the best obtainable advice. For vision matters, this "best" source was often Professor Hecht. I remember well the patience with which he explained to me the reason for removing our blue battle lights and replacing them with red lights. (The problems of red lighting in the chart room were more difficult to solve!) He was equally generous in giving advice as to settings for fixed-focus binoculars and telescopes, magnifications to employ in search and fire control optical instruments, and the like. I remember his saying in a moment of candour that what persons in our predicament should do was to go to an appropriate scientist, ask for his best advice, and then follow it. He went on to say that the educated guess of an expert in such matters was likely to be at least 90 per cent correct - it might take a year or two to fill in the other 10 per cent by an extended research programme, the only useful result of which would be to convince his scientific colleagues that he had been right in the first place!

Professor Hecht attended the second meeting of our Vision Committee, held in May 1944, and after that he seldom missed a meeting although they came frequently. The last meeting he attended was our nineteenth, held in late May of 1947. At our twentieth meeting, held in October of that year, the Committee noted with sorrow his death on 18 September. I would like to close by reading part of the memorial that was prepared by Professor Walter R. Miles, read before our Committee, and incorporated into our Minutes; a copy was sent to Mrs. Hecht.

"Dr. Hecht's contributions as a scientist were by no means restricted to his work in the laboratory. From the beginning of the recent war he gave unsparingly of his time and knowledge to the solution of a wide range of military problems. He served as a distinguished member of our Committee and of other national advisory committees. He was counsellor and expert consultant on numerous occasions and he directed many researches, individually conducting both field and laboratory investigations, to answer specific critical military problems. At the end of the war he enthusiastically embraced the role of educator of the public in basic concepts and implications of atomic energy. The role of education was not new to him; he had long been recognized as a great teacher effective in training and stimulating workers in diverse scientific fields. As colleague, friend, and critic, he was unique, and he has left an impress both personal and scientific that will endure."

SESSION II

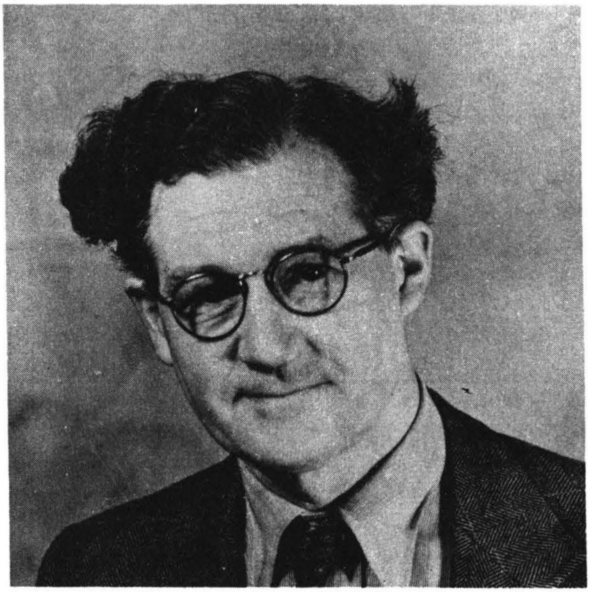
VISUAL PIGMENTS, PARTICULARLY IN RELATION TO COLOUR VISION

Chairman: PROFESSOR GEORGE WALD, HARVARD UNIVERSITY

PAPER I

THE CONE PIGMENTS OF THE HUMAN FOVEA IN COLOUR BLIND AND NORMAL

By W. A. H. RUSHTON



Dr. W. A. H. Rushton is Reader in Physiology in the University of Cambridge, where he has spent practically all his academic life. He entered, and later obtained a Research Fellowship at Emmanuel, the College of Thomas Young; and, after qualifying in medicine, became Staff Fellow and Director of Medical Studies at Trinity, the College of Isaac Newton.

Most of his work has been upon the theory of nerve excitation for which he was elected F.R.S. in 1948. The next year he spent in Stockholm with Professor Granit, and this turned his interests towards the nervous and chemical organisation of the retina.

1. THE CONE PIGMENTS OF THE HUMAN FOVEA IN COLOUR BLIND AND NORMAL

By W. A. H. RUSHTON

SUMMARY

1. A technique is described for measuring foveal pigments by analysing light reflected from the fovea in an ophthalmoscope.
2. The fovea of the protanope becomes bleached upon bright exposure and darkens during the next few minutes. This is quantitatively what would be expected of a visual pigment.
3. The difference spectrum on partial bleaching only reveals one pigment in the protanope, and the spectrum is similar to the luminosity curve.
4. The normal fovea contains two visual pigments one of which is that of the protanope, and one more red sensitive.
5. The photosensitivity and regeneration rate is about the same for the two pigments. The red sensitive one is present in somewhat greater amount.
6. The deuteranope contains both normal pigments with a greater preponderance of red.
7. The green pigment mechanism appears to be twice as sensitive as the red pigment.
8. Stiles' π_4 sensitivity could correspond to the green sensitive pigment. His π_5 cannot correspond to the red-sensitive pigment.

I. PRINCIPLES AND TECHNIQUES

(a) Principles of Measurement

Light seen reflected from the *fundus oculi* has passed twice through the retina and consequently suffered absorption from any visual pigments encountered. Thus light received in an ophthalmoscope contains a signal which involves absorption by visual pigments. The problem is to abstract that absorption from the many other factors upon which the extreme attenuation of the reflected light depends.

It is known that after rhodopsin has been bleached there is a very great change in its absorption spectrum, and the amount of the photolysis has long been measured in this way *in vitro*. The same thing can be done in the periphery of the human eye *(ref. 1)* for green light reflected from the *fundus* returns brighter after bleaching. Now it is also found that light reflected from the human fovea is brighter after exposure to a strong light and the possibility arises that this might be the result of bleaching the pigments in the cones, which are the sole photoreceptors of the fovea. If this supposition were true we should possess a means of measuring some properties of human cone pigments *in vivo*, so a technique has been developed for looking into the matter.

The aim of the present paper is first to substantiate the claim that the density of cone pigments constitutes an important parameter in the reflexion measurements made, and second to extract from the measurements some information about the cone pigments in colour blind and normal.

There are two grave limitations in the present treatment which must be stated at the outset. One is that nothing will be said about the "blue" receptors and their pigment. The technique so far used gives very weak and unreliable signals reflected from the fovea in blue light, and nothing has been noted that can obviously be attributed to a specific blue-receiving pigment. I wish therefore neither to affirm nor deny anything about foveal blue receptors. The second admission lies in the difficulty I have had in doing these experiments with proper accuracy, or in knowing where systematic errors might be creeping in. I have therefore preferred to perform a considerable range of experiments in order to obtain a surer perspective. So in almost every result to be quoted it will be seen that more and better work needs to be done.

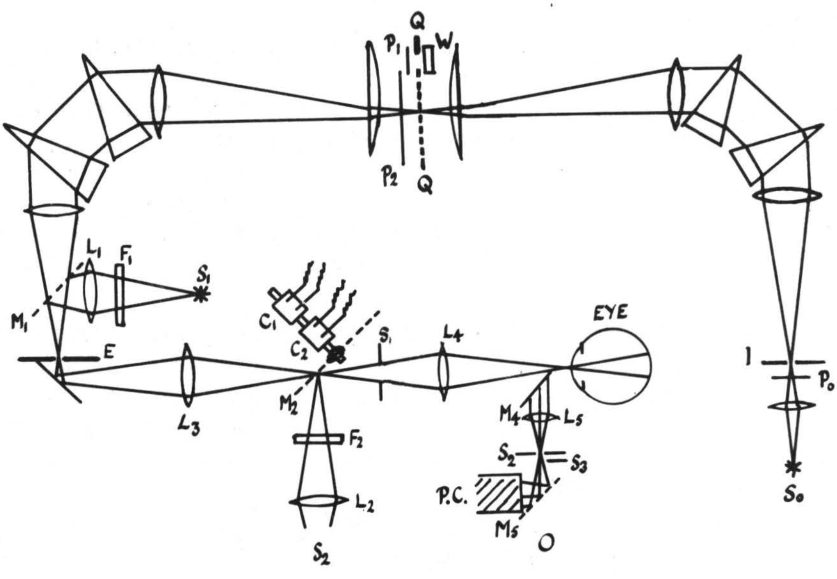


Fig.1. Stiles' double monochromator adapted for the measurement of foveal pigments

(b) The technique

The apparatus and methods are very similar to those already described *(ref.2)*. Measurement of reflexion from the fovea is made in light of narrow wave bands obtained from a fine double monochromator *(fig.1)* designed by Stiles and built by the N.P.L., being essentially one deck of his great trireme 'N.P.L. Trichromator' *(ref.3)*.

In the median plane of the instrument Q, Q where the spectrum is formed for wave length selection, a metal screen is placed in which are cut 8 slits. Each slit is provided with a shutter so that one or more wave bands may be selected to emerge from the exit slit E of the instrument. In addition there is a broad slot in the far red from about 700 mμ upwards. The change in foveal reflectivity upon bleaching which may be observed in yellow light, is not detectable in light of wave length 700 mμ, so the deep red is used as a control against which to check fluctuations in the intensity of the light source etc.

The comparison is made by placing, in the far red beam, a photometric wedge W and a polaroid P_1. A second polaroid P_2 crossed with respect to the first is placed in front of the 8 slits so that when a third polaroid P_0 is rotated in the common beam at I, the inlet slit of the instrument, it alternately cuts off the 700 mμ and the other wave lengths from emergence at the exit slit. In fact if the polaroid rotates at p radians/sec, the light reflected from the eye will fall upon a photomultiplier tube so as to generate an electrical output of magnitude

$$r \sin^2 pt + g \cos^2 pt \quad . \quad . \quad . \qquad (1)$$

where r is the contribution from the far red, and g from some other wave length in whose light the pigment density is to be studied.

If the wedge is shifted it will change the intensity of the far red light, so the value of r will alter, and when $r = g$, expression (1) becomes simply g, and the a.c. output of the photocell will be exactly zero.

All measurements are made by reading the wedge setting required for this null output, which setting is nearly independent of fluctuations either in the light source or in the amplifying system but is sensitive to changes in the density of visual pigments. The signal is extremely weak and hence 'noisy'. A very great improvement in the precision of measurement results from passing the output of the photocell through a phase-sensitive rectifier which rejects all components of the noise unless they have exactly the same frequency and approximately the same phase as the pure signal.

The light from the spectrum was too weak to produce any great change in foveal reflectivity, so two separate bleaching lights were used. Near the exit slit E of the spectroscope is a straight filament 6V 6A car headlamp S_1 similar to the spectral source S_0. This filament was focussed by L_1 upon the exit slit after reflexion in M_1, a mirror mounted upon a pivot so that light from the spectrum could instantly be exchanged for light from S_1. This bleaching light was varied in colour and intensity by filters F_1.

Since M_1 allowed either bleaching light or measuring light to reach E, it was impossible to measure the reflectivity while bleaching was actually going on. This was more or less achieved by the alternative bleaching source S_2, a car lamp similar to S_1. This was focussed by L_2 upon a mirror M_2 so that the image of the filament coincided with that of the exit slit projected there by L_3. M_2 consisted of two mirrors mounted upon the shaft of a velodyne motor like sails of a windmill. As the windmill rotated the sails alternately intercepted the light from S_2 or allowed light from the spectrum to pass. S_2 occupied a quarter of the cycle which was about 0.1 sec in total duration. Mounted upon the axis of the motor were two commutators C_1 C_2 upon which contacts suitably phased operated an electric shutter S_3 which excluded from the photocell P.C. light reflected from the eye during the bleaching phase, and short-circuited the electric signal except while the measuring lights were being received.

The light reaching the Maxwellian lens L_4 was limited by the stop S_1 which was a circle with cross hairs, placed so as to be sharply in focus for the subject (that is in the focal plane of L_4 for a homatropinized emmetrope). It illuminated about 2.7^0 of fovea, but only the central 1.8^0 was accepted by the photocell. After reflexion in the ophthalmoscopic mirror M_4 the retinal image was formed at S_2 in the focal plane of L_5 where it could be observed from O when M_5 was swung aside. The image of the cross hairs could be clearly seen and the subject told if they were not properly in focus (though naturally a good subject was a better judge of this). A tiny bright spot about 30 μ in size could be seen dancing around the fixation spot. It was the real image formed just in front of the retina by reflexion in the concavity of the foveal depression. It occupied the exact spot fixated by the subject at the moment and could be used to estimate the steadiness of his fixation when requested for short intervals.

An iris diaphragm S_2 in the plane of the retinal image was closed down to accept about 1.8^0 of the central part of this retinal image. Finally the mirror M_5 was swung round to deflect the light on to the large surface cathode of the E.M.I. 11-stage photomultiplier cell P.C.

The subjects were nearly all young male undergraduates of Cambridge, mainly medical students. The left pupil was dilated with homatropine which also paralyzed accommodation. Head fixation was achieved by dental impression and a moulded forehead rest. But in this work everything depends upon the self-control and co-operation of the subject.

The sessions lasted 3 hours and the inconvenience of the drug lasted all day. In spite of this exacting and unrewarded work my volunteers came time after time. The surprising precision of the measurements obtainable is due to their generosity and steadfastness.

II. FOVEAL PIGMENTS IN THE PROTANOPE

THE protanope is a dichromat who confuses red and green and is rather insensitive to red light. Plotted upon the colour triangle, the loci of tints which all appear to him identical are lines radiating from a point which is generally agreed to be near that corresponding to the primary red sensation *(ref.4)*. As Helmholtz and Maxwell have pointed out this is the geometry of normal colour vision reduced by simple loss of the red sensory mechanism, but there is no implication as to what part of the sensory mechanism is defective - pigment, nerves, or brain centre.

The evidence now to be presented shows that the red-sensitive *pigment* is in fact absent. This of course greatly simplifies both the measurements and the inferences to be drawn from them, and therefore it will be easiest to begin our analysis by considering the light reflected from the fovea of protanopes.

(a) Regeneration after bleaching

The protanope was first properly aligned in the apparatus; he then came out and 'dark adapted' after the bright alignment light by looking at nothing stronger than the ordinary lighting of the room. Then he took up position again and one or two wedge settings were made giving the reflectivity of the fovea in the dark adapted state. For these settings the

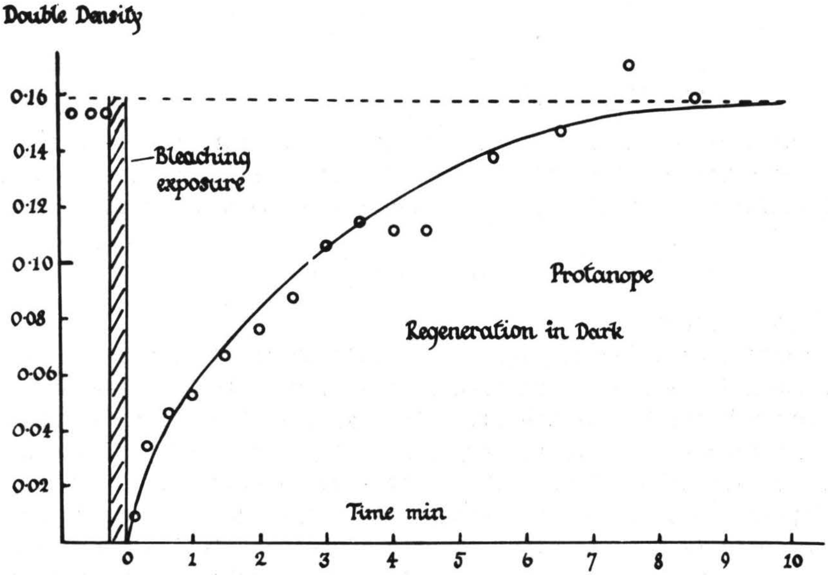


Fig.2. Protanope. The change in foveal reflectivity upon 'bleaching' and dark adaptation

light used was obtained by opening slits at 535 and 555 mμ and balancing the combined beam against the control at 680 mμ. This will be symbolized by (535 + 555)/680.

A very bright white light was now shone upon the fovea for 15 seconds and immediately afterwards the subject was instructed to fixate as best he could, and remain fixated for a minute or so. During this time wedge settings were being made as rapidly as seemed reliable - some 4 per minute; later measurements were made every 30 seconds with the subject relaxing attention between whiles.

The results of one experiment are shown in *fig.2* where it is seen that the apparent double density of the fovea falls by 0.15 upon bleaching and rapidly returns, reaching the initial 'dark adapted' level or a little higher in 7 or 8 minutes.

(b) Light intensity and the fraction bleached

The change recorded in *fig.2* might well be the bleaching and regeneration of a photosensitive pigment. But if so there will be a strict relation between the change we measure and the intensity of the bleaching flash we use.

For if y_0 is the density of the dark adapted pigment, and y_t the density after exposure for time t to light of intensity I, then

$$-\frac{dy}{dt} \propto Iy$$

$$\therefore \log(y_0/y_t) \propto It$$

Now in the experiment to test this relation the light source was fixed and so was the exposure time (10 seconds) and the intensity was varied by interposing different neutral filters of density D, thus we obtain

$$\log\log(y_0/y_t) = K - D \quad . \quad . \qquad (2)$$

where K is constant.

The continuous curve of *fig.3* shows this theoretical relation which should hold between (y_t/y_0), the fraction of the pigment left unbleached after a flash, and $-D$, the log of the light intensity in the bleaching flash.

To investigate the relation experimentally, the procedure was similar to that in the former section. The first wedge setting (x_1) was taken in the dark adapted state; then a 10 seconds bleaching exposure was given and a second measure (x_2) taken after exactly 10 seconds in the dark. Finally a further 10 seconds bleach was given with a very bright light and again a measure (x_3) taken after exactly 10 seconds in the dark. The fraction of pigment remaining unbleached after the first exposure

$$= \frac{y_t}{y_0} = \frac{x_2 - x_3}{x_1 - x_3}$$

The subject was allowed to rest and dark adapt for some 5-8 minutes and then the procedure was repeated with a different neutral filter in the bleaching light. Since the full bleach $(x_1 - x_3)$ was about the same when redetermined, it was not necessary to perform the second half of the procedure every time.

In the theoretical treatment above nothing has been said about regeneration, but from *fig. 2* it is seen that this is rapid and cannot be neglected. Fortunately the rate is nearly proportional to $y_0 - y$ for any value of y and thus the procedure adopted eliminates its effects. For by keeping the *time* course of operations fixed, regeneration at the moment of measurement reduces y_t and y_0 in the same proportion and hence their ratio, which alone enters expression (2), remains unaffected.

The circles in *fig. 3* give the experimental values measured in this way upon the same subject as in *fig. 2*, using a white bleaching light.

(c) The difference spectrum

Figs. 2 and 3 support the belief that one or more photosensitive pigments are being measured, but they give little indication of their number or

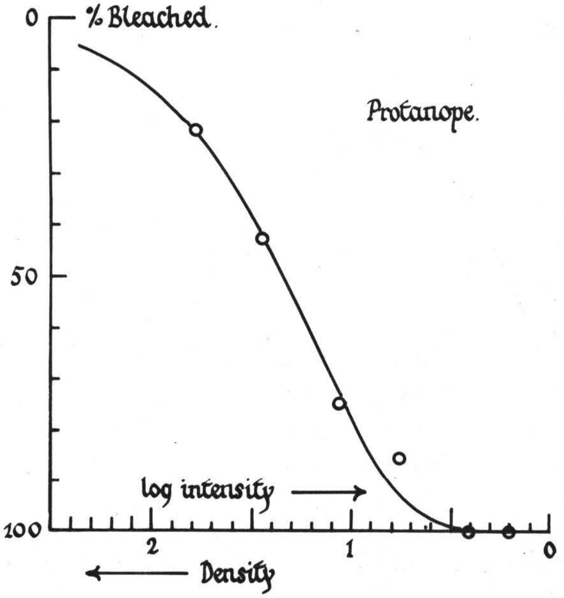


Fig. 3. Protanope. Relation between % pigment bleached in 10-second exposure and log intensity of exposure. Curve is theoretical relation.

nature. To investigate this we may use the difference spectrum. Hitherto the measurements have been made in a broad spectral band around 545 mμ. Now measurements are made in narrower bands at 4 different wave lengths as indicated by the horizontal rectangles of *fig.4*.

In this experiment bleaching is performed by light reflected from the sails of the mirror windmill (M_2 *Fig.1*) so that measurements may be carried out during a steady state. This is a difficult experiment and an inexperienced subject could not maintain fixation sufficiently for more than 4 wave lengths to be satisfactorily measured. The procedure was as follows.

The dark adapted wedge settings were taken by light of 4 different wave lengths and these were repeated and checked again if necessary until reliable readings were obtained correct to a density of 0.01 or better. Now bleaching was begun by applying a strong orange light intermittently as the windmill arms rotated (20 flashes/second). After this had been going on a minute or two and was still continuing, the subject was exhorted to fixate steadily despite the flashing light, and new measurements were taken at all 4 wave lengths, and these were confirmed as carefully as possible. The subject then left the apparatus, rested and dark adapted for 5-8 minutes and then the experiment was repeated using a green bleaching light.

The results of one experiment are plotted in *fig.4* where the white circles show the difference spectrum due to bleaching with an orange light (Ilford filter 203) and the black circles with a green light (filter 625).

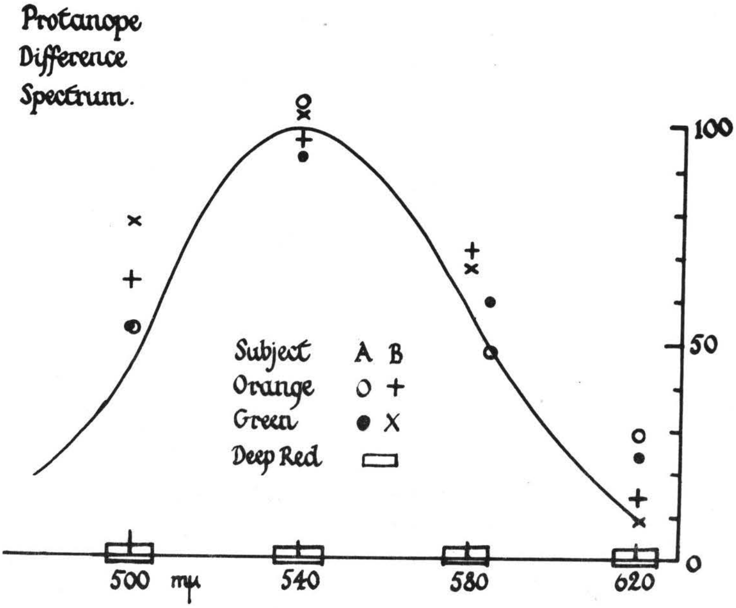


Fig. 4. Protanope difference spectrum from partial bleach with various colours.

'Bleaching' with the deep red filter 206 never causes any change in density (rectangles). The figure also includes results from another subject obtained slightly differently. The (+) marks were determined just like the white circles, but then the bleaching light was changed and filter 625 substituted. After waiting a few minutes, the new equilibrium was measured (x). These results are somewhat more reliable but otherwise typical of all difference spectra obtained from the 6 protanopes and severe protanomalous subjects that I have examined. The two subjects of *fig.4.* are actually protanomalous and can distinguish with certainty between red and green lights if these are clear and bright. But they have only one photosensitive pigment that can be measured by this technique, and this pigment is clearly not rhodopsin. For it can be measured upon the rod-free area of the fovea, and it regenerates so fast that it is half restored in 1.5 minutes whereas rhodopsin takes 5 times as long *(ref.1)*. Moreover the maximum of the difference spectrum lies not at 500 mμ but at 540 mμ.

(d) Action spectrum and visibility

Evidence has been advanced that one and only one photolabile pigment can be measured upon the fovea of the protanope, but so far no observation has been described which relates it to vision.

When, however, vision depends upon only one pigment a relation is to be expected which has long been known for the scotopic mechanism. In twilight vision lights of different wave lengths which appear equally bright are those which equally bleach rhodopsin. Do lights which appear to the protanope equally bright bleach equally his pigment? The experiment consists of two parts, first the coloured lights have to be matched, and second the amount of bleaching produced by the matched lights must be measured.

The bleaching lights were obtained by interposing coloured and neutral filters at F_1 *fig.1.* Each of these lights was matched against a fixed yellow or green light in a bipartite field, by the protanope or protanomalous subject who was told to match brightness and to disregard the blueness or other colour difference in the two fields. Each of these coloured lights was also matched against a narrow band from the spectrum both in colour and brightness and the energy of the spectral beam was determined in quanta/second by a Rb photocell calibrated by the N.P.L.

The amount of bleaching produced was determined as in the experiment of *fig.3.* A level of bleaching intensity was chosen for one coloured light which bleached about 40% of the pigment. Then the % bleaching produced by the 8 other matched coloured lights was measured in a series of successive bleaches with intervals between for regeneration. The average results from two subjects are given in *Table I*, in the column % bleach. The remaining columns give the wave length which appeared the same colour to the protanomalous subject, and the reciprocal energy which appeared equally bright.

TABLE I

Bleaching in the protanope with coloured lights of matched brightness

Ilford filter	Colour	% bleach	λ	Rel. sensitivity Quanta^{-1} sec
205	red	45	650	2.5
204	deep orange	43	605	28
203	light "	41	585	55
626	yellow	40	570	76
625	yellow-green	37	530	100
624	green	38	520	93
623	blue-green	41	500	51
622	blue	39	480	12
-	white	41	-	-

The results are about as good as can be expected of this double measurement which includes direct brightness matches by an untrained subject. As far as they go, the measurements support the belief that the photolabile pigment investigated is a *visual* pigment, and indeed is that pigment upon which the sensation of brightness in the protanope principally depends.

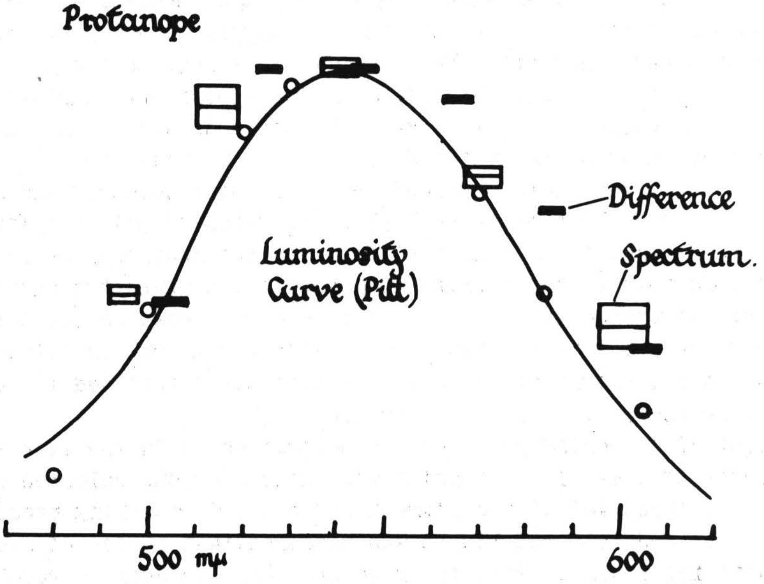


Fig. 5. Protanope. White and black rectangles, difference spectrum (white bleach). Circles, luminosity; curve, Pitt's luminosity for protanopes.

The spectral absorption of this pigment should therefore correspond to the spectral sensitivity of the protanope just as that of rhodopsin corresponds to twilight visibility. Now Pitt *(ref.4)* gives a luminosity curve for the protanope which Wright *(ref.5, p.322)* states is very constant from one protanope to another and *(p.307)* similar to the protanomalous. This then should be the absorption spectrum of the pigment of the protanope uncorrected for absorption in the eye media. It is plotted in *fig.4* with energy expressed in quanta instead of ergs *(ref.6)*, and also in *fig.5*. The circles in *fig.5* show the luminosity curve of my most reliable protanomalous subject, *(Table I, columns 4 and 5)* given not to support the results of Wright and his colleagues, but to derive support from them in my own attempts at photometry. *Fig.5* includes difference spectra from two protanomalous subjects, where white light was used in bleaching, the rectangles being the average ± the standard deviation of the mean of 3 determinations taken at one sitting.

There is clearly a fair correspondence between the action spectrum (luminosity curve) and the difference spectrum, but there is a marked tendency for the action spectrum to be sharper, and for the difference points to lie above it.

Absorption by the macular pigment must cause some steepening of the luminosity curve towards the blue; the difference spectrum would not be so directly affected by a photostable pigment and this might account for the divergence of the two curves seen in the blue-green region. The difficulty of estimating the magnitude of this effect discourages any statement about the spectrum of the cone photoproducts, which in theory constitute the principal divergence between action and absorption spectrum. But it hardly looks as though the photoproducts could be very absorbing and very stable in the part of the spectrum investigated.

(e) Photosensitivity

This is the reciprocal of the retinal illumination (quanta/cm^2) in a flash which reduces the pigment to $1/e$ of its former value. It was measured as follows.

In the previous section it was shown that the set of bleaching lights used each reduced the pigment to about 60% of its former value. From *fig.3* we know that if 0.3 density had been removed from each of these bleaching lights, a fraction $1/e$ of the pigment would have been left. To measure the number of quanta/cm^2 in a 10 second flash of the broad band passed by these bright filters it was assumed that a pure spectral light which looked identical would bleach the same. Moreover since a protanope accepts the normal match I matched the green bleaching light against a spectral band at 550 mμ and measured the energy of the spectral light with a Rb photocell calibrated by the N.P.L. This gives the total number of quanta (550 mμ) falling upon the cornea, in a flash which bleaches all but $1/e$ of the pigment. It is assumed that only half this light reaches the retina where it covers an area determined by the 2.7° angle subtended by the circular bleaching patch. (Note: though 2.7° of retina are bleached, only the 1.8° at the centre are accepted by the photocell for analysis).

From these assumptions and measurements the photosensitivity at 550 mμ was found to be

$$6 \times 10^{-16} \ \mathrm{cm}^2$$

This is about 7 times as great as the maximum photosensitivity of rhodopsin in solution and suggests that the cone can concentrate light upon a very restricted area of pigment.

III. THE NORMAL FOVEA

(a) A red-sensitive pigment

The foregoing section has shown that the essential loss of the protanope is a visual pigment, which in the normal is presumably sensitive to red. If so, a deep red light too little absorbed by the protanope pigment to cause measurable bleaching, might cause a change in the normal eye. The Ilford filter (206) transmits practically no light of wave length shorter than 630 mμ and bleaching by the mirror windmill with this filter interposed has never produced any detectable change in protanope reflectivity at any measured wave length (rectangles *Fig. 4*).

Table II gives the complete set of readings for one such experiment with a normal subject. The readings show the degree and limits of reliability which can be attained. Each column measures reflectivity in light of the wave length indicated at the top. All other numbers are mm of wedge setting

TABLE II

Bleaching with deep red light and recovery.

Figures give wedge setting (mm). 1 mm = 0.005 double density

λ mμ	655	630	610	590	565	545	525	505
Dark	36	46	44	56	66.5	69	64	61
repeat	34	46	43	58	67	68	62	60
Red bleach.	35.5	42	38	49	62	63	60	58
repeat	35	42.5	37	49	60.5	61	56	56
Dark	36	46	44	56.5	67	68	62	59
Difference	0	4	6	8	6	6	5	3

where 1 mm corresponds to 0.005 decadic density unit. The eye was dark adapted and wedge settings taken for each of the wave lengths in turn. This was immediately repeated with similar readings and then the red bleaching light (206) was applied. After about 2 minutes when equilibrium was nearly attained, the third line of readings was taken, and immediately confirmed (line 4). Finally the red light was extinguished and after 7 minutes in the dark the fifth line of readings was obtained. This so nearly coincided with the initial set that no confirmation was made, for the subject had been in position for about 20 minutes and was fixating most of this time.

The final line gives the difference between the dark and red-bleached equilibriums. This difference which in the protanope is always zero throughout, exhibits in the normal a reasonable spectral distribution. The maximum is not at 540 mμ as was found for the protanope pigment but near 590. It constitutes clear evidence that the normal has a measurable pigment absent from the protanope and more red-sensitive than his.

(b) Two pigments present

The last experiment showed that the normal fovea contains a pigment absent from the protanope, but it gives no information as to the presence also of the protanope pigment or indeed any other measurable pigment. This can be learnt by making in quick succession measurements of reflectivity at wave lengths 605/700 and 545/700. If only one visual pigment is present the wedge settings made in these two lights will change in a constant proportion for

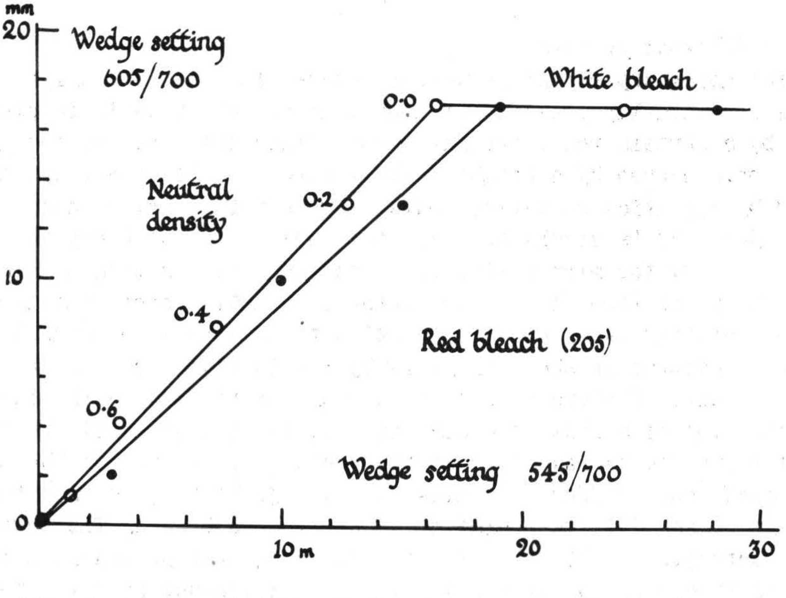


Fig. 6. Black and white circles two normal subjects. Change of foveal density measured in light of 545 mμ plotted against measurements in light of 605 mμ when a red bleaching light of various strengths was used followed by a strong white bleach.

any bleaching manoeuvre, so if one setting is plotted against the other they will lie on a fixed straight line.

The results of such an experiment are plotted in *fig.6* where circles and dots represent the same experiment performed on two different subjects. The change measured in light of 605 mμ is plotted vertically against the change in light of 545 mμ horizontally, the zeros of the scales being the dark adapted settings. The manoeuvre was to use the 'windmill' for bleaching to various equilibrium values at each of which wedge settings were taken at the two wave lengths. The bleaching light was from the red filter 205 with neutral densities 0.8, 0.6, 0.4, 0.2 and 0.0 successively interposed. As may be seen from *fig.6* there is a linear relation between the two wedge settings for each equilibrium level, of the red light. But now a white bleaching light was applied bright enough to bleach completely the remaining pigments, for increasing its brightness caused no further change. The final point is seen to lie well to the right of the line which had been followed up till now; thus two pigments must be present and be bleached by the white and red lights in different proportions. Indeed, the total bleach does not appear to cause any change in density measurements at 605 mμ. This can only mean that the pigment whose density was measured at this wave length had already been completely bleached by red, so no further bleaching by white was possible. Measurement at 545 mμ on the other hand shows that white light does produce a substantial change so this must occur in a second pigment nearly transparent to light of 605 mμ.

(c) The difference spectra

The foregoing experiment is useful because it shows not only that two pigments are normally present but also that one of these is completely removed by a certain red bleaching light. Since the other pigment alone may then be bleached by a bright white light, its difference spectrum can be found by measuring at various wave lengths this final change.

The experiment is simply to adapt the subject to the bleaching light (205) flashed by the mirror windmill, and take wedge settings at 8 wave lengths. Then the (205) filter is replaced by a 0.4 density neutral, and new wedge settings taken after adaptation to this total bleach. The difference spectrum is shown in *fig.7* by the large rectangles which represent the results of 5 experiments made upon 4 normal subjects. The horizontal dimension shows the wave band of the measuring lights and the vertical shows the average value ± the standard deviation of the mean of the *unscaled* wedge differences after bleaching. It is seen that the amount of pigment bleached is very similar in all four subjects. The curve joining the rectangles is Pitts luminosity curve for the protanope which was seen *(figs. 4 and 5)* to fit the protanope's difference spectrum rather closely. It fits the rectangles of *fig.7* also and hence justifies the belief that one of the normal pigments is that found alone in the protanope.

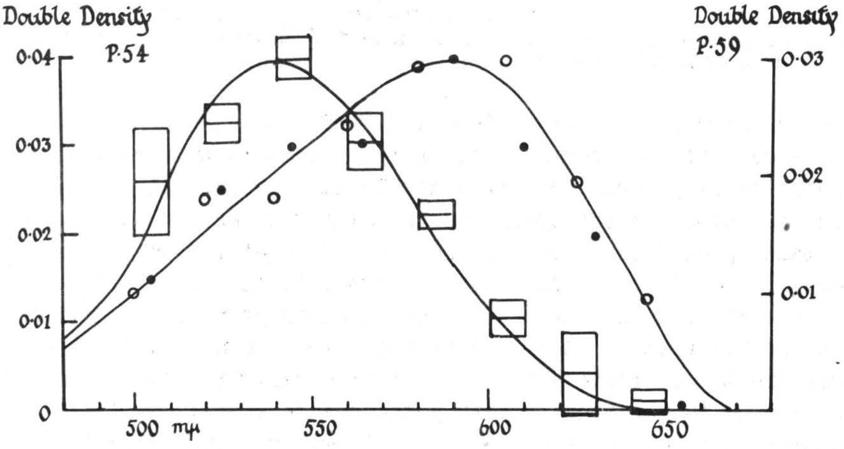


Fig. 7. Normal. Difference spectrum of the two foveal cone pigments.

This is not surprising. It is known that protanopes accept normal colour matches and so the protanope pigment must have an absorption spectrum which is a linear combination of the absorption spectra of the three normal pigments (self-screening neglected). On chemical grounds this would be very unlikely unless the mathematical relation degenerated into an identity with one of the normal pigments, and from *fig.7* this appears to be the case.

In order to find the difference spectrum of the other pigment it will not do simply to measure the difference between the (205) red adaptation and complete dark adaptation. For there is so far no indication of how much protanope pigment was also bleached by the red light which bleached the other pigment entirely. Certainly the straight lines of *fig.6* are consistent with the bleaching of a single pigment, but they cannot be used to exclude some bleaching of both. And in fact both *are* bleached by (205). But having established that one pigment is the protanope pigment we may now turn again to *Table II* which gave the results of a bleach with the light (206) which is without effect upon the protanope. Clearly the difference spectrum of *Table II* must concern the red-sensitive pigment only. It is plotted in *fig.7* as dots, and the circles are the average of two similar experiments upon a different subject scaled to the same maximum. The curve is drawn free-hand among the points.

It will be convenient to have labels for the two foveal pigments. That with maximum absorption at 0.54 μ will be designated P·54, the other P·59. This is essentially Dartnall's labelling system.

Dartnall would write V.P. 540. In this the 'V' appears unnecessary. It stands for 'visual' but he does not use it for visual as opposed to other photolabile pigments, nor is it always easy to know what visual function if any is subserved by an extracted photolabile substance. The advantage of expressing the wave length in μ is that one is not committed to 3-figure accuracy when (as here) the work only justifies 2. The full label should be written P_1·54 if it is considered established that the pigment is derived from vitamin A_1.

(d) The relative quantities of P·54 and P·59

If the dark adapted fovea is entirely bleached with strong white light the double density change measured at 550 mμ is about 0.14. Only half of this can be accounted for by the curves of *fig.7*. We may consider the total bleach made up of first a change from dark to equilibrium in the deep red of (206). *Table II* or the dots *fig.7* shows this change is 0.03 and is due to P·59 entirely. Then there is an intermediate step from equilibrium at (206) to equilibrium at (205), about which we have no information. Finally there is the total bleach which is seen from *fig.7* to involve only P·54 and to be 0.04 in magnitude. Clearly the change from (206) to (205) must account for half the total pigment, and if we knew what fraction of this was due to P·54 we should know the proportions of the two pigments (as measured) upon the fovea. Our problem is to measure the density of P·54 by a method which is indifferent to any changes in P·59 which may be taking place simultaneously. It is easily solved. Hitherto the comparison beam has been light of about 700 mμ to which all pigments are transparent. If however this comparison light is made 620 mμ and the measuring light is 540 mμ, since these wave lengths have equal ordinates on the P·59 difference curve, any change in the density of P·59 can produce no alteration in the null position of the wedge. On the other hand P·54 may be measured with nearly full sensitivity.

It is important to get the comparison beam to balance the other exactly so as to be indifferent to the bleaching of P·59. This was done by trial using bleaching by (206) as a condition affecting P·59 only. If this bleach caused a shift of wedge in the direction usual with bleaching, the comparison beam must be too far out in the red. After recovery another similar bleach was performed with both measuring and comparison beams at slightly shorter wave lengths. When the position had been found which gave no wedge shift on bleaching with (206), the change of wedge setting was noted after bleaching with (205) and after a total bleach. It was found that about 0.4 of the total amount of P·54 was bleached by (205).

The proportion of the two pigments is illustrated by the experiment of *fig.8* in which the difference spectrum was first measured from a total bleach following dark adaptation, circles, and again following adaptation to (205), crosses.

The crosses represent P·54 only, and from what has been said this is about 0.6 of all the P·54 present. The dots in *fig.8* then, give the total

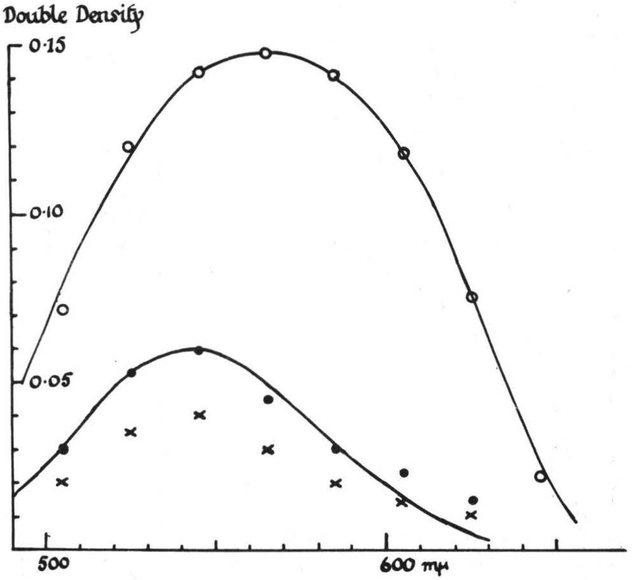


Fig. 8. Normal. Circles, difference spectrum with total bleach. Crosses, difference spectrum of P·54 only. Dots, total amount of P·54 present. Thus the difference between circles and dots is the total amount of P·59 present. Curves replotted from *fig.7*.

density of P·54 and the curve through them is the protanope luminosity. Clearly the difference between this curve (total P·54) and the circles (total P·54 + P·59) is due to P·59. Now the difference spectrum of P·59 is given in *fig.7* so we may add this curve suitably scaled to the protanope curve in *fig.8*. The curve through the circles is the result of this plot where the density of P·59 is 1.5 that of P·54.

(e) Regeneration rate of P·54 and P·59.

Fig.9 shows the regeneration in the dark in two experiments. The results are similar to those from the protanope in *fig.2*, and lead to the conclusion that each of the normal pigments regenerates at the same rate.

In curve A bleaching was produced by 10 seconds of bright white light, and crosses represent measurement at 605 mμ, circles at 545 mμ. In curve B the bleaching was a bright orange light (Ilford 203) for 30 seconds where triangles are measurements at 625 mμ, dots at 585 mμ.

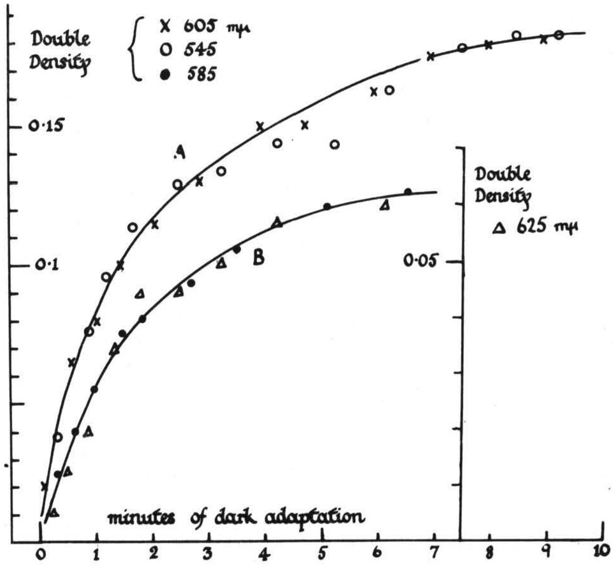


Fig. 9. Normal: recovery after total bleach.
A. Measurements at 605 and 545 mμ show same amount and rate of recovery at all stages. Thus regeneration rate of both pigments equal.
B. Measurements at 585 and 625 mμ are superposable when 625 is plotted at twice the scale. No evidence of self-screening.

The crosses and circles represent the recovery of mixtures of P·59 and P·54 in which P·54 contributes 15% to the crosses but some 50% to the circles. If in the normal eye these two pigments regenerated at very different rates, the circles and crosses would diverge more than they do.

(f) Pigment density

Brindley *(ref. 7)* in his striking analysis of the change of a colour match which follows the adaptation to a very bright yellow light has deduced the remarkable figure of 0.98 for the density of pigment in the red receptor. Now in measuring the density of foveal receptors by reflected light what is observed is essentially the % change in the total reflected light due to change in transparency of the cones. So the actual double density in the 'red' cones might be much greater than the 0.1 recorded from the fovea if a substantial proportion of the light received by the photocell had not passed through the red receptors. The question arises whether these reflection measurements are compatible with the light passing through Brindley's double density of 2. *Fig.9B* shows that during regeneration from complete bleaching the density as measured at 625 mμ (triangles) and at 585 mμ (dots) keeps almost precisely in the ratio 1:2. This constant ratio is exactly what would be expected of a dilute pigment. It is not at all what would be expected of a very dense pigment. A possible interpretation might be that at these two wave lengths the absorption is heavy and equal but the dilution by stray light is very different. This is excluded by the fact (see later *fig.11)* that the photosensitivity as measured by the rate of bleaching is twice as great at 585 as at 625 mμ. A quantitative analysis of *fig.9B* results in the conclusion that though the double density may well be considerably greater than the 0.1 recorded, it can hardly be higher than 0.8, and (what is most important for the present treatment) there are no serious errors introduced into the density measurements by self-screening.

These results then do not support Brindley's high cone densities, but neither do they refute them. For my catch may be from the shallows of the pigment pool while he traps quanta in the depths.

(g) The bleaching rate and photosensitivity

As these experiments have recently been published *(ref.8)* and are similar to the corresponding measurements described earlier upon the protanope, they will be treated quite shortly. The pigment was measured in lights (605 + 625)/690, where P·54 contributes about 12% to a total bleach. *Fig.10* shows

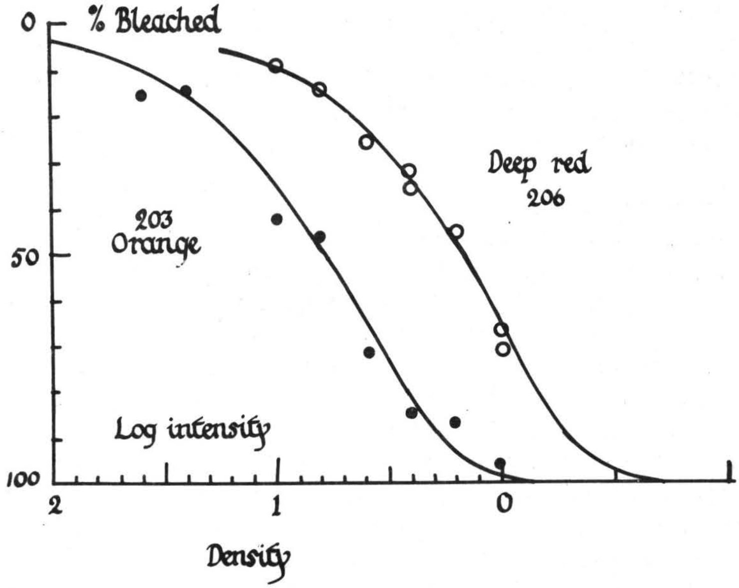


Fig.10. Normal. Relation between % pigment bleached and log intensity (compare *fig.3)*. Curves are theoretical relation.

(56753)

the results of two experiments where bleaching was performed in the deep red light (206) which does not affect P·54, and at (203), a bright orange which at full intensity probably bleaches most of P·54. The results, like those of *fig.3* fit the theoretical curve, which is not sensitive to detect the admixture of a pigment small in amount and similar in photo sensitivity.

The photosensitivity was measured at 580 mμ by the method described for the protanope and the value found was 4 x 10^{-16} cm^2, which may be compared with 6 x 10^{-16} found (at max. λ) in the protanope, and 16 x 10^{-16} found by Brindley *(ref.7)* for the red receptor in his measurements upon the alteration in colour matching after strong adaptation. The photosensitivity of rhodopsin is only 0.9 x 10^{-16} *(ref.9)* and the high value found from the cones is probably due to their focussing light upon a small pigment area, which would also account for the Stiles-Crawford effect. The similar values for photosensitivity for P·59 in the normal and for P·54 in the protanope might mean that there exist special focussing cones which in the normal are filled with P·59 and in the protanope with P·54, so the 'green' cones in the normal might have a much lower photosensitivity. This possibility was contradicted by some rather inaccurate experiments where the relative photosensitivity of P·54 was measured in the normal eye. Measurements were made at 547/607 which was carefully adjusted to give no change of equilibrium upon bleaching P·59 alone with light (206). A 10 second flash with light of equivalent wave length 545 mμ produced a change of wedge setting which thus was therefore entirely due to P·54.

The bleaching produced by this flash was compared with the total white bleach and found to be 60%. This then is the fraction of P·54 removed. The same measurements were made upon the same green and white exposures using measuring lights 545/700 where 60% of the measured change is due to P·59. In this case the pigments showed a 67% bleach. Thus the photosensitivities of the two pigments in the normal eye are of the same order.

(h) Action spectrum of P·59

The difference spectrum of P·59 shown in *fig.7* might deviate from the absorption spectrum through the possible presence of coloured photoproducts and admixture with stray light of unknown quantity and composition. It is important to have some indication of the magnitude of these factors, and this is provided by the action spectrum into which they do not enter.

The method was the same as that described earlier upon the protanope; it has recently been published *(ref.8)* but not properly analysed. Measurements were made at (605 + 625)/690 and the pigment was bleached by 10 second flashes of lights of various colours adjusted in intensity to give about 50% of total bleaching. The actual % bleach was measured and then from *fig.10*, one can read off the intensity of light which would have produced exactly 50% bleaching. This intensity needed to be expressed as quanta/sec of a particular wave length. The (attenuated) bleaching light was matched

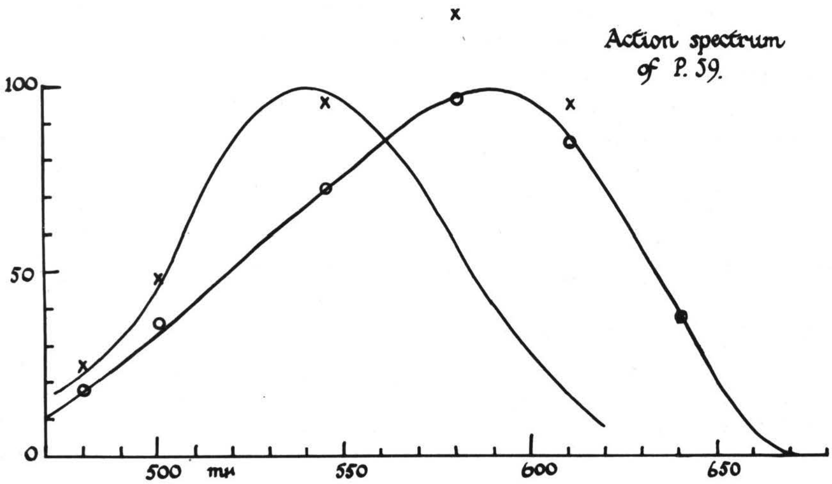


Fig.11. Normal. Action spectrum; crosses, bleaching of both pigments; circles action spectrum of P•59 after correcting for effect of P•54.

against a spectral light in a bipartite field, and it was assumed that lights which look alike bleach alike. The bleaching effects were thus attributed to the spectral light whose wave length and quantum energy were measured.

The results obtained are shown in *fig.11* where the crosses give the reciprocal quantum energy at various wave lengths necessary for a 50% bleach. Now these results though mainly the action spectrum of P•59 are necessarily contaminated with some bleaching of P•54. This may be approximately corrected as follows. P•54 contributes about 12% to the total bleach at the wave length measured. At 545 mμ the photosensitivities of the two pigments are about the same and hence P•54 contributes 12% to the bleach which occurred there, that is the 50% total observed was made up of 44% P•59 and 6% P•54. At other wave lengths the contribution of P•54 may be found from the action spectrum of the protanope and thus the proper correction made to the action spectrum of P•59. The results are shown as circles; the curves are the same as given in *fig. 7* for the difference spectrum of P•59 and P•54.

The corrected action spectrum coincides sufficiently well to suggest that neither stray light nor absorption by photo-products enter as serious errors in the measurement of the difference spectrum.

(i) The relation of P•59 and P•54 to vision

So far nothing has been said to justify the belief that the pigments measured constitute the basis of colour vision. But if this is so there is one (and I think only one) relation to colour measurement which follows without additional hypothesis. The dichromatic spectral coefficient curves for the central fovea should be related directly to the absorption spectra of P•59 and P•54.

Thomson and Wright *(ref.10)* have matched spectral lights against a mixture of 0.46 μ and 0.65 μ in a 20' field centrally fixated. Their results *(ref.5, fig.216, p.343)* show for each wave length the fraction of the 0.65μ light in the mixture, expressed in units such that at wave length 0.5825 μ this fraction was 1/2. If the foveal dichromacy is due entirely to the two pigments P•59 and P•54, then the condition of the colour match is necessarily that P•59 absorbs light equally from the spectral field of wave length λ, and from the mixed field of radiations 0.65 and 0.46 μ; and the same applies to P•54. This follows, even if the cone population consists of members containing all sorts of mixtures of the two pigments (self-screening neglected) and are interconnected through any kind of nerve net with non-linear properties.

Now from *fig.11* we can measure off the relative absorptions x_λ, y_λ of P•59, P•54 at every wave length λ. It is seen that $y_{0.65}$ is zero, and that we have no information about $x_{0.46}$ or $y_{0.46}$; fortunately that lack may be circumvented.

Let $I_{0.65}$, $I_{0.46}$ be the light energies (quanta) of the "D units" used by Thomson & Wright. Let r be the spectral coefficient for red in their curve *(fig.216, p.343)*, and let D_x, D_y be the densities of P•59, P•54 on the fovea, (or alternatively in any one cone).

Then for any wave length λ the match is obtained when

$$\frac{x_\lambda D_x}{y_\lambda D_y} = \frac{I_{0.65}\; x_{0.65}\; D_x\; r \;+\; I_{0.46}\; x_{0.46}\; D_x\; (1-r)}{I_{0.46}\; y_{0.46}\; D_y\; (1-r)} \tag{3}$$

$$\text{or} \left(\frac{x}{y}\right)_\lambda = \frac{I_{0.65}\; x_{0.65}\; r/(1-r) \;+\; I_{0.46}\; x_{0.46}}{I_{0.46}\; y_{0.46}}$$

Since the densities D_x, D_y have been eliminated from expression (3) the relation is valid for a heterogeneous population of cones containing various mixtures of P•59 and P•54.

Now in (3) inserting the condition that at 0.5825 μ $r = 1 - r = 0.5$ we obtain

$$\left(\frac{x}{y}\right)_{0.58} - \left(\frac{x}{y}\right)_{\lambda} = \frac{I_{0.65} \quad x_{0.65}}{I_{0.46} \quad y_{0.46}} \cdot \frac{1-2r}{1-r} \quad . \quad . \qquad (4)$$

The fraction r may be measured upon Wright's graph and the value $(1-2r)/(1-r)$ computed for each wave length. The resulting curve is shown in *fig. 12.* The ordinate ratio x/y is now measured from the curves of *fig. 11* and at $\lambda = 0.5825$ μ it is found to be 1.78. The difference from this of the other ratios is plotted in *fig. 12* and when reduced 1.55 times gives the results shown by the circles. These fit the curve sufficiently to encourage the belief that P·59 and P·54 are the visual pigments of the fovea.

If we put into equation (4) the numerical values obtained we get

$$\left(\frac{x}{y}\right)_{\lambda} = 1.78 - 1.55 \; \frac{1-2r}{1-r}$$

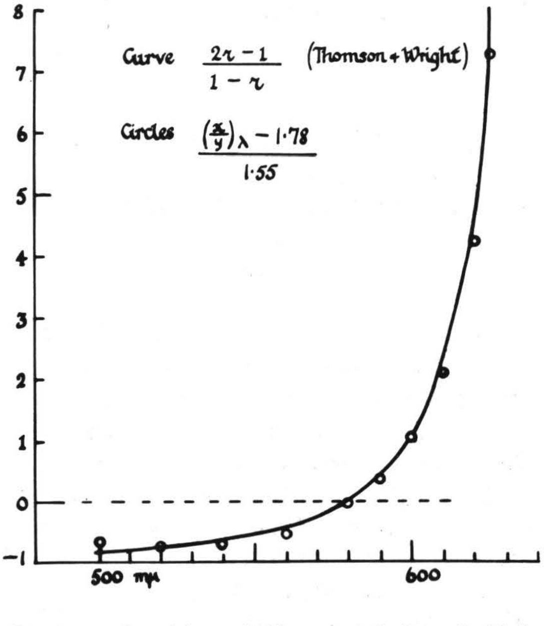


Fig.12. Curve, a function of the normal foveal dichromatic spectral coefficients (Thomson and Wright). Circles, the corresponding relation from P·54 and P·59.

Now at $\lambda = 0.46\ \mu$, by definition $r = \infty$, hence

$$\left(\frac{x}{y}\right)_{0.46} = 1.78 - 1.55 = 0.23$$

We thus deduce from measurements made in the yellow-green where they are relatively reliable, that at 0.46 μ the extinction of P·59 is about 1/4 that of P·54.

(j) Luminosity

Thomson & Wright *(ref.10)* found that when blue and red lights were combined to match 0.5825 μ on a 20' foveal field, the red light was 0.73 times as bright as the blue. Now we have seen that the relative extinction of P·59/P·54 at 0.5825 μ is 1.78, at 0.46 μ is 0.23 and that there is about 1.5 times as much of P·59 present as of P·54. We do not know, however, the relative contribution of the photolysis of these two pigments to the physiological process measured as luminosity. Suppose P·54 is α times as effective as P·59. Then the blue component of the match on 0·5825 μ contributes to luminosity.

$$\alpha + 1.78 \times 0.23 \times 1.5$$

and the red component

$$1.78 \times (1-0.23) \times 1.5$$

Since the red is 0.73 as bright as the blue we deduce that

$$\alpha = 2.2$$

The simplest way in which P·54 could be twice as 'luminous' as P·59 is for the photosensitivity to be double. This was investigated as follows.

Measuring and comparison wave lengths were chosen so that on *complete* bleaching there was no change in balance. In this position a partial bleach with a red light threw the balance one way, with a green light the other. A bleach at 555 mμ favoured green, 575 favoured red; it was concluded that 565 mμ bleached P·54 and P·59 equally. As this is the wave length of equal absorption *(fig.11)*, the photosensitivities of the two pigments must be nearly the same and a 2:1 ratio is unlikely.

In the experiment above, regeneration from a total bleach did not show any imbalance, confirming that both pigments have the same relative regeneration rate. As I know almost nothing of the way in which photolysis generates nerve impulses, or how impulses interact to contribute to brightness, it is not profitable to speculate further on why P·54 is about twice as effective as P·59.

IV. THE DEUTERANOPE

THE deuteranope is a dichromat who confuses red and green but who is not insensitive to red light and indeed his spectral sensitivity curve lies a little to the red side of the normal. The loci of confusion colours plotted upon a chart using Wright's primaries gives a set of nearly parallel lines. This was interpreted by Pitt *(ref. 4)* who found it as meaning that the green sensation was not absent, but that there was a fusion of red and green mechanisms so that both participated indistinguishably. MacAdam pointed out that the projective properties of the colour triangle cause lines which are parallel upon one system of primaries to be concurrent upon another. Pitt's results are therefore compatible with the absence of a green mechanism if the green point is situated far away on Wright's diagram.

We have seen that the protanope lacks the normal red-sensitive pigment, and the question now arises whether the deuteranope lacks the green.

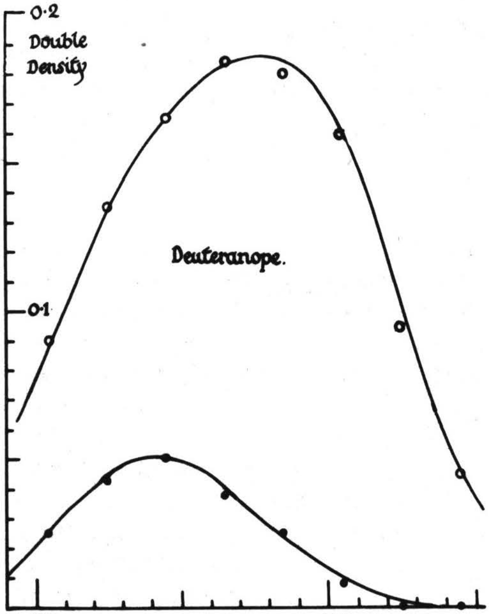


Fig.13. Deuteranope. Circles, difference spectrum with total bleach, dots, total amount of P·54 present (compare *fig.8*). The difference between circles and dots represents the amount of P·59 present. Curves are replotted from *fig.11*.

(a) Difference spectrum

The subject of *fig.13* was a dichromat who matched a spectral green with a very precise mixture of red and blue, and was eventually satisfied that these were identical. He was not a protanope for the red was not brighter than the green in my judgement.

The circles of *fig.13* show the difference spectrum upon total bleaching, the dots show the difference between the red bleach (205) and total. As has been shown earlier, (205) bleaches about 0.4 of P·54 and the subsequent total bleach is due entirely to the remaining 0.6 of P·54. This difference has therefore been multiplied by 1.7 to give the dots in *fig.13* which should represent the total P·54 present. The curve is the protanope sensitivity taken from *fig.11* and suitably scaled. *Fig.13* may be compared with *fig.8* which shows the same results upon the normal. P·54 is less in the deuteranope, the total pigment is more, and since the difference is composed of P·59 the pigment maximum is at a longer wave length. The curve through the circles is a suitable value of P·59 added to the given amount of P·54. There is about 3 times as much P·59 as P·54 in this deuteranope as compared with 1.5 times in the normal.

It is clear that the deuteranope is monochromatic upon the fovea not because he lacks normal pigments but because he lacks the normal organization for discriminating between their effects. This is precisely the Pitt-Wright interpretation. The normal two pigments might be mixed in a single class of cone; or the two normal cone types might be connected to a single class of nerve fibre.

(b) Deuteranope Luminosity

A satisfactory feature of the protanope analysis was that the luminosity curve fitted the difference spectrum. The maximum of the deuteranope difference spectrum lies at about 680 mμ whereas the quantum luminosity maximum is at 560 mμ. This might be thought to exclude the hypothesis that both pigments were mixed in one class of cone, and so it would if it were clear that both pigments there contributed equally to nerve excitation. But in discussing luminosity in the normal eye it was necessary to postulate that P·54 was twice as effective as P·59, and if we assume that in the deuteranope cone the pigments are mixed with the proportions shown in *fig.13* but that P·54 is 1.8 times as effective in stimulating, then the luminosity curve will be as shown by the uppermost curve of *fig.14*. The crosses are the deuteranope luminosity values given by Wright *(ref.5, fig.205)*.

V. CONCLUSIONS

THE most penetrating analysis into the mechanism of colour vision is that of Stiles *(ref.11)* by the method of increment threshold. In photopic central vision there appear to be at least 5 more or less independent colour mechanisms which preserve a remarkable stability and uniformity of analytic structure in a wide range of colour and intensity adaptations. I cannot conclude this paper without raising the question of the relation of Stiles' red and green sensitivities $\pi5$ and $\pi4$ respectively, to the visual pigments of the fovea.

In *fig. 14* Stiles' $\pi4$ is shown by the dots which are seen to lie in fairly close agreement with the protanope luminosity curve. To a first approximation then we may say that $\pi4$ represents the sensitivity of the green receptors which contain only P·54 and are present alone in the protanope.

The same cannot be said for $\pi5$ which is shown by the circles in *fig. 14* and is clearly quite different from the curves of P·54 and P·59. Stiles has suggested that $\pi5$ might be a mixture, and in fact it lies close to the mixture given by the upper curve of *fig. 14*. To be sure, there are difficulties in reconciling a mixture with the constant sensitivity spectrum found by Brindley *(ref. 12)* in extremes of adaptation. But I have found no pigment like $\pi5$ and Stiles has found no sensitivity like P·59, and the particular mixture of *fig. 14* corresponds to the luminosity curve of the deuteranope.

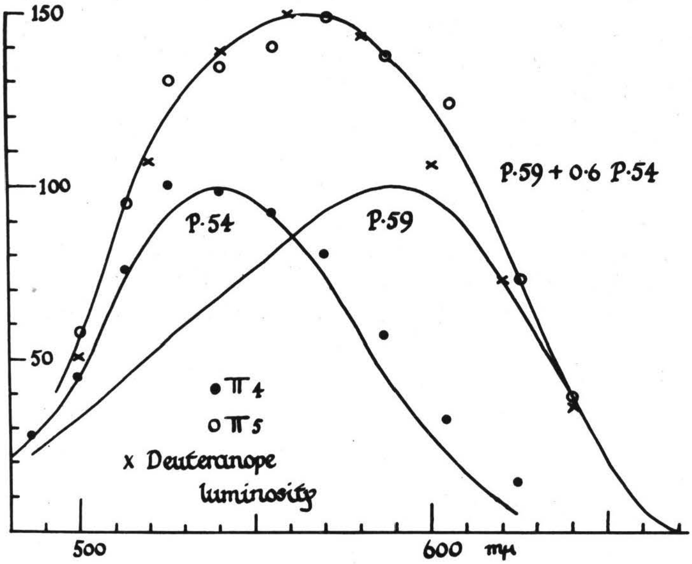


Fig. 14. Curves of P·59, P·54 and (P·59 + .7 P·54). Dots, Stiles' $\pi4$, circles $\pi5$, crosses Wright's deuteranope luminosity curve.

It is possible then that the normal fovea has two classes of cone, one contains P·54 only and the other contains a mixture of 3 parts P·59 and 1 part P·54, this being about twice as effective as P·59 per quantum in exciting vision. The sensitivities of these two cone types are given by Stiles' π4 and π5. The protanope has only the first type: the deuteranope has only the second.

DISCUSSION

WHAT is the relation of these two foveal pigments to the photosensitive extracts which have been obtained from animals' eyes, and especially to Wald's cone pigment iodopsin *(ref.13)*? What is the relation to the spectral sensitivity of retinal elements so widely studied by Granit, and especially to his red and green modulators *(ref.14)*? What is the relation to the foveal thresholds of Willmer *(ref.15)* to the humps upon the luminosity curve noted by many investigators, and to the great mass of other important and relevant observation?

If I do not plunge forthwith into a discussion upon all these topics it is not that I fail to appreciate their importance. It is because this paper is long enough already, and because I hope that what there is to say in discussion will be said in the Discussion at the Symposium. This will have the great advantage that comment will be made not by me but by the various authorities themselves.

ACKNOWLEDGEMENT

IT is a pleasure to express my indebtedness to Dr. Stiles and the N.P.L. for the monochromator, to the Medical Research Council for apparatus grants, and to Clive Hood for help in all practical aspects of the work.

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PRESENTATION AND DISCUSSION OF PAPER 1

DR. W. A. H. RUSHTON (Cambridge) made three general comments before summarising the main points of his paper. The paper described experiments which were essentially of a preliminary nature, and although he thought the main conclusions were right, some details had yet to be checked. Secondly, he had made few attempts to find a possible foveal pigment sensitive in the blue, and he would say nothing about such a pigment. Finally, he would record the indispensable assistance of the medical students who acted as subjects under the irksome conditions of observation required.

THE CHAIRMAN (PROF. G. WALD) opened the discussion, with the comment that though Dr. Rushton had faithfully refrained from saying anything about a foveal blue-sensitive pigment it was none the less there. Experiments by Auerbach and himself indicated a foveal pigment with maximal sensitivity at 450 mμ, in accordance with Dr. Stiles' earlier work. This appeared even for foveal test fields as small as one eighth of a degree in diameter. No foveal field could be made so small that violet light was not perceptible but apparently the violet receptors in small foveal fields made no significant contribution in the colour-matching process and very little to luminosity.

PROF. Y. LE GRAND (Paris) recalled that it was Thomas Young 150 years ago who first assigned the trivariance of colour to retinal processes. It was gratifying that another Englishman, Dr. Rushton, had, in his (Prof. Le Grand's) opinion, actually proved that there were at least two retinal mechanisms in action in colour vision. The experiments also went far to establish that for deuteranopes, Fick's conception of the fusion of two mechanisms was correct, while for protanopes, König's idea of the lack of one mechanism remained valid. The work also provided some evidence that actual mixtures of pigments may be present in single cones.

DR. W. S. STILES drew attention to the assumption underlying Dr. Rushton's determination of the absorption (difference) spectrum of the second or red-sensitive pigment in the normal trichromatic eye (*Table II* and *fig. 7*). The calculation assumed that all the light in the test beam passed through both the protanopic and the red-sensitive pigment, so that if t_p and t_d were the respective transmissions of these pigments before bleaching, and if, after bleaching with deep red, t_p remained the same but t_d became t'_d, the measured quantity $t_p t_d / t_p t'_d$ would in fact give t_d / t'_d. But if, to take another extreme case, the two pigments were located in separate cones covering respectively fractions α and $1 - \alpha$ of the retinal patch illuminated, and if every reflected ray that was measured entered and emerged through the same cone, the measured quantity would be

$$(\alpha t_{\lambda p} + 1-\alpha\ t_{\lambda d}) / (\alpha t_{\lambda p} + 1-\alpha\ t'_{\lambda d}).$$

A correction would then be necessary to determine t_d/t'_d. But all the transmissions t_p, t_d, t'_d were fairly near unity, and it would seem that the correction could have little effect on the shape of the derived absorption curve for the red-sensitive pigment. It would be interesting to have Dr. Rushton's views on this point. Dr. Stiles also referred to the support given to Fick's hypothesis by Dr. Rushton's results. The application of the hypothesis to subjective data of normal and defective colour-vision led (for example, in F. J. H. Pitt's well-known analysis) to a red fundamental curve with a secondary maximum in the blue. This seemed to him (Dr. Stiles) not very plausible for a photochemical pigment, and he hoped that the further development of Dr. Rushton's technique to give absorption data in the blue would throw light on the point.

PROFESSOR R. A. MORTON said that as a chemist he could accept the existence of two pigments, with maximal absorptions at 540 and 590 mμ respectively, and presumably they were both iodopsins. Hitherto we had thought that the λ_{max} of iodopsins was at about 560 mμ. A current hypothesis, a plausible one, was that vitamin-A deficiency could not only affect the supply of retinene, but could also produce defects in the structure of the cones. Another hypothesis was that we had to apply to the cones the physics of the solid state, and on this view there might be only one iodopsin pigment, giving different spectra depending on the orientation of its molecules. If vitamin-A deficiency did cause deterioration of cone structure it might be expected that one of the two "iodopsin" pigments found by Dr. Rushton would be affected first, and the early stages of deficiency would be marked by some degree of colour-blindness. He did not know if this was so.

DR. E. BAUMGARDT pointed out that yet another hypothesis on defective colour-vision was provided by Segal's 3-layer theory. This postulated that there was only one pigment, which might be in the solid or liquid states, or in a "decomposition" state; a protanope would have a deficiency of photosensitive material and a deuteranope would have an excess of it. This conception was compatible with Dr. Rushton's findings and Dr. Baumgardt felt that we should give more consideration to Segal's theory.

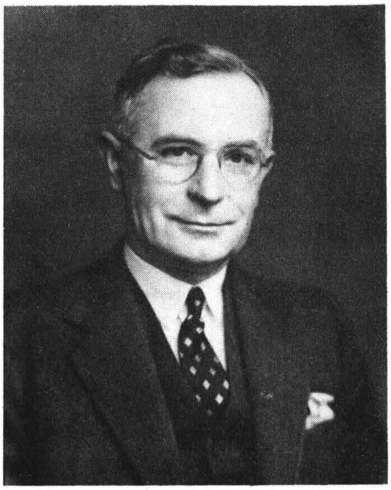
In reply, DR. RUSHTON referred Prof. Morton's question on the iodopsins to the Chairman. As regards Segal's theory, he felt that it was interesting, but as the theory was developed in Segal's book hypothesis was piled on hypothesis until one seemed to lose all touch with reality, and he (Dr. Rushton) had not yet succeeded in finishing the book. Dr. Stiles' first point he believed was a valid one, and he had considered whether it could be applied to the deuteranope - where the expected effects would be most pronounced - to decide whether the two pigments were present as a mixture in every cone or occurred separately in two sets of cones. The distinction rested on second order differences in the measurements and the practical examination of the point had not yet been possible.

In concluding the discussion, the CHAIRMAN referred to Prof. Morton's comments and recalled the work of F. Weigert on the production of gelatine films, containing only one pigment in different states of orientation, which gave different spectral absorptions.

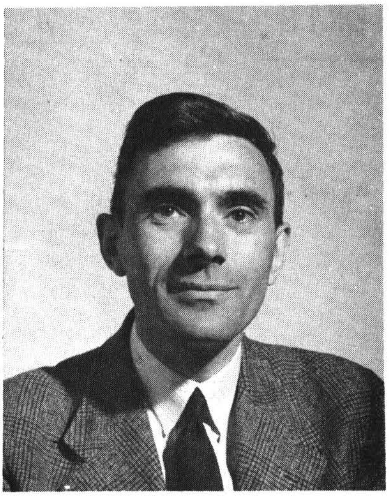
PAPER 2

THE MULTIPLICITY OF VISUAL PIGMENTS

By R. A. MORTON and G. A. J. PITT



R. A. Morton, F.R.S., Johnston Professor of Biochemistry in the **University** of Liverpool since 1944, approached biochemistry through the study of ultra-violet absorption spectra. He has worked on vitamin A and D, retinenes and visual pigments; present activities include studies on the systemic modes of action of vitamin A.



G. A. J. Pitt, after war-time service in the Indian Army, read biochemistry at the University of Liverpool, where since 1951 he has been Assistant Lecturer and subsequently Lecturer in Biochemistry.

2. THE MULTIPLICITY OF VISUAL PIGMENTS

By R. A. MORTON and G. A. J. PITT

SUMMARY

IT has been possible in only a few cases to isolate solutions of visual pigments free from light-absorbing impurities and showing absorption spectra corresponding to the spectral sensitivity of the animal, but other pigments have been investigated by means of difference spectra. Reports of new pigments obtained by this means are summarized. Some possible complications are discussed, together with the criteria which should be applied before accepting as genuine a reported new pigment. Recent work has nevertheless provided clear evidence of a multiplicity of visual pigments, and this may have implications for colour vision.

THE MULTIPLICITY OF VISUAL PIGMENTS

ANY light effect mediated by the retina must involve selective absorption and as all known visual pigments are photolabile, photodecomposition ('bleaching') is assumed to be a necessary component of the visual process. The sensitivity of the eye can be determined directly on man and indirectly on animals by studies of behaviour but these physiological responses are the end-result of considerable 'processing' by the nervous system. Chemical and biochemical studies have been concerned with the less complex end of the chain of events, namely the chemical structure of the light-absorbing molecules and the processes regenerating the visual pigments.

The orthodox chemical approach to a natural product of biochemical interest involves two stages: first, isolation of the active principle; and second, the determination of its structure. On these standards, chemical knowledge of visual pigments is still unsatisfactory, despite the many advances made in recent years. Even the major structural components of many of the most important pigments are uncertain; we know of the existence of other pigments only because many creatures possess sight although measurable amounts of visual pigments are not readily obtained from their retinas. The isolation of pigments is hampered by paucity of material, by their insolubility (dispersing agents have to be used to bring them into solution), and by their instability. As a result only rhodopsin (visual purple) has been obtained in a state approaching purity; other pigments have been obtained in less pure form; but some which are known to exist, and may even be demonstrated in the eye by *in situ* bleaching, have never been detected *in vitro*.

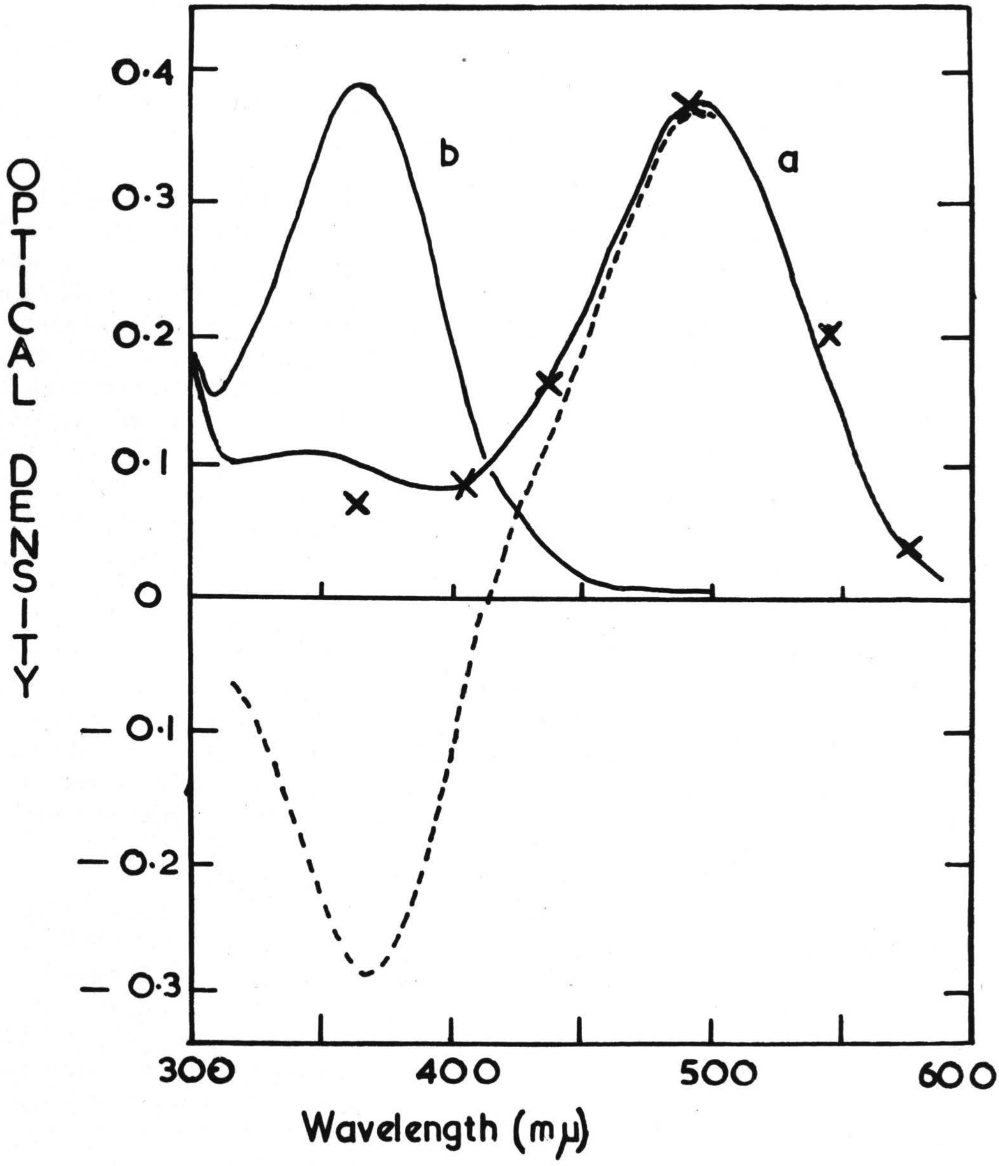


Fig.1. Absorption spectrum of cattle rhodopsin in digitonin solution (pH 9.2) (a) before bleaching (b) after bleaching *(ref.19)*. The dotted line is the difference spectrum. The crosses represent the scotopic sensitivity of the aphakic eye, according to Wald *(ref.20)*.

The chemists' goal is to isolate in 'pure' solution a visual pigment showing an absorption spectrum (in the visible region) which corresponds to a spectral sensitivity curve. This has been achieved only with rhodopsin *(fig.1)*. Many other visual pigments have been obtained in solutions containing light-absorbing impurities. The contribution of visual pigments to such solutions can be detected by difference spectra. *(fig.1)*. The absorption spectrum of the retinal extract is determined (a) before and (b) after exposure to light. Curve (b) is subtracted from curve (a) to give a difference spectrum which has two portions: one positive, the other negative, indicating respectively the disappearance of the visual pigment and the appearance of the photoproduct. As the absorption spectra of the pigment and the product always overlap, the difference spectrum is a function of the two.

The interpretation of difference spectra is not always simple. Other photolabile substances may be present; the photoproduct may be unstable (thermally or photochemically) and the absorption of the photoproduct may not differ greatly from that of the visual pigment so that the difference spectrum will bear no obvious relationship to the absorption of the original pigment. It is therefore essential that difference spectra be scrutinized warily.

The absorption curves of the recognized visual pigments are rather similar in shape but differ in position on the wave-length scale. All the visual pigments of which we have chemical knowledge bleach to give either retinene$_1$ or retinene$_2$, the aldehydes of vitamins A_1 and A_2 respectively. If a visual pigment revealed or characterized by a difference spectrum exhibits an absorption curve of about the expected shape and liberates a retinene, it will be reasonable to consider it a genuine visual pigment. Several pigments have been recognized in this way and have gained wide acceptance.

The use of difference spectra has been extended in recent years (a) to the study of pigments in retinal end organs (or fragments thereof), (b) to intact retinas removed from the animal and (c) to the living eye itself. Bleaching *in situ* seems at present to be the best way to obtain knowledge of the spectral absorption of cone pigments. These pigments are present in most eyes in exceeding quantities, but the distribution is such that the amount of pigment registered by ophthalmoscopic densitometry is high. It is very difficult, however, to apply to any putative visual pigment the strict criteria mentioned above. Moreover, complications come to light and have to be fitted into the developing pattern.

Rhodopsin is the only visual pigment for which the path of breakdown is understood. The following sequence summarizes the present state of knowledge:-

TABLE I

Some visual pigments reported on evidence obtained by means of difference spectra

Name of pigment	Approx. λmax. (mμ)	Species	Methods by which detected (see foot of table)	Chemical basis of Chromophore	Reference
Green-rod pigment	430	Frog	D	-	Denton and Wyllie *(21)*
-	430-440	Frog	B	-	Dartnall *(22)*
Euphausiopsin	462	Shrimp	A	not a retinene	Kampa *(23)*
Visual pigment 467	467	Tench	B	-	Dartnall *(24)*
-	460-470	Guinea pig	E	-	Weale *(25)*
Pigment 478_1	478	Argyropelecus affinis (b)	A	retinene$_1$	Munz *(18)*
		Bathylagus wesethi (b)	B	retinene$_1$	Munz *(18)*
Pigment 485_1	485	Sternoptyx obscura (b)	A	retinene$_1$	Munz *(18)*
'Chrysopsin'	below 490	deep sea fish	D	-	Denton and Warren *(26)*
-	487	Conger eel	A	-	Denton *(27)*; Walker *(28)*
Pigment 488_1	488	Melamphaes bispinosus (b)	A	retinene$_1$	Munz *(18)*
Pigment 490_1	490	Stomias atriventer (b)	A	retinene$_1$	Munz *(18)*
		Lampanyctus mexicanus (b)			
-	500-510	Guinea pig	E	-	Weale *(25)*
Visual pigment 507(a)	507 ± 2	Rainbow trout	B	probably retinene$_1$	Bridges *(29)*
Visual pigment 510(a)	510 ± 3	Bleak	B	-	Dartnall *(30)*
Mudsucker rhodopsin	512	Mudsucker	A	retinene$_1$	Munz *(31)*

TABLE 1 (Continued)

Name of pigment	Approx. λmax. (mμ)	Species	Methods by which detected (see foot of table)	Chemical basis of Chromophore	Reference
Visual pigment 523 (porphyropsin)	523	Carp	A	retinene$_2$	Crescitelli and Dartnall *(32)*
"	523	Xenopus	B	retinene$_2$	Dartnall *(12)*
-	524	Gecko	A	retinene$_1$	Crescitelli *(33)*
-	525-530	Marine scup	B	retinene$_1$	Wald, Brown and Smith *(34)*
Visual pigment 533 (porphyropsin)	533	several fresh-water fish	A and B	retinene$_2$	Dartnall *(24,30)* Bridges *(29)*
Narrow-banded pigment	535	Squirrel	E	-	Weale *(16)*
Narrow-banded pigment	535	Frog	C	-	Arden *(35)*
-	540	Guinea pig	F	-	Weale *(25)*
-	540	Man (protanope)	E	-	Rushton *(5,6)*
-	550	some fresh-water fish	B	retinene$_2$	Wald, Brown and Smith *(34)*; Dartnall *(30)*
-	560	Cat	G	-	Weale *(8)*
Iodopsin	562	Chick	B and H	retinene$_1$	Wald, Brown and Smith *(9)*
Human cone pigment	about 570	Man	E	-	Rushton *(36)*
-	600-610	Guinea pig	F	-	Weale *(25)*
Cyanopsin	620	artificial *in vitro* preparation from chick cones	B	retinene$_2$	Wald, Brown and Smith *(37)*

(a) probably identical
(b) a deep sea fish

METHODS USED FOR DETECTION

A : Simple difference spectrum of solution
B : Differential bleaching (that is successive bleaches using different wavelengths) of solution.
C : Differential bleaching of a suspension of retinal end organs.
D : Difference spectrum of intact retina removed from animal.
E : Simple difference spectrum of eye.
F : Differential bleaching of eye.
G : Regeneration spectrum of eye (that is increase of absorption in dark after bleaching visual pigments).
H : Regeneration spectrum of solution.

Rhodopsin ($\lambda_{max.}$ approx. 500 mμ)
↓
Lumi-rhodopsin ($\lambda_{max.}$ approx. 490-495mμ)
↓
Meta-rhodopsin ($\lambda_{max.}$ approx. 480-485mμ)
↓
N-retinylideneopsin ($\lambda_{max.}$ 440 mμ in acid
(indicator yellow) 365mμ in alkali)
↓
All-*trans*-retinene ($\lambda_{max.}$ 389mμ) + opsin.

Recent work *(ref.1)* has shown that even in dilute solutions, the bleaching of rhodopsin is not as simple as was formerly believed and in the living eye further complications may arise. Bridges *(ref.2)* has found that changes in the environment can greatly slow down the rate of bleaching around the meta-rhodopsin stage, and meta-rhodopsin itself shows species differences in stability *(ref.3)*. Rushton *(ref.4)* has found that orange intermediates may accumulate to a detectable extent in some eyes, and there is no certainty that *in the eyes of all species* rhodopsin will rapidly decompose to colourless products. Nothing is known of the pathway of breakdown of cone pigments, and analogous complications cannot be ruled out. Where cone pigments exist in mixtures, the interpretations of results may be difficult.

In the last few years difference spectra have been determined on retinal extracts, on retinas or fragments *in vitro*, and in the living eye, and have led to reports of a surprisingly large number of visual pigments. Most of these are summarized in *Table 1*.

A few in this formidable list merely confirm results obtained from gross absorption spectra of solutions; others seem to be genuine new pigments; but some are very doubtful. A number do not comply with the criteria previously mentioned and so should not be readily accepted. To reject them on the sole ground that they do not fit tidily with current conceptions is plainly risky, but it is even more risky to accept them as true pigments on the basis of difference spectra alone.

For some results obtained by *in situ* bleaching insistence on chemical criteria is unfair as most cone pigments are not extractable into solutions on the scale needed for spectroscopic investigation. If, however, the absorption curve detected *in situ* coincides with the spectral sensitivity curve of the animal, then this is evidence for the existence of a true visual pigment. Thus Rushton *(refs.5,6)* has found in the fovea of a protanope a pigment with $\lambda_{max.}$ near 540 mμ, corresponding with the foveal sensitivity curve. Such compelling evidence cannot usually be obtained from the sensitivity curves of animals other than man. For example, Gunter's photopic curve for the cat *(ref.7)*, determined by a behavioural method merely confirmed that the animal shows a sensitivity maximum substantially above 500 mμ, which bears no obvious relationship to Weale's rapidly regenerating pigment with $\lambda_{max.}$ 560 mμ (probably iodopsin) *(ref.8)*. At their best, the relationships between such spectral sensitivity curves of animals and known visual pigments are somewhat approximate, probably owing to the low accuracy attainable; see, for example, the absorption spectrum of chick iodopsin *(ref.9)* and the spectral sensitivity of the bird *(ref.10)*. At the worst, the sensitivity curve may not connect up with any known visual pigments, as in Xenopus *(refs.11,12)*.

By measuring the electrical responses of the retina and optic nerve to light, the visual process can be investigated at a stage intermediate between the changes of light absorption by retinal molecules and the spectral sensitivity of the intact animal. Electrophysiological 'sensitivity curves' are more precise than those obtained by behavioural methods but share the disadvantage of having undergone some processing by the nervous system and thus may bear no simple relationship to any single pigment.

Electrophysiological work has confirmed that rhodopsin and porphyropsin are the pigments of scotopic vision; it gives strong indications that pigments akin to iodopsin and cyanopsin are involved in cone vision (see *refs.13,14)*. Perhaps the most striking recent result has been a response from the squirrel retina *(ref.15)* coinciding within experimental error with the narrow-banded curve obtained by Weale *(ref.16)* by *in situ* bleaching; but many other electrophysiological data remain uncorrelated with any reported visual pigments - in particular Granit's modulators *(refs.13,14)*.

It is clear that we are very far from the ideal state of affairs in which pigments can be obtained in solutions with spectra corresponding to visual sensitivities.

To some extent this is an illusory target. Certainly the approach can scarcely expect to succeed with the pigments involved in colour vision, and the prospects are not good with any cone pigment. The excellent correlation obtainable between scotopic vision and rhodopsin is due largely to a number of fortunate factors. Rhodopsin occurs in rods in vastly larger quantities than are necessary for its function. Although all visual pigments are unstable chemically, rhodopsin is less unstable than iodopsin, the only cone pigment on which there is any chemical information. Probably most important, rhodopsin operates in conditions in which no other pigment is affected and it occurs in man, whose spectral sensitivity can be determined accurately using parts of the aphakic eye where other pigments will not interfere.

For no other pigment are the circumstances so fortunate and we cannot normally expect so satisfying a fit between the absorption characteristics of a pigment in solution and the spectral sensitivity of a subject.

Workers trying to sort out the visual pigments by spectroscopic techniques have tended to look to the physiologists for guidance, hoping for a directive such as: 'to explain vision we shall need x pigments, each with defined absorption characteristics, your task is to find *x* appropriate chemical structures'. Such a hope has not yet been realized; even to entertain it is probably naive.

On the other hand, it is perhaps also true that physiologists have a plethora of phenomena and look hopefully to the chemists to simplify their ideas by defining the available substances. At one time this seemed an attractive possibility. All the visual pigments so far elucidated are based on the retinenes; in the light of present knowledge, this may be true for all other pigments. A very valuable working hypothesis has been that all visual pigments are built up from four components: two vitamin A aldehydes, retinene$_1$ and retinene$_2$; and two proteins, scotopsin and photopsin in Wald's nomenclature *(ref.17)*, which give rise to rhodopsin and iodopsin respectively. This simple approach has probably served its purpose and more attention is being paid to the complications now apparent.

The best studied set of pigments, those clearly based on retinene$_1$, show great variation in their absorption maxima (see *Table 2*). Some of these have been shown to be formed from the specific isomer, 11-*cis*-retinene; it is probable that all are, and very unlikely that any isomerization of the retinene moiety can be invoked as an explanation of these differences. It must be accepted that the protein molecule itself is responsible in some as yet unknown way for these changes in absorption maxima.

TABLE 2

Absorption maxima of some pigments based on retinene$_1$

Source	$\lambda_{max.}$	Reference
Argyropelecus	478	Munz *(18)*
Sternoptyx	485	Munz *(18)*
Conger eel	487	Denton *(27)*; Walker *(28)*
Melamphaes	488	Munz *(18)*
Squid	490	St. George and Wald *(38)*
Opossum	493	Crescitelli, cited by Munz *(18)*
Cattle	498	Wald *(39)*
Frog	502	Wald *(39)*
Mudsucker	512	Munz *(31)*
Gecko	524	Crescitelli *(33)*
Chicken (iodopsin)	562	Wald, Brown and Smith *(9)*

The retinene_2 set of pigments has not been studied to the same extent as the retinene_1 family. Owing to a more restricted distribution in nature, retinene_2-pigments are likely to be fewer but a number have nevertheless been reported and they cover a substantial range of the spectrum (see *Table 1)*.

To the spectroscopist, the general situation is somewhat disquieting. The cardinal chemical problem of a visual pigment is to explain why it is a pigment - a matter of colour and constitution. Accepted spectroscopic theory will predict approximately the absorption of the retinenes themselves, and indeed this gave the clue to their structure, but why combination with the opsins causes the retinene absorption peak to move up to the visible remains unexplained. Furthermore, the small variations in $\lambda_{\text{max.}}$ of similar pigments are commonly ascribed to minor differences in the opsins for example frog rhodopsin 502mμ, cattle rhodopsin 498mμ. This seems reasonable, but it is difficult to dismiss as species variations the larger displacements now found. For example, the difference between the pigments of the deep-sea fish (478mμ) and gecko 'rhodopsin' (524mμ) warrants a more definite explanation. Wald's division of the opsins into scotopsins and photopsins *(ref. 17)*, is now perhaps less clear-cut.

To the question - how many visual pigments? - the chemist must answer that even the four known components, the two retinenes and the two opsins, can account for pigments absorbing between 478mμ and 620mμ. If we assume that photopsin is as versatile as scotopsin, then there could be a large number of pigments with different absorption maxima spread over that range of the spectrum.

Chemistry offers no immediate hope of simplifying the problem (so far as we can tell) but these recent indications of a multiplicity of pigments based on one retinene and somewhat variable scotopsins may aid anyone seeking to 'explain' colour vision by a plurality of pigments.

The only characterized iodopsin is that from the chick with $\lambda_{\text{max.}}$ 562mμ. It seems very probable that iodopsins are present in the cones of other species although in quantities too small to permit detection after extraction. Arguing from experience with rhodopsins which have absorption maxima scattered over 46 millimicrons of the spectrum, there is reason to expect species differences among iodopsins over at least as wide a range. If an animal possessed in its own cones more than one of these iodopsins - just as the deep sea fish *Bathylagus wesethi* has two rhodopsin-type pigments *(ref. 18)* - it could have the potentialities needed for the discrimination of colour. The simplest assumption is that some such phenomenon is involved among cone pigments, that the key to colour receptors and hence colour vision lies in the auxochromic properties of these protein molecules. The biochemists' task is to find what these properties are.

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PRESENTATION AND DISCUSSION OF PAPER 2

MR. G. A. J. PITT presented the paper. He mentioned that since the manuscript had been prepared, several further visual pigments had been reported which would add to the already considerable number listed in *Tables 1* and *2*. He emphasised the existence in some species of two kinds of scotopsin - besides the case of *Bathylagus wesethi* quoted in the paper, another fish species with two rhodopsins had been found by Dr. Dartnall. The possibility of the existence of several kinds of photopsin in the same species was the important thing for colour theory.

THE CHAIRMAN (PROF. G. WALD) quoted in support of Mr. Pitt's view the analogy of the globins, several varieties of which, combined with the same prosthetic group, occurred in man and other species. He made a suggestion on nomenclature, namely that the cone visual pigments of man might be called "chromopsins R, G and V".

DR. R. A. WEALE believed that the multiplicity of cone pigments could be reduced by one, as his data for the cone pigment of the squirrel were not really distinguishable from Dr. Rushton's results for his P·54 pigment.

DR. RUTH HUBBARD considered that the statement that iodopsins were less stable than rhodopsins (Morton and Pitt, p.115) was a dangerous generalisation. She hoped that some conclusion would be reached as to whether visual pigments with different λ_{max} but of fundamentally the same chemical constitution should be called by the same name.

DR. H. J. A. DARTNALL put the question "How constant are the visual pigments in any one species?" He quoted a case where, some years ago, he had examined the retina of Tench and found, quite definitely, two pigments, one with λ_{max} at 467 mμ and the other with λ_{max} at 533 mμ. Earlier workers had found only the latter, and he himself in later work found only the latter. On the other hand Dr. Kampa had obtained results which indicated that there were in fact two pigments present. He could quote other examples. He had been unable to find iodopsin in the chick. In an extreme case, he (Dr. Dartnall) had found no visual pigment whatever in 36 Australian geckoes, apparently in normal condition, whereas Prof. Crescitelli had previously reported a visual pigment with λ_{max} of 524 mμ. Was it that workers never reported their failures? Moreover, how should cases of "interbred" fish be dealt with?

DR. C. D. B. BRIDGES emphasised that we should not only consider the configuration of the retinenes and of the opsins but also the linkages between the retinene and protein chains. In the case of indicator yellow where it was established that the linkage was between the NH_2 group of the protein and the aldehyde group of the retinene, the λ_{max} in acid solution was about 440 mμ, whereas in visual purple it was about 502 mμ, and we had to account for the difference. Dr. Dartnall and Dr. Hubbard had separately suggested that the p2 electrons of the carotenoid chain were linked with p2 electrons in the protein. Dr. Bridges thought these linkages might offer many more modes of resonance of the molecule, energy levels between them would be diminished, and the absorption maximum moved towards longer wavelengths. The variety of possible linkages might produce with one protein and one retinene almost any desired value of λ_{max}. It might well be that there were really many fewer distinct pigments than would appear from this paper.

DR. L. R. FISHER referred to euphausiopsin, which was mentioned in *Table 1*. It was the only invertebrate pigment listed, and it was stated that the chemical basis of the chromophore was not a retinene. He had tried to repeat Dr. Kampa's work, with assistance from Dr. Dartnall, and they had found indications that there was a retinene there.

MR. PITT replied to these comments by asking two questions. In the case of Dr. Weale's squirrel pigment, had not Dr. Tansley shown by electrophysiological means that this was a narrow-band type of pigment?

DR. WEALE said that in his opinion most workers attached far too much weight to the region of the maximum of their curves and the interpretation of the narrowness of a curve tended to depend on the way in which it was drawn. Dr. Rushton's P.54 could equally be considered a narrow-band pigment if drawn on a suitable scale.

MR. PITT continuing, said that he feared that standardisation of nomenclature for visual pigments at this stage might lead to an undesirable rigidity, and he would advise against it.

THE CHAIRMAN said that he and his co-workers had not found considerable variations (as far as λ_{max} was concerned) in their preparations; an accuracy of 1 mμ for λ_{max} was invariable for any one pigment.

DR. DARTNALL agreed that any one pigment was remarkably constant; he had meant to convey that not all the pigments that had been observed for a given species appeared on all occasions. When they did appear they had the right λ_{max}.

PAPER 3

THE SPECTRAL VARIATION OF THE RELATIVE PHOTOSENSITIVITIES OF SOME VISUAL PIGMENTS

By H. J. A. DARTNALL

Dr. H. J. A. Dartnall is Head of the Visual Research Division of the Ophthalmological Research Unit (M.R.C.). For many years he has been interested in the photosensitive retinal pigments. He devised the method of partial bleaching with which the homogeneity of retinal extracts can be tested. The application of this method, in his own and in other laboratories, has led to the discovery of several new photopigments.

Lately he has measured the relative photosensitivities of some of these pigments, a study forming the subject of his contribution to the present Symposium.

Dr. Dartnall's publications include papers to the Royal Society and the Physiological Society, and a book, *The Visual Pigments* (1957).

3. THE SPECTRAL VARIATION OF THE RELATIVE PHOTOSENSITIVITIES OF SOME VISUAL PIGMENTS

By H. J. A. DARTNALL

SUMMARY

1. It has been shown that the mathematical analysis developed to describe the bleaching kinetics of frog visual purple (method of photometric curves) is applicable also to the retinal photopigments of the carp (pigment 523), the tench (pigment 533), the conger eel (pigment 487) and the gurnard (pigment 497).

2. The photosensitivities of these four photopigments have been measured relative to that of frog pigment 502 (visual purple or rhodopsin) over the wavelength range 440 - 580 mμ.

3. From the ratios so determined and from Schneider, Goodeve and Lythgoe's values for frog pigment 502 (*ref. 3*), the photosensitivities of the carp, tench, conger and gurnard pigments have been calculated in absolute units. The wavelength dependence of these photosensitivities parallels that of the extinctions of the relevant pigments. This suggests that in all cases the quantum efficiencies of the bleaching processes are independent of wavelength.

INTRODUCTION

ABOUT twenty years ago, Dartnall, Goodeve and Lythgoe (*refs. 1,2*) made a quantitative analysis of the photic bleaching of frog visual purple in monochromatic light. Using a new method (the method of photometric curves), which involved observation of the changing transmissivities of solutions as they bleached in lights of known quantum intensities, they were able to calculate the *photosensitivity* of visual purple. Photosensitivity - which is the product of the two quantities extinction coefficient and quantum efficiency - has a significance for photochemical reactions similar to that which the reaction-velocity constants have for thermal reactions.

In 1939, Schneider, Goodeve and Lythgoe (*ref. 3*), using the same method, determined the spectral variation of the photosensitivity between 436 and 560 mμ. They found that the variation paralleled that of the extinction. This indicated that the quantum efficiency was independent of the wavelength of the bleaching light.

Since these investigations there have been no attempts to measure the photosensitivities of visual pigments other than visual purple.

In the present experiments the method of photometric curves has been applied to a number of visual pigments. Instead of measuring the light intensities in absolute units, however, the bleaching rates for each pigment have been referred to those obtained with frog visual purple when subjected to the same treatment. In this manner the photosensitivities of pigment 487 from the conger eel (*refs. 4,5*), pigment 497 from the gurnard (*ref. 4*), pigment 523 from the carp (*ref.11*) and pigment 533 from the tench (*ref. 7*) have been compared with the photosensitivities of pigment 502 (rhodopsin or visual purple) from the frog over the wavelength range 440–580 mμ.

METHODS AND APPARATUS

Extraction of the visual pigments

Extracts of photosensitive retinal pigments were prepared from five different species, an amphibian, two river fish and two marine fish. The species used were the frog (*Rana temporaria*), the carp (*Cyprinus carpio*), the tench (*Tinca tinca*), the conger eel (*Conger vulgaris*) and the gurnard (*Trigla cuculus*).

Gurnard extracts G1, G3, G4 and conger extracts E1, E2 were prepared in Plymouth from retinae which had been freshly dissected out under teleost Ringer solution. Gurnard extract G2 and conger extracts E3, E4 and E5 were made in London from retinae which had been removed at Plymouth and stored frozen. All the other extracts (see *Table 1*) were prepared in London from fresh retinae.

The dark-adapted retinae were washed in McIlvaine's pH 4.6 buffer until the washings were colourless, or nearly so. (Conger and gurnard retinae were washed in teleost Ringer, except in the case of Conger extract E5 when the pH 4.6 buffer was used). The washed retinae were then extracted twice with 2% aqueous solutions of digitonin. The extracts were combined and brought to the desired alkalinity (pH 8–9) by the addition of 1/10th volume of saturated sodium borate solution. Three of the carp extracts (C2, C5 and C6) were prepared in a different way, namely by extraction of the rod outer-segments after these had been separated from the rest of the retinae by Saito's method (*ref. 8*) of flotation in (35%) sucrose solution. These extracts also were made alkaline with saturated sodium borate solution. All operations were conducted in the light of a ruby dark-room lamp.

The extracts were stored in darkness in a deep-freeze operating at about -30°C. When a sample was required, the frozen extract was thawed, well mixed to ensure homogeneity, and then centrifuged e.g. at 4000 r.p.m. for

18

20 minutes. The sample was then withdrawn carefully from the top of the solution to avoid disturbing any sediment.

Apparatus and technique for the bleaching experiments

The source of light was a 108 watt strip filament lamp run off a Westat constant potential power unit (8.6 volts D.C., 310 watt) and series rheostat. The light from the filament was focussed on to the (straight) entry slit of a Hilger Barfit monochromator. The dominant wavelength of the light emerging from the (curved) exit slit of this instrument could be varied by rotation of the constant-deviation prism. The spectral purity of the emergent beam depended on the widths of the entry and exit slits and on the wavelength setting of the prism. With the slit widths shown in *Table 2* the beams were of sufficient intensities to bleach the visual pigment solutions in reasonable times (20 mins. - 2 hrs.).

Immediately after emerging from the exit slit, the light passed through a lens to form an image of the monochromator telescope lens on the aperture of a cell holder. The cell holder, which was of similar construction to one previously described (*ref. 7*) accommodated two optical cells either of which, by a lateral movement of an inner cell carrier, could be brought into accurate alignment with the axis of the light beam. After passing through one or other of the cells the light was received on the sensitive surface of a barrier-layer photocell. The degree of spreading of the slightly divergent light beam in its passage (0.5 cm) through the cell was negligible.

The output terminals of the photocell were connected to a Cambridge 'spot' galvanometer of sensitivity about 17 cm scale deflection per microamp. Tests showed that the photocell-galvanometer combination had a satisfactorily linear response to light intensity. For the experiments with lights of dominant wavelengths 540, 560 and 580 mμ a neutral filter of density 0.24 was interposed between the cell holder and the photocell.

The exit slit of the monochromator, the cell holder, and the photocell were enclosed in a light-proof box. During the experiments water from a thermostat was circulated through the cell holder to maintain a temperature of 25 ± 0.2°C.

The experiments were carried out in the following way. One of the optical cells was filled with a sample of the photosensitive solution under test, the other with distilled water. The cells were then placed in the holder so that the water-filled cell was in the path of the light beam. The light was then switched on and, after a suitable warming up period, was adjusted in intensity (by means of the series rheostat) until the galvanometer deflection was slightly less than full scale (16.0 cm).

The cell carrier was then moved into its other position so that the light beam now passed through the photosensitive solution. At the same time a stop-clock was started. As the solution bleached its density decreased and

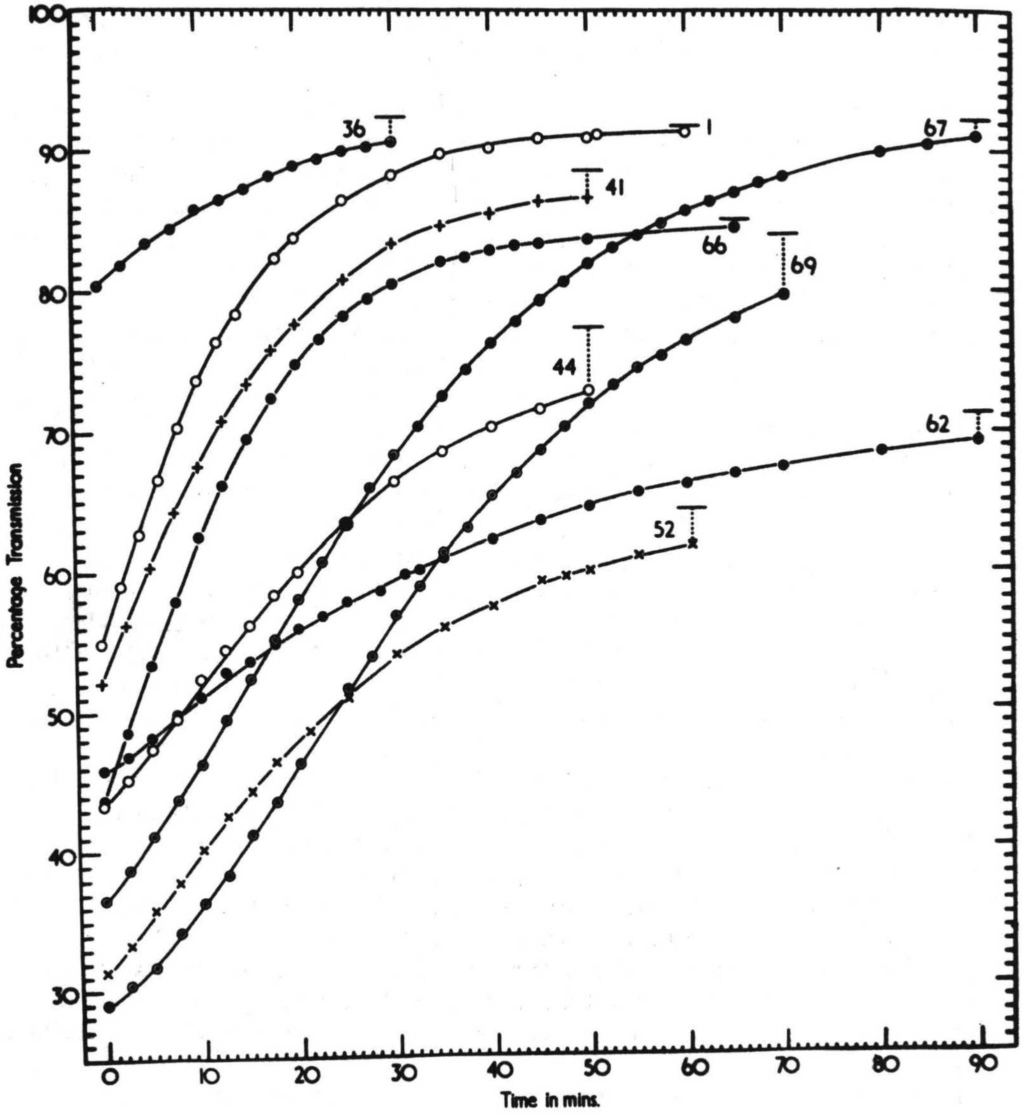

Fig.1. Examples of transmission/time curves for visual pigment solutions from the frog (O), the carp (+), the tench (•), the conger (⊙), and the gurnard (X). T = 25°C. The numbers identify the experiments *(Tables 3-7)*. The short horizontal lines, connected by dots to the last readings, indicate the final transmissions, that is after complete bleaching.

there were corresponding increases in the light transmitted (I_t). Readings of I_t were taken, initially and every 2½ minutes thereafter (every 2 minutes in a few of the earlier experiments), until bleaching was practically complete. The cell was then removed from the holder, exposed for 5 minutes to the light of a 15W lamp, and replaced for measurement of the final transmission I_f. Typical transmission/time curves for a few of the experiments are shown in *fig. 1*.

The intensities of the bleaching lights were not quite constant, there being slow fluctuations of the order of ± 2% of the mean. These changes were followed during the course of each experiment by observing, at regular intervals, the galvanometer scale deflections (I) caused on passing the light through the *control* cell. This was done immediately after each transmission reading (I_t) had been taken, the operation of moving the control cell into the light beam, taking the reading and moving the cell out again entailing an interruption of about 5 seconds in the bleaching. Every transmission reading was corrected for the intensity value so determined.

No account required to be taken of the 5 second interruption in every 2½ minutes (2 minutes in a few cases) of bleaching since the procedure described was followed in all cases and the results were needed only for comparative purposes. For the same reason no allowance was made for the light reflected back into the solution from the rear surfaces of the optical cell and the surface of the photocell.

All the experiments were carried out with precisely the same apparatus, the arrangement of which was not altered in any way. With this precaution it was found that the results, for example for a particular photosensitive pigment and wavelength of bleaching light, could be repeated accurately over the whole of the 7-month period of the investigation.

THEORY

THE following symbols and definitions are used in this paper.

I, intensity of light (number of quanta per second) incident on the front surface of a photosensitive solution contained in an optical cell with plane parallel faces at right angles to the light beam;

I_t, intensity of light transmitted by the bleaching solution at a time t.

I_f, intensity of light finally transmitted by the solution, that is after bleaching has been completed;

t, time (seconds) from the initial exposure of the solution to the light;

A, exposed area (cm^2) of the solution;

γ, quantum efficiency of the bleaching, a ratio defined as

$$\frac{\text{number of chromophores destroyed}}{\text{number of quanta absorbed}}$$

α, extinction coefficient (cm^2) per chromophore defined by the equation

$$\log_e \frac{I}{I_t} = \alpha c l$$

where c is the chromophore concentration (number per cm^3) and l is the length (cm) of light path through the solution.

It has been shown *(refs. 1,2,9)* that the equation

$$\log_{10} \frac{I_t}{I_f - I_t} = \emptyset \cdot \frac{\alpha \gamma I}{2.303A} \cdot t + \text{constant} \tag{1}$$

accurately describes the bleaching kinetics of visual purple under certain conditions.

The validity of equation (1) is not affected when there are stable light-absorbing impurities in the photosensitive solution, nor when the bleaching lights are of such wavelengths as are absorbed by the progressively-increasing amounts of end product. The reduction in reaction rate arising from either or both these causes is allowed for by the slope-compensating function, $\emptyset$.

The function $\emptyset$ is given by

$$\emptyset = \frac{I_f}{I_f - I_t} \cdot \frac{I - I_t}{I} \cdot \frac{\log I_f / I_t}{\log I / I_t}$$

and hence, since it contains I_t, is a variable. In practice, however, its variation throughout any one experiment is negligible, and the mean value can be used without introducing significant error (*refs. 2,10*).

Equation (1) describes the kinetics of any photic reaction. In the bleaching of visual purple the photoreaction is the formation of a transient substance (transient orange). This substance is not stable but decomposes spontaneously to form a stable product (indicator yellow). A consecutive thermal reaction, if comparable in rate with that of the original photoreaction, would invalidate the use of equation (1). For this reason it is important to choose experimental conditions such that the thermal decomposition of transient orange is either negligibly slow or extremely fast when compared with the photochemical process. In the present experiments the temperature was controlled at 25°C. At this temperature the decomposition of transient orange is very rapid indeed. Consequently, at the low bleaching light intensities used, the photic breakdown of visual purple to transient

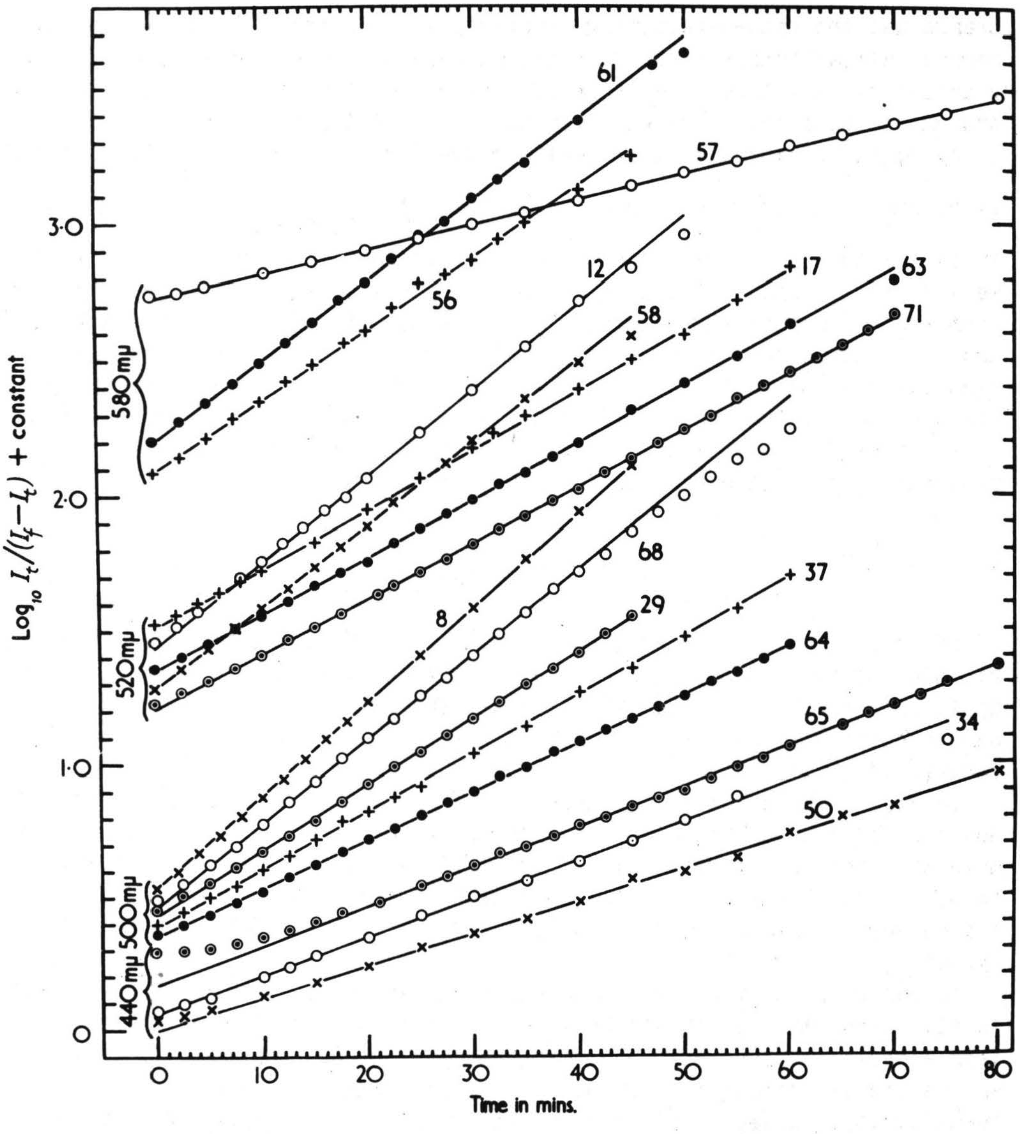

Fig.2. **Examples of the straight lines** obtained by **plotting**, as functions of time, the expression $\log_{10} I_t/(I_f - I_t)$ + constant for visual pigment solutions from the frog (O), the carp (+), the tench (•), the conger (⊙), and the gurnard (X). The numbers identify the experiments *(Tables 3-7)*. Different arbitrary constants have been added to each set of ordinate values, to avoid undue overlapping.

orange was the rate-determining reaction, all the transient orange being removed almost immediately after it had been produced. The secondary reaction did not interfere, therefore, and the bleaching proceeded as though visual purple were directly changed into indicator yellow.

By plotting experimental values for the L.H.S. of equation (1), straight lines are obtained with slopes having the value $\frac{\phi\alpha\gamma I}{2.303A}$. In the previous investigations on visual purple the light intensities per unit area (I/A) were directly measured in absolute units, and the values of ϕ were calculated from the transmissivity readings. Consequently, it was possible to calculate absolute values for $\alpha\gamma$, the photosensitivity. In the present work the photosensitivities of various visual pigments have been *compared* by carrying out all the bleaching experiments (at any given wavelength) with lights of very nearly the same intensities, and correcting the data for the small departures from standard, and for the relevant values of ϕ.

RESULTS

IT has been tacitly assumed that the equation (1) describing the bleaching kinetics of frog visual purple under certain conditions would be equally applicable to the bleaching kinetics of other visual pigments under the same conditions. That this assumption was justified was shown by the results (*fig.* 2).

In *fig.* 2 the quantity $\log_{10}I_t/(I_f-I_t)$ is plotted as a function of time. Results for four of the nine bleaching wavelengths employed are shown, not more than one result from each of the five species studied being included in each group. Apart from this limitation the sixteen experiments illustrated were chosen at random from the total of seventy-two and are quite typical.

In general, as *fig.* 2 shows, straight lines fairly described the results in all cases. Mention should be made, however, of two kinds of divergence from linearity which were observed. The first kind, occurring towards the end of the experiment, is exemplified by experiment 68 (*fig.* 2). Now the quantity $\log_{10}I_t/(I_f-I_t)$ is very sensitive to I_f as I_t approaches it in value, that is as the bleaching nears completion. Small errors in the value of the final transmissions could easily account for the ultimate divergences from linearity in all the (few) cases in which they were observed (*ref.* 2). The second kind, in which the divergence was at the beginning of the experiment, is illustrated by experiment 65 (*fig.* 2). This case was the most pronounced one, but in most of the experiments with conger eel extracts - and in some of the others - this effect was observed, though usually to a much less degree. Other instances, for example, can be seen in *fig.* 2 by holding the page horizontal and glancing along the lines. The reason for these divergences, which were always in the same sense, namely suggesting that the bleaching took some time to get under way, is not known.

Apart from this the general result is clear, namely that the equation (1) describing the bleaching kinetics of frog visual purple is applicable also to extracts of visual pigments from the carp, tench, conger eel and gurnard.

The results obtained for each species are considered in detail below.

The frog, (Rana temporaria)

The light-sensitivity of frog retinal extracts is essentially, if not entirely, due to the well-known visual pigment 502 (frog rhodopsin or visual purple). If any other light-sensitive pigments were present in the extracts they were in too small a concentration to have any appreciable effect on the measurements.

The results obtained are tabulated in *Table 3*. When the investigation was originally planned it was feared that changes might occur over long periods to the sensitivity of the photocell/galvanometer combination. Since the frog results were intended to provide data to which the results on other species could be referred it was consequently thought advisable to match every experiment with a similar one on a frog extract. It soon became evident, however, that this was unnecessary, the results for the frog (and for the other species too) being satisfactorily reproducible over the whole 7-month period of the investigation. In other words only occasional checks were necessary. Consequently, of the total of 21 experiments on frog extracts (*Table 3*), 16 were done in the first half of the investigation and only 5 in the latter half; an indication of increased confidence in the constancy of the apparatus.

Another satisfactory feature shown in *Table 3* is that the results were independent of the particular frog extracts used, and of their age. Thus, considering especially the measurements at 500 mμ, the corrected slopes of the straight lines are the same, within experimental error, for four different extracts sampled at times ranging from 0 to 182 days after they had been prepared. Similar examples can be seen in *Table 3* for other wavelengths of bleaching.

We may conclude that the photosensitivity of frog visual purple in aqueous digitonin solution at 25^{0}C is a constant and easily reproducible property, not appreciably affected by the age of the extract, nor by the particular circumstances of its preparation.

The carp, (Cyprinus carpio)

Crescitelli and Dartnall (*ref. 11*) have shown that the light-sensitivity of extracts of carp retinae is due to a single photopigment having maximum extinction at 523 mμ. The extracts prepared for the present investigation appeared to be identical with those of Crescitelli and Dartnall.

The carp pigment 523 ("porphyropsin") is a retinene$_2$ derivative. (Recently Crescitelli *(ref. 12)* has reported that retinal extracts of the Australian gecko, *Phyllurus milii* contain a photopigment (λ_{max} = 524 mμ) of very similar light-absorbing properties, but based on retinene$_1$).

The results obtained in the bleaching experiments with the present carp extracts are listed in *Table 4*.

The final column of *Table 4* gives the ratios of the mean corrected slopes to those obtained with the frog extracts *(Table 3)* at the same wavelengths. These ratios, which are the relative photosensitivities of carp pigment 523 to frog pigment 502 are plotted against wavelength in *fig. 3*.

In *fig. 3* the smooth curve drawn through the 523/502 ratios intersects the zero axis at 540 mμ, indicating that at this wavelength the photosensitivities of the two pigments are equal (log. ratio = 0). This was confirmed using an artificial mixture of frog and carp extracts (F.6A and C.6) in proportions such that the maximum extinctions of the two pigments were roughly equal. A sample of this mixture was bleached with light of wavelength 540 mμ (experiment 54). The plot of $\log_{10} I_t/(I_f-I_t)$ against time was found to be linear with a slope (corrected) of 7.80×10^{-4} per second. This may be compared with slopes of 7.88×10^{-4} per second for the frog pigment alone *(Table 3)* and of 7.92×10^{-4} per second for the carp pigment alone *(Table 4)*. Thus at 540 mμ the mixture behaved as though only a single photopigment were present and could not have been distinguished by this experiment from either the frog or the carp photopigments alone.

For interest's sake another sample of the mixture was bleached with light of 500 mμ (experiment 55). At this wavelength the photosensitivity of the carp pigment is only two-thirds that of the frog. As expected, the plot of $\log_{10} I_t/(I_f-I_t)$ against time diverged sensibly from linearity, particularly towards the end of the experiment. Nonetheless, the earlier data were approximately linear and yielded a line with slope (corrected) of 4.88×10^{-4} per second, that is between the values for the frog and carp pigments at 500 mμ *(Tables 3 and 4)*.

The tench, (Tinca tinca)

Dartnall *(ref. 7)* has reported that extracts of dark-adapted tench retinae contain two photopigments, with maximum extinctions at 533 mμ and 467 mμ respectively. The proportion of pigment 467 in his extracts was variable, and averaged about one-third that of the 533 pigment (in terms of maximum extinctions). Owing to the presence of these two pigments the total difference spectra had λ_{max} = 520-525 mμ.

The tench had been previously examined; by Köttgen and Abelsdorff in 1896 *(ref. 13)* and by Bayliss, Lythgoe and Tansley in 1936 *(ref. 14)*, but the results, when studied in the light of the modern findings, afforded no evidence for the presence of pigment 467. In both cases the difference spectra, which had been obtained by unselective bleaching of the extracts, had λ_{max} = 535-540 mμ and in this, and in other, respects were similar to those which had been obtained by Dartnall on bleaching his extracts with long wave light (which does not appreciably affect the pigment 467). This suggests that the extracts of the earlier workers had contained only the pigment 533. Recently Kampa (*ref. 15*) has reported λ_{max} = 525 mμ for the tench total difference spectrum which suggests that her extract contained pigment 467.

For the present investigation an extract of tench retinae was prepared in the expectation that it would contain both the 533 and 467 pigments. On analysis by partial bleaching, however, the extract was found to contain virtually only one photosensitive substance, namely pigment 533. The amount of pigment 467 was negligibly small.

The reason for these variable results is not known. The answer to the question whether only certain tench possess the pigment 467 or whether its presence in extracts depends on some uncontrolled factor in the extraction process must await the results of future work. The absence of pigment 467 from the present extract was, in a sense, fortunate for it allowed the measurement of the photosensitivity of pigment 533 relative to that of the frog pigment 502 to be made without the complication which the presence of another pigment would have caused.

The results obtained with the tench extract are shown in *Table 5*. The ratios of the photosensitivities of tench pigment 533 to those of frog pigment 502 (last column of *Table 5*) are plotted against wavelength in *fig. 3*. The photosensitivity ratios lie on a curve (*fig. 3*) which, like the carp curve, intersects the zero axis at about 540 mμ. Also, like the carp curve, the tench curve is convex to the wavelength axis, as would be expected of pigments whose maximum photosensitivities are on the long wave side of 502 mμ. In other respects, however, the tench and carp curves are quite distinct.

The conger, (Conger vulgaris)

The retina of the dark-adapted conger eel is golden yellow in colour and has an absorption spectrum similar in shape to those of 'visual-purple' retinae, but displaced by about 16 mμ towards the violet end of the spectrum (*ref. 5*). Aqueous digitonin extracts of conger retinae contain a photosensitive pigment having maximum extinction at 487 mμ. This pigment has been shown to be homogeneous by the method of partial bleaching (*ref. 4*). (Similar photopigments, for which the names 'visual golds' or 'chrysopsins' have been proposed, have recently been found in four species of deep-sea fish (*ref. 16*) but their homogeneity, unlike the conger photopigment, has not yet been tested).

The conger pigment 487 is probably a retinene$_1$ derivative, for on bleaching it gives rise to a product with maximum extinction at about

380 mμ. It is remarkably similar in light-absorbing properties to the 'iso-rhodopsin' (λ_{max} = 487 mμ) which Hubbard and Wald (*ref.* *17*) have prepared by adding excess *iso*-retinene$_1$ *a* to cattle opsin in digitonin solution and leaving the mixture to incubate in darkness for 2¾ hr.

The results obtained with the present extracts of conger retinae are shown in *Table 6*. The ratio of the photosensitivity of the conger pigment to that of the frog (last column of *Table 6*) is plotted as a function of wavelength in *fig.* *3*. The data lie on a curve which is concave to the wavelength axis, that is opposite in sense to that for the carp and tench curves. This is to be expected for a pigment having its maximum photosensitivity on the short-wave side of 502 mμ.

The gurnard, (Trigla cuculus)

Walker (*ref.* *4*) has reported that aqueous digitonin extracts of dark-adapted gurnard retinae contain a homogeneous photopigment having maximum extinction at 497 mμ. This pigment, which is apparently a retinene$_1$ derivative, has similar light-absorbing properties to human visual purple (*ref.* *6*).

The results obtained with the present gurnard extracts are shown in *Table 7*. The ratio of the photosensitivities of the gurnard and frog pigments (last column of *Table 7*) is plotted as a function of wavelength in *fig.* *3*. As *fig.* *3* and *Table 7* show, the photosensitivities of the gurnard and frog pigments are practically indistinguishable between 440 and 520 mμ. At longer wavelengths, however, the photosensitivity of the gurnard pigment 497 decreases more rapidly than that of the frog pigment 502.

DISCUSSION

Calculation of light intensities

From Schneider, Goodeve and Lythgoe's values for the photosensitivity of the frog pigment 502 (*ref.* *3*), it is possible to calculate the absolute values of the light intensities used in the present bleaching experiments. Thus from equation (1), the slope of the straight line obtained by plotting $\log_{10} I_t/(I_f - I_t)$ as a function of time is, after dividing by the compensating function $\emptyset$, equal to

$$\frac{\alpha\gamma}{2.303} \cdot \frac{I}{A}$$

Consequently since A, the area exposed to light was in the present experiments 0.785 cm^2 (circle of diameter 1 cm) we have

$$I \text{ (quanta per sec.)} = \frac{2.303 \times 0.785 \text{ (cm}^2\text{) X corrected slope (sec}^{-1}\text{)}}{\alpha\gamma \text{ (cm}^2\text{)}}$$

The results of the calculations are set out in *Table 8*.

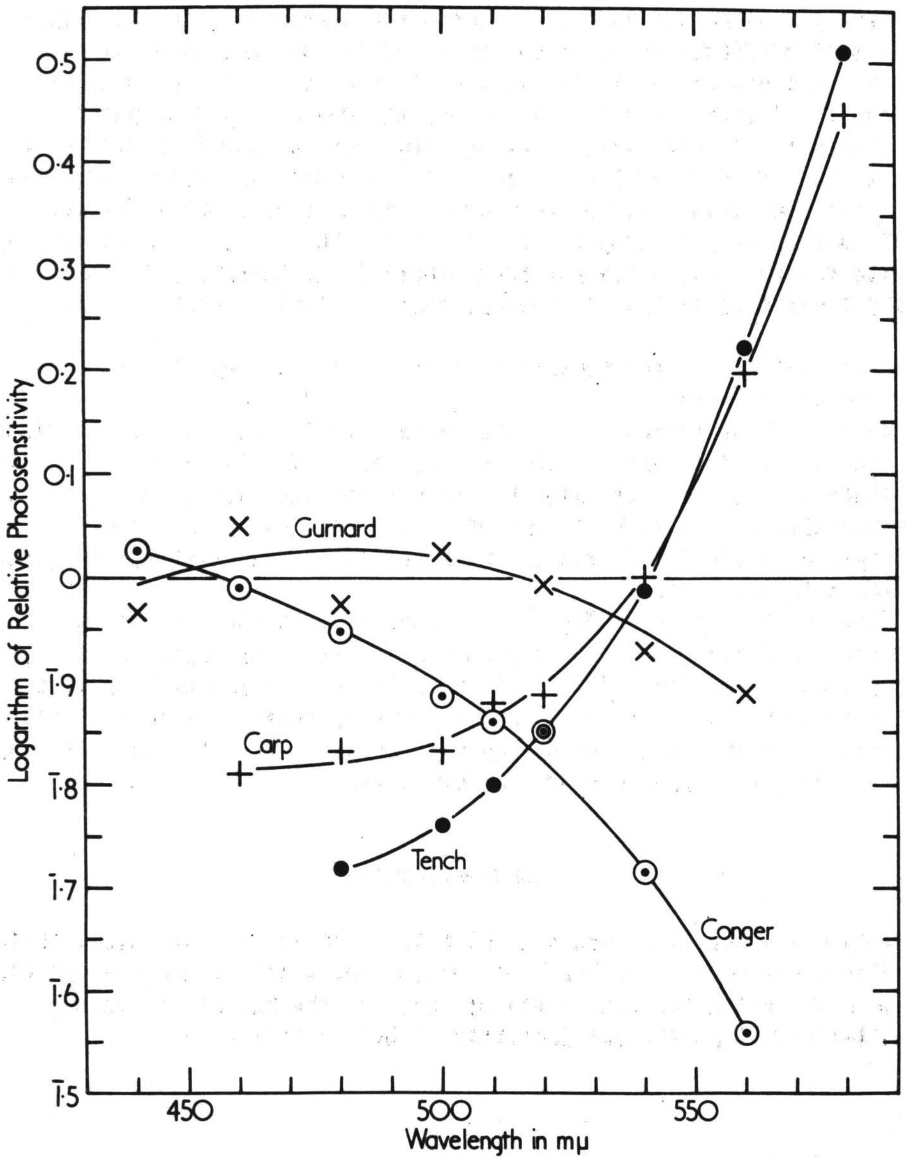

Fig.3. The logarithms of the photosensitivities of carp, tench, conger and gurnard photopigments relative to that of the frog, plotted as functions of the wavelength. The points of intersection of the curves with the zero axis give the wavelengths at which the relevant pigments have the same photosensitivity as that of the frog pigment 502 (visual purple or rhodopsin).

The fact, shown in the present investigation, that the photosensitivity of frog visual purple is such a constant and reproducible property suggests that this substance would be suitable for the determination of light intensities in absolute units. In certain circumstances, for example where the intensity of light is excessively low, the use of a visual purple solution for intensity measurements might be advantageous, for it could be left in the light for long periods of time until appreciable photodecomposition had occurred. This would be permissable since visual purple is very thermostable at room temperature and below. Thus a solution of frog visual purple buffered at pH 7.7 has been stored in darkness for 14 months at $3^{O}C$ without perceptible loss of photopigment (Dartnall, unpublished).

Absolute values for the photosensitivities of the carp, tench, conger and gurnard photopigments

In *fig. 3* the ratios of the photosensitivities of the various pigments to that of the frog pigment 502 are plotted as functions of the wavelength. If these ratios are multiplied by the appropriate values for the absolute photosensitivity of frog pigment 502, as determined by Schneider, Goodeve and Lythgoe (*ref. 3*), we obtain the absolute photosensitivities of the pigments in question.

This has been done in *fig. 4*. The curves drawn through the data so obtained have maxima within experimental error of the expected positions for pigments 523, 533, 487 and 497 and, in other respects, parallel the extinction curves for these pigments. This suggests that in all cases the quantum efficiencies of the bleaching processes are, like that of frog visual purple, independent of the wavelength.

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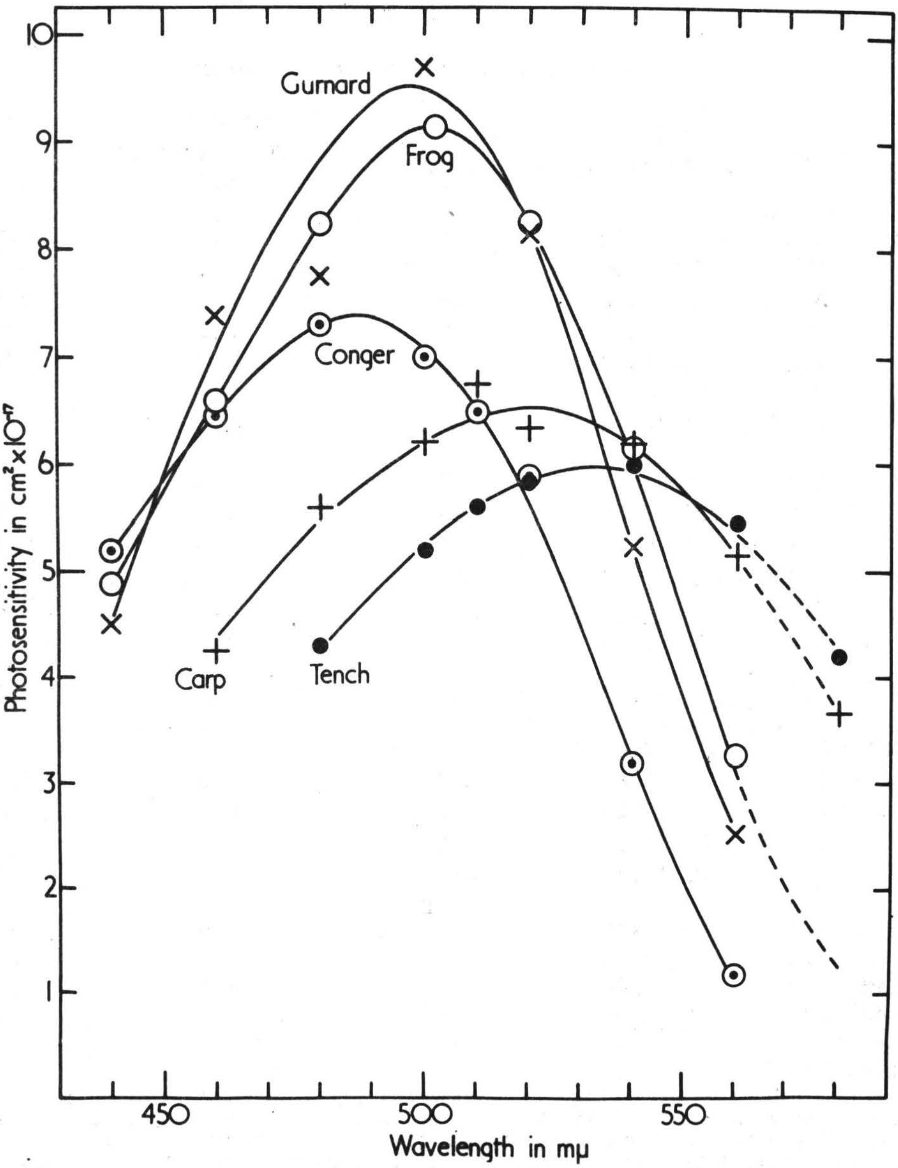

Fig.4. The photosensitivities of the frog, carp, tench, conger and gurnard photopigments in absolute units, plotted as functions of the wavelength. Values for the frog pigment 502 (visual purple or rhodopsin) from Schneider, Goodeve and Lythgoe, *(ref.3)*; values for the other pigments obtained by multiplying the values for the frog pigment by the relevant ratios plotted in *fig.3*.

TABLE 1

Preparation details of the extracts

	Extract	Date of preparation	No. of retinae	Volume of buffered extract (ml)	ΔD^{*}_{max} (approx.)
Frog	F. 1	13.12.55	36	3.3	0.28
	F. 2	28. 5.56	16	2.2	0.36
	F. 3	12. 6.56	48	4.4	0.44
	F. 4	26. 6.56	28	2.2	0.40
	F. 5	1. 8.56	40	4.4	0.41
	F. 6	28. 9 - 2.10.56	248	34.1	†
Carp	C. 1	5.12.55	18	3.3	0.25
	C. 2	30. 5.56	24	4.4	0.23
	C. 3	3. 7.56	18	3.3	0.32
	C. 4	30. 7.56	24	4.4	0.27
	C. 5	10. 9.56	20	3.3	0.31
	C. 6	24.10.56	30	3.3	0.28
Tench	T. 1	16.11.56	18	3.3	0.31
Conger	E. 1	not recorded	2	2.2	0.38
	E. 2	"	2	2.2	0.38
	E. 3	20. 7.56	4	2.2	0.43
	E. 4	25. 7.56	3	2.2	0.54
	E. 5	29.10.56	not recorded	3.3	0.96
Gurnard	G. 1	11. 5.56	4	2.2	0.26
	G. 2‡	4. 9.56	8	3.3	0.38
	G. 3§	18.10.56	6	3.3	0.85
	G. 4	5.12.56	6	2.2	0.68

* The loss, caused by bleaching, in optical density of the extracts at the wavelengths of maximum change, namely at about 504 mμ, 525 mμ, 535 mμ, 489 mμ and 499 mμ respectively for the frog, carp, tench, conger and gurnard extracts.

† The major portion (23 ml) of this extract was evaporated to dryness using a vacuum pump. The residue was taken up with 1 ml of distilled water in which it was completely soluble. The resulting solution (F.6A) was thus extremely concentrated, both in digitonin (about 50% w/v) and in visual purple (optical density at 560 mμ = c. 1.8), and was diluted as required for the photosensitivity experiments (see footnotes to *Table 3*).

‡ In the preparation of this extract photosensitive pigment was lost in the retinal washings.

§ In addition to the photosensitive gurnard pigment (λ_{max} = 497 mμ) this extract contained an appreciable amount of a photostable contaminant (λ_{max} about 450 mμ) of retinal origin.

TABLE 2

Band widths of the bleaching lights used
in the experiments

Wavelength setting (mμ)	Width of entry and exit slits (mm)	Approximate band width (mμ)
440	1.00	16
460	1.00	18
480	0.90	20
500	0.60	15
510	0.60	18
520	0.50	14
540	0.60	20
560	0.40*	15
560	0.50†	19
580	0.40	17

* For experiments Nos. 49, 51 and 59.

† For experiments Nos. 24, 25, 41, 42 and 60.

TABLE 3

The results, at 25°C, for the frog extracts (pigment 502)

Bleaching wavelength (mµ)	Experiment No.	Date 1956	Extract No.	Age (days)	Initial density	Final density	Slope* X 10^4 (sec^{-1})	Mean† intensity %	Meanϕ	Corrected‡ slope	Mean corrected slope
440	34	24/8	F. 4	59	0.296	0.183	2.43	96.8	0.820	3.07	3.07
460	5	7/6	F. 2	10	0.301	0.082	3.93	98.2	0.916	4.35	4.33
460	44	27/9	F. 3	107	0.363	0.111	3.77	98.7	0.888	4.30	
480	19	10/8	F. 5	9	0.426	0.070	4.88	99.2	0.929	5.30	5.30
480	45	28/9	F. 3	108	0.486	0.119	4.57	97.6	0.883	5.30	
500	7	8/6	F. 1	178	0.336	0.057	5.52	98.6	0.940	6.02	6.07
500	9	12/6	F. 3	0	0.544	0.076	5.47	96.9	0.925	6.10	
500	10	12/6	F. 1	182	0.336	0.053	5.45	99.1	0.944	5.83	
500	11	14/6	F. 3 ‖	2	0.485	0.090	5.52	98.3	0.911	6.17	
500	27	20/8	F. 5 ¶	19	0.360	0.064	5.92	98.3	0.934	6.45	
500	29	21/8	F. 5	20	0.487	0.062	5.58	97.2	0.938	6.12	
500	68	6/12	F. 6A**	67	0.532	0.062	5.30	98.3	0.938	5.73	
510	3	6/6	F. 2	9	0.401	0.057	5.75	97.7	0.941	6.25	6.25
520	12	29/6	F. 4	3	0.400	0.029	5.30	98.6	0.969	5.55	5.62
520	16	3/7	F. 4	7	0.412	0.043	5.28	97.4	0.956	5.68	
540§	1	30/5	F. 2	2	0.260	0.037	7.12	98.3	0.960	7.53	7.88
540	22	16/8	F. 5	15	0.278	0.035	7.32	97.8	0.962	7.78	
540	32	23/8	F. 5	22	0.326	0.055	7.53	98.0	0.942	8.17	
560§	24	17/8	F. 5	16	0.160	0.027	3.97	98.4	0.971	4.15	4.10
560	42	26/9	F. 3	106	0.164	0.031	3.87	98.7	0.967	4.05	
580§	57	8/11	F. 6A††	39	0.151	0.039	1.53	95.7	0.958	1.67	1.67

* The slope of the straight line obtained on plotting log $I_t(I_f - I_t)$ as a function of time.

† The light intensity in arbitrary units (galvanometer scale deflections) expressed as a percentage of the full scale deflection (16.0 cm). Owing to the variation with wavelength of the response of the photocell, the figures given are mutually comparable only within each wavelength group.

‡ Corrected by dividing the slope by $\frac{\text{mean intensity} \times \phi}{100}$

§ For the experiments at 540, 560 and 580 mµ a neutral filter of density 0.24 was fixed between the cell holder and the photocell so that the light intensity could be raised to a sufficient level to cause reasonably rapid bleaching.

‖ This sample was diluted with 1/10th volume of distilled water.

¶ This sample was inadvertently exposed to the bleaching light for about 5 minutes before the start of the experiment.

** A sample of extract F. 6A, as diluted for experiment No. 57, further diluted with about an equal volume of distilled water.

†† A sample of extract F. 6A diluted with four volumes of distilled water.

TABLE 4*

The results, at 25°C, for the carp extracts (pigment 523)

Bleaching wavelength (mμ)	Experiment No.	Date 1956	Extract No.	Age (days)	Initial density	Final density	Slope X 10^4 (sec^{-1})	Mean intensity %	Meanϕ	Corrected slope	Mean corrected slope	Ratio†
460	4	7/6	C. 2	8	0.267	0.191	2.20	98.6	0.811	2.75	2.80	0.65
460	43	26/9	C. 5	12	0.274	0.178	2.38	99.0	0.825	2.85		
480	20	14/8	C. 4	15	0.318	0.146	3.10	99.8	0.854	3.63	3.60	0.68
480	40	24/9	C. 5	10	0.325	0.130	3.08	99.1	0.870	3.57		
500	26	20/8	C. 4	21	0.355	0.116	3.65	96.7	0.883	4.27	4.12	0.67
500	37	19/9	C. 5	5	0.342	0.075	3.63	99.2	0.923	3.97		
510	2	6/6	C. 2	7	0.345	0.136	4.03	99.2	0.863	4.72	4.72	0.75
520	15	3/7	C. 1	211	0.365	0.117	3.63	99.2	0.883	4.13	4.32	0.77
520	17	4/7	C. 3	1	0.501	0.180	3.63	97.6	0.827	4.50		
540	21	16/8	C. 4	17	0.352	0.109	7.00	98.3	0.890	8.00	7.92	1.00
540	31	23/8	C. 4	24	0.355	0.086	7.03	98.4	0.912	7.83		
560	25	17/8	C. 4	18	0.280	0.077	6.03	98.9	0.920	6.63	6.45	1.57
560	41	25/9	C. 5	11	0.283	0.053	5.90	99.6	0.943	6.28		
580	56	7/11	C. 6	14	0.176	0.027	4.45	98.1	0.970	4.68	4.68	2.81

* The first four notes to *Table 3* apply also to this table.

† The ratio of the mean corrected slope to that for frog extracts (*Table 3*) at the same bleaching wavelength. This gives the ratio of the photosensitivity of pigment 523 to that of pigment 502.

TABLE 5*

The results, at 25°C, for the tench extracts (pigment 533)

Bleaching wavelength (mμ)	Experiment No.	Date 1956	Extract No.	Age (days)	Initial density	Final density	Slope X 10^4 (sec^{-1})	Mean intensity %	Mean∅	Corrected slope	Ratio†
480	62	20/11	T.1	4	0.338	0.147	2.33	98.6	0.853	2.77	0.52
500	64	27/11	T.1	11	0.362	0.114	3.02	97.6	0.885	3.50	0.58
510	70	10/12	T.1‡	24	0.302	0.089	3.55	99.3	0.908	3.93	0.63
520	63	23/11	T.1	7	0.393	0.090	3.52	97.1	0.909	3.98	0.71
540	66	29/11	T.1	13	0.358	0.070	6.90	97.1	0.927	7.67	0.97
560	60	16/11	T.1	0	0.355	0.059	6.25	97.5	0.939	6.83	1.67
580	61	19/11	T.1	3	0.246	0.057	4.97	98.2	0.939	5.38	3.23

* The first four notes to *Table 3* apply also to this table.

† The ratio of the corrected slope to that for frog extracts (*Table 3*) at the same bleaching wavelength. This gives the ratio of the photosensitivity of pigment 533 to that of pigment 502.

‡ After slight dilution with 2% digitonin solution in order to provide sufficient material to fill the optical cell.

TABLE 6*

The results, at 25°C, for the conger eel extracts (pigment 487)

Bleaching wavelength mμ	Experiment No.	Date 1956	Extract No.	Age (days)	Initial density	Final density	Slope X 10^4 (sec^{-1})	Mean intensity %	Mean φ	Corrected slope	Mean corrected slope	Ratio†
440	35	24/8	E. 4	30	0.421	0.164	3.00	97.0	0.840	3.68	3.27	1.07
440	65	27/11	E. 5⁊	29	0.335	0.097	2.48	96.9	0.900	2.85		
460	6	7/6	E. 2		0.379	0.100	3.75	97.1	0.898	4.30	4.25	0.98
460	48	5/10	E. 3	77	0.379	0.108	3.67	97.8	0.893	4.20		
480	18	9/8	E. 4	15	0.592	0.057	4.85	99.9	0.943	5.15	4.70	0.89
480	69	7/12	E. 5⁊	39	0.538	0.076	3.87	98.3	0.925	4.25		
500	28	21/8	E. 3	32	0.469	0.063	4.15	98.1	0.936	4.52	4.65	0.77
500	47	4/10	E. 3	76	0.441	0.057	4.45	99.0	0.941	4.78		
510	36	27/8	E. 4§	33	0.095	0.034	4.80	99.7	0.964	5.00	4.53	0.73
510	67	30/11	E. 5⁊	32	0.433	0.035	3.78	96.2	0.962	4.08		
520	13	29/6	E. 1		0.333	0.065	3.70	98.9	0.932	4.02	3.98	0.71
520	14	2/7	E. 1§		0.283	0.061	3.92	99.4	0.936	4.22		
520	71	12/12	E. 5⁊	44	0.412	0.064	3.47	99.2	0.935	3.73		
540	23	16/8	E. 4	22	0.200	0.028	3.88	97.6	0.970	4.10	4.10	0.52
560	59	15/11	E. 5	17	0.149	0.029	1.42	98.3	0.969	1.48	1.48	0.36

* The first four notes to *Table 3* apply also to this table.

† The ratio of the corrected slope to that for frog extracts (*Table 3*) at the same bleaching wavelength. This gives the ratio of the photosensitivity of pigment 487 to that of pigment 502.

⁊ After dilution on 27/11/56 with an equal volume of buffered 2% digitonin solution.

§ After dilution with buffered 2% digitonin solution in order to provide sufficient material to fill the optical cell.

TABLE 7*

The results, at 25°C, for the gurnard extracts (pigment 497)

Bleaching wavelength mμ	Experiment No.	Date 1956	Extract No.	Age (days)	Initial density	Final density	Slope X 10^4 (sec^{-1})	Mean intensity %	Meanϕ	Corrected slope	Mean corrected slope	Ratio†
440	50	23/10	G. 3‡	5	0.433	0.301	2.02	97.6	0.728	2.83	2.83	0.92
460	39	20/9	G. 2	16	0.568	0.313	3.87	98.8	0.725	5.42	4.87	1.12
460	53	31/10	G. 3‡	13	0.492	0.266	3.20	97.6	0.758	4.33		
480	52	29/10	G. 3‡	11	0.503	0.191	4.05	98.9	0.819	5.00	4.99	0.94
480	72	13/12	G. 4	8	0.733	0.142	4.23	98.0	0.868	4.98		
500	8	8/6	G. 1	28	0.313	0.055	5.82	98.6	0.943	6.28	6.43	1.06
500	38	19/9	G. 2	15	0.527	0.161	5.48	98.4	0.846	6.58		
520	58	12/11	G. 3‡	25	0.368	0.064	5.13	99.3	0.934	5.53	5.53	0.99
540	46	2/10	G. 2§	28	0.314	0.142	5.65	98.4	0.857	6.70	6.70	0.85
560	49	22/10	G. 3	4	0.298	0.100	2.85	99.3	0.897	3.20	3.17	0.77
560	51	25/10	G. 3‡	7	0.122	0.043	2.97	99.4	0.952	3.13		

* The first four notes to *Table 3* apply also to this table.

† The ratio of the mean corrected slope to that for the frog extracts (*Table 3*) at the same bleaching wavelength. This gives the ratio of the photosensitivity of pigment 497 to that of pigment 502.

‡ A diluted G. 3 extract of ΔD_{max} = 0.35. (2.5 ml of distilled water added to 1.5 ml of G. 3 on 23/10/56).

§ After filtration to reduce opalescence.

TABLE 8

The absolute intensities of the bleaching lights used in the present experiments

Wavelength (mμ)	2.303 X Corrected slope* X 10^4 (sec^{-1})	Photosensitivity of frog visual purple X 10^{17}† (cm^2)	Calculated light intensity X 10^{-13} (quanta/sec)
440	7.07	4.88	1.14
460	9.97	6.59	1.19
480	12.2	8.25	1.16
500	14.0	9.15‡	1.20
520	12.9	8.26	1.23
540	18.1	6.16	2.30
560	9.44	3.28	2.26

* The corrected slopes given in *Table 3* multiplied by 2.303 in order to express them in natural logarithms.

† From Schneider, Goodeve & Lythgoe (*ref. 3*).

‡ At 502 mμ.

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PRESENTATION AND DISCUSSION OF PAPER 3

DR. H. J. A. DARTNALL gave a brief resume of his paper, emphasising that his results showed the quantum efficiency of bleaching to be independent of wavelength for fish species just as for the frog, and established material differences between the absolute photosensitivities; for example, the conger had a lower photosensitivity than the frog.

DR. RUTH HUBBARD questioned whether Dr. Dartnall's results really showed that on a molecule for molecule basis there was a difference between the absolute photosensitivities of conger and frog visual pigments. He had measured the product $\gamma\alpha$ and seemed to be assuming α was the same for the different pigments.

DR. E. DENTON said that the actual density of the pigment in the conger eel was extremely high; the effective density for one traverse of the retina was about 1.0. He was not altogether satisfied with this kind of argument, which took no account of the size of the molecules; some insect pigments had very small molecules. He referred to Dr. Barlow's work on noise, and the idea that the conger pigment might be more stable than the rhodopsin pigment. If it was accepted that pigments with lower values of λ_{max} were more stable than ones with higher λ_{max}, then it would seem that deep-sea fish received more benefit from the stability of their pigments than from an adjustment of the λ_{max} to suit the blue-green light of the natural environment.

DR. M. H. PIRENNE thought the paper important because it drew attention to the level of sensitivity rather than to the position of λ_{max}. Factors of thousands or millions in sensitivity were clearly as important as considerations of the value of λ_{max}. If in a series of pigments from animals of high sensitivity the values of α did not vary widely, then it was possible that bleaching of the pigment might have nothing to do with visual excitation, but might be only a concomitant effect. The action of the light on the molecule might effect some transformation in the receptor structure, which might be responsible for vision. If this was so, only the absorption would be of consequence, and not the photosensitivity.

This hypothesis might help on the question of thermal decomposition and spontaneous excitation. Why should nature make use of a system sensitive to heat when light quanta were so much more energetic than kT : most biological structures were extremely efficient. If light and heat acted in different ways then the splitting of molecules in Brownian motion would not affect the visual process.

PROFESSOR R. W. DITCHBURN considered some serious difficulties in treating the action in visual end-organs as a case of solid state physics. With ordinary solids, electrostatic fields of several thousand volts/cm were required to cause any kind of photoconductive effect whatever, and he thought it unlikely, though not impossible, that such fields could be produced in any animal structure. Moreover such photoconductive effects gave spectral response curves with a sharp threshold unlike visual sensitivity curves. But perhaps the importance of surface effects in the visual case meant that we were dealing with an intermediate state between liquid and solid.

DR. DARTNALL, replying to Dr. Denton, said he did not question the great sensitivity of the conger, but his photosensitivity measurements gave no support for the view that a low λ_{max} was beneficial. He was more inclined to Dr. Barlow's suggestion of the greater stability associated with lower λ_{max}. To Dr. Hubbard he would reiterate that what he had actually measured was the product $\gamma\alpha$.

GENERAL DISCUSSION ON PAPERS 1, 2 and 3

PROFESSOR LE GRAND commented on Dr. Stiles' point concerning the location of the pigment in foveal cones; was there a mixture in any one cone or did each cone contain only one pigment? He thought it would not be possible to distinguish in the way suggested. Light entering through one cone might or might not pass back through the same one after reflexion. The probability of its doing so was very small. If the light was reflected back through other cones it would not be possible to locate the position of the pigments. Moreover, the dimensions of the foveal cones were of the same order as the wavelength, and it was not possible to apply geometrical optics to the problem.

DR. STILES considered that Prof. Le Grand's first objection might well apply to the light on its way back through the cones after reflexion but not to light on its way in, so that perhaps "half" the suggested effect would persist. On the second objection, it was questionable whether the smallness of the cones would vitiate completely the argument from geometrical optics.

THE CHAIRMAN, referring to Dr. Ditchburn's comment, did not think there could be a simple explanation of vision based on photoconductivity phenomena. The outer segments of the rods and cones were not true crystals but were in some semi-crystalline or liquid crystal phase. It was certain however that they were highly oriented assemblies of molecules.

PAPER 4

ON THE CHROMOPHORES OF THE VISUAL PIGMENTS

By RUTH HUBBARD

Ruth Hubbard was born in Vienna, Austria. She received her Ph.D. degree from Radcliffe College, Cambridge, Massachusetts. She has held fellowships from the United States Public Health Service with Professor C. Rimington in London, and from the Guggenheim Foundation at the Carlsberg Laboratory, Copenhagen. Her research, performed mostly in association with Professor George Wald at Harvard University, has dealt primarily with the biochemistry and physiology of vision. Specifically, she has studied the enzymatic interconversion of retinene and vitamin A, the synthesis of rhodopsin *in vitro*, geometrical isomers of vitamin A and retinene and their role in vision, and related problems.

4. ON THE CHROMOPHORES OF THE VISUAL PIGMENTS

By RUTH HUBBARD*

SUMMARY

A HYPOTHESIS is proposed for the structures of the chromophores of the visual pigments. The chromophores are derived from neo-*b* (11-*cis*) retinene, combined with opsin as the conjugate acid of a Schiff base: $\mathrm{R.}\overset{H}{C}\overset{+}{=}\underset{H}{N}\mathrm{.opsin}$, where R stands for $C_{19}H_{27}$ (in retinene$_1$) or $C_{19}H_{25}$(in retinene$_2$). The opsin surface fits the shape of the neo-*b* chromophore, establishing strong interactions with the entire retinene molecule. These stabilize the attachment of the chromophore to opsin, and shift the absorption spectra of the conjugate acids toward longer wavelengths.

Light bleaches vertebrate visual pigments by isomerizing their chromophores, primarily to the all-*trans* configuration. All-*trans* retinene does not fit the geometry of the chromophoric site on opsin. Isomerization thus disrupts the stabilizing interactions, and results in the release of free all-*trans* retinene and opsin.

The chromophore of squid rhodopsin also is isomerized by light. Yet squid rhodopsin does not release all-*trans* retinene$_1$, and hence does not bleach in neutral solution. This is due to the fact that squid opsin can accept also all-*trans* retinene$_1$ as a chromophore, forming a new stable chromoprotein -- squid metarhodopsin. Light re-isomerizes the chromophore of squid metarhodopsin to rhodopsin. Thus in the light, a steady-state mixture is formed which contains both metarhodopsin and rhodopsin.

For the process of vision, therefore, the eye has selected a reaction in which light affects *directly* the shape of a protein chromophore, so changing its relationship with the protein surface. It thus achieves a mechanism for translating the absorption of quanta instantaneously into a chemical reaction.

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* This research was supported in part by grants to Professor G. Wald from the Division on Neurology and Blindness of the U.S. Public Health Service (Grant No. B-568 C-2, C-3). I am indebted to Professor Wald and the members of his laboratory for many helpful discussions and suggestions. In particular, I want to thank Dr. Allen Kropf for critical discussions of the present paper.

ON THE CHROMOPHORES OF THE VISUAL PIGMENTS

DURING the ten years which have elapsed since the last colour vision conference, our understanding of the chemistry of the visual pigments has advanced considerably. Following the identification of retinene as vitamin A aldehyde *(refs.1,2)*, it has become possible to describe in chemical terms all the reactions involved in the bleaching and synthesis of four visual pigments, and to synthesize them from well-defined precursors in aqueous solution. These investigations have been described *(refs.3,4,10;* for reviews see *refs.5,6,7)*. I shall therefore only summarize them briefly, preparatory to a discussion of the structure of the chromophores of the visual pigments.

I. THE BLEACHING AND SYNTHESIS OF THE VISUAL PIGMENTS

THE reactions involved in the bleaching and synthesis of rhodopsin are summarized in *fig.1*. Rhodopsin bleaches over a series of orange intermediates -- lumi- and metarhodopsin -- to all-*trans* retinene and opsin. The retinene is either reduced to all-*trans* vitamin A by the enzyme alcohol dehydrogenase collaborating with the reduced form of the coenzyme, DPN; or

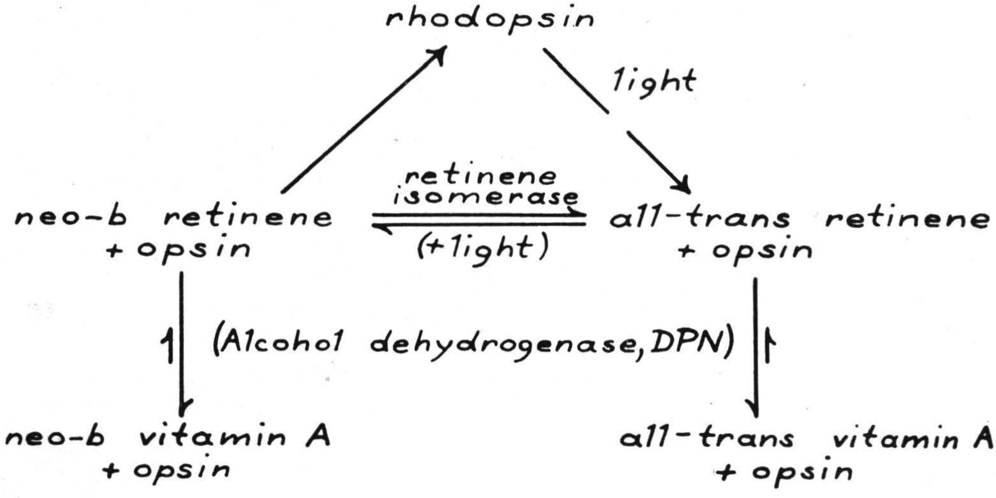

Fig.1. Bleaching and synthesis of rhodopsin (modified from Hubbard and Wald, *(ref.3)*).

isomerized by an enzyme, retinene isomerase, which catalyzes the interconversion of all-*trans* and neo-*b* retinene *(ref.8)*. Neo-*b* is the only isomer of retinene capable of acting as a rhodopsin precursor. Neo-*b* retinene, in turn, may be reduced to neo-*b* vitamin A and stored in the pigment layers, primarily as a fatty acid ester *(ref.9)*. Alternatively, it may condense with opsin to form rhodopsin. When these reactions are studied with purified systems in solution, the neo-*b* isomer is in short supply. The eye, however, accumulates considerable stores of neo-*b* vitamin A ester *(ref.9 and unpublished observations by G. Wald and P. S. Brown)*. It therefore probably has additional mechanisms for converting all-*trans* retinene or vitamin A to the neo-*b* isomer.

Similar reaction cycles have been demonstrated for three other visual pigments — porphyropsin, iodopsin, and cyanopsin *(refs.4,5,10)*. These four visual pigments are built on the same pattern, differing only in the type of opsin — rod or cone — and the type of retinene — retinene$_1$ or retinene$_2$ — which they contain. These relations are summarized in *Table 1*[*] *(compare refs.5,7)*. It is clear that *different opsins* (rod or cone) *combine with the same retinene to yield different visual pigments;* just as *different retinenes* (retinene$_1$ or retinene$_2$) *form different visual pigments with the same opsin.*

TABLE 1

Composition and absorption maxima[†] of the four visual pigments derived from rod and cone opsin, and retinene$_1$ and retinene$_2$.

Visual pigment	Opsin	Retinene	Absorption maximum (mμ)
Rhodopsin	rod opsin	retinene$_1$	about 500
Porphyropsin		retinene$_2$	about 522
Iodopsin	cone opsin	retinene$_1$	562
Cyanopsin		retinene$_2$	620

[*] The absorption maxima of rhodopsin and porphyropsin differ somewhat from one species to the next. Since all the pigments which have been analyzed contain neo-*b* chromophores, these differences must be due to species differences in the opsin. Dartnall *(ref.37)* suggests that these variations render the names rhodopsin and porphyropsin obsolete and urges that the pigments be named on the basis of their absorption maxima: visual pigment 498, 502, 523, etc. It seems to me that, as physiologists and biochemists, the things we most want to know about a visual pigment are whether it functions in rod or cone vision, and whether it contains retinene$_1$ or retinene$_2$ — in other words, what it is made of. The precise position of the absorption maximum is of less vital interest. It therefore seems both valid and useful to retain the conventional terminology, granting that it does not *precisely* define the absorption spectrum.

A specific *cis* isomer of retinene* is required to synthesize the visual pigments, while bleaching always liberates predominantly all-*trans* retinene. Cycles of isomerization are therefore fundamental to the chemistry of the four pigments.

We shall now pass on to a discussion of the structure of the chromophores of the four visual pigments. This must necessarily be based in part on speculation. It may, however, be useful to propose a concrete model for discussion and future investigation.

II. THE CHROMOPHORES OF THE VISUAL PIGMENTS

Stereoisomeric configuration.

All four visual pigments derive their chromophores from a specific *cis* isomer of retinene. In the case of retinene$_1$, this isomer (neo-*b*) has been crystallized and its configuration established by synthesis as 11-*cis* *(refs.11,12)*. The opsins accept only one other isomer of retinene$_1$, iso-*a*, to form the iso-pigments,† isorhodopsin (λ_{max} 487 mμ) and isoiodopsin (λ_{max} 510 mμ) *(refs.3,10)*. Iso-*a* retinene has also been crystallized and its structure determined by synthesis as 9-*cis* *(ref.13)*. The configurations of the isomers of retinene$_1$ are summarized in *fig.2*.

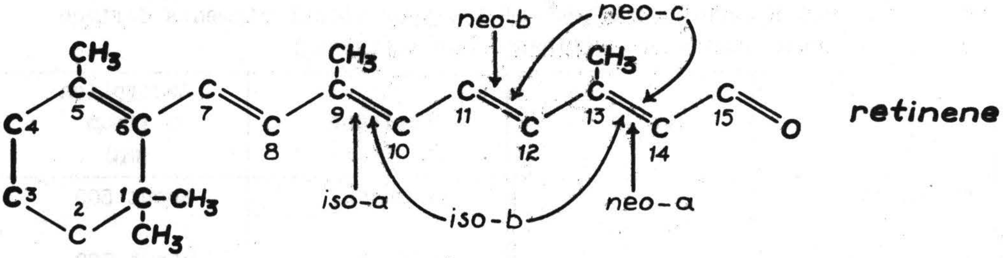

Fig.2. Configurations of the stereoisomers of retinene$_1$. The structure as drawn has the all-*trans* configuration. Arrows indicate the double bonds, which are in *cis* linkage in the various *cis* isomers.

The chemical analysis has not proceeded so far with the stereoisomers of retinene$_2$. Here also only two *cis* isomers react with opsin: one forms the visual pigments, porphyropsin and cyanopsin; the other, the corresponding

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* We are using the term "retinene" to include retinene$_1$ and retinene$_2$, and shall specify whenever we refer only to one or the other.

† There is no evidence for the occurrence of iso-pigments *in vivo*.

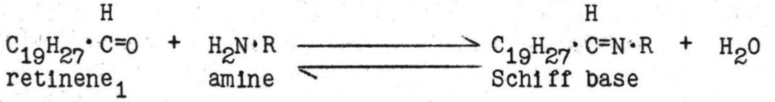
iso-pigments* *(ref. 7 and Wald, Brown and Smith, in preparation)*. Retinene$_2$ differs from retinene$_1$ only in the possession of an extra double bond located between carbon atoms 3 and 4 in the β-ionone ring *(ref. 14)*. The side chains are identical. The isomer specificity of the opsins as well as direct spectroscopic observations on the two relevant *cis* isomers of retinene$_2$ suggest that these two *cis* isomers have the 11-*cis* (neo-*b*) and 9-*cis* (iso-*a*) configurations.

The fact that two *cis* isomers of retinene condense with opsin, yielding *two sets* of pigments with distinct spectroscopic properties (the visual pigments and the iso-pigments) indicates that the two sets of chromophores must in some measure retain the stereoisomeric configurations of their retinene precursors.

Nature of the linkage of retinene to opsin.

The attachment of neo-*b* retinene to opsin shifts the absorption spectrum about 120 mμ toward longer wavelengths in rhodopsin and porphyropsin, and about 175 and 215 mμ in iodopsin and cyanopsin. Wald *(ref. 15)* and Collins and Morton *(ref. 16)* first proposed that the shift in spectrum between retinene$_1$ and rhodopsin might be achieved by combining two molecules of retinene$_1$, thus doubling the length of the conjugated system. This hypothesis, however, was unsatisfactory for a number of reasons *(ref. 6)*, and was finally ruled out by the observation that the rhodopsin molecule contains only one molecule of retinene$_1$ *(ref. 17)*. The colour of the rhodopsin chromophore is therefore produced by attaching a single molecule of retinene$_1$ to opsin.

How is retinene attached to opsin? Collins *(ref. 18)* and Morton and Pitt *(ref. 19)* have shown that in rhodopsin, the chromophore is attached to an amino group on opsin, probably as a Schiff base. Morton and co-workers have explored the spectroscopic properties of Schiff bases of retinene$_1$ and retinene$_2$ with a variety of amino acids and amines *(refs. 2, 19, 20, 21)*. The reaction involves the condensation of the aldehyde group of retinene with the amino group of amines or amino acids, with the elimination of a molecule of water:


(1)

$$\underset{\text{retinene}_1}{C_{19}H_{27}\cdot\overset{H}{C}{=}O} + \underset{\text{amine}}{H_2N\cdot R} \rightleftharpoons \underset{\text{Schiff base}}{C_{19}H_{27}\cdot\overset{H}{C}{=}N\cdot R} + H_2O \qquad (1)$$

The formation of Schiff bases of retinene$_1$ with aliphatic amines shifts the absorption maximum about 15 to 20 mμ toward *shorter* wavelengths, due to the replacement of the carbonyl group (-C=O) of retinene by a C=N double bond. Acidification, however, shifts the absorption maximum to about 440-450 mμ, due to the formation of the conjugate acid:

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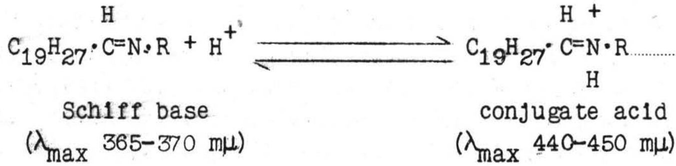
* There is no evidence for the occurrence of iso-pigments *in vivo*.



$$C_{19}H_{27}\cdot\overset{H}{C}{=}N\cdot R + H^{+} \rightleftharpoons C_{19}H_{27}\cdot\overset{H}{C}{=}\underset{H}{\overset{+}{N}}\cdot R \qquad (2)$$

Schiff base (λ_{max} 365-370 mμ) conjugate acid (λ_{max} 440-450 mμ)

In aqueous solution, Schiff bases are readily hydrolyzed to the component aldehyde and amine (equation (1) from right to left), but the conjugate acids are stable *(ref. 19)*.

Lythgoe *(ref.22)* and Wald *(refs.23,24)* found twenty years ago that rhodopsin, on bleaching in acid solution, yields a first product with an absorption maximum at 440 mμ* (acid indicator yellow, in Lythgoe's terminology). Morton and Pitt *(ref.19)* have shown that it is the conjugate acid of the Schiff base of retinene$_1$ still combined with the specific amino group to which it is attached in rhodopsin. It is stable below about pH 3; in less acid solution, it loses a hydrogen ion and is hydrolyzed, releasing retinene$_1$ (viz. equations (2) and (1) above).

Morton and Pitt *(ref.19)* have therefore suggested that the rhodopsin chromophore is somehow derived from the conjugate acid of a Schiff base of retinene with a specific amino group on opsin. We should like to extend this hypothesis to all four visual pigments.

Spectroscopic properties.

We mentioned above that the conjugate acids of Schiff bases of retinene$_1$ have their absorption maxima at about 440-450 mμ. The absorption maxima of the corresponding compounds of retinene$_2$ are at about 460-465 mμ *(ref.20)*. Both fall far short of the respective rod and cone pigments. Furthermore, Schiff bases of retinene are necessarily pH indicators, whereas the visual pigments are not. If the Schiff base hypothesis is correct, the opsins must therefore: (1) shift the absorption spectra of the conjugate acids to longer wavelengths; and (2) stabilize these acids in neutral and alkaline solution, in which model Schiff bases lose a hydrogen ion, and are consequently hydrolyzed to retinene and the amine.

Before proceeding further with attempts to interpret the spectroscopic properties of the visual pigments, let us briefly consider the physical meaning of the shift in spectrum between a Schiff base of retinene and its conjugate acid. The absorption of a quantum of light in the visible or near ultra-violet leads to the electronic excitation of a molecule from its ground state (that is the most probable state) to an excited state. This transition requires energy, which is supplied by the absorbed quantum. A shift in spectrum towards longer wavelengths (smaller quanta) therefore implies that less energy is required to raise the molecule from the ground

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* Lythgoe and Wald were working with frog rhodopsin. Their observations have since been confirmed using rhodopsins prepared from cattle *(ref.19)* and squid *(ref.36)*.

state to the first excited state. In other words, the energy of the transition has been decreased. We can therefore re-word the above spectroscopic information, and say that the transformation of a Schiff base of retinene to the conjugate acid decreases the difference in energy between the ground state and the first electronically excited state. The magnitude of this decrease can be obtained to a first approximation from the shift in absorption spectrum. The Schiff bases of retinene$_1$ have absorption maxima at about 365 mμ; quanta of this wavelength contain 78.2 kcal. per mole. The absorption maxima of the conjugate acids are at about 440 mμ, corresponding to an energy of 65.0 kcal. per mole of quanta. Addition of a hydrogen ion to a Schiff base of retinene$_1$ therefore decreases the energy of the first electronic transition by about 13 kcal. per mole.

We have made similar calculations for the Schiff bases of retinene$_2$, and for the rod and cone pigments derived from the two retinenes. These are summarized in *Table 2*. With the Schiff bases of both retinenes, formation of the conjugate acid lowers the energy of the first transition by 13 to 14 kcal. per mole. Combination with rod opsin causes a further decrease of about 7 kcal. per mole, and combination with cone opsin a decrease of about 14 kcal. per mole, compared with the energy required to excite the conjugate acid. The energies of the transitions are decreased by the same amount for both types of retinene, suggesting that opsin interacts in the same way with both.*

How can opsin affect the electronic transitions of the retinene chromophore? To a first approximation, we may picture the ground and first excited states of the chromophore as follows. The actual state of a molecule containing a number of alternating double and single bonds cannot be described accurately by a unique chemical formula. It is a composite -- a resonance hybrid -- of a number of limiting states. The molecule corresponds to none of these singly, but receives contributions simultaneously from them all. However, only states of equal or similar energy contribute appreciably to the resonance hybrid. *Fig.3* shows the most stable limiting states of the conjugate acid of a Schiff base of retinene$_1$. Resonance distributes the positive charge from the quaternary nitrogen atom (structure I) to the adjacent carbon atom (structure II), and thence to alternate carbon atoms along the conjugated system (structures III to VII). However, the distribution of electrons is by far most stable (that is of lowest energy) in structure I, which carries the positive charge on the nitrogen atom. The internal energies of the structures containing a positively charged carbon atom are much higher and more nearly equal to one another. There is

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* Observations on the synthesis of the visual pigments also indicate that the opsins cannot distinguish between retinene$_1$ and retinene$_2$, and hence react the same way with both. Thus rod opsin from frog, cattle, perch, or bass, forms rhodopsin with retinene$_1$ and porphyropsin with retinene$_2$ *(Wald, Brown and Smith, in preparation)*; and cone opsin from chickens forms iodopsin with retinene$_1$ and cyanopsin with retinene$_2$ *(ref.4)*.

TABLE 2

Absorption properties of Schiff bases of retinene$_1$ and retinene$_2$, their conjugate acids, and the corresponding rod and cone pigments. The energies of the electronic transitions have been calculated by the equation,

$$\text{energy} = Nh\nu = \frac{Nhc}{\lambda},$$

where N is Avogadro's number, h Planck's constant, c the velocity of light, and ν and λ, the frequency and wavelength at the absorption maximum.

	Retinene$_1$		Retinene$_2$	
	λ_{max}	Quantum energy (kcal/mole)	λ_{max}	Quantum energy (kcal/mole)
Schiff base	365–370[a]	77.2–78.2	380[b]	75.0
Conjugate acid	440–450[a]	63.5–65.0	460–465[b]	61.4–62.0
Rod pigment	500[c]	57.1	523[c]	54.6
Cone pigment	562[c]	50.8	620[d]	46.1
Differences in quantum energy (kcal/mole)				
Schiff base *minus* conjugate acid	13.2–13.7		13.0–13.6	
Conjugate acid *minus* rod pigments	6.4–7.9		6.8–7.4	
Conjugate acid *minus* cone pigment	12.7–14.2		15.3–15.9	

(a) Ball *et al.*, *(ref.29)*; (b) Cama *et al.*, *(ref.20)*; (c) Wald, *(ref.5)*;
(d) Wald *et al.*, *(ref.4)*

therefore little interaction between structure I and the other six structures, which interact primarily with each other. To a first approximation, therefore, structure I describes the molecule in the ground state, while the first excited state is a resonance hybrid of structures II to VII.

It clearly follows that stabilization of structures II to VII would lower the energy of the first excited state, thus shifting the absorption spectrum toward longer wavelengths. This could be achieved by stabilizing the positively charged carbon atoms. The stereospecificity of opsin for neo-*b* retinene shows that retinene interacts with opsin at more than one point. This is further indicated by the fact that bleaching exposes not just one, but a number of reactive groups on opsin which were masked by the chromophore *(refs.25,26,27)*. It is therefore likely that the chromophore fits the opsin surface in a definite geometry. Interaction of opsin with the entire conjugated system of retinene could stabilize the resonance structures of higher energy by polarizing the positive charge toward the β-ionone ring, possibly by bringing it close to negatively charged groups, such as, for example, $-COO^-$.

To summarize: we have tried to explain the spectroscopic properties of the visual pigments by assuming that retinene and opsin form a Schiff base, which is stabilized against hydrolysis by being converted to the conjugate acid. Opsin protects the conjugate acid against the loss of a hydrogen ion in neutral and alkaline solution, and shifts its absorption spectrum toward longer wavelengths.

Site of attachment of retinene in rhodopsin.

In rhodopsin, we can pinpoint more closely the site of attachment of the chromophore to opsin. Cattle rhodopsin and opsin do not have any N-terminal amino groups *(ref.28)*. Lysine and arginine therefore probably offer the only amino groups available for combination with retinene$_1$. Arginine would add one double bond to the effective length of the chromophore. It would therefore yield an "acid indicator yellow" at longer wavelengths than is found on bleaching rhodopsin *(ref.29)*. In rhodopsin, the chromophore is therefore probably attached to the ε-amino group of a lysine residue. The same must be true for porphyropsin. With cone opsins, the analysis has not proceeded so far.

Mechanism of bleaching.

The above formulation suggests a plausible mechanism for the bleaching of the visual pigments. We know that light isomerizes retinene *(refs.3,8)*. With the visual pigments, bleaching by light also involves an isomerization, since light always releases predominantly all-*trans* retinene, while the chromophores have the 11-*cis* configuration. We should therefore like to suggest that *light bleaches the visual pigments by isomerizing their chromophores to the all-trans configuration.*

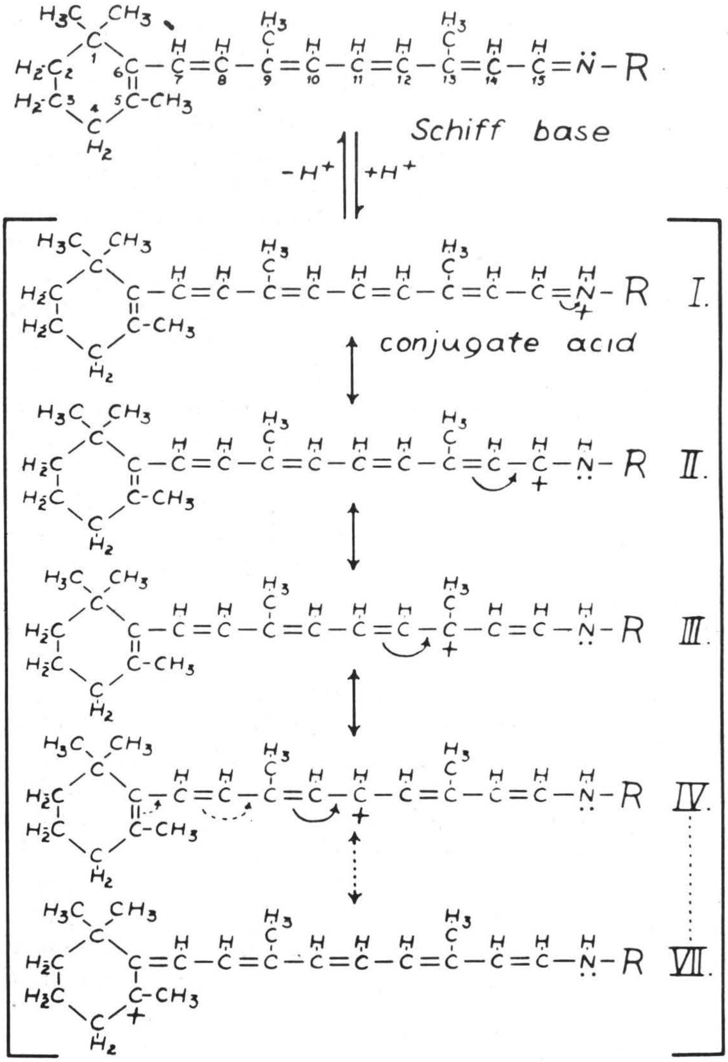

Fig. 3. Schiff base of retinene$_1$, and the most stable limiting states of its conjugate acid. The conjugate acid is not represented accurately by any one of these structures, but is a resonance hybrid of them all.

Why should isomerization of the chromophore bleach the visual pigments? We have just seen that the colour and stability of the neo-*b* chromophores presuppose strong steric interactions with opsin. Isomerization destroys the steric fit between the chromophore and the opsin surface. This is shown diagrammatically in *fig. 4*. The Schiff base linkage to opsin is at the right;

the opsin surface complements the shape of the neo-*b* chromophore all the way to the β-ionone ring at the left. Isomerization of the chromophore to the all-*trans* configuration pulls the β-ionone ring away from the opsin. The all-*trans* chromophore, attached to opsin only by the Schiff base linkage, is now an ordinary conjugate acid of a Schiff base. In neutral solution, it loses a hydrogen ion and is hydrolyzed to retinene and opsin (viz. discussion above). *Fig.4* also shows that the shapes of neo-*b* and iso-*a* retinene are sufficiently similar to allow the iso-*a* isomer to fit the chromophoric site on opsin. However, the stabilizing interactions of opsin with the chromophore must be weaker with iso-*a* retinene than with the neo-*b* isomer, since the absorption maxima of the iso-pigments all lie at shorter wavelengths than the maxima of the corresponding visual pigments *(refs.4,5)*.

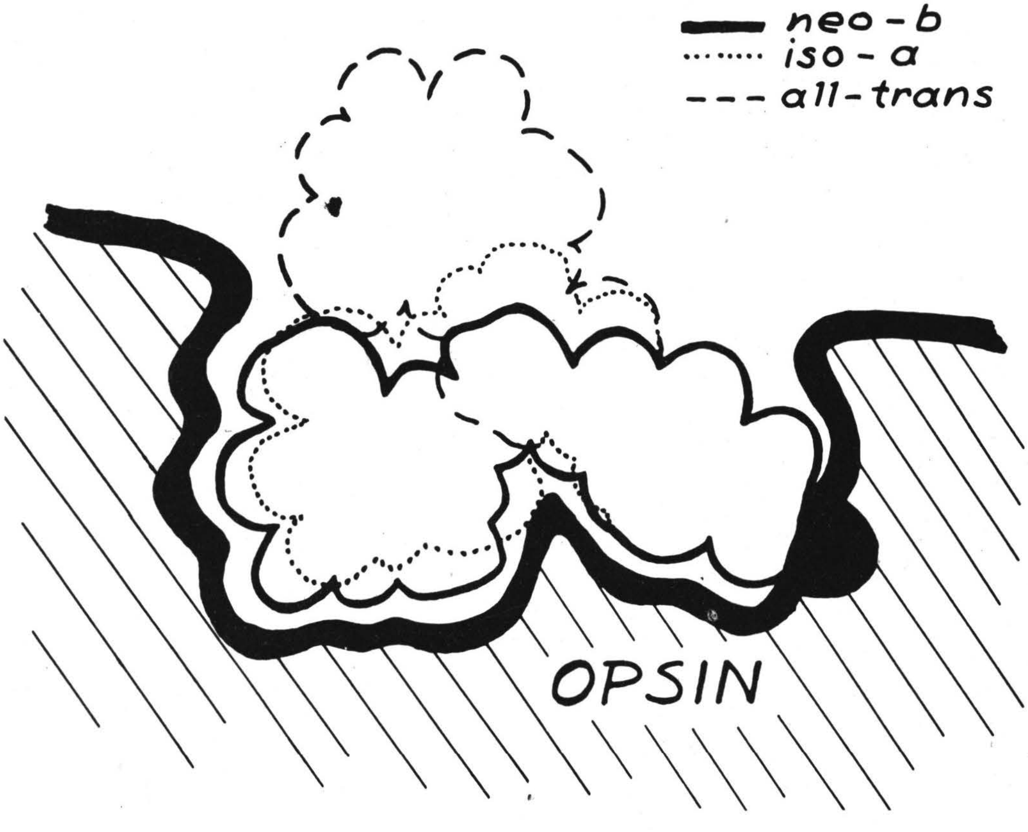

Fig.4. Postulated configuration of the chromophoric site on opsin. The Schiff base linkage is shown at the right. Opsin complements the shape of the neo-*b* chromophore all the way to the β-ionone ring at the left. Iso-*a* retinene fits the site less well; all-*trans* retinene does not fit at all.

We have previously shown that light isomerizes neo-*b* retinene more rapidly than iso-*a* retinene *(ref.30)*. If light bleaches rhodopsin and isorhodopsin by isomerizing their chromophores, one might expect the photosensitivities of the two pigments to differ in a similar manner. This is in fact the case. We have recently found that, under identical conditions of illumination, rhodopsin bleaches about three times as fast as isorhodopsin. The relative photosensitivities for bleaching rhodopsin and isorhodopsin therefore parallel the relative photosensitivities for isomerizing the neo-*b* and iso-*a* isomers of retinene.

While light liberates predominantly all-*trans* retinene, we have recently found that in a thermal bleach (in the dark) at least two-thirds of the liberated retinene retains the *cis* configuration of the chromophore — neo-*b* when bleaching rhodopsin, iso-*a* when bleaching isorhodopsin. Furthermore, whereas light attacks rhodopsin more rapidly than isorhodopsin, heat attacks both at about the same rate.

These and other experiments *(Hubbard, unpublished observations)* have led us to conclude that light bleaches rhodopsin by isomerizing the chromophore, while heat bleaches by attacking the configuration of the opsin (denaturation). Though for different reasons, both therefore disrupt the stabilizing interactions between the chromophore and opsin, and thus cause the release of retinene from the chromophoric site.

III. THE CHROMOPHORE OF SQUID RHODOPSIN

EARLIER investigators noticed that squid rhodopsin does not bleach in the light (in neutral solution), and therefore concluded that it was not photosensitive *(refs.31,32)*. St. George and co-workers, however, established the photosensitivity of squid rhodopsin by showing that while it did not *bleach* (that is, turn yellow), its spectrum was *changed* upon absorption of a quantum *(refs.33,34)*. We have re-examined the properties of the squid pigment *(refs.35,36)*, and shall briefly summarize them here.

The chromophore of squid rhodopsin, like that of vertebrate rhodopsins, is isomerized by light. However, the isomerized chromophore, instead of being expelled from the chromophoric site, remains attached to opsin.

If squid rhodopsin (λ_{max} 493 mμ) is irradiated in alkaline solution, it bleaches to a yellow compound, which we call *alkaline metarhodopsin* (λ_{max} 380 mμ). It contains retinene$_1$ still attached to opsin in such a way as to prevent its reaction with hydroxylamine. If the solution is neutralized in the dark, alkaline metarhodopsin adds one hydrogen ion and is converted to a bright red compound, which we call *acid metarhodopsin* (λ_{max} 500 mμ). This reaction sequence is illustrated in *fig.5;* the absorption properties are summarized in *Table 3.* The interconversion of acid and alkaline metarhodopsin is reversible and involves only the reaction of one hydrogen ion with an acid binding group of pK 7.7. When squid rhodopsin is

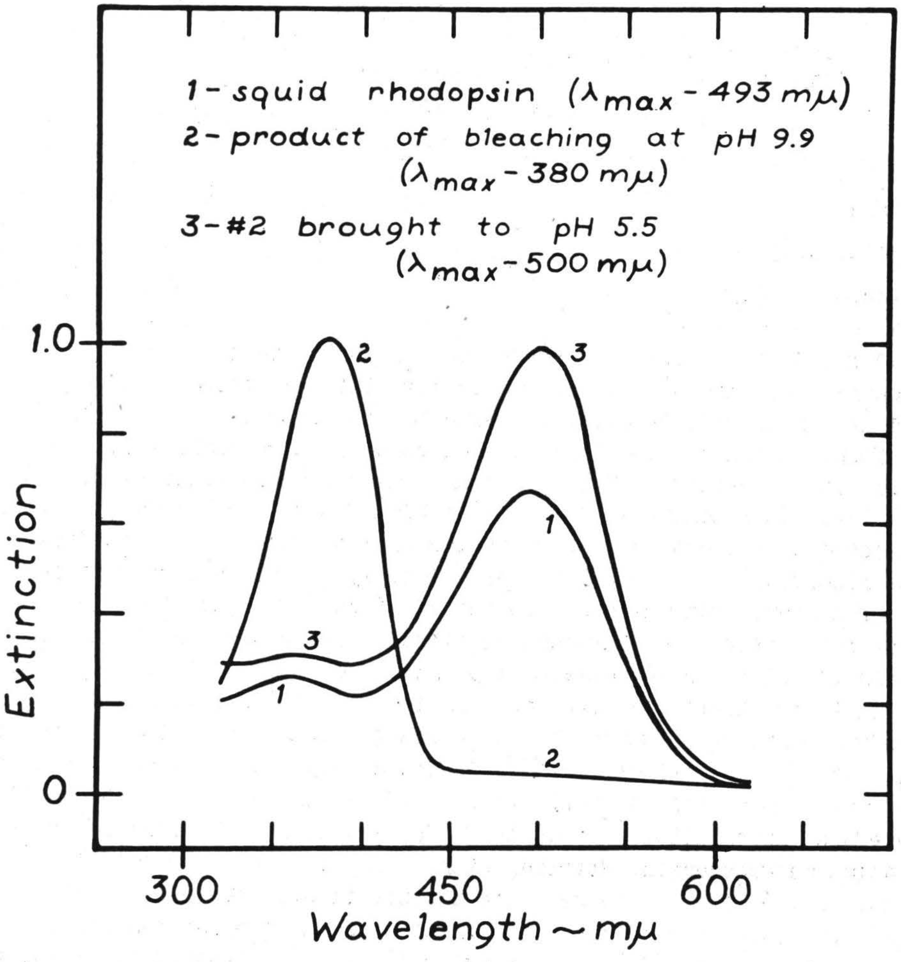

Fig. 5. Reactions of squid rhodopsin. Squid rhodopsin in alkaline solution (curve 1) is bleached by orange light, yielding alkaline metarhodopsin (curve 2). If the pH is now lowered in the dark, this is converted reversibly to acid metarhodopsin (curve 3).

irradiated in neutral or slightly acid solution, it is converted directly to acid metarhodopsin.

TABLE 3

Absorption properties of squid rhodopsin, and acid and alkaline metarhodopsin in aqueous digitonin. The extinction coefficients are expressed as ε (1M, 1cm).

Compound	Absorption properties	
	Absorption maximum (mμ)	Extinction coefficient
Rhodopsin	493	40,600
Acid metarhodopsin	500	59,800
Alkaline metarhodopsin	380	61,000

Squid rhodopsin contains neo-*b* retinene$_1$, while the two forms of metarhodopsin contains the all-*trans* isomer. This has recently been established by thermal denaturation experiments (viz. p. 164 above). Squid rhodopsin and the two forms of metarhodopsin are completely denatured in 30 seconds at 70°C., but whereas rhodopsin releases at least 70% of its retinene in the neo-*b* configuration, both forms of metarhodopsin release all-*trans* retinene. The conversion of rhodopsin to metarhodopsin, therefore, involves primarily the stereoisomerization of the chromophore from the neo-*b* to the all-*trans* configuration.

Light reisomerizes the chromophore of squid metarhodopsin to rhodopsin. Rhodopsin therefore is regenerated from metarhodopsin *in the light*. When rhodopsin is irradiated in alkaline solution, the isomerization of metarhodopsin can be prevented by using orange light, which is not absorbed by alkaline metarhodopsin (viz. *fig.5*). The absorption spectra of rhodopsin and acid metarhodopsin, however, overlap widely. In neutral solution, it is therefore impossible to irradiate rhodopsin without simultaneously irradiating metarhodopsin. Starting with either rhodopsin or acid metarhodopsin, in the light a steady state mixture is produced which contains about 45% rhodopsin and 55% acid metarhodopsin. The composition of this mixture is independent of light intensity and of pH, although pH of course affects the relative concentrations of acid and alkaline metarhodopsin. The compositions of steady state mixtures at various pH's are shown in *Table 4*. The experimentally determined values should be compared with the values in brackets. The latter have been calculated on the assumption that in orange light the reaction is described completely by the coupled equilibria,

$$\text{rhodopsin} \underset{\text{light}}{\overset{\text{light}}{\rightleftharpoons}} \text{acid metarhodopsin} \underset{+\,H^+}{\overset{-\,H^+}{\rightleftharpoons}} \text{alkaline metarhodopsin,} \quad (3)$$

in which the ratio of the concentrations of rhodopsin and acid metarhodopsin at the photo-steady state is 0.9 *(Table 4)*, and the interconversion of acid and alkaline metarhodopsin has a pK of 7.7. The agreement between the observed and calculated values shows that these assumptions are valid to a first approximation.

TABLE 4

Compositions of steady-state mixtures produced by irradiating squid rhodopsin or acid metarhodopsin with orange light at various pH's. The values in brackets have been calculated on the basis of assumptions described in the text.

pH	Rhodopsin %	Acid Metarhodopsin %	Alkaline Metarhodopsin %	Ratio of rhodopsin to acid metarhodopsin
6.1	44 (47)	56 (52)	negligible (1)	0.8
6.1	47 (47)	53 (52)	" (1)	0.9
6.2	42 (47)	58 (52)	" (1)	0.75
7.6	33 (34)	37 (38)	30 (28)	0.9
8.35	21 (19)	19 (22)	60 (59)	1.1
			Average :	0.9

Since the interconversions of squid rhodopsin and the two forms of metarhodopsin proceed without the intermediate release of retinene$_1$, the attachment of the chromophore to opsin must remain intact throughout. With squid rhodopsin, isomerization of the chromophore therefore does not result in hydrolysis of the Schiff base in the manner described for the vertebrate pigments. It does, however, introduce pH indicator properties.

The term "metarhodopsin" was introduced by Wald *et al.* *(ref. 38)* to designate an initial meta-stable product formed by irradiating vertebrate rhodopsins in the cold or in dry gelatin films. These authors reported that vertebrate metarhodopsins above about 0° C. were transformed in the dark into mixtures of all-*trans* retinene and opsin, and of regenerated rhodopsin and isorhodopsin. Recent experiments in this laboratory *(Kropf, unpublished observations)* suggest that the metarhodopsin described by Wald *et al.* was not a single molecular species but rather a mixture containing an all-*trans* chromoprotein (similar to squid acid metarhodopsin) along with regenerated rhodopsin and isorhodopsin. It appears that the initial light reaction in vertebrates, as in the squid, transforms rhodopsin into an all-*trans* chromoprotein, and that irradiation of this initial photoproduct re-isomerizes it to rhodopsin and isorhodopsin. Prolonged irradiation therefore results in a photo-steady state containing the all-*trans* chromoprotein, regenerated rhodopsin, and isorhodopsin. And it is this steady state mixture which has until now been called metarhodopsin. The present analysis suggests that it would be better to restrict the term "metarhodopsin" to the all-*trans* chromoprotein in vertebrates as well as in the squid.

To summarize: We have assigned three roles to opsin in its interactions with retinene. It must (1) form a Schiff base; (2) stabilize the conjugate acid of this Schiff base in neutral and alkaline solution; and (3) shift the absorption spectrum of the conjugate acid towards longer wavelengths. The first of these requires no special assumptions. The other two, however, presuppose strong stabilizing interactions between the chromophore and opsin.

IV. CONCLUSION: THE ROLE OF STEREOISOMERIZATION IN VISION

STEREOISOMERIZATION of the chromophore is involved in the chemistry of all four visual pigments. Is this a chemical accident, or does it have significance for vision? We need not labour the point that a pigment, in order to be effective in vision, must *be changed* by visible light in some way which can be translated into a nervous excitation. Wald and co-workers have shown that light exposes new reactive groups on opsin which may be responsible for the initiation of the nerve impulse *(refs. 25,26,27)*. We are suggesting that *the primary effect of light is the stereoisomerization of the chromophore.*

Proteins are large molecules with a complicated and specific geometry. We have come to expect them to impose stringent steric requirements in their interactions with other molecules. We now find that for the process of vision, the eye has selected a reaction in which light affects directly the *shape* of molecules. By coupling it with the spatial requirements of a protein surface, it has thus provided a mechanism for translating the absorption of quanta, which is instantaneous and hence difficult to communicate as a physiological event, into a chemical reaction.

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PRESENTATION AND DISCUSSION OF PAPER 4

IN presenting her paper, DR. RUTH HUBBARD devoted her remarks mainly to the configuration of the chromophore and the mechanism of bleaching.

MR. G. A. J. PITT opening the discussion asked Dr. Hubbard if she could comment further on the remark in her paper (the unrevised preprint), "We have as yet insufficient evidence to decide whether squid metarhodopsin is chemically related to the vertebrate metarhodopsin." In what way would Dr. Hubbard's general picture affect Prof. Wald's conception of bleaching and the "classical story", i.e. the lumi-rhodopsin, meta-rhodopsin, indicator yellow, retinene sequence? What had happened to lumi-rhodopsin and was it possible to obtain indicator yellow from squid rhodopsin? Dr. Hubbard had stated in the first part of her paper that opsins must have two effects on Schiff bases - firstly to stabilize the conjugate acids of the Schiff base in neutral alkaline solution and secondly to shift the absorption spectra of the conjugate acids to longer wavelengths and, it should be added, to shift the absorption of alkaline metarhodopsins towards longer wavelengths. How could these shifts be accounted for?

Mr. Pitt referred to Dr. Bridges' comment on π-electrons, and to Dr. Hubbard's suggestion that ionised carboxyl groups on the protein might be concerned. Could Dr. Hubbard make any specific hypothesis? Might not SH groups play a part?

DR. C. D. B. BRIDGES had the impression that the squid photoproduct resembled an indicator yellow rather than a metarhodopsin. Cattle and frog rhodopsins, which were not pH sensitive were capable of regenerating iso-rhodopsin, based on iso-*a* retinene. If this were due to irradiation of the all-*trans* metarhodopsin we would expect to produce also some of the neo-*b* form, and to obtain a mixture of the two. This mixture would not in fact bear the definite stoichiometric relation which it apparently did to metarhodopsin.

He had examined metarhodopsin from monkeys which was rather more stable than frog metarhodopsin. On regeneration by warming this gave the parent pigment and not the iso-form. He thought it was only in the indicator-yellow stage that we could see there was any all-*trans* there at all. He thought that the only difference between the squid and frog cases was that the squid photoproduct could combine with the protein surface, thus giving an absorption maximum at a longer wavelength.

DR. W. A. H. RUSHTON wanted to know about the action spectrum of the isomerisation process; did it resemble that of retinene in solution or metarhodopsin? His own work with human eyes had shown that yellow and blue lights of apparently equal brightness in twilight vision, which should be equally absorbed by rhodopsin, had equal effects on bleaching and regeneration. This might mean that there was no isomerisation, or that there was some in both processes. When very bright lights were used for bleaching the regeneration rate during bleaching increased with the intensity of the bleaching light. He hoped that Dr. Hubbard would say that this was due to isomerisation, and in this case the action spectrum of the isomerisation process should closely resemble the absorption spectrum of rhodopsin.

DR. H. B. BARLOW considered that Dr. Hubbard's finding that the thermal reaction produced neo-*b*, did not mean that none of the *trans*-isomer was produced. If one accepted the concept that the sensitivity of the eye was limited by thermal reactions, the thermal reaction concerned would be the production of the *trans* not the neo-*b* isomer. It might be that the scheme suggested by Dr. Hubbard greatly reduced the rate at which the reaction went on in the end-organ as compared with the rate of the thermal reaction in the test-tube.

PROF. R. A. MORTON expressed his gratification that Dr. Hubbard had found his Schiff base hypothesis useful, but was not himself wholly satisfied with the situation. One had to explain a shift in absorption maximum from 445 mμ to 500 mμ in the rhodopsin system and a larger shift in the system of the photopic pigment. If, as Dr. Hubbard's scheme implied, there was no "missing piece" in the chromophoric system, yet to be discovered, which might help the explanation, then the whole onus was thrown on the protein - scotopsin or photopsin - which would have to be unique among proteins in producing a large auxochromic effect on the Schiff base. Although this had been made very plausible it raised new chemical problems. The lightest amino-acid residue to intervene in the union between protein and retinene was, he believed, lysine which had its amino-group insulated from the rest of the system by two or three CH_2 groups. This was important because the entire responsibility for the shift of absorption maximum would then fall on the secondary valencies - which would be most remarkable. If the amino-group had been attached to an aromatic group, we should not be forced to this conclusion.

DR. M. H. PIRENNE enquired about the quantum efficiency of bleaching. It seemed that in the majority of rhodopsins it was about 0.5, but apparently in the case of squid rhodopsin it was nil.

DR. HUBBARD, in her reply, agreed with Dr. Pirenne's facts but thought that the essential quantum efficiency for vision was the transformation from rhodopsin to metarhodopsin, which in the particular case of vertebrates was identical with, or rather immediately followed by, bleaching. On Prof. Morton's point she would observe that the onus being thrown on a simple amino-acid rather than on an aromatic one arose from Prof. Morton's own work. In the case of iodopsin as distinct from rhodopsin she had been careful to avoid any assertion: it might be that there we had an additional piece of conjugated system thrown in. Regarding more specific hypotheses about the secondary valencies, Dr. Hubbard said she had referred to carboxyl groups as a basis for discussion but at this stage had nothing to add on this, or on the possible role of sulphahydryl groups.

In speaking of the connexion between squid rhodopsin and indicator yellow it was important to make clear exactly what was meant by "indicator yellow". She took it to mean the compound described by Lythgoe which could be taken apart in the presence of hydroxylamine. Squid metarhodopsin could not, and its spectral absorption properties were different, especially when considered over a wide pH range. Indicator yellow could be produced from squid rhodopsin, but only by denaturing the protein.

It was always easier to produce, by light, retinenes of the neo-*b* type than those of the 'iso-*a*' type, and Dr. Hubbard was not surprised by Dr. Bridges' result. As far as the action spectrum of the isomerisation process was concerned she had no information, but shared Dr. Rushton's hopes.

THE CHAIRMAN agreed with Prof. Morton's view that the proteins involved must have peculiar properties if the shift of the absorption spectrum was to be explained. He thought that the action of the protein on the retinene might have some parallel with the antimony chloride test for retinene, where a much larger shift of absorption spectrum occurred, and the reaction products were highly photosensitive. This led to the idea that the protein might behave in some way like a metal, and perhaps it contained a metal. With this in view he had asked a student of his to prepare and examine very pure specimens of rhodopsin, but no metals had been found in significant amounts.

PAPER 5

LIGHT ABSORPTION BY THE INTACT RETINA

By E. J. DENTON

Eric James Denton, born in Yorkshire in 1923, was educated at St. John's College, Cambridge and University College, London. He spent some time during the war at the Telecommunications Research Establishment, Malvern. His research on the physiology of sense organs has been carried out in the Biophysics Research Unit, University College, London, in the University of Aberdeen and at the Museum National d'Histoire Naturelle, Paris when he was a Carnegie Fellow. He is at present working on physiological problems in marine biology, at the Marine Biological Station, Plymouth.

5. LIGHT ABSORPTION BY THE INTACT RETINA

By E. J. DENTON

THE experiments which are described here were all made on intact retinae dissected from the eye. Such preparations were much used by Boll *(ref. 1)* and Kühne *(ref. 2)* in their classical work on the retinal photosensitive pigments. A photograph of such a retina, that of *Salamandra maculosa,* is shown in *fig. 1.*

GENERAL METHOD

A retina is dissected under Ringer's solution and floated into a small chamber made by fixing a ring of wax to a glass slide. The chamber is filled with Ringer's solution and closed by a cover glass. In most experiments a magnified image of the retina is thrown onto a diaphragm and then using visual observation, the preparation is moved until the image falling on the diaphragm corresponds to a suitable part of the retina, the eye of the observer is replaced either by a camera or by a photo-multiplier tube (P1) and measurements are made. Although the measurements have not been pushed to the experimental limits, very small areas of retina can be used. Here areas of about 4/100 and 1/100 sq.mm. were used. The apparatus is shown diagrammatically in *fig. 2,* whilst on *fig. 1* a ring circumscribes a typical area of retina on which measurements were made.

This method usually gives difference curves, that is the difference in density between unbleached and bleached retina as a function of wavelength. It can, however, be slightly modified so as to give a very good approximation to the absorption curve. After a suitable area of retina has been chosen microscopically, the microscope body is removed and an E.M.I. photomultiplier, the end of which is the photosensitive surface, is lowered on the preparation so as to collect almost all the light leaving the retina *(fig. 2b).* Using this method losses of light caused by scattering in the preparation are largely avoided and the difference in absorption between the retina and an equal thickness of Ringer's solution can be measured. *Fig. 3a* shows the results of such experiments made on the conger eel and the brill. The absorption of the retina is dominated by the photosensitive pigments and the absorption of the layers of retina between rods and internal limiting membrane is trivial over the visible spectrum.

The method is an extremely quick one and numbers of animals can be easily examined. Two interesting new groups of pigments have been found:

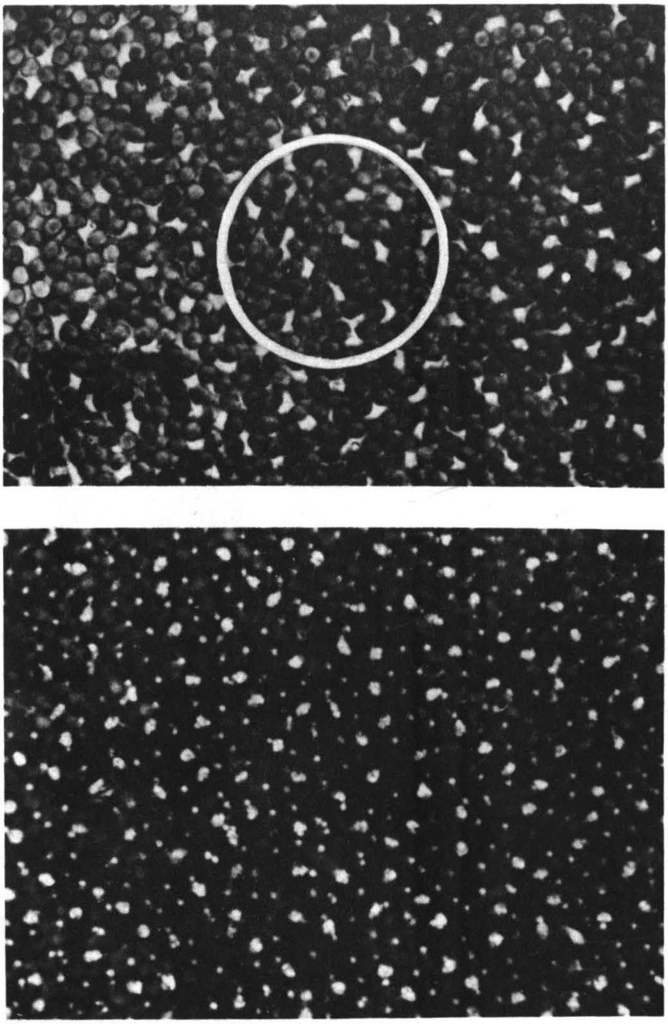

Fig.1. Photographs of the retina of a dark adapted *Salamandra Maculosa.* (a) focussed on the tops of the rods. (b) focussed on the tips of the cones. The cones can be seen to be arranged both in clusters and singly. The ring shows the area used for measuring the absorption curves. The individual rods are about 13μ in diameter.

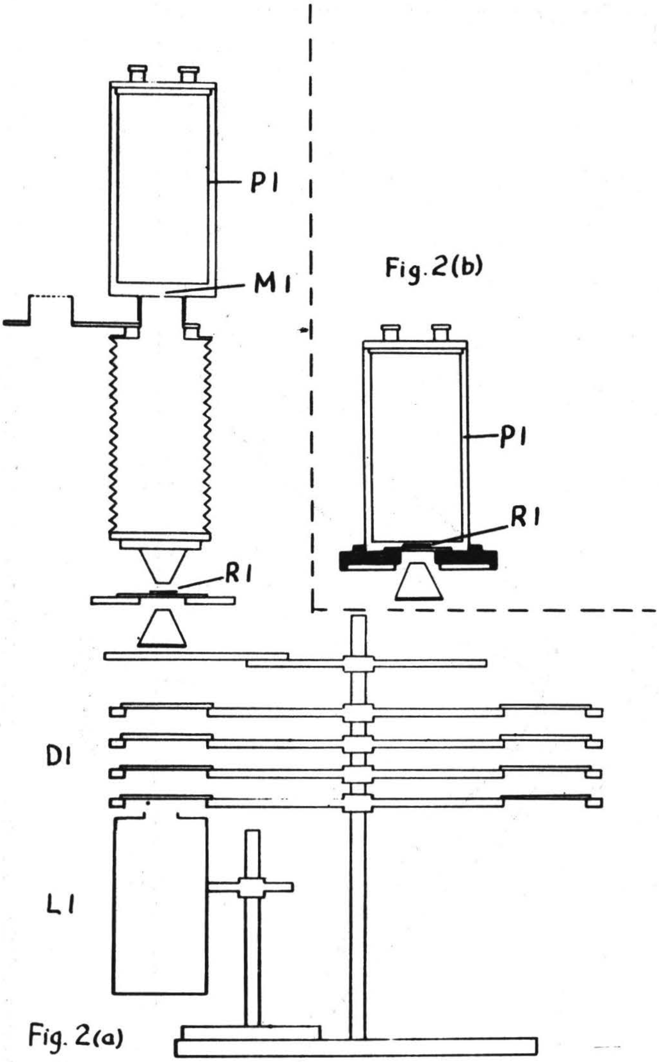

Fig.2. Diagrams of apparatus. **(a) An enlarged image of a small** area of the retina R1 is thrown on to a diaphragm (M1) at the other side of which is a photomultiplier tube P1. D1 represents four discs carrying neutral and colour filters. The discs can be rotated to change the spectral composition and intensity of light reaching the retina. L1 is the light source. (b) Adaptation of the same apparatus to avoid losses by scattering.

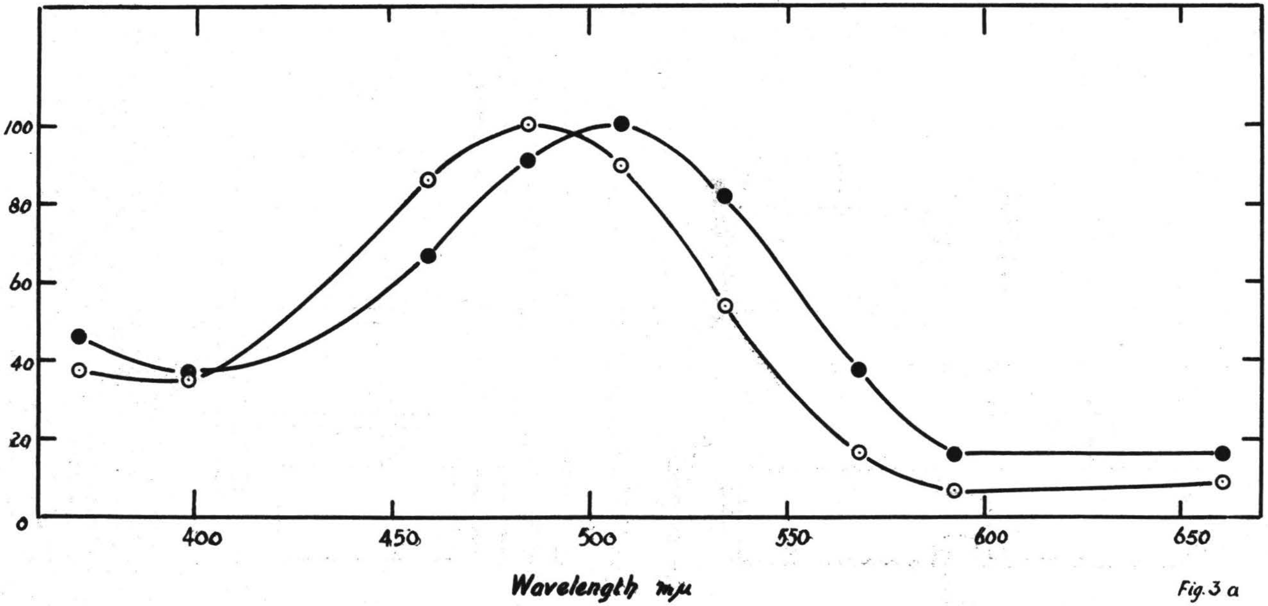

Fig. 3 a

Fig. 3a. Curves of densities of the dark adapted unbleached retinae with respect to equal thicknesses of Ringer's solution. The densities are plotted against wavelength in mμ. The maximum densities have been made equal to 100%.

○ ———— ○ *Conger vulgaris*
● ———— ● *Scophthalmus rhombus*

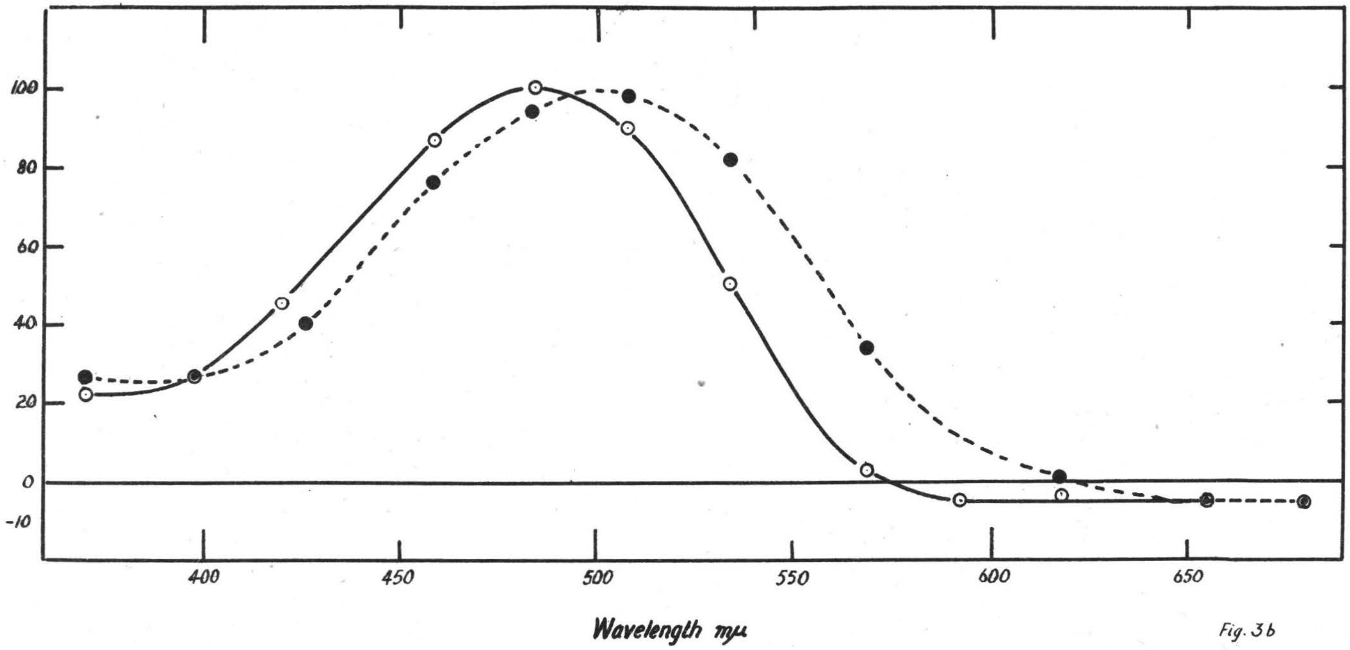

Fig. 3b

Fig. 3b. Curves for unbleached dark-adapted retinae showing difference in densities of the preparation (that of *Fig. 5*) for light polarized across and along the axes of the rods. The density difference of the unbleached preparation for that wavelength for which this difference is greatest is made equal to 100% and other density differences are expressed relative to this value.

o ——— o *Conger vulgaris*
● ——— ● *Salamandra maculosa*

(1) the geckoes, which are terrestrial animals, have been shown *(refs. 3,4)* to have purple coloured retinae which become colourless on bleaching. Crescitelli has shown, moreover, that these retinae contain vitamin A_1 scotopic pigment with an absorption curve like that of a porphyropsin (porphyropsins are all compounds based on vitamin A_2 *(ref. 5)*). (2) The retinae of deep sea fish have been shown *(ref. 6)* to contain principally golden coloured photosensitive pigments (chrysopsins) absorbing maximally around 485 mμ. This property is shared by the conger eel *(ref. 7)* and by the fresh water eel (Carlisle and Denton, unpublished observations). Both the conger and fresh water eels begin their lives in the deep sea and return there to spawn when mature.

OBSERVATIONS ON RETINAL DENSITIES

ONE of the advantages of the measurements upon intact retinae is that they give directly the retinal density, or density change on bleaching of the photosensitive pigments. Such densities are often very high. Examples of typical retinal densities are:

	Wavelength mμ	Measured density change on bleaching	Estimated unbleached retinal density
frog	508	0.45	0.50
salamander	508	0.52	0.57
geckoes	534	0.51	0.56
conger	484	0.60	0.80
some deep sea fish	484	1.00	1.30

These densities *in situ* have been shown for the frog *(ref. 8)* and the conger *(ref. 7)*, to be twice as high as the densities estimated on the assumption that the original average density of ~~visual~~ pigment is that which a complete extract would have if spread over the area of retina from which the extract has been made. This has been discussed by Denton and Wyllie and by Rushton *(refs. 9,10)*. They attributed it to the arrangement of the visual pigments in the rods in preferred positions for absorbing light incident along the axes of the rods, and to the condensing of light into the external segments of the rods which collect a larger fraction of the light incident on the retina than the fractional retinal area which they cover.

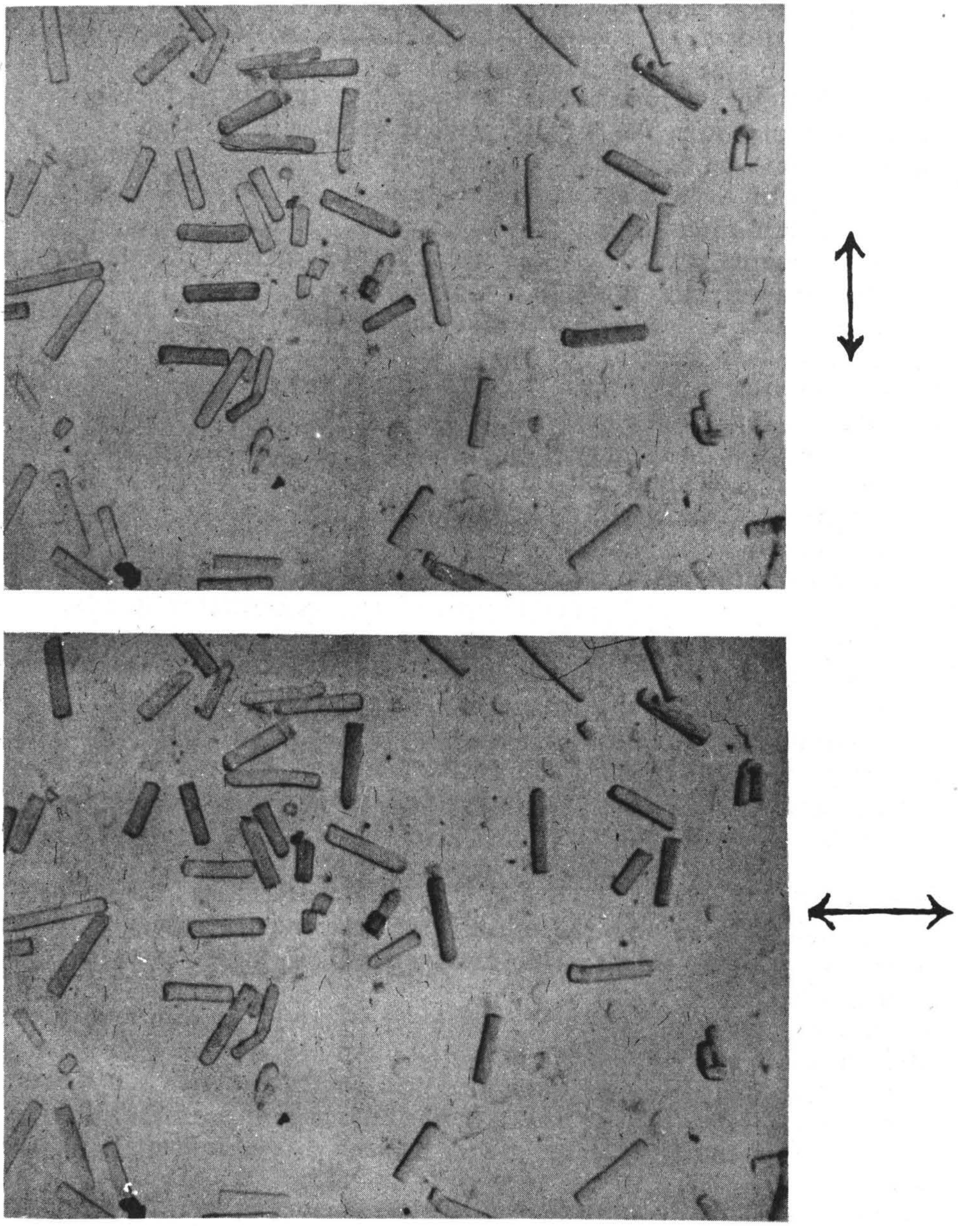

Fig. 4. Two photographs of the same microscopic field made with green light showing the dichroism in the unbleached rods of *Salamandra maculosa*. The arrow at the side of a photograph shows the plane of polarization of the light used.

OBSERVATIONS ON THE DICHROISM OF THE RODS

W. J. SCHMIDT *(ref. 11)* showed that the retinal rods are dichroic and if laid on their side absorb light more heavily if the light is polarized (electric vector) across rather than along their axes. A photograph of this effect obtained with the very large rods of *Salamandra maculosa* is shown on *fig. 4*. This is generally accepted as being a consequence of the orderly arrangement of the visual pigment molecules in a crystalline structure in the rods. This might affect the relationship between the absorption curve of the extracted pigment and the spectral sensitivity curve in three ways:

(1) The absorption curve in the crystalline rods may differ from that in solution. It would be expected that its curve would be displaced towards the red end of the spectrum.

(2) The orderly arrangement of the molecules will make the photosensitive pigment 3/2 times more effective *in situ* than in the extract.

(3) The molecule of visual purple might have axes of absorption in different directions for its absorption in different parts of the spectrum and the possibility exists that some axes of absorption might be arranged along the axis of the rods and, therefore, be ineffective in the absorption of light coming as it does in life along these axes. Dr. W. S. Stiles who first thought of this possibility (private communication) suggested that it might explain the difference in the deep blue and near ultra violet between the absorption curve of visual purple in solution and the human scotopic visibility curve.

A suitable preparation for studying the dichroism of the rods was obtained by carefully folding a retina and using the region at the edge of the fold where the rods are stacked in piles with their axes perpendicular to the light axis of the microscope *(fig. 5)*. By moving the retina and making visual observations an image of such a region which shows a marked dichroic effect is thrown onto a slit diaphragm. The observer's eye is now replaced by a photomultiplier tube and the change of absorption with plane of polarization of light can be studied as a function of wavelength.

Fig. 3(b) shows curves of difference in density, of such preparations, between light polarized along and across the rod axes as a function of wavelength. The close correspondence between these curves and those of absorption spectra both in solution and intact retinae *(Fig. 3a)* shows that the axes of absorption of the visual pigment molecules must be in the same plane or very nearly in the same plane in the rod for all wavelengths from 380–720 mμ and no discrepancy between extract and spectral sensitivity is to be expected on this account.

A more surprising result comes from a study of the products of bleaching and the changes which they undergo, in the dark, in the time following bleaching. During the first 40 minutes after bleaching the sense of the

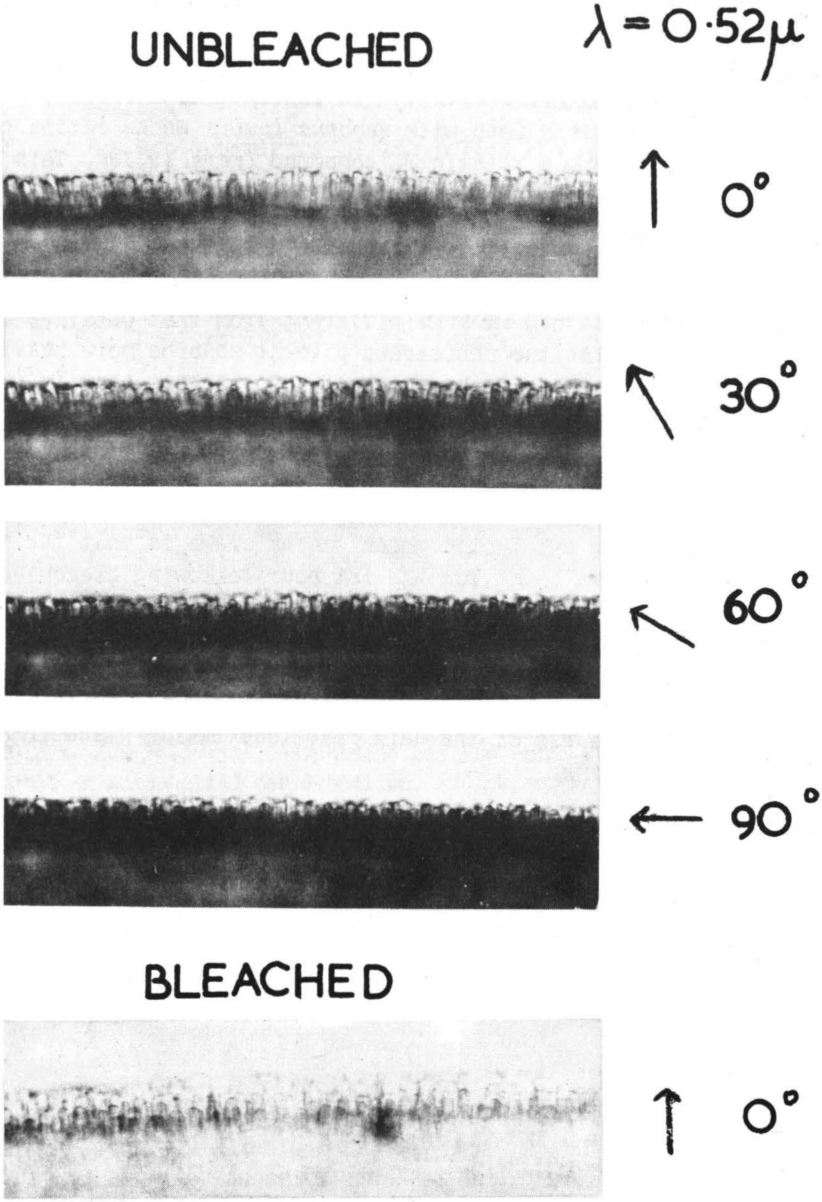

Fig. 5. *Salamandra maculosa*. Photographs of a fresh retina all made with the same exposure to green light (λ = 520 mμ). The retina is folded so that at the edge of the field the rods lie on their side in piles, so that together they show a marked dichroic effect. The arrows show the planes of polarization used.

dichroism reverses. The final product visual white which absorbs strongly in the near ultra violet shows a dichroism in the opposite sense to that of the unbleached pigment and first products of bleaching. With the present apparatus in which measurements were confined to the range 365 - 720 mμ the effect was most easily seen with *Xenopus laevis* whose retina contains principally porphyropsin, a vitamin A_2 compound *(refs. 12,13)*. This change in the sense of the dichroism means that the effective axis (that is, that for the absorption of light) of the molecule of visual white is turned more towards the axis of the rods than that of both the unbleached visual pigment and the first products of bleaching. Such a movement would make the 'difference curve' obtained *in situ* different from that obtained in extracts because, whilst the unbleached pigment absorbs more heavily *in situ* than in extract, the bleached product absorbs less heavily *in situ* than in extract. This conclusion is confirmed by the fact that for *Xenopus laevis* the increase in absorption in the near ultra violet is much less than would be expected from a simple change from a porphyropsin to vitamin A_2.

Another interesting conclusion which can be drawn is that the products of bleaching remain (at least for the 1st hour following bleaching) in an organised state and we have the interesting view of a series of reactions going on presumably without the displacement of the molecule. This evidence is strongly against any chemical theory which demands from the intact retina the liberation of the chromophore from the protein part of the molecule in the course of the dark reactions taking place in the first hour following bleaching.

ACKNOWLEDGEMENT

I wish to thank Dr. W. S. Stiles for much help and encouragement in the course of this work.

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PRESENTATION AND DISCUSSION OF PAPER 5

DR. E. DENTON summarised his paper and added some further observations on fluorescence, and dichroism before and after bleaching of fish retinae. He concluded that the product of bleaching, (vitamin A), remained firmly attached and definitely oriented in the rods but with the axis of absorption turned round so as to reduce further absorption and transformation. He also put forward the view that the purpose of the pigment in the crystalline lens of the human was to protect the vitamin A in the retina from destruction by ultra-violet radiation rather than to reduce the effects of chromatic aberration. Supporting evidence for this idea was provided by the different ultra-violet absorptions of the eye lenses of different species of fish, depending on the advantage to the species of eliminating ultra-violet.

THE CHAIRMAN (PROF. G. WALD) opened the discussion with a description of several cases where the chemistry of the visual pigments was different in different stages of the life history, paralleling the results reported by Dr. Denton. He quoted an interesting case where Dr. Crescitelli and he had obtained apparently different visual pigments but where the discrepancy was resolved when it was realised that the specimens (lampreys) had been taken in one case on their way up-stream, in the other on their way down.

DR. W. A. H. RUSHTON referred to some experiments - not his own - made about 20 years ago, which appeared to contradict the vitamin A story as put forward by Dr. Denton. Two sets of albino rats were used, one set being kept in the dark as controls while the others were exposed to very strong light; the retinae were dissected out, and were examined in ultra-violet light, in which vitamin A fluoresces. It was found that in the bleached set the vitamin A had left the rods and was present in the pigment epithelium. If this were confirmed it would suggest that the carotenoids on bleaching left the rods and moved to the pigment epithelium where the isomerising enzymes might be situated which would bring about the change to the neo-*b* form before the return to the rods.

DR. DENTON said that the orientation of the vitamin A within the rods lasted several hours, and there was no indication that the vitamin A ever left them.

THE CHAIRMAN pointed out that the conditions were quite different, as the epithelium was detached in Dr. Denton's experiments and so the migration, if it occurred in Nature, could not occur there. There was some other experimental evidence for such a migration.

He thought that Dr. Denton's last sentence (in his paper) was much too strong, as there was a high degree of orientation of all the molecules in the outer segments of the rods; he was not altogether convinced by the observations on blue and green fluorescence; moreover, opsins were not the only molecules in the rods with which retinene would combine with a definite orientation.

GENERAL DISCUSSION ON PAPERS 4 AND 5 AND ON PROFESSOR WALD'S "SELIG HECHT LECTURE"

(CHAIRMAN: DR. STILES)

PROF. R. A. MORTON referred to the Sheffield experiment on conscientious objectors. It had *not* ended in a muddle; it was carried out for the British and American governments, primarily to determine the amount of vitamin A necessary for reasonable health in humans. It was first found that in England most of the subjects had sufficient vitamin A for at least 2 years stored in their bodies, so any experiment had to go on for at least that time. After two years the blood vitamin A of some subjects was down to 50% of the original level and some changes in rod and cone thresholds and rod-cone transition times had been found. These could be reversed by dosing with vitamin A or carotene, and the results so obtained for human requirements of vitamin A had not yet been disproved. Experiments on humans could not be continued beyond a certain danger point. In animals, some died of vitamin A deficiency before showing visual symptoms. In rats, deficiency could not strictly be said to occur before there was cessation of growth and the appearance of xerophthalmia. But a different criterion of deficiency might be when the supply of rhodopsin or opsin was impaired, and this would be an earlier criterion. The true deficiency symptoms would only appear after exhaustion of the animal's stocks. He would ask Prof. Wald whether perhaps in his experiments his subjects or test animals were made nearly vitamin-A deficient but never quite.

PROF. WALD replied that in his rat experiments the opsin was lost at the same time as the xerophthalmia appeared. He would like to know if other proteins in the body were similarly affected or whether opsins were special in this respect.

DR. E. BAUMGARDT made two points in connexion with the lecture. Prof. Wald had said the ratio of the sensitivities of the rods and cones was of the order of 7 or 10. Dr. Baumgardt had not been able to use test fields of very small diameters, but if he had been, he might have found lower values for this ratio. The important point was that it was low, of the order of units, and not 50 or 100 which was required by some hypotheses.

Secondly he could not accept the view that the range of dark adaptation was linked to the number of rhodopsin molecules in each rod. It had been shown that at levels of illumination of say 100 to 1000 times the absolute threshold, the concentration of rhodopsin would not be perceptibly affected by photochemical destruction of the molecules, and at 1 million times the absolute threshold, which was a level in the photopic range, the concentration would not be greatly affected after several minutes exposure, even if there was no regeneration. It seemed that Prof. Wald's slide showing the variation of log sensitivity with percentage of rhodopsin was not straight but curved at low levels. This meant that Wald's photochemical explanation failed below a certain level which was photopic. An attempt had been made by Wald in 1954 to explain this by a special hypothesis, but this was now abandoned because it led to results not in quantitative agreement with experiment. PROF. WALD replying, referred to the reaction kinetics of the system. How small in fact were the operative portions of the rods? Dr. Baumgardt had assumed that the entire rod was involved, but if the vision process only went on in some smaller portion of the outer segments this basis of argument failed. The fact was that we knew too little about the reaction kinetics in the actual eye (the processes proceed much faster in the test-tube) and we ought to refrain for the present from attempts at "curve-fitting" in this problem.

PROF. R. GRANIT observed that it was essential to appeal to electrophysiological results in discussing dark-adaptation. ERG measurements showed a different time course of dark adaptation for normal and colour-blind subjects and Dr. Rushton's observations appeared consistent with the latter but not the former.

PROF. C. HAIG mentioned recent work which indicated that if the curves of the log rod and log cone thresholds against log stimulus area were taken to very small areas they remained parallel and separate i.e., they did not converge.

DR. W. A. H. RUSHTON was concerned with the difficulty of extracting the cone pigments, which in his own work were shown to give optical densities perhaps two thirds that of rhodopsin in the rods. Presumably there was little cone pigment there and it must be compacted into a very small volume to give the observed optical density, that is, it must be a solid.

DR. H. B. BARLOW, stressing that dark-adaptation changes included both photochemical and neural effects, pointed out that cats regularly showed changes some three hours after being put in the dark and this must almost certainly be neural.

On the cone pigments, PROF. WALD said he had had no opportunity to extract pigments from a human fovea and he thought there was no information on their amounts.

SESSION III

BRIGHTNESS MATCHING AND COLOUR MATCHING

Chairman: PROFESSOR W. D. WRIGHT, IMPERIAL COLLEGE, LONDON

PAPER 6

THE QUESTION OF THE METRICS OF BRIGHTNESS

By S. G. YUROV

S. G. Yurov was born in 1915. After graduating from the Moscow Power Institute, he worked in the Photometric Laboratory of the All-Union Electrotechnical Institute. In 1952, he became chief of the Light Measurements section and in 1955, Deputy-Director of this Institute. Master of Sciences. He is the author of 40 works on photometry, physiological optics, industrial lighting and physics.

6. THE QUESTION OF THE METRICS OF BRIGHTNESS

By S. G. YUROV

SUMMARY

BRIGHTNESS and equivalent luminance represent the values characterizing radiation according to the light sensation formed by this radiation. These values at the same time can be considered as the conditional measures of the light sensation.

The quantitative evaluation of brightness and the measurement of the equivalent luminance of any radiation are based on the determination of the equality of the light sensations formed both by the given radiation and the usual radiation of comparison.

The lower metrics of the light sensations may be based on the principle of equality.

The main questions of the metrics are those concerning the distributivity and transitivity of the equality of the light sensations.

The results of the experiments showing the non-distributivity of brightness are given. They mean that the spectral sensitivity of the eye depends on the spectral composition of the radiation perceived by the eye.

Deviations from non-distributivity at the different adaptation levels of scotopic and mesopic vision as well as of photopic vision have different signs ($\gtrless 1$). These deviations are subject to a definite law.

Our conceptions of the mechanism of the transformation of radiant energy in the light-sensitive cells of the retina (that is the initial stage of formation of the light sensation) explain the deviations from distributivity, founded experimentally by many authors.

THE QUESTION OF THE METRICS OF BRIGHTNESS

THE light sensation is a process of transformation of radiant energy in a visual apparatus, transfer of the transformed energy to the brain and registration of the received signals by the brain.

Brightness is a notion which characterizes radiation, evaluated according to the light sensation which it causes. Thus, this evaluation is based on the criterion of "brighter-darker".

In so far as radiation is the cause of the light sensation, the notion of brightness may also serve for the evaluation and characterization of the sensation.

One of the possible methods for the quantitative evaluation of brightness of any radiation and consequently of the light sensation is the following:

(1) a certain radiation of comparison is chosen;

(2) luminance of the radiation of comparison is taken as a conditional quantitative characteristic of the brightness of this radiation and consequently as a conditional measure of the light sensation, caused by this radiation; and

(3) brightness of any radiation is considered to be equal to the brightness of the radiation of comparison, on condition that an observer or a group of observers have stated that the comparable radiations generate exactly the same light sensation, that is, if it is impossible to say which of these radiations is brighter and which is darker.

The equivalent luminance *(ref. 1)* is determined and measured in the same way and consequently the equivalent luminance may be considered as a measure of the light sensation as well as brightness.

The necessity of using two different terms is caused by the fact that during the measurements of the equivalent luminance a definite customary or agreed radiation of comparison must be used, and this radiation is equalized with the measured radiation under definite customary or agreed conditions (the size and the form of the photometric field, adaptation, the number of observers etc.).

The notion of brightness has a wider sense and therefore may be utilized for characterizing any radiation viewed by the eye in any conditions.

The metrics of the light sensation, which is one of the main problems of theoretical photometry and colorimetry, is naturally divided into two parts:

(1) lower metrics of the light sensation are the metrics of the equality of light sensations;

(2) higher metrics must answer the question by how many times one light sensation differs from another, that is, higher metrics are the metrics of the ratio of light sensations. The higher metrics of the light sensation are at present in the first stage of their development *(ref. 2)*. It is quite clear that the principle of the measurement of brightness outlined above is quite useless for forming ratios between the light sensations.

However, the basis of the lower metrics of the light sensation (the principle of equality) is an item of great interest taking into consideration that:

(1) the principle of equality is widely used in the practice of visual photometry and colorimetry;

(2) the development of the same problems of higher metrics of colour was carried through on the basis of the lower metrics of colour and in

indissoluble connexion with the latter; the lower metrics of colour were based on the principle of equality of colours and on the linear laws which exist between the spectroenergetic distributions of radiations and the equality of colours of these radiations.

In speaking of the laws of the equality of brightness, it is necessary to bear in mind above all the laws of distributivity and transitivity* *(ref. 3)*.

This article deals with the question of the distributivity of brightness.

As applied to the notion of brightness the law of distributivity is represented by three identities:

$$\text{if } B_1 \equiv B_3 \text{ and } B_2 \equiv B_3 \text{ then}$$

$$mB_1 + nB_2 \equiv B_3 \qquad \text{where } n + m = 1. \tag{1}$$

Here B_1, B_2, and B_3 are the luminances of radiations, which are viewed by the eye, m and n are fractions of these radiations, and the sign $\equiv$ means identity of brightness radiations, that is equality of brightness of these radiations.

The investigation of the law of distributivity is conducted in the following manner:

(1) a definite value of the luminance of the radiation of comparison B_3 is chosen. This luminance expressed in some photometric units standardizes the adaptation level of the eye. For practical convenience, the adaptation level is expressed in apostilbs;

(2) the observer in turn equalizes B_1 (var.) with B_3 (const.) and B_2 (var.) with B_3 (const.) according to the brightnesses of the radiations;

(3) the radiations B_1 and B_2 are then mixed in fractions m and n. The brightness of the mixed radiation $mB_1 + nB_2$ is equalized with the same radiation B_3 (constant in luminance).

If it is found that $m + n > 1$, then the sensitivity of the given observer under the given conditions to the more saturated radiations (B_1 and B_2 respectively) is greater than the sensitivity to the less saturated radiation (the so-called Helmholtz-Kohlrausch effect).

If $m + n < 1$, the reverse is true.

It is easy to show that the deviation of the sum of the fractions $m + n$ from unity means that the spectral sensitivity of the eye depends on the spectral composition of radiation, acting on the eye *(ref. 4)*.

The investigation needs to be made in the conditions of photopic vision as well as in the conditions of mesopic and scotopic visions. These notions are not exactly defined. Scotopic and mesopic vision are principally produced by the action of the rods, whereas photopic vision is produced by the

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* The law of distributivity is termed the law of additivity in many publications (see REFERENCES).

action of the cones. Rods play the main role in the process of vision when the adaptation levels are below 1 apostilb and the field of vision is not less than 10°. Cones play the main role in the process of vision when the adaptation levels are above 1 apostilb and the field of vision is about 2°. Therefore the more the given adaptation level differs from 1 apostilb (in either way) the less the size of the field of vision influences the type of vision.

For each of the two types of vision mentioned, the adaptation levels can be divided on certain conditions into low, average and high levels. Numbers, approximately defining the limits of these ranges on the scale of adaptation levels, are given in the summary table. This table represents the results of investigations on distributivity of the light sensation.

The results of the investigations given in the table, show that the light sensation is not distributive, that is, the spectral sensitivity of the eye depends on the spectral composition of radiation.

THE TABLE OF VALUES $m + n$

Investigator	Reference	Year	The adaptation levels (apostilbs)					
			Scotopic and mesopic vision			Photopic vision		
			low $10^{-4}-10^{-3}$	average $10^{-3}-10^{-2}$	high $10^{-2}-1$	low	average	high
1 Ives	*Ref.6* *	1912	–	–	–	>1	–	–
2 Kohlrausch	*Ref.7*	1935	–	–	–	>1	–	–
3 Dresler	*Ref.8* *	1937	–	–	–	>1	–	–
4 Weaver	*Ref.9*	1949	–	–	<1	–	–	–
5 MacAdam	*Ref.10* *	1950	–	–	–	>1	–	–
6 Tessier and Blottiau	*Ref.11* *	1951	–	–	–	>1	–	–
7 Yurov	*Ref.12*	1953†	>1	–	<1	–	–	–
8 Yurov	*Ref.17*	1954	–	–	–	>1	–	–
9 Chapanis and Halsey	*Ref.13*	1955	–	–	–	>1	–	–
10 Yurov	*Ref.17*	1956	>1	1	<1	>1	1	?

Notes: * Paper is analysed in Dresler's article *(ref.5)*.
† Investigations carried out in 1949.

Our experiments were carried out in 1954 and 1956 under the following conditions.

		1954	1956
1	Number of observers	6	6 - 7
2	Photometric field	Total quantity of the round holes; 10' each situated on the square of 2°.	Round 2°
3	Radiation of comparison (radiation B_3)	$T_c = 2850^{\circ}$K	$T_c = 2850^{\circ}$K
4	Number of measurements, made for every adaptation level	48	20 - 25
5	Comparable radiations	B_1 - orange, Schott's filter OG-2 B_2 - blue, Schott's filter BG-23	

It should be remembered that there are some publications in which the results obtained were opposite. These results show the distributivity of the brightness *(ref. 14)*.

In appraising these results one should bear in mind the following:

(1) at the present moment there are many fewer publications dealing with the distributivity of brightness than those stating the non-distributivity of brightness;

(2) in some of these publications there are methodological errors. For example, Bouma, who investigated the distributivity for only one observer, has averaged the data, obtained for low, average and high adaptation levels under conditions of mesopic vision. The table of values (items 7 and 10) shows that in this case the average value can equal 1, although for separate adaptation levels $m + n \lessgtr 1$;

(3) in no publications were explanations given of the results obtained, confirming or denying the distributivity of brightness.

The explanation of the non-distributivity of brightness (see below) was given by the author in 1950 *(ref. 15)*. This explanation is based on the supposition that the light sensation is formed as the result of the joint action of at least two photo-sensitive substances with different spectral "sensitivity", existing in the cells of the retina. At the present moment the joint action of two photo-sensitive substances in mesopic vision is an established fact. From this it follows that the explanation of distributivity of brightness under this type of vision would require additional special hypotheses, whereas the explanation of non-distributivity is naturally a result of the mechanism of the visual processes within the retina *(ref. 16)*.

In what way can one explain the non-distributivity of brightness?

The increase of sensitivity at high adaptation levels of mesopic vision for the mixed radiation in comparison with the sensitivity for more saturated radiations *($m + n < 1$, (refs. 9,12,17))* can be explained as follows; if the light sensation is formed only by the blue or by the orange radiation, either rods or cones are mainly loaded. On formation of the same light sensation (brightness is constant) by the mixed red-blue radiation the load on rods and cones is more evenly distributed, that is, a smaller fraction of the radiation falls on each of these two elements of the visual apparatus. This results in an increase of the molecular concentration of photo-sensitive substances existing in the rods and cones. Owing to this fact less power of radiation for the formation of the given light sensation is required.

If one adheres to the widespread view that there are three different photo-sensitive substances in the cone apparatus, it is possible to suppose that at the high adaptation levels of photopic vision $m + n$ will also be less than 1. This hypothesis has not been completely proved but it is confirmed by the fact that in our experiments carried out in 1956, $m + n = 1$ (see the table of values) at the level of 10^3 apostilbs.

The reduction of sensitivity to the mixed radiation ($m + n > 1$) at the low adaptation levels of scotopic vision *(refs. 12,17)* as well as of photopic vision *(refs. 5,10,13)* is explained in the following way. The experiments of Hecht, Vavilov and others show that for the formation of the single light sensation in near-threshold conditions, it is necessary to accumulate some dissociated (by means of radiation acting on the eye) molecules of photo-sensitive substances. The process of accumulation is accompanied by the process of recombination. The slower the process of accumulation of the dissociated molecules (that is, the lower the adaptation level) the greater the probability that some of the dissociated molecules will have time for recombination and will be unable to take part in the formation of the light sensation.

Thus at the low adaptation levels and with the visual apparatus subjected to the effect of the mixed radiation, the probability of the recombination of the dissociated molecules is increased owing to the reduction of the load on the separate photo-sensitive substances. This results in reducing the sensitivity towards the mixed radiation.

Such is the qualitative explanation of the non-distributivity of brightness. This picture is not vivid enough and needs further work but its main traits hardly contradict our modern conception of the mechanism of the visual process.

Thus the overwhelming majority of the empirical data concerning photopic vision, and most of the data concerning scotopic and mesopic vision, indicate that the light sensation as measured by brightness and equivalent luminance does not conform to the law of distributivity. On the other hand the comparatively few empirical data, showing the applicability of the laws of additivity to equivalent luminance have not up to now received an explanation from the point of view of the mechanism of the visual process.

ACKNOWLEDGEMENT

THE author is much indebted to Mrs. L. G. Ksentickaja for carrying out the measurements, leading to the conclusion of the non-distributivity of brightness.

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PRESENTATION AND DISCUSSION OF PAPER 6

PROFESSOR S. G. YUROV prefaced his presentation with the following remarks -

> "On behalf of myself and of my colleagues, those present here and those who have sent their papers, allow me to express our deep gratitude to our English colleagues for organising this Symposium on the Visual Problems of Colour. It is very important that the Symposium takes place in the N.P.L., which has a very high scientific reputation throughout the world."

Proceeding to his paper, Professor Yurov said that recently in the U.S.S.R. there had been published "An Optical Treatise on the Gradation of Light", written by P. Bouguer who was indisputably the founder of scientific photometry. On the first page of this classic work appeared the statement: "It (the light) affects us more or less powerfully according as its action on our eyes is greater or less: but we will ignore all the metaphysical aspects of sensation; and confining ourselves to finding out the relationship which exists between its external and incidental causes, we will consider only the measurement of this relationship." This statement, if it did not define, certainly formulated the main tendency in the development of light measurement for almost two hundred years.

For a long time the eye was practically the only receiver of radiation, used in light and colour measurements. Nevertheless its measuring properties ("metaphysics of sensations" as Bouguer expressed it) had not been specifically investigated with enough accuracy and understanding. The measuring properties of the receiver defined the properties of the numbers which expressed the results of any particular measurement. The main question then was one of the additivity, distributivity and transitivity of these numbers. In discussing the problems of additivity and other similar laws, it had to be made clear whether one was concerned with the application of these laws to the causes which produced various reactions or to the reactions themselves. For example, the additivity of forces was quite evident but the deformations caused by the forces in the general case were non-additive because the so-called law of elastic deformation (Hooke's law) was correct only to a first approximation.

The distributivity law, when used in connexion with the equality of light sensations, could be formulated in the following way:

$$\text{if } B_1 \equiv B_3, \text{ and } B_2 \equiv B_3, \text{ then } mB_1 + nB_2 \equiv B_3$$

$$\text{where } m + n = 1.$$

In these expressions B_1, B_2, B_3 were luminances, m and n were fractions of unity and the sign $\equiv$ meant that the brightnesses of the radiations were identical. The distributivity law had been investigated in just that form by Dresler (Germany), Blottiau (France), Weaver (U.S.A.), Volkenshtein (U.S.S.R.), and by other authors.

He (Professor Yurov) had been carrying out during the last nine years a number of investigations on the distributivity of the equality of light sensations. The results of these investigations agreed within the limits of comparison with the results found by other investigators.

There were very few experimental data about the metrics of equality of brightness. That was why the coincidence of results obtained by different investigators using different measuring instruments had essential significance.

We could make the following further statements, relating to our investigations.

1. It was known that in direct matches of coloured radiations the readings of an observer showed a wide scatter. We made a statistical analysis of the scatter of several thousand readings obtained by seven observers. It appeared that even for the measurements of such saturated radiations as red or blue the average scattering of readings obtained by observers having very poor training did not exceed ± 5 or 6%. Such a result was a very important one as it justified us in attributing to $(m + n)$, obtained by averaging hundreds of readings, a high degree of certainty.

2. We made measurements of the sum $(m + n)$ for a certain group of radiations (B_1, B_2, B_3) at different levels of adaptation (from 10^{-4} up to 10^3 apostilbs) using one and the same instruments and the same observers. We were able to show that deviations of $(m + n)$ from unity at different levels of adaptation had different signs (+ and -).

3. Measurements of the sum $(m + n)$ at a certain level of adaptation for different groups of radiations showed that the sign of the deviation of $(m + n)$ from unity (+ or -) depended upon the spectral composition of the radiations B_1, B_2 and B_3. This result agreed with the data, obtained by Dr. MacAdam, Dr. Chapanis and Mrs. Halsey in their works published in recent years in the Journal of the Optical Society of America.

4. We attempted an explanation of the way the sum $(m + n)$ depended upon the level of adaptation.

The proposed explanation was based upon the hypothesis that the light sensation was produced by the combined activity of several photo-sensitive substances differing in their spectral characteristics. In mesopic vision, the presence of two such photo-sensitive substances (rods and cones) was beyond doubt. In photopic vision the presence of the three photo-sensitive substances was postulated in the Young-Helmholtz theory of colour vision and confirmed by experiments (for instance Dr. Rushton's experiments). The explanation given in his (Prof. Yurov's) communication could not be considered as a complete one. It was quite possible that specialists of physiology would have criticisms. Perhaps the explanation could be regarded as exemplifying the fact that unusual and complicated properties of our visual apparatus might be explained by the physical processes taking place in it.

DR. D. L. MACADAM asked what was the dividing line between low and average photopic levels. He also asked Prof. Yurov to say something more on the direction of the non-additivity found by Weaver.

Professor Yurov replied in these terms.

We knew that the Helmholtz-Kohlrausch effect (Farbenglühe) was the phenomenon that saturated colours were estimated by an observer to be brighter than less saturated ones. This effect could be expressed quantitatively in two ways.

In the first way, we had,

$$\text{if} \quad B_1 \equiv B_3 \text{ and } B_2 \equiv B_3 \text{ then}$$

$$mB_1 + nB_2 \equiv B_3 \text{ with } m + n > 1.$$

Here B_1, B_2, B_3 were luminances (not brightnesses!), m and n were fractions of unity and the sign $\equiv$ denoted that the brightnesses (not luminances!) of the radiations were identical!

The Helmholtz-Kohlrausch effect had been investigated by Dresler (Germany), Tessier and Blottiau (France) and by the speaker and his colleagues (see *Table* on *p. 200* Paper 6) at adaptation levels of some tenths of an apostilb. The results obtained could be formulated as follows.

For equality of brightness, the luminance of the mixed, that is, the less saturated radiation should be taken greater than the luminance of the more saturated colour radiations ($m + n > 1$).

In the second way, an observer had several coloured radiations (1, 2, 3, etc.) and one white radiation of constant luminance B_0, in his field of vision, and he changed the luminances of the coloured radiations B_1, B_2, B_3, etc. until all the radiations had equal brightnesses. That is, the observer obtained the brightness identity:

$$B_1 \equiv B_2 \equiv B_3 \equiv \ldots\ldots \equiv B_0 \,.$$

The numerical values of the *luminances* B_1, B_2, B_3 and so on, were then determined with the help of the photopic luminosity function V_λ.

Dr. MacAdam, as well as Dr. Chapanis and Mrs. Halsey, performed such experiments and discovered that $B_1 < B_0$, $B_2 < B_0$ and so on except for colours in the yellow region. Such results were in agreement with the data, obtained by Dresler, Tessier and Blottiau and by the speaker and his colleagues. The conclusion could be formulated as follows.

For equality of brightness, the luminance of the less saturated radiation (of the white radiation B_0) should be taken greater than the luminance of the coloured radiations $(B_1 < B_0)$. It was easy to see that this conclusion did not differ from the one mentioned before. Consequently in order to facilitate comparison, the results obtained by Dr. MacAdam, Dr. Chapanis and Mrs. Halsey were expressed as $(m + n) > 1$.

The foregoing related to low adaptation levels of photopic vision.

The investigations carried out by Mr. Weaver and the speaker had shown that $(m + n) < 1$ for *mesopic vision at the levels of adaptation* $(1 \text{ to } 10).10^{-2}$ *apostilbs*. Weaver himself probably did not pay great attention to this result as in his paper, published in 1949 in the Journal of the Optical Society of America, he wrote: "These results indicate that the restricted additivity law holds quite closely under the conditions of these experiments. The small failure found may be ascribed to a tendency on the part" (the part was six observers among seven) "of some observers to estimate saturated colours a little brighter than the less saturated colours of the mixtures." The last sentence taken from Mr. Weaver's article describes the phenomenon inverse to the Helmholtz-Kohlrausch effect because the sum $(m + n) < 1$.

PAPER 7

THE AVERAGE COLOUR-MATCHING FUNCTIONS FOR A LARGE MATCHING FIELD

By W. S. STILES

DR. W. S. Stiles joined the staff of the National Physical Laboratory in 1925 and was originally concerned with problems bearing on photometry and illuminating engineering. His later work has been mainly devoted to the study of visual response by psychophysical methods. His best known contribution is the discovery with Dr. B. H. Crawford of the directional sensitivity of the retina (Stiles-Crawford Effect). He has recently been elected to fellowship of the Royal Society for his work on vision.

7. THE AVERAGE COLOUR-MATCHING FUNCTIONS FOR A LARGE MATCHING FIELD

By W. S. STILES

SUMMARY

THE colour-matching functions for a group of 50 subjects were determined in the range 14,000 to 25,500 cm^{-1} using a 10° bipartite field and red, green and blue spectral primaries. In the long-wave region of the spectrum ($^{1}/\lambda < 17{,}000$ cm^{-1}) the usual green primary was replaced by a yellow primary to reduce interference by rod vision. In addition, the location of the blue primary was changed for half the subjects from wave-number 22,450 to 21,250 cm^{-1}. All the results were finally transformed to reference primaries at 15,500, 19,000 and 22,500 cm^{-1}.

Some small differences in the mean colour-matching functions, depending on the location of the blue primary and the intensity level used in the matches, were observed. These probably represent deviations from strict additivity in full colour-matching. Comparison of the observed tristimulus values for a continuous white with the values calculated from the subject's own colour-matching functions showed on average certain small but significant deviations which again indicate imperfect additivity. The additivity defects are smaller than the average differences between the matches of different subjects and probably can be ignored in defining the colour-matching properties of a standard average eye.

An extension of the measurements from 14,000 to 12,000 cm^{-1} confirmed Brindley's discovery of the yellowing of the spectral colours for wavelengths greater than about 700 mμ.

INTRODUCTION

THE results of a pilot investigation of the colour-matching functions in 2° and 10° matching fields for a group of 10 subjects were presented in two previous publications *(refs. 1* and *2)* and were discussed at the Zürich meeting of the Commission Internationale de l'Éclairage in June, 1955. Following the discussions at that meeting, it was decided to concentrate on the determination of average colour-matching functions in the 10° matching field for a large group of about 50 subjects. In the present paper the results of this work by my colleague Dr. J. M. Burch and myself will be considered.

According to the trichromatic generalisation in its stronger form *(ref. 1)* it is possible to find three functions of wavelength $\bar{x}_\lambda$, $\bar{y}_\lambda$, $\bar{z}_\lambda$ such that the necessary and sufficient condition for a complete match, i.e., a match in colour and brightness, of two visual stimuli of spectral energy distributions $\{E_\lambda \, d\lambda\}$ and $\{E'_\lambda \, d\lambda\}$ respectively takes the form: $\int E_\lambda \bar{x}_\lambda \, d\lambda = \int E'_\lambda \bar{x}_\lambda \, d\lambda$, and two similar relations with $\bar{y}_\lambda$ and $\bar{z}_\lambda$ replacing $\bar{x}_\lambda$. The colour-matching functions $\bar{x}_\lambda$, $\bar{y}_\lambda$, $\bar{z}_\lambda$ are derivable from colour-matches made with a series of spectral stimuli of known energy and measured quantities of three fixed stimuli (primaries) suitably mixed - the spectral stimulus being generally mixed with a quantity of one of the primaries and matched with a mixture of the other two. All the steps in the derivation involve assumptions - additivity assumptions - which are valid if the strong generalisation is true. The generalisation is certainly true as an approximation and it is almost essential to assume it in reducing the quantities given immediately by the colour-matches to a form in which they can be discussed. Some light is thrown on the extent to which the generalisation is true, from the consistency of colour-matching functions and other results obtained in different ways and reduced to comparable form by its aid.

In the pilot investigation the red, green and blue spectral primaries were at wave-numbers 15,420, 19,000 and 22,450 cm^{-1} approx. From a comparison of the results for 2^{0} and 10^{0} matching fields and other evidence it appeared that at long wavelengths the colour-matching functions for a 10^{0} field were slightly different from those that would have been obtained if the matching conditions had been such as to reduce possible interference by the rod mechanism to a negligible amount. To obviate the need for a correction to remove distortion by rod vision, the present measurements were made using for test stimuli of longer wavelength ($\frac{1}{\lambda} < 17,000$ cm^{-1}) a yellow (17,000 cm^{-1}) instead of the green (19,000 cm^{-1}) primary. The way this reduces rod intrusion is explained below. For test stimuli of shorter wavelength ($\frac{1}{\lambda} > 17,000$ cm^{-1}), the green primary was used (the "green" run) and from the matching quantities of a yellow at 17,000 cm^{-1} obtained in this run the results of the "yellow run" could be transformed to refer to the green primary.

The matching of blue test stimuli in the approximate wave-number range 21,500 (465 mμ) to 24,000 (417 mμ), which gave most subjects difficulty in the pilot investigation, was found to be facilitated by reducing the field intensity then used, and a reduction to about one third was made for the present measurements. A possible difference in the colour-matching functions depending on the location of the blue primary had previously been studied *(ref. 2)*. Little effect was obtained for the 10^{0} field when the blue primary was moved from 22,450 to 21,750 cm^{-1}. It was decided however to include a further examination of this point by making the measurements for one half of the large group of subjects with the original blue primary at 22,450 cm^{-1} (445 mμ), and substituting, for the remaining subjects, a blue primary of considerably longer wavelength at $\frac{1}{\lambda}$ = 21,250 cm^{-1} (471 mμ). Another change in conditions was also made for this second group: the field intensity for the measurements in the green, yellow and orange was considerably increased - by a factor of about 5 - with the object of still further reducing any rod intrusion and probing a possible change in the apparent matching functions derived from spectral matches made at high field intensities.

The measurements just considered were terminated in the red at the wave-number 14,000 cm^{-1} (714 mμ). Beyond this wave-number it has usually been considered that there is no further variation in the colour of a spectral stimulus. Thus the colour-matching functions could be continued to longer wavelengths by using a single primary of long wavelength, say 700 mμ, in the comparison half of the field and making straight brightness matches with spectral stimuli of longer wavelength, the measurements being extended as far in the red as the source energies available would allow. However, it was shown by G. S. Brindley *(ref. 3)* that, in fact, beyond about 700 mμ, spectral stimuli become slightly but progressively more yellow. This has been confirmed in the present work, but to obtain satisfactory colour matches in the deep 'red', increased intensity obtained by a considerable increase in spectral band width was necessary. This was so, even though a Xenon arc was substituted for the ribbon filament source. In addition to full colour-matches made by a group of 9 subjects using wide spectral bands, straight heterochromatic brightness matches with a primary at 14,250 cm^{-1} were made for another group of 16 subjects using the normal, fairly narrow band-widths. By a combination of the results a fairly adequate extension of the colour-matching functions down to about 12,000 cm^{-1} (833 mμ) can be made.

The main blocks of measurements are summarised in *Table 1*.

In addition to the measurements on spectral test stimuli, colour-matches were made for a "white light" test stimulus provided in the N.P.L. trichromator by removing the slit in the central spectrum and allowing the rays of all wavelengths to recombine in the second stage of the monochromator. The insertion of a blue and a neutral filter gave a "white" of suitable brightness and colour. The energy distribution of the resultant white was substantially the same as that used in the preliminary work *(ref. 2)*. Finally, observations were made of the relative brightnesses of three spectral stimuli, corresponding to the "green run" spectral primaries, using heterochromatic, direct comparison brightness-matching, with white as the comparison stimulus.

TABLE 1

	Instrumental Primaries (cm^{-1})	Test Stimuli (cm^{-1})
Part I (24 subjects)		
Green Run	15,420, 19,000, 22,450	15,500, 17,000 to 25,500
Yellow "	15,420, 17,000, 22,450	14,000 to 17,000 (to 19,000), 22,500
Part II (29 subjects)		
Green Run	15,420, 19,000, 21,250	*15,500, 16,000, 16,500, 17,000 to 25,500
Yellow "	15,420, 17,000, 21,250	14,000 to 17,000 17,500, 18,500, 19,000 21,250, 22,500
PART III		
(a) Deep red, brightness matching (16 subjects)	14,250 - -	12,000 to 15,000
(b) Deep red, colour-matching (9 subjects)	14,250, 17,000, 21,250	12,133, 13,081, 14,000 15,000 (Nominal)

* In the latter part of the Pt.II measurements one of the trichromator slits was accidentally displaced by a small known amount and as a result the measurements for the final eight subjects were made at slightly smaller wave-numbers. To facilitate processing and final averaging, the instrumental colour-matching functions for these eight subjects at the exact wave-numbers were determined by interpolation from the values at the displaced wave-numbers: the subsequent treatment of the results was then identical with that of the main group.

APPARATUS AND TECHNIQUE

NO more than minor changes to the N.P.L. trichromator - already described *(ref. 1)* - were made for the present measurements. Quick change from "green run" to "yellow run" primaries made it necessary to install a fourth wedge for the yellow primary in the central spectrum planes of the upper and lower tiers. This fourth wedge was fixed to the wedge normally used for the green primary, on its long-wave side, and shared the motion and position scale of the latter. The wedge used for the blue primary was sufficiently wide to intercept the beam from the blue primary slit in either the 22,450 or the 21,250 cm^{-1} position. Also the wedge for the normal red primary was wide enough to intercept the beam from the slit for the deep red primary - needed only in the mixture tier.

For each primary in the mixture and desaturation tiers an independent wedge calibration was required, entailing eleven wedge calibrations in all. These calibrations were made - and checked several times during the work - using the photoelectric photometer incorporated in the trichromator set-up. Tests of the linearity of the photometer response showed possible deviations of 0.001 log unit on a density of 1 but were not sufficiently certain to warrant any attempt to apply correction. The slight variations in wedge densities shown up in the check calibrations have been allowed for in computing the mean results for Pts. I and II. It is estimated that in the mean results the inaccuracies in wedge densities from all causes are unlikely to exceed 0.003 log unit on a difference of wedge density of up to 1 log unit and 0.005 on differences between 1 and 2 log units. When a supplementary filter in the primary beam was needed to extend the density range to more than 2, the possible error is greater ($\leqslant$0.008).

The other essential physical measurement is the relative energy for different wavelengths of the spectral stimulus delivered to the eye from the half of the matching field occupied by the test colour. For the main light source - a tungsten ribbon-filament lamp run at constant current - a basic relative spectral energy distribution was determined initially from p.e. photometer measurements at 250 cm^{-1} wave-number intervals. A running correction to this distribution was made by repeat energy measurements at six selected wave-numbers, taken at intervals of 30 to 40 burning hours of the lamp. By a combined wave-number and time interpolation, the distribution appropriate to the measurements on a particular subject was derived and used in processing that subject's results. The error in the ratio of the energies at any two wave-numbers arising from the interpolation procedure and from possible inaccuracy in the density of a neutral filter required for some wave-numbers to give the desired field intensity, is estimated not to exceed 0.005 log unit for the mean data. There is in addition a possible error in the calibration of the relative spectral sensitivity of the photocell against a thermopile, determined both before and after the colour-matching measurements. With this may be coupled any variations in the true relative spectral sensitivity of the photocell in the course of the work - variations which were certainly very small. The energy ratio for any two wave-numbers is subject to an estimated inaccuracy of at most 0.008 log unit from these causes.

For the extreme wave-numbers of Pts. I and II ($\geqslant$24,500 and $\leqslant$14,500 cm^{-1}) a 100W Zirconia concentrated arc lamp, and for the whole of Pt. III, a 1,000W Xenon arc was substituted as test tier source. During the energy calibration and the actual measurements, the intensity of the radiation passing through the test tier entrance slit was monitored by placing a rectifier photocell in the light reflected from the surface of the first dispersing prism. Using the observed currents of the monitor photocell, relative energies were obtained with an average error probably not exceeding 0.010 log unit, exclusive of the calibration error of the main photoelectric photometer.

The direct results of a spectral match gives the intensity U_λ of the spectral stimulus (T_λ) in energy units which mixed with a quantity of desaturating primary - say D_R of the "red" primary (R) - and exposed in one half-field - say the upper - matches a mixture of quantities M_G and M_B of the other primaries (G) and (B), exposed in the other (lower) half-field.

$$U_\lambda\ (T_\lambda) + D_R\ (R) \overset{\mathrm{uM1}}{=} M_G\ (G) + M_B\ (B) \qquad \ldots(1)$$

To derive the instrumental colour-matching functions $\bar{a}_\lambda$, $\bar{b}_\lambda$, $\bar{c}_\lambda$ for this spectral stimulus, an auxiliary match is required between the desaturating and mixture red primaries:

$$D'_R\ (R) \overset{\mathrm{uM1}}{=} M'_R\ (R) \qquad \ldots(2)$$

By the general additivity assumption, (1) and (2) give

$$1\ (T_\lambda) \overset{\mathrm{uM1}}{=} -\frac{D_R}{U_\lambda}\frac{M'_R}{D'_R}\ (R) + \frac{M_G}{U_\lambda}\ (G) + \frac{M_B}{U_\lambda}\ (B)$$

$$= \bar{a}_\lambda\ (R) + \bar{b}_\lambda\ (G) + \bar{c}_\lambda\ (B) \qquad \ldots(3)$$

It will be noted that matching is not assumed to be symmetrical with respect to the two halves of the field, i.e., one cannot move a quantity through the match symbol "uM1" without using an auxiliary match similar to (2).

If the instrumental colour-matching functions of any three spectral stimuli are written as the rows in a 3 x 3 matrix, E,

$$\begin{matrix} \bar{a}_1 & \bar{b}_1 & \bar{c}_1 \\ \bar{a}_2 & \bar{b}_2 & \bar{c}_2 \\ \bar{a}_3 & \bar{b}_3 & \bar{c}_3 \end{matrix}$$

the colour-matching functions referred to these stimuli as primaries (reference primaries) are obtained by multiplying the vector $(\bar{a}_\lambda, \bar{b}_\lambda, \bar{c}_\lambda)$ by the reciprocal of the above matrix. The calculation rests on the general additivity law, and the derived colour-matching functions should define the equivalence of a test stimulus and a mixture of reference primaries, both being assumed to be seen in the upper half of the field. To bring the yellow run results of Pt. I to the same basis as the green run results, the instrumental colour-matching functions were first transformed independently to intermediate reference primaries at 15,500, 17,000, 22,500 cm^{-1}. From the intermediate colour-matching functions for the green run only, the matrix necessary to change the middle reference primary from 17,000 to 19,000 cm^{-1} was derived, and applied to the intermediate colour-matching functions of both yellow and green runs. This means simply that the green run match on 17,000 cm^{-1} was used to represent the yellow (17,000) primary of the yellow run in terms of the final reference primaries. A similar procedure was applied to the Pt. II data and the final colour-matching functions $\bar{\bar{r}}_\lambda$, $\bar{g}_\lambda$, $\bar{b}_\lambda$ for every subject were obtained in terms of reference primaries 15,500, 19,000, 22,500 cm^{-1} (645.2, 526.3, 444.4 mμ). Where unit co-ordinates (chromaticities) are required in discussing the results, these are always obtained on the W.D.W. system with the reference primaries just specified and with the red and blue colour-matching functions normalised to equal the green function at the normaliser wave-numbers 17,250 and 20,500 cm^{-1} respectively.

SUBJECTS AND OBSERVATIONAL CONDITIONS

THE Ishihara test was used to exclude dichromatic or strongly anomalous subjects. The measurements made on a few subjects who gave markedly erratic results were jettisoned. *Table 2* gives the sex and age distribution of the subjects actually used. Some 25 per cent of these were practised in colorimetric or photometric observations. The rest had little or no experience in colour-matching apart from one day's training on the trichromator.

TABLE 2

Age and Sex Distribution of Subjects

	16-23 incl.		: 24-32 incl.		33-42 incl.		: 43-55 incl.	
	Male	Female	Male	Female	Male	Female	Male	Female
Pt. I	0	4	10	1	3	1	4	1
Pt. II	5	6	4	3	5	0	6	0
Pt. III (a)	0	5	2	4	2	1	2	0
Pt. III (b)	1	0	3	1	0	0	3	1

(The subjects of Pt. III were a selection of those of Pts. I and II. Four subjects were common to Pts. I and II.)

The field conditions at match are specifiable (a) by the retinal illumination in trolands of the mixture half-field - computed on the conventional basis of C.I.E. V_λ values of the mixture primaries - and (b) by the chromaticity of the matched field in the W.D.W. system. The purity of the spectral test stimulus is specified by the total band width *(Table 3)*.

TABLE 3

Wave-number cm^{-1}	Total Band Width cm^{-1}
14,000 increasing to 20,750	188 decreasing to 108
21,000 " " 23,500	132 " " 83
24,000	131
24,500 Tungsten	226
" Zirconia	124
25,000	210
25,500	529

The retinal illumination curves *(fig. 1)* show the differences already noted in the intensity conditions of Pts. I and II. The pilot investigation had indicated that the stability of matching in the difficult spectral region 21,500 to 23,500 cm^{-1} was improved by increased desaturation with the green primary. In Pt. I, sufficient green primary was added to keep the resultant field chromaticity on the line from the spectrum locus at 21,500 cm^{-1} to the red primary, as shown by the diagram in *fig. 2*. In Pt. II, with the blue primary at 21,250 cm^{-1}, no difficulty was experienced in using the minimal desaturation. Throughout the work, a surround of the same brightness and colour as the mixture half-field extended the total field to a circle of 14° diameter. For approximately half the subjects, the test stimulus was presented in the upper semi-circle of the 10° matching field, and in the lower, for the rest. Each subject observed with his "best" eye, 14 left-eye and 39 right-eye sets being obtained. The match at each wave-number was based on two or four completely independent settings of the controls, certain wave-numbers being measured twice in the course of the set. To minimise in the final average any effect on a match, of the colour to which the eye had previously been exposed, the wave-numbers in the green run were taken for half the subjects in the order red to blue by "odd" wave-numbers, blue to red by "even" wave-numbers. For the remaining subjects the order was reversed. The white matches were obtained from two groups of five independent settings, one taken before, the other after, the spectral matches.

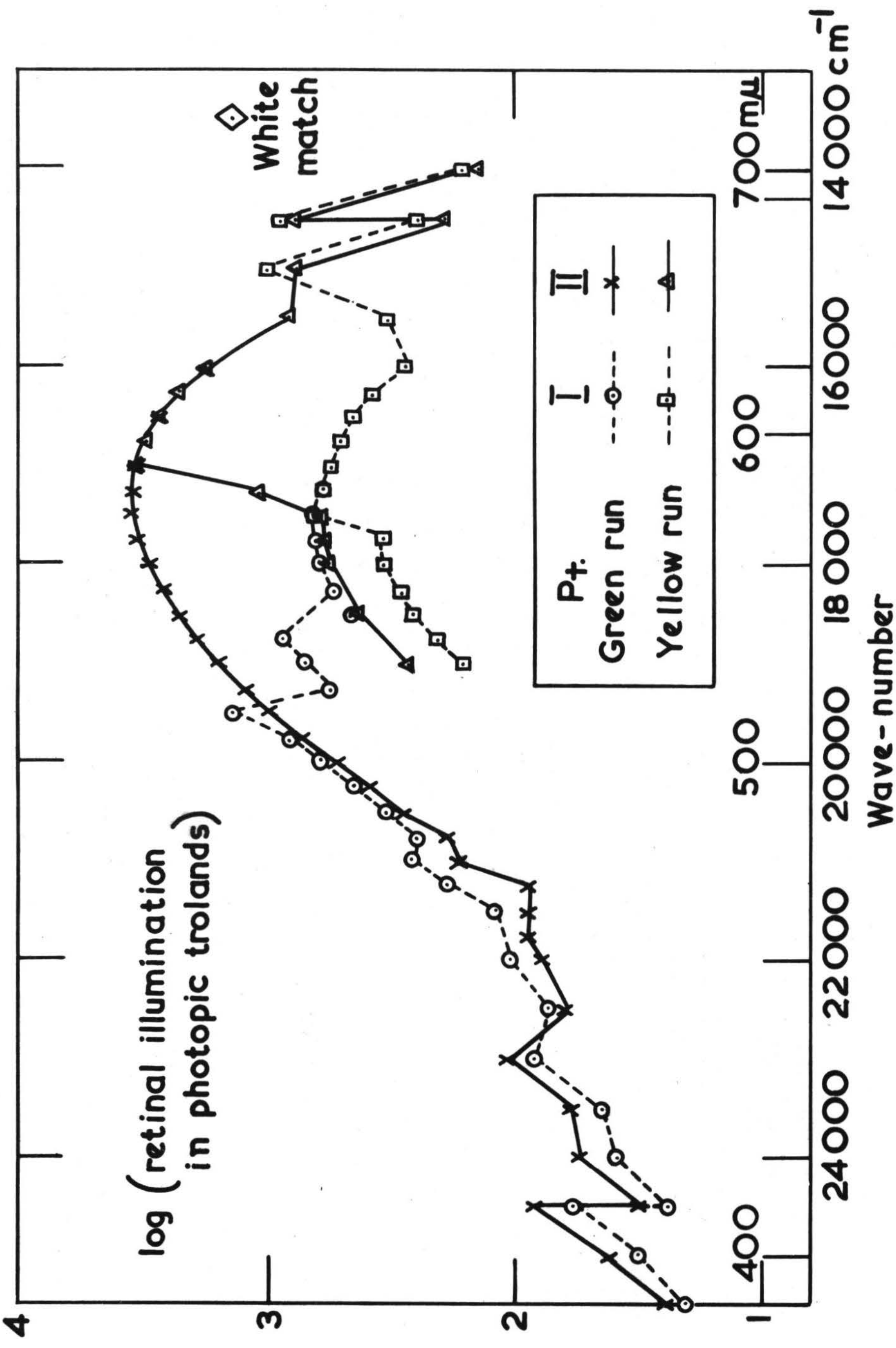
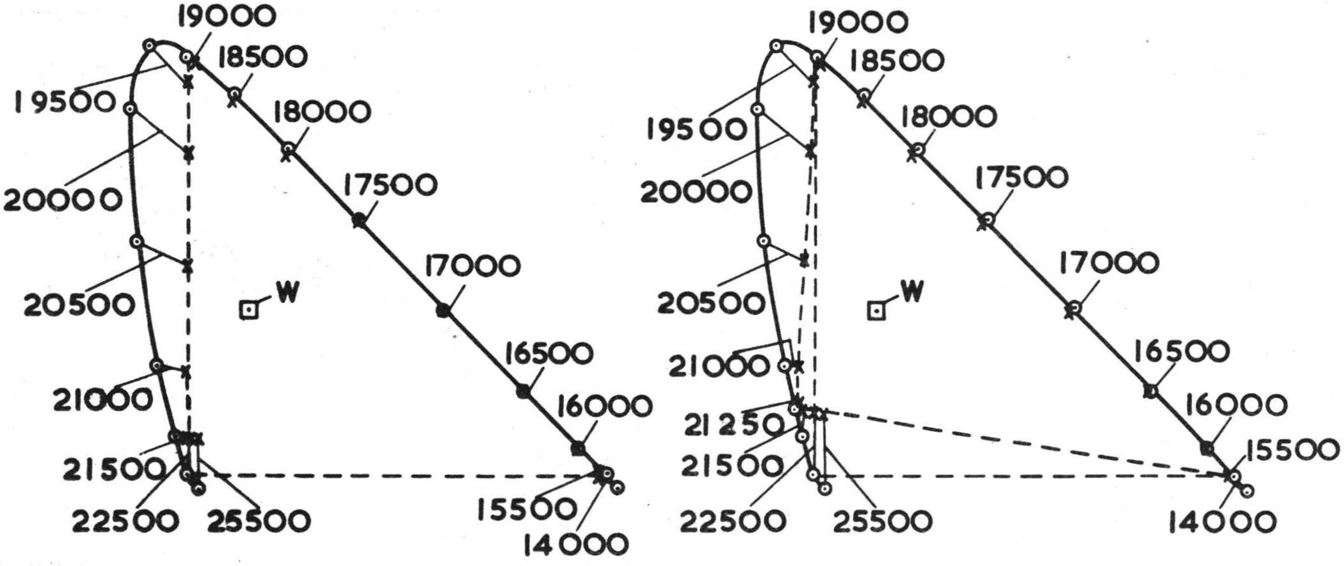

log (retinal illumination in photopic trolands)
4
3
2
1
400
500
600
700mμ
24000
22000
20000
18000
16000
14000 cm⁻¹
Wave-number
White match
Pt.
I
II
Green run
Yellow run

FIG. I.


19000
18500
19500
18000
20000
17500
17000
W
20500
16500
21000
16000
21250
15500
21500
22500
25500
14000

(a)

(b)

FIG. 2.

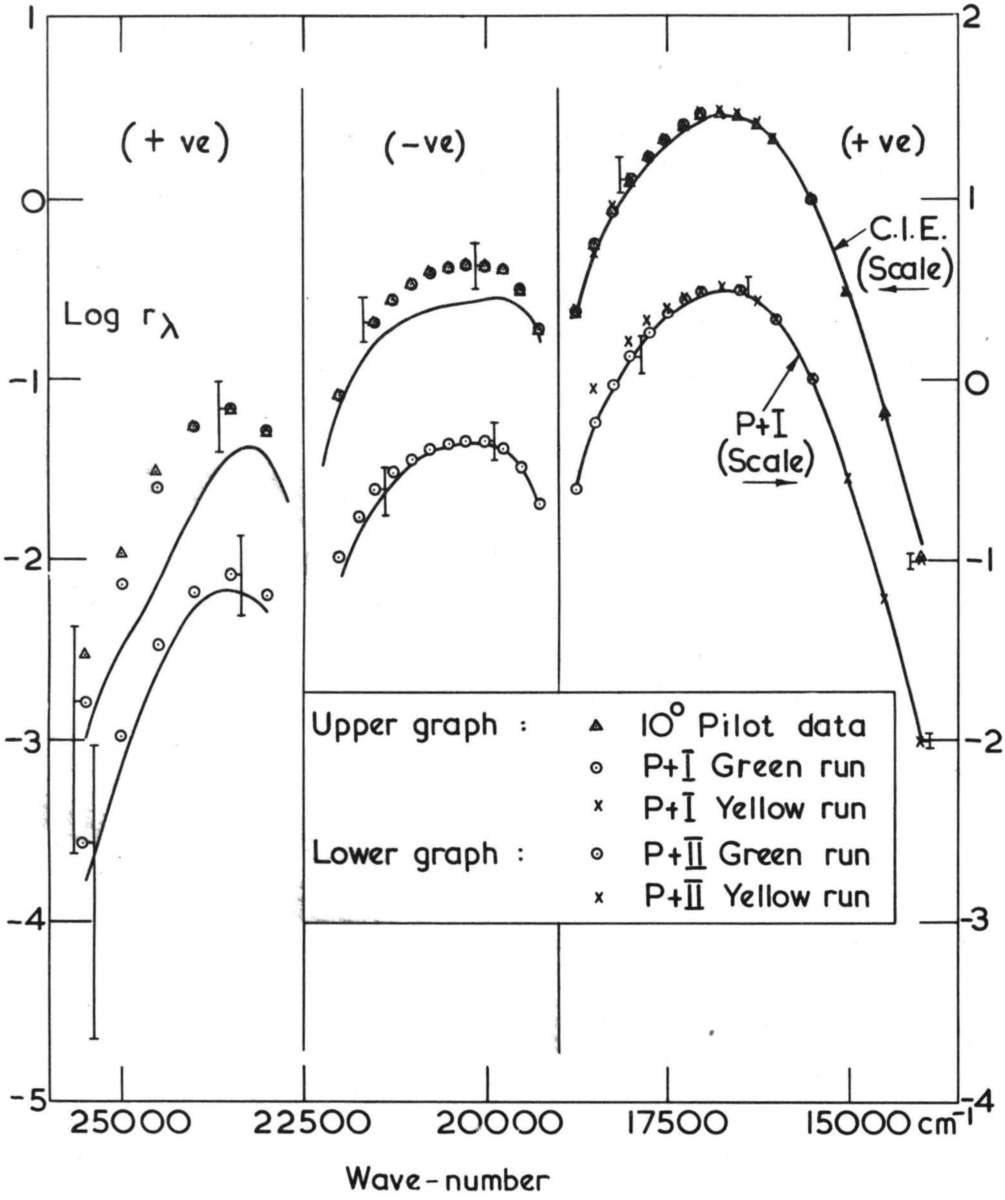

(+ ve)
(−ve)
(+ ve)
Log r_λ
C.I.E. (Scale)
P+I (Scale)
1
0
-1
-2
-3
-4
-5
2
1
0
-1
-2
-3
-4
25000
22500
20000
17500
15000 cm⁻¹
Wave-number
Upper graph : ▲ 10° Pilot data
⊙ P+I Green run
x P+I Yellow run
Lower graph : ⊙ P+II Green run
x P+II Yellow run

FIG. 3.

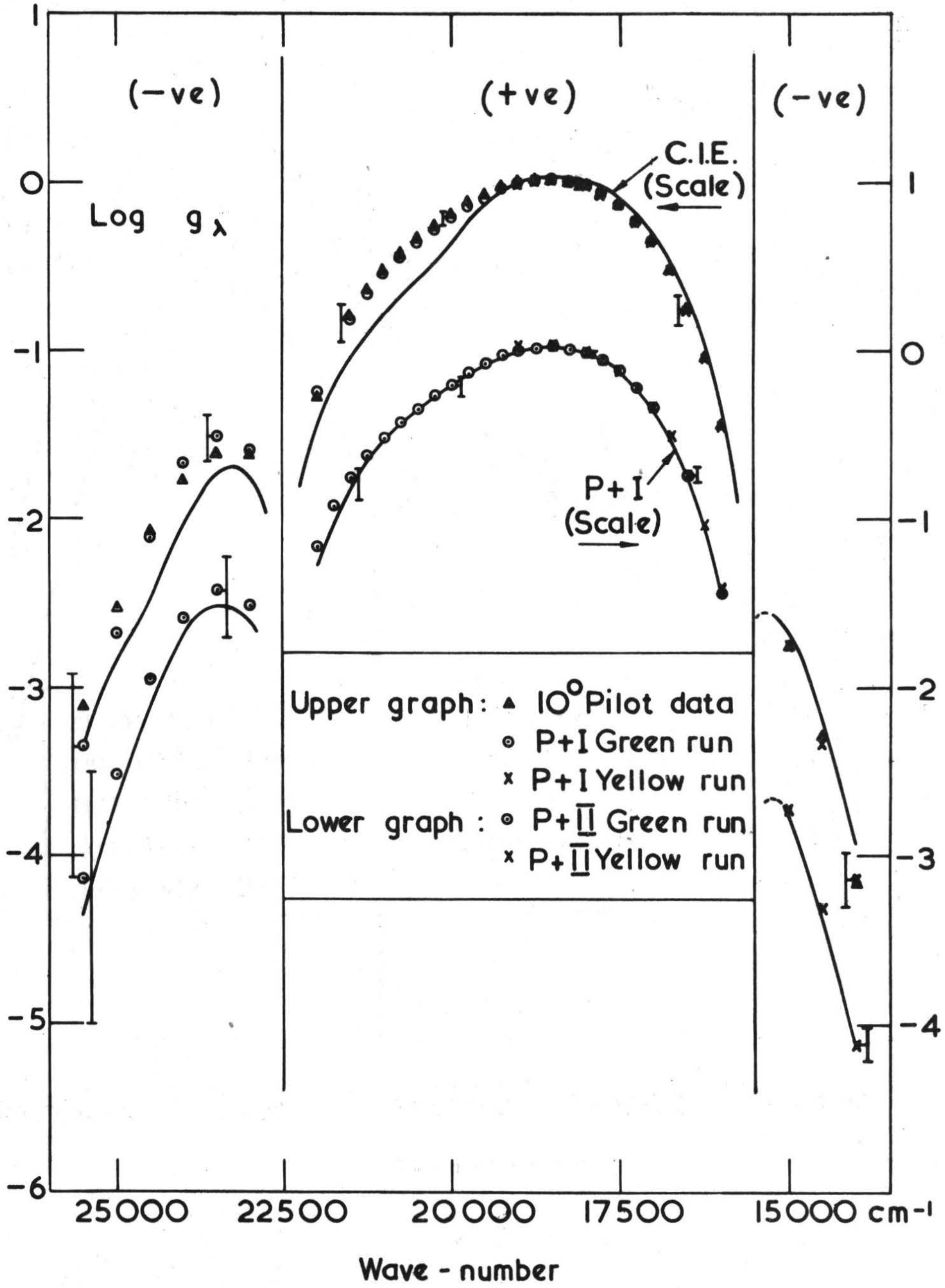
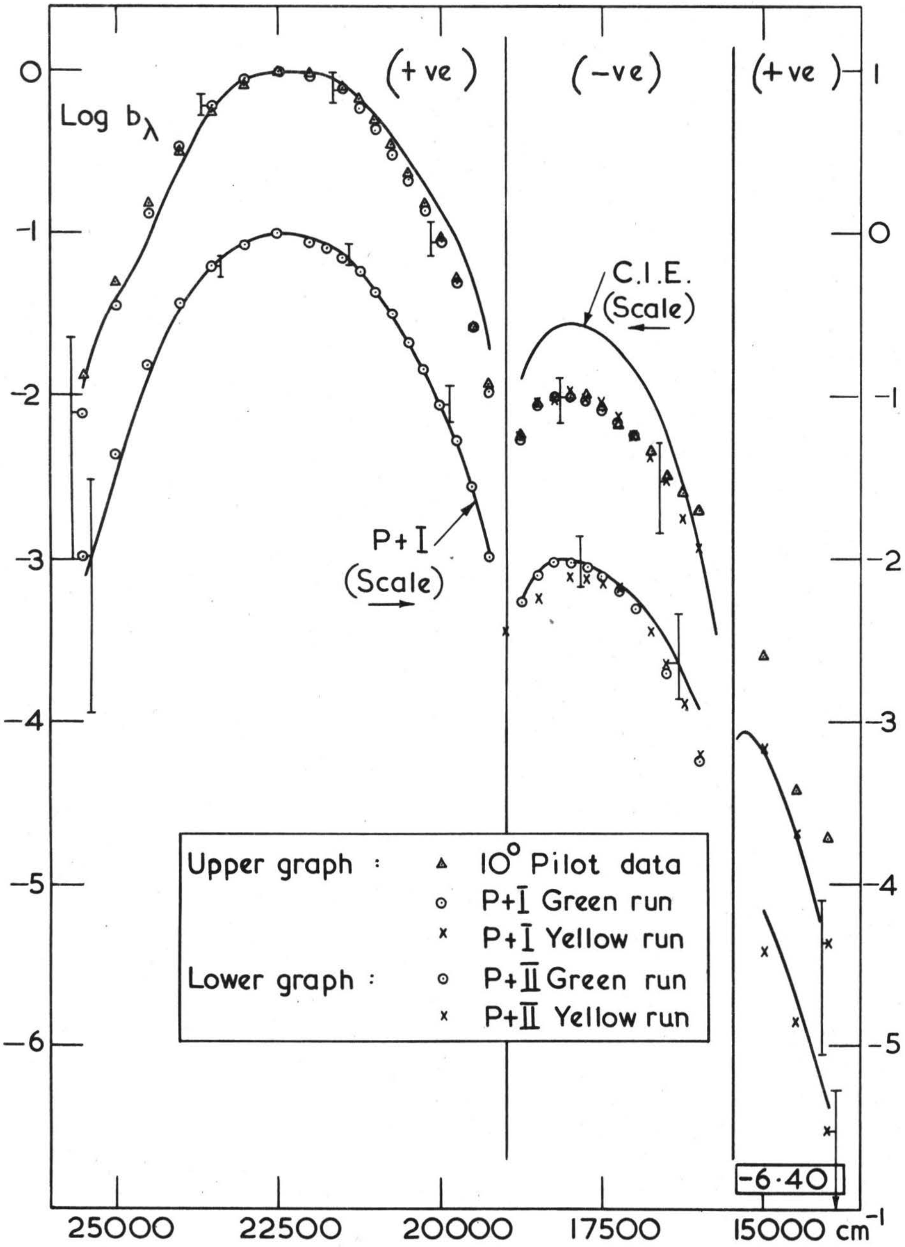

(−ve)
(+ve)
(−ve)
Log g_λ
C.I.E. (Scale)
P+I (Scale)
1
0
-1
-2
-3
-4
-5
-6
1
0
-1
-2
-3
-4
Upper graph: ▲ 10° Pilot data
⊙ P+I Green run
× P+I Yellow run
Lower graph: ⊙ P+II Green run
× P+II Yellow run
25000
22500
20000
17500
15000 cm-1
Wave - number

FIG. 4.



(+ve)
(−ve)
(+ve)
Log b$_\lambda$
0
-1
-2
-3
−4
−5
−6
1
0
−1
−2
−3
−4
−5
C.I.E. (Scale)
P+I (Scale)
-6·40
Upper graph : ▲ 10° Pilot data
⊙ P+I Green run
x P+I Yellow run
Lower graph : ⊙ P+II Green run
x P+II Yellow run
25000
22500
20000
17500
15000 cm^{-1}
Wave-number

FIG. 5.

RESULTS: PT. I. BLUE PRIMARY AT 22,450 cm^{-1}

THE upper curves in *figs. 3,4* and *5* represent the log (colour-matching function) for the reference primaries at 15,500, 19,000 and 22,500 cm^{-1} respectively, as calculated from the current C.I.E. standard colorimetric data. The circle points show the mean Pt.I green run measurements ($^1/_\lambda \geqslant 17{,}000\ \mathrm{cm}^{-1}$), and the cross points the mean Pt.I yellow run measurements linked to the green run result at 17,000 cm^{-1} in the way already explained. Comment on the differences from the C.I.E. curves is deferred. The agreement of the Pt.I and the 10° pilot group results shown as triangle points is fairly close except in the extreme blue where there are deviations of 0.2 to 0.3 log unit for all three functions and, in the orange and red, where the pilot results give a blue function appreciably higher in absolute magnitude.

The second of these differences results from the use in Pt.I of the yellow primary introduced to reduce rod intrusion in the measurements for $^1/_\lambda < 17{,}000\ \mathrm{cm}^{-1}$. The way the difference comes about is probably as follows. Suppose that, using for the middle primary a green at 19,000 cm^{-1}, spectral matches are made in a retina whose rod mechanism has in some way been prevented from acting. For any particular match, say the one represented by the matching equation (1), the quantitative effect which the two halves of the matching field would have on the rod mechanism can be estimated by multiplying the quantity in energy units of each stimulus by the scotopic (rod) luminous efficiency function V'_λ and the constant factor K' required to express the effect on the rod mechanism in scotopic trolands:

$$\begin{array}{c|c} S_u = U_\lambda V'_\lambda\ K' + D_R V'_R K' & S_l = M_G V'_G\ K' + M_B V'_B K' \\ \text{total scotopic effect} & \text{total scotopic effect} \\ \text{in upper field} & \text{in lower field} \end{array} \quad \ldots\ldots (4)$$

If the scotopic effects in the two halves come out equal then we may assume that even if the rod mechanism were not out of action, the match would be unaffected. Otherwise the possible disturbance of the match may be expected to depend to a first approximation on the difference of scotopic effect $\Delta S = S_u - S_l$, divided by the smallest perceptible or threshold difference, T_S, for a scotopic mechanism adapted to a level somewhere between S_u and S_l, a level for which the geometric mean $S = \sqrt{S_u\ S_l}$ is probably the best estimate. An approximate calculation of the ratio $\Delta S/T_S$ for spectral matches through the spectrum under the intensity conditions of the 10° pilot group data has been made using the threshold data for rod vision obtained by Aguilar and Stiles *(ref. 4)*. It is apparent *(fig. 6* circle points) that over most of the spectrum $\Delta S/T_S$ is below unity - largely because the scotopic level is high enough to lift the rod mechanism to the first stages of saturation - but in the orange and red, on either side of the red primary, it reaches a rather high value of nearly 12.

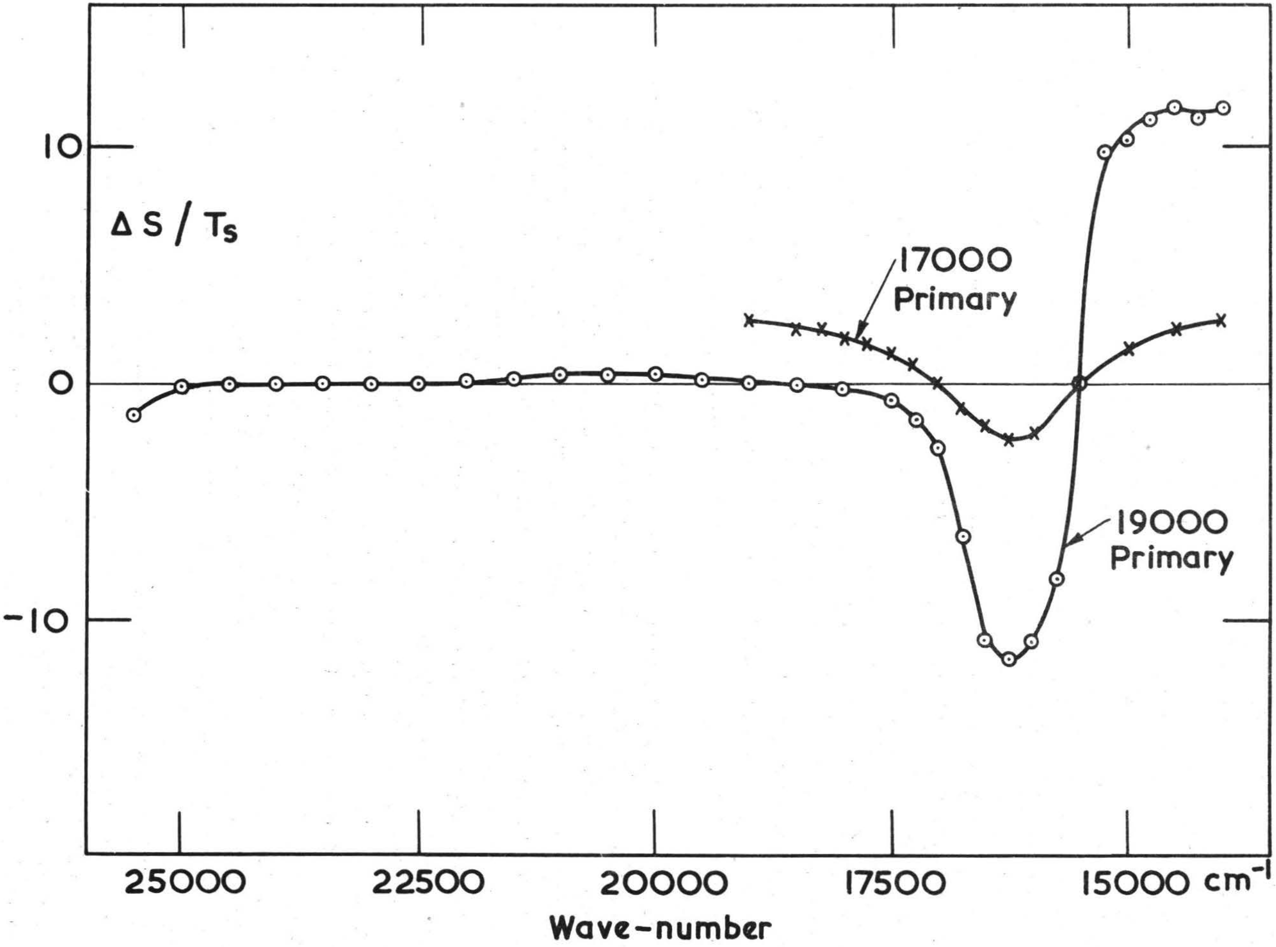

10
0
-10
Δ S / Ts
17000 Primary
19000 Primary
25000
22500
20000
17500
15000 cm⁻¹
Wave-number

FIG. 6.

While the scotopic imbalance specified by the ratio $\Delta S/T_S$ is not the sole factor determining the disturbance of the colour match - others may be considered as defining the extent to which the eye will allow an imbalance of the *cone* mechanisms in the compromise match it is forced to make in a three-control matching system - the tentative calculation just given indicates the spectral regions where rod intrusion is potentially important. The ratio $\Delta S/T_S$ is high in the orange and red because the only stimulus in either field of high scotopic value is the green primary and at the same time the mean scotopic level S is not high. The main action of the green primary is to stimulate the green cone mechanism, which has maximal sensitivity at about 18,500 cm^{-1}. To produce the same effect on this mechanism with a yellow stimulus at 17,000 cm^{-1} and a green stimulus at 19,000 cm^{-1}, the energy of the former stimulus must be about twice that of the latter. On the other hand, for the same effect on the scotopic mechanism, the energy of the 17,000 stimulus must be about thirteen times that for the 19,000. Thus for the same effect on the green cone mechanism, the yellow primary at 17,000 cm^{-1} produces a less effect on the scotopic mechanism, corresponding to an energy ratio of 1 : 6.5. A calculation of $\Delta S/T_S$ for colour-matches made with a middle primary at 17,000 cm^{-1} gives the cross points of *fig. 6*, the intensity level assumed being approximately the same as for the circle points. Potential intrusion by rod vision is materially reduced.

The modification produced by the use of the yellow primary appears principally in the blue colour-matching function, and is best seen by plotting the blue unit co-ordinate on a large scale *(fig. 8)*. Between 16,500 and 14,500 cm^{-1} the buckling of the pilot results (triangle points) away from the Pt. I values (circle points) is in general accord with what might be expected from the above discussion. In the extreme red, the diminishing field luminance will reduce the resistance of the cone mechanisms to imbalance, so that the same scotopic imbalance can produce greater distortion of the match. *Fig. 8* compares mean results for different groups of subjects, but similar differences have been established for two subjects each of whom made measurements with the green and yellow middle primary. Another device, the addition of equal amounts of green primary to both fields to raise the scotopic level, and an increase in the intensity of the matching field (see Pt. II results below) also reduce rod intrusion in a way conforming with the general picture given above.

The differences in the blue between the mean colour-matching functions of the pilot and Pt. I measurements are almost certainly unrelated to the lower field intensity used in Pt. I. They arise in the main from a difference in average properties of the two groups of subjects of a kind which would be produced by differences in lens pigmentation, i.e., they would be produced by placing in front of the same eye a colour filter

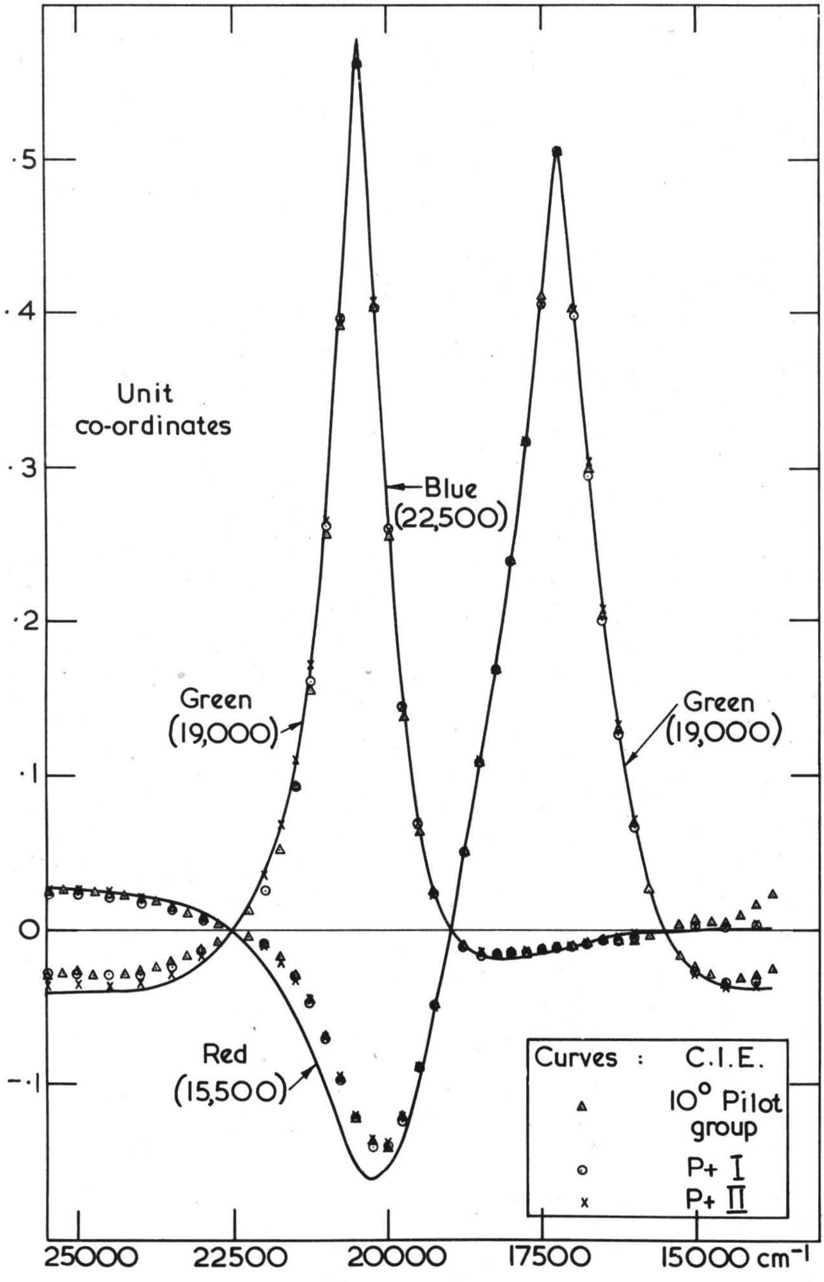

·5
·4
·3
·2
·1
0
−·1
Unit co-ordinates
Blue (22,500)
Green (19,000)
Green (19,000)
Red (15,500)
Curves : C.I.E.
▲ 10° Pilot group
○ P+ I
x P+ II
25000
22500
20000
17500
15000 cm⁻¹
Wave-number

FIG. 7.

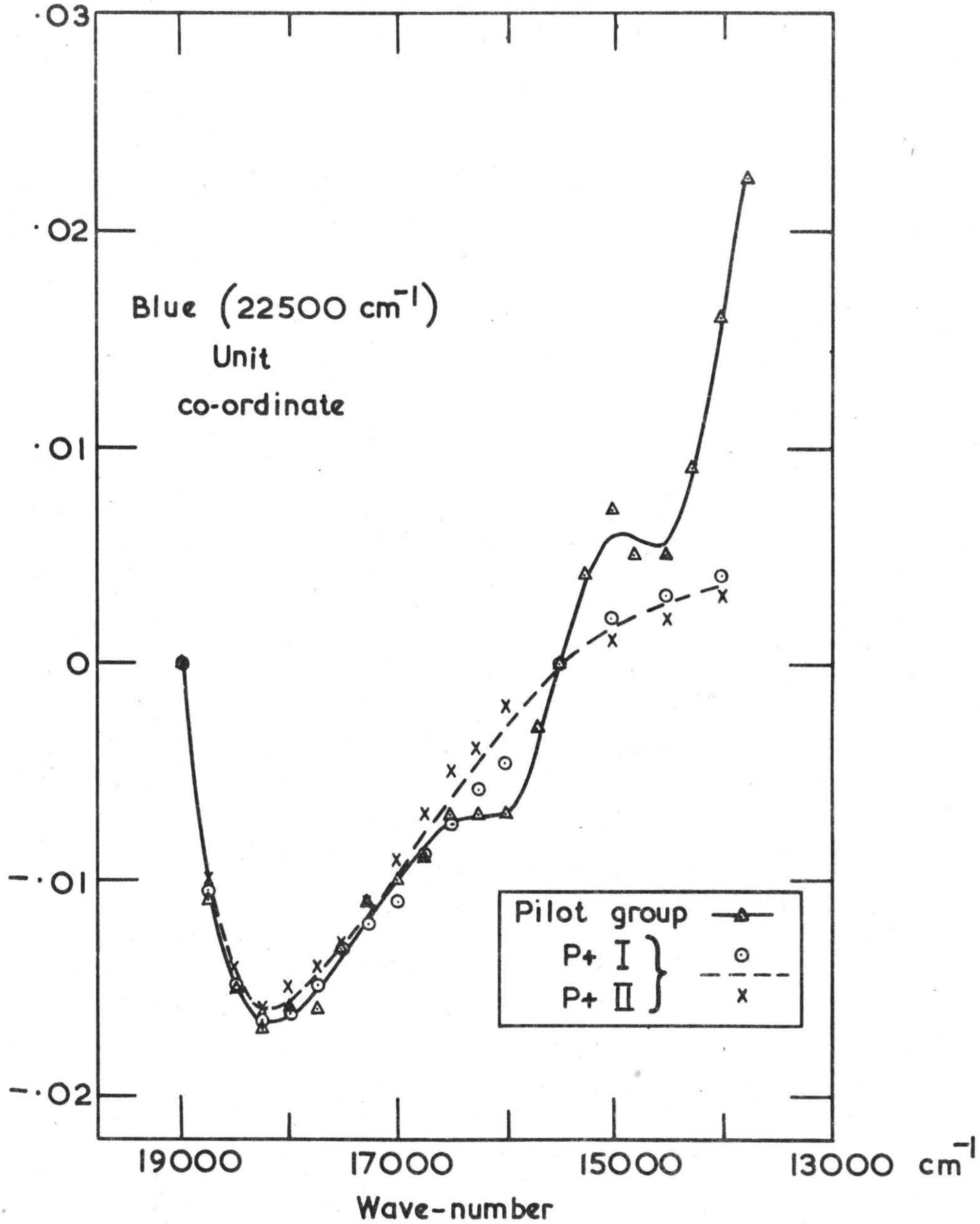

·03
·02
·01
0
-·01
-·02
Blue (22500 cm^{-1})
Unit
co-ordinate
Pilot group
P+ I
P+ II
19000
17000
15000
13000 cm^{-1}
Wave-number

FIG. 8.

which modified similarly the light reaching all the retinal receptors. This is apparent from the close agreement of the unit co-ordinates on the W.D.W. system for the two sets of data (triangle and circle points in *fig. 7*). The large individual variations of the colour-matching functions in the extreme blue can be seen from *figs. 3* to *5* where the extreme spread of the Pt. I (and Pt. II) values has been indicated by the vertical lines linked to the plotted points at selected wave-numbers. It should be noted that for each function the spread is necessarily nil at the wave-number of the corresponding reference primary.

If the general additivity law is valid it should be possible to predict the quantities of the primaries required to match the white light stimulus from the energy distribution of the latter and the colour-matching functions derived from the spectral matches. The energy distribution $\{W_\lambda \, d\lambda\}$ appropriate to the tungsten ribbon-filament lamp at the time of each subject's measurements - the distribution actually used in computing this subject's colour-matching functions - was inserted in the integrals $\int W_\lambda \, t_\lambda \, \bar{r}_\lambda \, d\lambda = \bar{r}_w$, $\int W_\lambda \, t_\lambda \, \bar{g}_\lambda \, d\lambda = \bar{g}_w$, $\int W_\lambda \, t_\lambda \, \bar{b}_\lambda \, d\lambda = \bar{b}_w$, where t_λ represents the spectral transmission of the OB9 and neutral filters placed in the test field beam when making the white light measurement. The tristimulus values $\bar{r}_w$, $\bar{g}_w$, $\bar{b}_w$, calculated in this way should equal those derived by applying to the quantities of the instrumental primaries (green run) obtained by the subject in his white light match, the transformation from instrumental to reference primaries used in deriving his green run colour-matching functions. The physical intensity of the white light stimulus was only approximately the same for each subject and the tristimulus values will vary on that account. It is convenient therefore to multiply the six quantities - $\bar{r}_w$, $\bar{g}_w$, $\bar{b}_w$, observed and calculated - by a single factor, different for each subject, which reduces the tristimulus values to those corresponding to a white light of fixed intensity. The slight changes in relative energy distribution of the white light stimulus are not taken into account by this factor which it should be noted leaves unaffected the ratio of the observed to the calculated tristimulus values. In *fig. 9* the logarithm of the ratio of the observed to the calculated tristimulus value for the *(R)*, *(G)* and *(B)* components is plotted against the logarithm of the calculated value. To consider first how far the additivity law for the group as a whole is borne out by the results for the white match, we examine the mean log ratios summarised in *Table 4*.

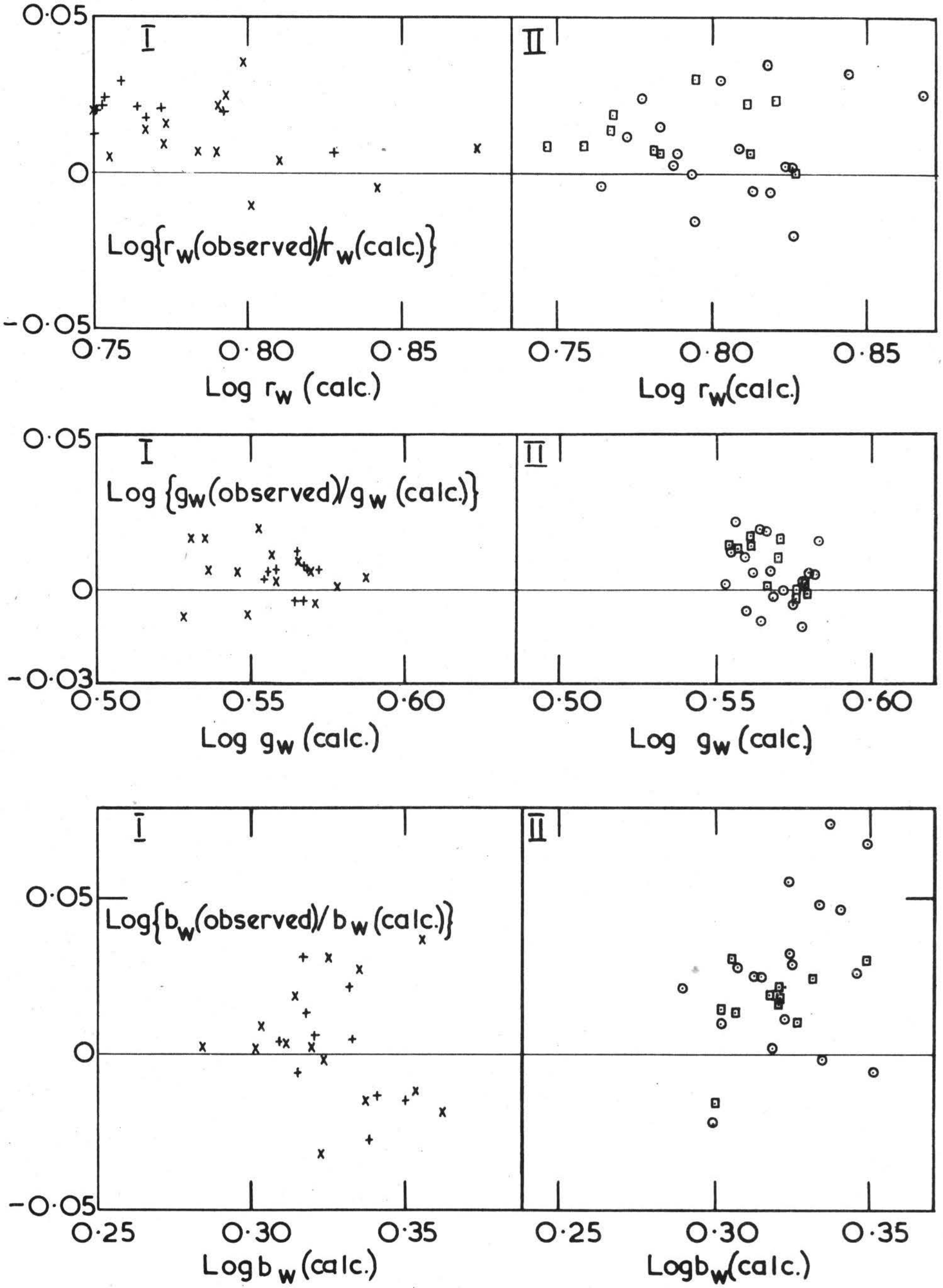

0·05
I
II
Log{r_W(observed)/r_W(calc.)}
0
-0·05
0·75
0·80
0·85
Log r_W (calc.)
0·05
Log{g_W(observed)/g_W(calc.)}
0
-0·03
0·50
0·55
0·60
Log g_W (calc.)
Log{b_W(observed)/b_W(calc.)}
0·25
0·30
0·35
Log b_W (calc.)

FIG. 9.

TABLE 4

Tristimulus values for the white stimulus

Log {ratio (observed/calculated value)}

	$(\bar{r}_w)$		$(\bar{g}_w)$		$(\bar{b}_w)$	
	Mean	Standard Deviation of the Mean	Mean	Standard Deviation of the Mean	Mean	Standard Deviation of the Mean
Pt. I	+0.0144 (0.0091)	0.0021	+0.0053 (0)	0.0015	+0.0030 (- 0.0023)	0.0037
Pt. II	+0.0094 (0.0029)	0.0026	+0.0065 (0)	0.0017	+0.0229 (0.0164)	0.0040

Instrumental errors are not negligible here but it is easy to see that some sources of such errors, notably those affecting the *relative* energy distribution of the ribbon filament, are automatically eliminated by the method of producing the white light stimulus from the recombination of the spectral stimuli used in the spectral colour-matching observations. There remain residual errors in the calibrated densities of the primary wedges and in the filters (OB9 and neutral) inserted in the white light beam. The effective width of the slit used in the central spectrum of the test tier for the spectral energy measurements is also involved in the calculated tristimulus values. This width was determined by a photometric comparison in the apparatus of the slit actually used (0.66 mm) with a larger slit (3.1 mm) for which the effective width and the easily measured geometric width could be assumed to agree closely. The total instrumental error from all causes in the log ratio of observed to integrated tristimulus values is estimated as unlikely to exceed 0.008 log unit. On taking also into account the effect of individual differences on the means, indicated by the standard deviations of the latter, it is only for the red tristimulus value that there is fairly strong evidence of failure of the additive law for the group as a whole. If the estimated maximum instrumental error acts to reduce the discrepancy for the red tristimulus value there remains a difference of (0.0145 - 0.0080) = 0.0065 log unit (or 1.5%) which differs significantly from zero at about the 0.001 level.

Although the amount of the deviation from the additive law is not striking for the group taken as a whole, the individual deviations, particularly for the blue component, are large for some subjects - up to about 0.04 log unit, and will be considered after the Pt. II results have been given.

RESULTS: PT.II. BLUE PRIMARY AT 21,250 cm^{-1}

IN *figs. 3-5* the lower curves represent the logarithms of the mean colour-matching functions of Pt.I while the associated circle and cross points are the logarithms of the mean values obtained respectively in the green and yellow runs of Pt.II.

In both Pts.I and II, some yellow run measurements were made at wavenumbers in the range 17,000 to 19,000 cm^{-1} although they are not strictly required, and in fact cannot be expected to give as satisfactory results as the green run values in this range. This is because large amounts of desaturating red primary have to be used in these matches and in transformation to the reference primaries the red colour-matching function is determined by a difference of large quantities, small errors in which become important. Taking the averages for all subjects in each group, we should not expect any considerable systematic difference from the green run values and little is observed in the results for Pt.I (see upper graphs in *figs. 3-5*). But for Pt.II there is a marked discrepancy in the red colour-matching function, a discrepancy which increases as 19,000 cm^{-1} is approached so that at this wavenumber the yellow run value of $\bar{r}_\lambda$ instead of being zero equals 0.23. To assess the importance of the discrepancy suppose that, in the green run, unit energy at 17,000 cm^{-1} matches the mixture $\alpha(R) + \beta(G) - \gamma(B)$ and, in the yellow run, unit energy at 19,000 cm^{-1} matches the mixture $-u(R) + v(Y) + w(B)$. The mixture of (R), (G) and (B) to match unit energy of 19,000 cm^{-1} as derived from the yellow run match is then, by substitution,

$$-u(R) + v\{\alpha(R) + \beta(G) - \gamma(B)\} + w(B)$$

or

$$\overline{\alpha v - u}\,(R) + v\beta(G) + \overline{w - v\gamma}\,(B),$$

and for complete consistency with the green run measurement at 17,000 cm^{-1} we should have

$$\overline{\alpha v - u} = 0, \quad \beta v = 1, \quad \overline{w - v\gamma} = 0.$$

Insertion of numerical values shows that $\overline{\alpha v - u}$ is the difference of two quantities each equal to about 6.6. In Pt.II, the mean value actually found for this difference - which is the value of the red colour-matching function at 19,000 cm^{-1} derived from the yellow run - is 0.23. If the inconsistency is attributable in its entirety to one of the quantities, v, u, α, it would be produced by a discrepancy in its value of $\frac{.23}{6.6}.100 = 3.5$ per cent. In Pt.I the corresponding discrepancy is only (-0.7) per cent. For $v\beta$, the mean Pt.II value is 1.029 instead of unity (0.991 in Pt.I), and again some inconsistency of the green and yellow runs of Pt.II is shown. The same is true for the blue colour-matching function at 19,000 cm^{-1} defined by $\overline{w - v\gamma}$.

Formally the match on 17,000 cm^{-1} in the green run and on 19,000 cm^{-1} in the yellow run are the same match. But in fact the intensity used at wave-numbers between 17,250 and 19,000 cm^{-1} in the Pt.II yellow run was considerably lower (0.7 to 0.8 log unit) than in the Pt. II green run. In Pt. I, the intensities used in both green and yellow runs were lower than in Pt. II and the intensity difference between green and yellow runs was smaller. The inconsistencies of the Pt .II yellow and green run values between 17,250 and 19,000 cm^{-1} are probably a consequence of the different field intensities. More direct evidence on this point is given below.

The observed and calculated tristimulus values for the white light stimulus obtained in Pt. II are compared in *fig. 9* and *Table 4*. If a maximum allowance of 0.008 log unit is made for instrumental error, there is no significant difference between the mean observed and mean calculated tristimulus values for the green and probably for the red component but for the blue component the observed mean exceeds the calculated mean by not less than $0.0228 - 0.0080 = 0.0148$ log unit (3.5 per cent), and is significant at the 0.001 level.

Additivity defect in the *relative* tristimulus values for white can be shown by normalising the log ratios to zero for the green component (bracketed figures in *Table 4*). Possible instrumental error will be less for these relative values and probably does not exceed 0.004 log unit.

If the mean white light results from Pts. I and II are considered together, it appears that in the metameric match of a continuous white with a mixture of red, green and blue spectral primaries, the white appears slightly redder than would be predicted from the spectral data, and also slightly bluer if the blue primary used is a greenish blue.

Apart from the deviations from additivity shown by the groups as a whole, there is an unexpectedly large spread in the individual values of the log ratio of observed to calculated tristimulus value, particularly for the blue component. As many of the subjects were new to colour-matching, a selection of about ten subjects who had considerable experience or who showed particular aptitude in colour-matching was made in each Part. Their values of the log ratio are distinguished by different point symbols in *fig. 9*. (plus-sign crosses in Pt. I, squares in Pt. II). There is perhaps a rather smaller spread in the log ratio for the "experienced" compared with the "inexperienced" subjects, notably in the blue component of Pt. II, but the conclusions on the average defect from additivity are substantially the same if based on the experienced subjects only.

RESULTS: COMPARISON OF MEAN COLOUR-MATCHING FUNCTIONS OF PARTS I AND II

A more detailed comparison of the colour-matching functions of Parts I and II is obtained by plotting for each function the logarithm of the ratio of the mean Pt. II to the mean Pt. I value *(fig. 10)*. (For clarity the curves for the green and red functions in *fig. 10* have been displaced upwards by 0.1 and 0.2 log unit respectively.) The log ratio necessarily equals zero

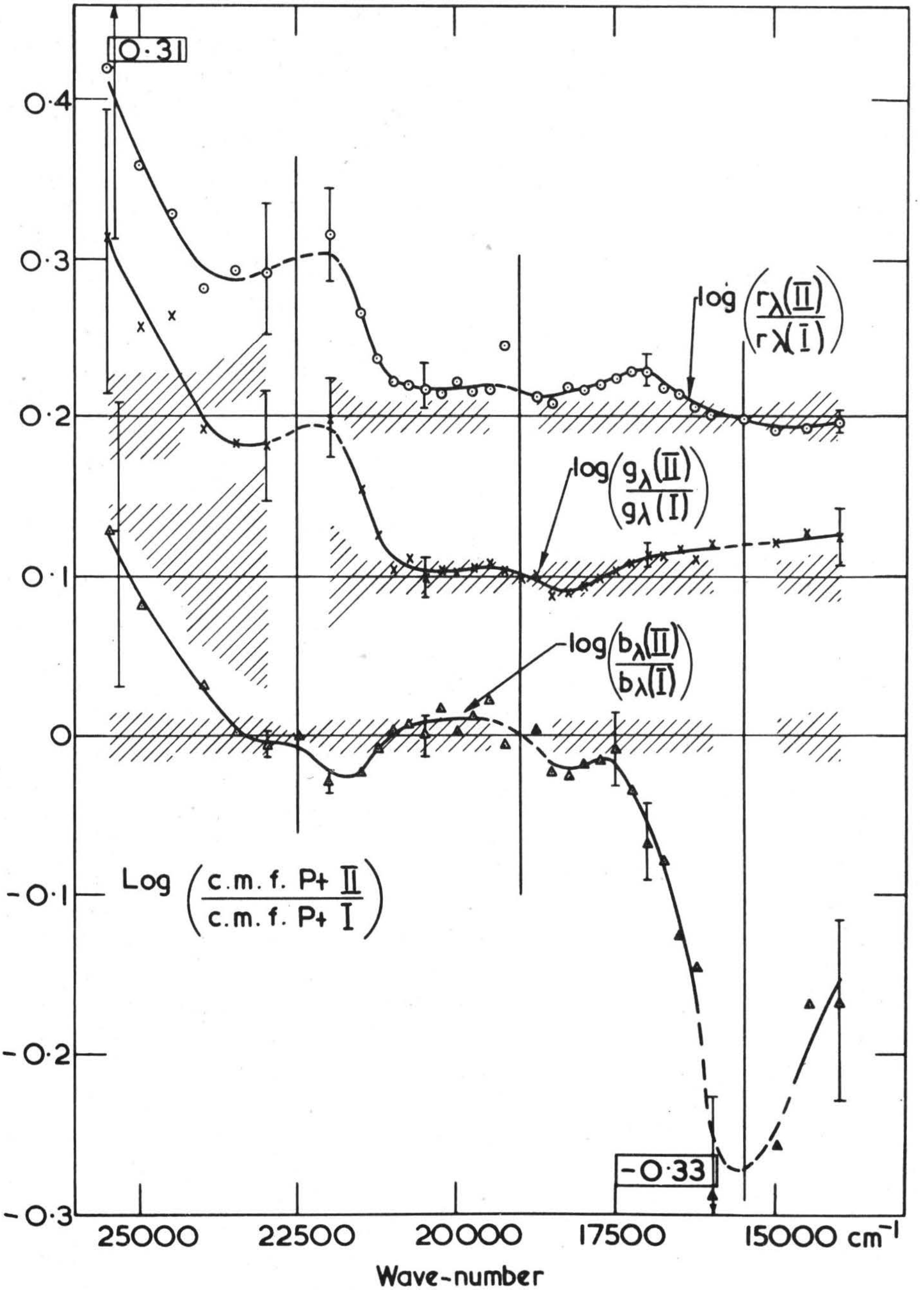

0·31
0·4
0·3
0·2
0·1
0
-0·1
-0·2
-0·3
$\log\left(\frac{r_\lambda(\mathrm{II})}{r_\lambda(\mathrm{I})}\right)$
$\log\left(\frac{g_\lambda(\mathrm{II})}{g_\lambda(\mathrm{I})}\right)$
$\log\left(\frac{b_\lambda(\mathrm{II})}{b_\lambda(\mathrm{I})}\right)$
$\text{Log}\left(\frac{\text{c.m.f. Pt II}}{\text{c.m.f. Pt I}}\right)$
-0·33
25000
22500
20000
17500
15000 cm⁻¹
Wave-number

FIG. 10.

at the corresponding reference primary and ideally it should remain zero through the spectrum except at the wave-numbers of the other two reference primaries where it becomes indeterminate. There are three factors to be considered in the present comparison: (a) instrumental errors, (b) differences in the average properties of the two groups of subjects, (c) differences in the matching conditions. While a statement of residual instrumental errors in statistical terms is difficult, an approximate estimate of the amount by which the log ratio might differ from zero as a result of such errors is shown by the shaded area. The considerable increase in the estimated possible instrumental error in the range 21,500 to 25,500 cm^{-1} for the green and red log ratios, is a consequence of an amplification of small errors in transforming the Pt. II data from the instrumental blue primary at 21,250 cm^{-1} to the blue reference primary at 22,500 cm^{-1}.

By computing for selected wave-numbers the standard deviation of the colour-matching functions in each Part and by then deriving the standard deviation of the ratio of the means (shown by the vertical lines through some of the points in *fig. 10*), the possible contribution of sampling error to the departures of the log ratios from zero, is obtained. In *Table 5* the statistical significance of these departures from zero has been evaluated (a) treating instrumental error as nil (b) assuming the observed deviation reduced by the full estimated instrumental error.

TABLE 5

Significance of the deviation from zero of
log (ratio of mean colour-matching functions Pt. II/Pt. I)

(A assumes instrumental error to be nil, B assumes the observed deviation to be reduced by the full estimated instrumental error. The figures give the level of significance, i.e., the probability that the deviation might occur by chance.)

$1/\lambda$	$\bar{r}_\lambda$		$\bar{g}_\lambda$		$\bar{b}_\lambda$	
	A	B	A	B	A	B
14,000	N.S.	N.S.	N.S.	N.S.	0.005	0.010
16,000	-	-	-	-	<0.001	<0.001
17,000	0.002	0.040	0.030	N.S.	0.008	0.020
20,500	0.030	N.S.	N.S.	N.S.	N.S.	N.S.
22,000	<0.001	0.003	<0.001	0.020	<0.001	0.020
23,000	0.050	N.S.	0.020	N.S.	N.S.	N.S.
25,500	0.050	N.S.	0.040	N.S.	N.S.	N.S.

(Probabilities greater than 0.05 are shown as N.S. - not significant.)

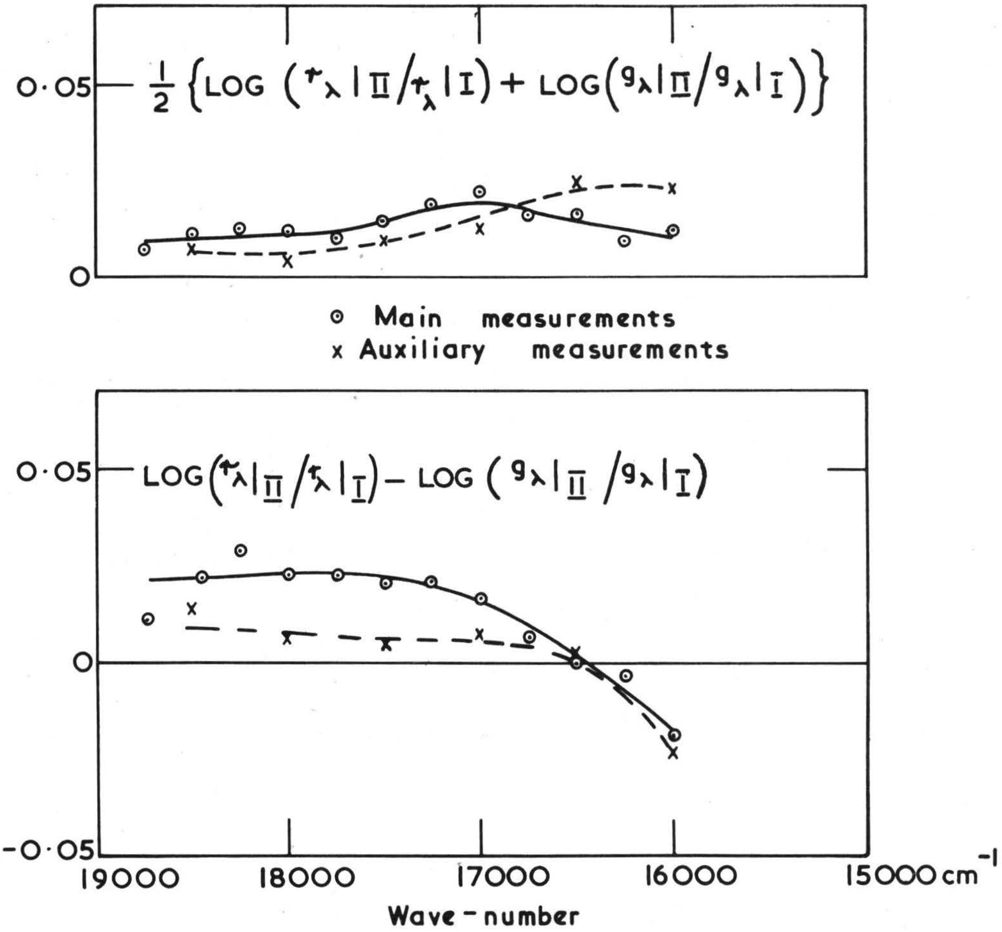

0·05
$\frac{1}{2}\left\{\mathrm{LOG}\left(t_\lambda|_{II}/t_\lambda|_{I}\right)+\mathrm{LOG}\left(g_\lambda|_{II}/g_\lambda|_{I}\right)\right\}$
0
⊙ Main measurements
x Auxiliary measurements
0·05
$\mathrm{LOG}\left(t_\lambda|_{II}/t_\lambda|_{I}\right)-\mathrm{LOG}\left(g_\lambda|_{II}/g_\lambda|_{I}\right)$
0
-0·05
19000
18000
17000
16000
15000 cm^{-1}
Wave-number

FIG. 11

The highly significant dip in the blue curve near 16,000 cm^{-1} indicates that in Pt. II where higher field intensities were used, the matches correspond to only about one half as much desaturating blue as in Pt. I, and this would be consistent with a further reduction of rod intrusion beyond that obtained merely by the use of the yellow primary at the lower intensities of Pt. I (see *fig. 8*).

More unexpected is the higher value of the red colour-matching function of Part II in the yellow, around 17,000 cm^{-1}, where it exceeds the Part I value by about 7 per cent. There is a smaller rise in the Pt. II green colour-matching function at 17,000 cm^{-1}, and it can be seen from *Table 5* that these effects are probably significant and not attributable to different average properties of the two groups. An auxiliary set of measurements was made on eight subjects to check directly whether the different matching conditions of Pts. I and II would produce the kind of effect observed. Each subject made matches in the range 15,500 to 19,000 cm^{-1} (a) under Pt. I (green run) conditions and (b) under Pt. II (green run) conditions. Thus the same red and green primaries were used throughout but the desaturating blue primary was changed from 22,450 to 21,250 cm^{-1} for the (b) measurements. The field intensities for the latter were set at the Pt. II values and the lower intensities for the (a) measurements were obtained by inserting between the eye and the complete field a rotating sector of density approximately 0.8 log unit. *Table 6* gives the means for the eight subjects of the log ratio of the red mixture components at match under the (a) and (b) conditions respectively, and the corresponding quantities for the green mixture component. A small correction to these log ratios is necessary to convert them to log ratios of colour-matching functions referred to the common basis of the usual reference primaries. This is because it follows from the transformation based on the usual additivity assumption that the amount of desaturating blue in the match, although small, is just large enough for the use of a 21,250 instead of a 22,450 cm^{-1} primary to alter the quantities of red and green in the match. An allowance can be made for this small difference on the basis of the mean Pt. I and Pt. II results. The corrected log ratios are included in *Table 6*. It is clear from this Table that the (b) measurements (Pt. II conditions) lead to red and green colour-matching functions which are a few per cent larger than for the (a) measurements (Pt. I conditions). The effect is rather smaller but in the same sense as that found in the main measurements.

The auxiliary measurements are not entirely in agreement with the main results and this is best seen by considering (i) the average of the red and green values of the log ratio given in *Table 6* and (ii) the difference of these values. *Fig. 11* shows how these quantities compare with the similar quantities derived from the main measurements. If the change of conditions left unaltered the proportions of red and green in the match the difference of the log ratios for the red and green components would be zero, and the

TABLE 6

Results of auxiliary tests on the red and green colour-matching functions in the range 15,500 to 19,000 cm^{-1}.

	Red Function			Green Function		
	$\text{Log}\left(\text{ratio } \frac{\text{(b) value}}{\text{(a) value}}\right)$			$\text{Log}\left(\text{ratio } \frac{\text{(b) value}}{\text{(a) value}}\right)$		
$^1/_\lambda$	Mean Log Ratio	Corrected Mean	Standard Deviation of the Mean	Mean Log Ratio	Corrected Mean	Standard Deviation of the Mean
18,500	0.010_3	0.013_5	0.003_8	0.001_6	0.000_3	0.002_8
18,000	0.005_1	0.006_7	0.003_3	0.002_6	0.000_9	0.003_2
17,500	0.010_2	0.011_0	0.006_9	0.007_8	0.006_1	0.002_8
17,000	0.016_1	0.016_5	0.004_4	0.011_2	0.009_2	0.003_0
16,500	0.025_3	0.025_5	0.004_3	0.025_2	0.022_8	0.005_9
16,000	0.010_3	0.010_4	0.002_8	0.037_2	0.033_5	0.006_5

average log ratio would represent the apparent increase in brightness of the yellow or orange test colour, compared with the mixture of primaries, in going from Pt.I or (a) to Pt.II or (b) conditions. The auxiliary measurements are in fair agreement with the main measurements as regards the "change of apparent brightness" but show a smaller change in the red-green ratio from 18,500 to 17,000 cm^{-1}. The main measurements give a change of red/green ratio of about 5 per cent in this range, but it must be recalled that here the comparison is being made for different groups of subjects. An independent calculation of the spread of the ratio of the red to green colour-matching functions at 17,000 cm^{-1} among the subjects of Pt.I and Pt.II, gave the following result:

$$\log \frac{\bar{r}_\lambda}{\bar{g}_\lambda} \quad \text{at } 17{,}000 \text{ cm}^{-1}$$

	Mean 0.823	Standard Deviation of the Mean
Pt.I	0.823	0.0078
Pt.II	0.808	0.0130

The difference of the means for Pts. I and II - 0.015 log unit - is not significant at the 0.05 level. The lower graph of *fig. 11* shows that both in the main and the auxiliary measurements the red/green ratio at the wave-number 16,000 cm^{-1} is relatively lower under Pt. II as compared with Pt. I conditions, and in the main measurements this trend continues to the red limit at 14,000 cm^{-1}. The result may be a consequence of the change of blue primary rather than of the change of intensity but insufficient tests have yet been made to judge.

The small apparent change of brightness of a yellow test stimulus compared with the red-green mixture used to match it, when the conditions are changed from Pt. I to Pt. II is of the right sign and right amount to explain the inconsistency of the green and yellow runs of Pt. II in the range 17,000 to 19,000 cm^{-1} which has already been discussed. This inconsistency corresponds to the red and green mixture components having values 3 to 4 per cent greater in a match on yellow at 17,000 cm^{-1} under the high intensity conditions of the green run than in the equivalent match (test colour at 19,000 cm^{-1}) at lower intensity in the yellow run.

Reverting to *fig. 10*, the rise in all three curves in the violet is largely the result of a chance difference of average pigmentation of the Pt. I and Pt. II groups of subjects. For the blue colour-matching function the log ratio at 25,500 cm^{-1} is not significantly different from zero when account is taken of the spread among the subjects (*Table 5*). For the red and green log ratios, however, subject spread does not account completely for the deviation from zero of the log ratio at 25,500 cm^{-1}, and at 22,000 cm^{-1} the log ratios for all three functions show significant deviations after subtracting the estimated instrumental error. These deviations must I think be attributed to the different location of the blue primary in Pts. I and II. Formally they imply imperfect additivity in complete colour-matching in the short-wave end of the spectrum for the conditions of the present measurements. It must be recalled that in this spectral range the Maxwell spot is clearly visible and the subject in making the large field match ignores as far as he can the non-uniformity of colour at the centre of the field. In fact, his settings may always be a compromise between a match over the main area and a match over the central patch of the field. It could be that additivity was accurately true for any small area, extrafoveal or foveal, but only approximately true when with a large matching field a compromise, conscious or otherwise, has to be made in equating areas in each of which the external stimulus is uniform while the retinal response varies in different parts.

In the above discussion of the mean colour-matching functions it was inferred that the differences between Pts. I and II were not solely the result of differences in average pigmentation. This is borne out by the plot of mean unit co-ordinates *(fig. 7)*. If subjects differed only in their pigmentation, they would give the same unit co-ordinates. The differences between the mean Pt. I and mean Pt. II values of the unit co-ordinates (circle and cross points in *fig. 7*) at short wavelengths are most apparent in the green and blue co-ordinates, the red being little changed. For Pt. II the green unit co-ordinate has an appreciably higher absolute value on either side of the blue primary. From the spread of the individual values and from the estimated effect of instrumental error, it is found that the difference is significant at better than the 0.05 level (full calculations of the significance of the differences in the unit co-ordinates are not yet complete). The rather close agreement in the same spectral range of the pilot group data (triangle points) with Pt. I results (circle points) - both obtained with the instrumental blue primary at 22,450 cm^{-1} - may be noted. In the previous measurements made on the effect of a change in location of the blue primary *(ref. 2)*, three subjects determined 10^0 instrumental colour-matching functions (a) with a blue primary at 22,450 cm^{-1} (b) with a blue primary at 21,750 cm^{-1}, the red and green primaries being in both cases at 15,420 and 19,000 cm^{-1} respectively. On transformation of the results to the standard reference primaries and computation of unit co-ordinates, the mean values showed a small change of very similar character to that found between the Pt. I and Pt. II results. The mean red unit co-ordinate was little changed but the green unit co-ordinate on either side of the blue reference primary was greater in absolute value for the "21,750" observations, by an amount equal very approximately to 0.6 of the excess of the Pt. II over the Pt. I value of the green unit co-ordinate at the corresponding wave-number. Thus these earlier results confirm that there is a small effect on the colour-matching functions when the wavelength of the instrumental blue primary is changed.

It is clear from *fig. 7* that the Pilot, Pt. I and Pt. II results all agree in showing a marked deviation of the red unit co-ordinate from the C.I.E. values, represented by the continuous curve, in the spectral range 20,000 to 22,500 cm^{-1}. The colour-matching data for the 10^0 field must differ from the C.I.E. data, which refer to a 2^0 field, in a more radical way than can be represented by a difference in pigmentation.

RESULTS: AGE EFFECTS; LUMINOSITY FACTORS

A fuller analysis of the colour-matching data particularly in respect of age of subject will be available at a later date. One result of interest is the following. In the yellow (17,000 cm^{-1}), the red/green ratio ($\bar{r}_\lambda/\bar{g}_\lambda$) tends to decrease with age. This trend appears in the results of both Pts. I and II. The red/green ratio diminishes both from an increase in $\bar{g}_\lambda$ and a decrease - rather smaller - in $\bar{r}_\lambda$. Between age groups 16-23 and 43-55 *(Table 2)* the ratio falls by about 13 per cent. The macular pigment will hardly have commenced to absorb at the wavelength of the green primary 526 mμ.

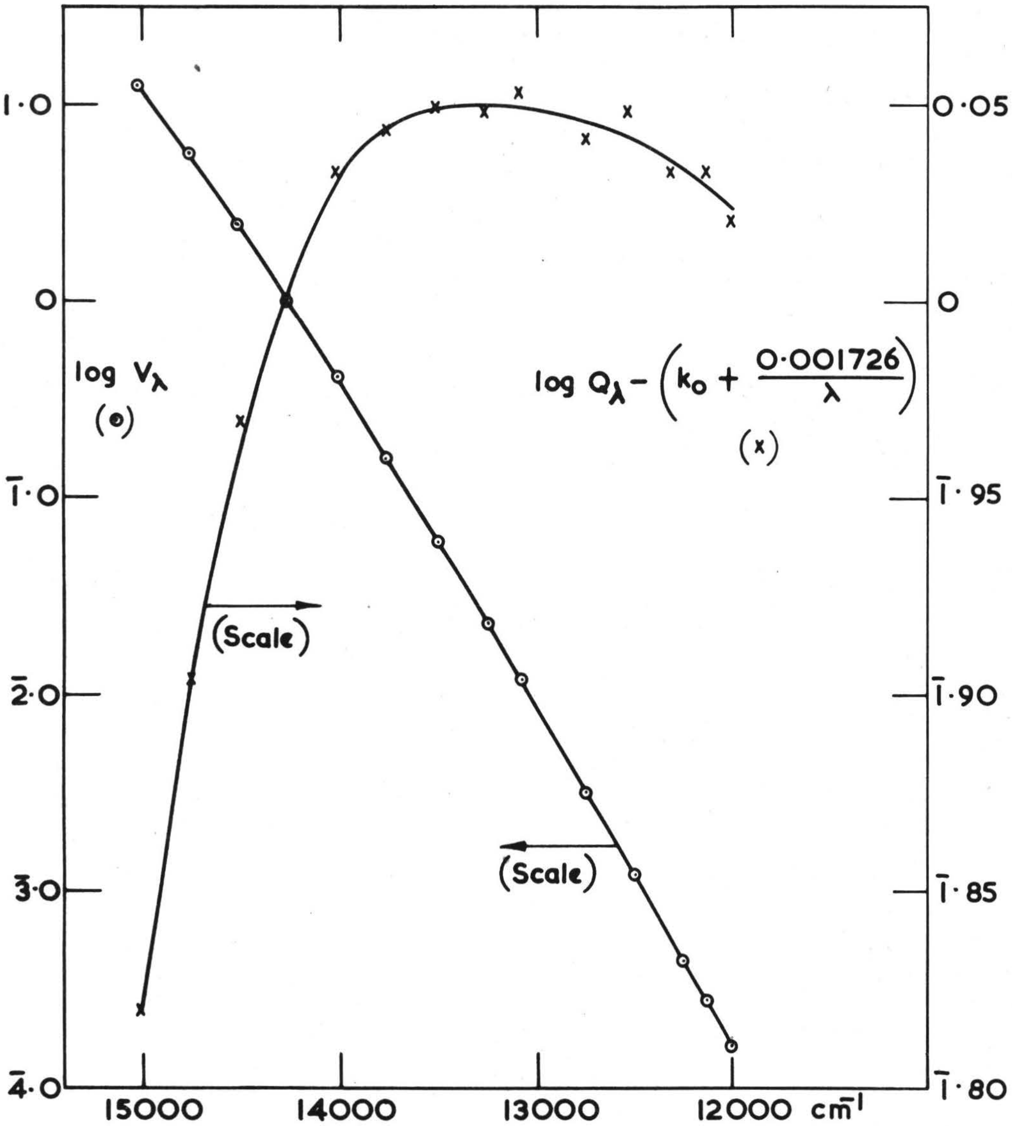

1·0
0
$\bar{1}$·0
$\bar{2}$·0
$\bar{3}$·0
$\bar{4}$·0
0·05
0
$\bar{1}$·95
$\bar{1}$·90
$\bar{1}$·85
$\bar{1}$·80
log V_λ
(⊙)
log $Q_\lambda - \left(k_0 + \frac{0\cdot001726}{\lambda}\right)$
(x)
(Scale)
(Scale)
15000
14000
13000
12000 cm^{-1}

FIG. 12.

but the absorption curve of the lens *(ref. 5)* has the form which would be roughly consistent with the observed change. Correlation of the variation of the red/green ratio at 17,000 cm^{-1} with the large variations in the colour-matching functions at 25,500 cm^{-1} should throw light on this point.

The relative luminosity factors of the primaries measured by direct comparison with white led to the following mean log values:

	log (L15,500/L19,000)	log (L22,500/L19,000)	log (L21,250/L19,000)
Pt. I	$\bar{1}.37$	$\bar{2}.87$	-
Pt. II	$\bar{1}.32$	-	$\bar{1}.36$

No great weight can be placed on these measurements, because relatively inexperienced subjects find the distinction between luminosity and vividness difficult to make in these violently heterochromatic matches.

RESULTS: PT. III. MEASUREMENTS AT LONG WAVELENGTHS

THE colour differences involved in the heterochromatic match of monochromatic stimuli in the range 15,000 to 12,000 cm^{-1} with a comparison stimulus at 14,250 cm^{-1} are too small to cause any difficulty. The mean results of the Pt. III(a) measurements are presented in *fig. 12* as a plot of log V_λ against $\frac{1}{\lambda}$, the value of V_λ at 14,250 cm^{-1} being taken as unity. V_λ is inversely proportional to the intensity in energy units of stimuli of different wavelengths producing the same sensation of brightness. If intensity is expressed in quantum units a related quantity $Q_\lambda = \text{const.} V_\lambda (1/\lambda)$ is obtained which has some theoretical advantage. The nearly linear descent of log Q_λ (or indeed of log V_λ) as $\frac{1}{\lambda}$ diminishes over a considerable range in the red is shown by forming the difference of log Q_λ and the quantity $(k_0 + k/\lambda)$ where k is chosen to make the difference constant over the widest possible range of wave-number. With $k = 0.001726$ the difference $\{\log Q_\lambda - (k_0 + k/\lambda)\}$ is constant within ± 0.01 log unit from 14,000 to 12,200 cm^{-1} *(fig.12)* but it tends to diminish at the longest wavelengths, i.e., the steepness of descent of log Q_λ increases. A feature of these measurements at long wavelengths is the smallness of the individual variations, in contrast to the very wide variations at short wavelengths. The standard deviations of the mean for log V_λ at several wave-numbers are given in *Table 7* which also shows the approximate intensities in C.I.E. trolands at which the measurements were made.

TABLE 7

$\frac{1}{\lambda}$	Field intensity C.I.E. trolands	Standard deviation of mean log V_λ
15,000	140	0.0038
14,250	110	(0.)
13,081	18	0.0038
12,133	1.6	0.0044

For three subjects observations were also made at a constant luminance level of the low value available at 12,133 cm^{-1}. This produced no material difference in the results.

In Pt.III(b) the following measurements were made for each of four test colours at 15,000, 14,000, 13,081 and 12,133 cm^{-1}: (i) a heterochromatic brightness match with the red primary at 14,250 cm^{-1}, (ii) a two-control colour match with a mixture of primaries at 14,250 and 17,000 cm^{-1}, (iii) a complete three-control colour match with the primaries 14,250, 17,000 and 21,250 cm^{-1}. The amounts of the red and yellow primaries required in (ii) and (iii) were not distinguishable. The addition of the blue primary in (iii) improved the match although at 12,133 cm^{-1} complete satisfaction was not obtained. In the two-control match at this wave-number the mixture field appeared too blue, but as desaturating blue was added to the test colour, only an optimum setting was possible before the test field appeared whiter (less saturated) than the mixture field.

To obtain sufficient intensity to make colour-matches at 13,081 and 12,133 cm^{-1} the slits had to be widened to give total band widths of 740 cm^{-1} and 890 cm^{-1} respectively. This entailed slit width corrections which were made by an iterative method in computing the results. Details of the calculation need not be given but the main steps were as follows.

(a) By comparison of the quantities of red primary required on the one hand in the heterochromatic match (i) and on the other in the colour matches (ii) and (iii), the ratio $\bar{r}'_\lambda/V_\lambda$ of the red colour matching function $\bar{r}'_\lambda$ in a system of primaries at 14,000, 17,000, 21,250 cm^{-1}, to the V_λ function, was determined. (Strictly the ratio obtained was $\bar{r}''_\lambda/V_\lambda$ where $\bar{r}''_\lambda$ is the red function in the primary system (14,250, 17,000, 21,250) but the difference of the ratios apart from a constant factor is negligible, and the primary system with 14,000 cm^{-1} is the one later required.) Using this ratio, which varies only by about 10 per cent between 15,000 and 12,000 cm^{-1}, the V_λ measurements of Pt.III(a) were converted into $\bar{r}'_\lambda$ values.

(b) From the quantities of red, yellow and blue primaries in the matches under (ii) and (iii) the ratio of the yellow to the red colour-matching functions, $\bar{y}'_\lambda/\bar{r}'_\lambda$, and the blue to the red functions, $\bar{b}'_\lambda/\bar{r}'_\lambda$, all in the system (14,000, 17,000, 21,250), were determined and corrected for slit width. As $\overline{r}'_\lambda$ was known from (a), $\overline{y}'_\lambda$ and $\overline{b}'_\lambda$ could then be determined.

(c) The values of the colour-matching functions in the reference system (15,500, 19,000, 22,500) for test stimuli at 14,000, 17,000 and 21,250 cm^{-1} were obtained from the mean of the Pt. I and Pt. II results and provided the transformation matrix for converting $\overline{r}'_\lambda$, $\overline{y}'_\lambda$, $\overline{b}'_\lambda$ to $\overline{r}_\lambda$, $\overline{g}_\lambda$, $\overline{b}_\lambda$.

The most interesting aspect of the results is shown in *fig. 13* where the mean green and blue unit co-ordinates in the standard reference system as obtained from the deep red colour matches are plotted, together with the overlapping, mean Pt. I and Pt. II values. At the link wave-number 14,000 cm^{-1} and, of course, at the reference primary 15,500 cm^{-1}, the Pt. III and the mean Pt. I and Pt. II results must coincide. The agreement at the intermediate point - 15,000 cm^{-1} - is satisfactory. The green unit co-ordinate has its greatest negative value at about 14,350 cm^{-1} (697 mμ) and at longer wavelengths it becomes progressively less negative. This means that the wavelengths beyond 697 mμ become yellower, a phenomenon discovered by Brindley *(ref. 3)*. Brindley, who used a bipartite field of 3° 20' by 6° 10', found the reddest wavelength at about 700 mμ and he determined pairs of wavelengths, one above and one below 700 mμ, which had the same colour. These have been included in *fig. 13* by placing the short-wave member of the pair on the curve of the green unit co-ordinate and plotting the long-wave member as a point at the same green co-ordinate value. Compared in this way our mean results show a rather larger effect than Brindley's but in view of the difference in field size and the higher intensities used by him, the agreement is not unsatisfactory. Our measurements were made at the following intensities:

$^1/_\lambda$ (cm^{-1})	15,000	14,000	13,081	12,133
Intensity in photopic trolands	150	150	150	12

With the intensities available from the trichromator it was not possible to make very satisfactory colour measurements down to 12,133 cm^{-1} when the field size was reduced to 2°. But several subjects made observations and obtained a yellowing at 12,133 cm^{-1}.

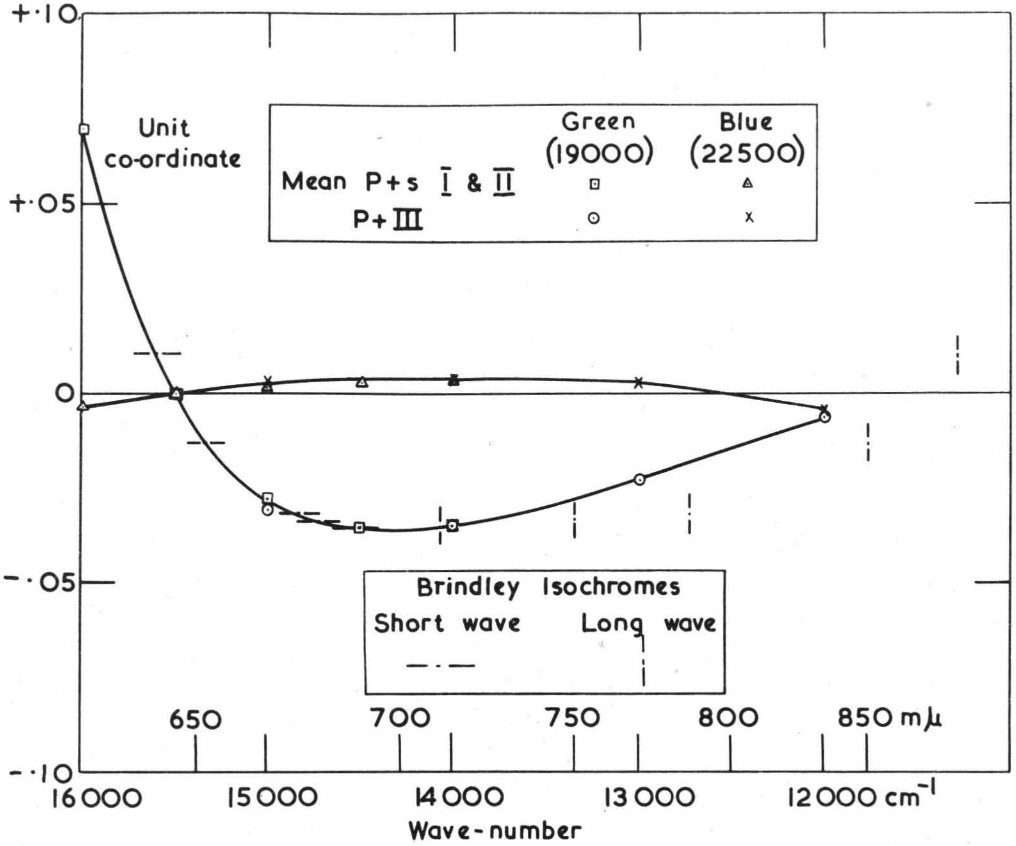

+·10
+·05
0
-·05
-·10
Unit co-ordinate
Green (19000)
Blue (22500)
Mean P+s I & II ⊡ △
P+III ⊙ x
Brindley Isochromes
Short wave —·—
Long wave ¦
650
700
750
800
850 mμ
16000
15000
14000
13000
12000 cm⁻¹
Wave-number

FIG. 13.

CONCLUDING REMARKS

THIS investigation was undertaken with the possibility in mind that the data obtained might be used, in conjunction with similar results by others, to define a standard system for large field colorimetry in a revision of the present C.I.E. system. Any recommendation in this direction would lie with the Working Party on Colorimetry of the C.I.E. and would have to take account not only of spectral measurements like the present ones but also of "field trials" under conditions nearer to those of every day practice. But some comment on the problem in the light of the results given above may be offered.

In several respects large field colour-matching has been found to deviate from an ideal linear three-variable system. In the first place, special measures (use of yellow primary and high intensity) were found necessary to obtain colour-matching functions free, or nearly so, from the effects of rod vision. There is no doubt that for certain pairs of stimuli, metameric according to these rod-free colour-matching functions, the inequality of rod stimulation may in fact produce small differences of colour even at luminances which evaluated on a photopic basis are moderately high, of the order of several hundred trolands. This seems to be an unavoidable limitation of large field colorimetry. But the metameric pairs for which this may happen are rather extreme and probably of little practical importance. A rough working rule, based on the method used in discussing the device of the yellow primary, could no doubt be applied to show when rod vision may modify a predicted match, although the determination of the amount of the modification would be less easy.

Certain defects of additivity have been observed both in the spectral matches and in the white light matches, and it may be asked to what extent such differences, assuming they are not the result of some undetected error in the work, should stand in the way of defining a standard observer for large-field colorimetry. Probably the criterion must be something on the following lines: the lack of additivity should not lead to worse discrepancies between the average observed and the calculated matches than would occur on the average by substituting one subject's match for another's. For the white match results of *fig. 9* this would correspond very roughly to comparing the displacement, above or below the horizontal axis, of the centroid of the cloud of points, with the root mean square of the displacement, measured parallel to the axis, of the individual points from the centroid. The use of the root mean square displacement of the *observed* values from their mean rather than of the calculated values would be closer to the proposed criterion. The results of this calculation for the data of *fig. 9* are given in *Table 8*.

TABLE 8

	Part I			Part II		
	log $\bar{r}_w$	log $\bar{g}_w$	log $\bar{b}_w$	log $\bar{r}_w$	log $\bar{g}_w$	log $\bar{b}_w$
Root mean square deviation of observed value from mean observed value	0.0278	0.0154	0.0224	0.0314	0.0110	0.0318
Mean deviation of observed value from integrated value	+0.0144	+0.0053	+0.0030	+0.0094	+0.0065	+0.0229

On the whole the conclusion from *Table 8* is that for the metameric match of a continuous white with a mixture of three spectral primaries, the defects of additivity are not large enough compared with individual differences to raise a serious objection to the adoption of a standard linear system of colour-matching functions.

For the non-additive effects associated with a change of the blue primary and with differences of intensity level, a preliminary calculation indicates that if the means of the Pt. I and Pt. II colour-matching functions are adopted as standard, the metameric matches corresponding to the observations on the spectral stimuli satisfy the criterion stated above. That is to say, in each spectral match the difference of the mean observed tristimulus value from the value computed from the standard functions is less than the root mean square difference of the observed tristimulus values. A further calculation on this point is in hand.

It is apparent from *figs. 3-5*, the upper curves of which represent the C.I.E. colour-matching functions, that at certain wave-numbers one or more of the mean observed colour-matching functions deviate from the C.I.E. value by more than the extreme subject variation. Thus the use of the C.I.E. curves for our large field matches would not satisfy the criterion laid down.

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PAPER 8

AN EXPERIMENTAL INVESTIGATION OF THE RELATIONSHIP BETWEEN COLOUR MIXTURE AND LUMINOUS EFFICIENCY .

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(56753)

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8. AN EXPERIMENTAL INVESTIGATION OF THE RELATIONSHIP BETWEEN COLOUR MIXTURE AND LUMINOUS EFFICIENCY

By H. G. SPERLING

SUMMARY

SIX observers' luminous efficiency functions for a 2^{0} foveal stimulus of 500 trolands illuminance, were determined by equality of brightness matching and flicker photometry. Each observer then made two sets of complete colour matches, one to the spectrum made equally bright on the basis of the brightness matching and the other on the basis of flicker photometry. V_λ's and equal energy colour mixture functions were derived from the data, and Abney's law, for metameric matches, was tested by comparing the sum of the mixture functions in luminance units, sV_λ, with the directly measured luminous efficiencies. Deviations of sV_λ from real V_λ amounted to 16 - 24% through part of the spectrum for the brightness matching but were small for flicker photometry. Comparisons with the real V_λ by brightness matching were made of three sV_λ's based on (a) brightness matching, (b) flicker photometry, and (c) weightings of the primaries which minimized the squared deviations of the brightness matching sV_λ from the real V_λ by brightness matching. The results were discussed in terms of the objectives of an International Standard Observer for Colorimetry and Photometry.

I.

AT the 1955 Congress in Zurich, the C.I.E. Technical Committee on Colorimetry passed a resolution recommending that any new colour mixture data be based on, "the results of procedures yielding in every case a complete match of the two fields being compared" *(ref. 1)*. This paper will report an experiment in which such a procedure was followed. In addition to colour mixture data, complete luminous efficiency functions were obtained for each observer, so that the arithmetic sum of the mixture functions expressed in luminance terms could be compared with the separately determined luminous efficiency functions. The results also contribute further evidence on the spectral distribution coefficients for the normal eye and on the V_λ function with regard to (a) the relative sensitivity of the disputed blue region as well as (b) the persistent question of local irregularities in several regions of the spectrum.

INTRODUCTION

THE C.I.E. resolution quoted above was made because evidence from several recent sources *(refs. 2-7)*, including preliminary data from the work to be described here, had cast doubt on the additivity assumption which underlies the structure of the 1931 standard observer system. According to Wright *(ref. 8)*, at the time his and Guild's trichromatic coefficient data were collected the V_λ curve had just been standardized and, following on Ive's work on heterochromatic photometry, no doubts then existed of the additivity of heterochromatic luminances for small fields. It therefore appeared adequate to determine only the proportions of three primaries which matched the spectrum. "The absolute amounts were derived by fractionating the standard luminous efficiency curve in accordance with those proportions on the assumption (known as Abney's law and which is specifically written into the definition of luminous flux) that the luminance of an additive combination of components is equal to an arithmetical sum of the luminances of those components" *(ref. 7)*. This procedure also avoided the difficulties inherent in the radiometric measurement of the comparison spectrum which would have been required in order to express the equal energy colour mixture curves directly. Wright *(ref. 9, p. 143)* mentions, however, that when the luminous efficiencies of his three primaries were separately determined they did not agree with the weightings which were required to make the distribution curves add up to the V_λ function. This discrepancy was taken at that time to be the result of the inherent variability of luminosity determinations. Viewed in the context of later findings, these differences might be interpreted as showing a failure of additivity. Granting that the sum of the resultant mixture functions agrees with the 1924 V_λ curve, this implies that in effect constant multipliers were found which altered the proportions differently from the luminosity factors of the three primaries, so that they would sum to the luminous efficiency function. Since, over the years, the reported failures of the system for predicting chromaticity have been few, one would presume that these adjustments were small. Chapanis and Halsey *(ref. 6)* have compared brightness matches of heterochromatic stimuli with the Y values computed from spectrophotometric data using the C.I.E. tristimulus values and have turned up discrepancies of the order of 30% in some regions. Thus, indications are that the initial corrections were not sufficient, or that the standard V_λ was incorrect, or both. By directly determining the equal energy colour mixture functions and comparing them with the same observers' luminous efficiency functions it will be possible to estimate the amount of the non-additivity and whether the application of constants to the three curves can reconcile the differences. It appears from Stiles' *(ref. 10)* preliminary data that the deviations of his summed distribution curves using experimentally determined luminous efficiencies for his primaries differed from Judd's revised luminous efficiency function by amounts which could not be accounted for by constant multipliers of the three curves. The comparison has not been made in terms of the variability of V_λ, however, so it is not clear that these differences are entirely due to the non-additivity of luminances rather than differences between the two groups in their luminous efficiency functions.

In his treatment of this problem, Dresler *(ref. 11)* shows from his own and earlier data, that while the equality of brightness matching technique reveals non-additivities for heterochromatic stimuli of the order of 30%, data from flicker photometry show very good additivity. He concludes that the photometrist can comply with the requirements of a linear additive standard by using flicker photometry. This implies that a C.I.E. standard using an additive combination of mixture functions could be made to serve both colorimetry and photometry, despite the non-additivity of heterochromatic equality of brightness matching. This solution, as aptly pointed out by the discussants of Dresler's paper, ignores the more important problems of physical photometry, which has largely supplanted the visual techniques in industry. The most frequent application of the standard observer is the evaluation of physical spectrophotometric data. In the 1931 system, by integrating spectrophotometric reflectance or transmittance data with the tristimulus values, both the chromaticity and the total luminance of a sample, for a specified illuminant, are obtained. Corrections for non-additivity in an improved standard observer could be employed as weightings of the integrals after the chromaticity coordinates of the sample are determined. Such a system would be useful for physical photometry. These weights could be in the form of multipliers of the three functions, as mentioned above.

Conceivably, of course, the entire system could be based on flicker photometric data, instead of the presently used V_λ which is based in part on flicker data and was claimed not to deviate from such determinations. Data taken by flicker photometry will be reported here and will serve to weigh this possibility. In fact, the sum of the mixture functions in luminance terms does agree better with our flicker V_λ function than with our equality of brightness determination. The original purpose in using flicker photometry, however, was largely to provide a check on the validity of the data, since we expected additivity by that technique. It was also intended to provide data for an analysis of the physiological side of the additivity problem. To base the standard observer on flicker photometric luminous efficiency data would require a careful re-study of the definition of luminance and a reconsideration of the objectives of the system, for the value of luminance in predicting brightness matching might be lost. On the other hand, it has been argued that luminance is an artificial quantity which cannot be expected to predict the multitude of subjective brightness situations in any case; so that an additive combination of the mixture curves which approximates a specified set of luminous efficiency data would be all that could be achieved. Were this the agreed-upon attitude of colorimetrists and photometrists, it might be better to base the standard observer on flicker photometry, providing greater additivity were achieved, than on wholly arbitrary weightings. The results of physical photometry could then, at least, be compared with direct visual measures based on standardized procedures of measurement. In any case, a careful comparison of the V_λ by both equality of brightness setting and flicker photometry with the same observers' colour mixture functions taking into account variability, should shed further light on the problem.

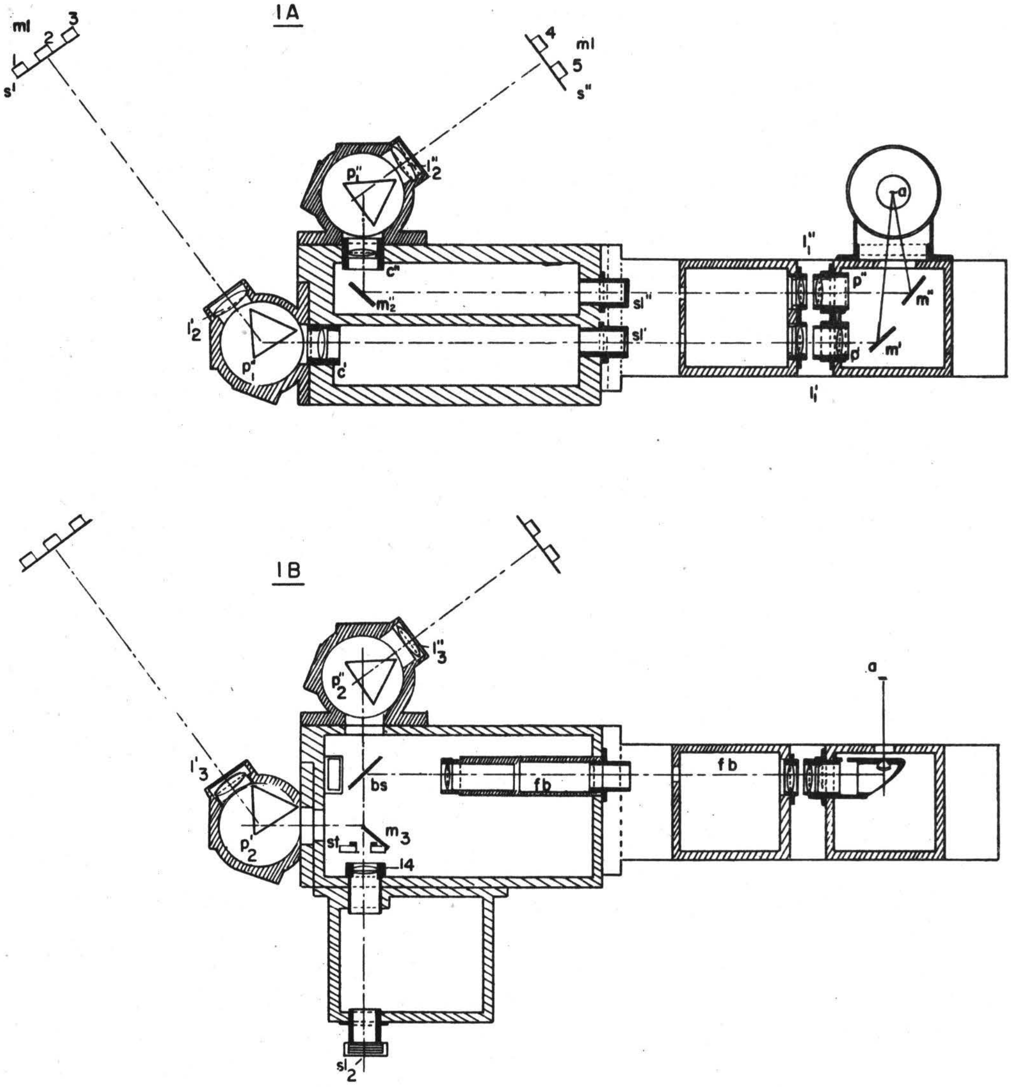

Fig. 1. Diagram of tristimulus colorimeter.

II. APPARATUS

THE apparatus used was a Wright type tristimulus colorimeter designed and constructed by Fry *(ref. 12)* for the U.S. Naval Medical Research Laboratory. Like the Wright colorimeter, it is essentially composed of two double monochromators, which receive light from a single ribbon filament source, a, in *fig. 1a*. The light is turned at right angles by mirrors m' and m'', and is condensed by lens assemblies l'_1 and l''_1, on two variable entrance slits, sl', and sl''. From there it fills a collimator lens c' for the first system and is dispersed by prism p'_1 and focused by lens l'_2 as a spectrum at s'. In the second monochromator it is turned at right-angles by mirror m''_2 into collimator c'', dispersed by prism p''_1, and focused by lens l''_2 to form a second spectrum at s''. The spectral bands used for mixture standards were selected from spectrum s'', to which could be added desaturating bands from the same spectrum. The three primaries came from the other spectrum s'. As in the Wright instrument, the return branch of the double monochromators lie on top of the dispersing branch. Wright used roof prisms to reverse the spectrum, while Fry employed a combination of three lenses and two mirrors which select, reverse, and reflect back each spectral band into the top half of the colorimeter. These mirror-lens assemblies ml_1 - ml_5 are sealed against dust and are located in the focus of spectra s' and s''. There are three in s' for the three primaries and two in s'' for the standard and desaturator. They move on curved tracks so that the optical centre of the assembly always remains in the focus of the spectrum. The tracks are moved against springs by high quality micrometer screws. The wavelength calibrations were made in terms of millimetres movement of the micrometer screws. Having been reflected back and reversed, the spectral bands retrace a path through a second set of lenses l'_3 and l''_3 in *fig.1b* which recollimate the light, and then through a second set of prisms p'_2 and p''_2 located on top of the first set. These prisms serve to superimpose the five bands for additive mixture, as well as to filter out stray light. Light from the s'' system travels straight through past a knife-edge mirror at m_3 thus filling one half of the field. Light from the other spectrum s' fills the other half of the field, being reflected at right angles from mirror m_3. At St is located a circular aperture which masks the field lens, l_4. This lens focuses the combined spectral bands on the exit slit, sl_2. The eye was held by a chin and head rest directly behind the exit slit so that it received a Maxwellian view of lens l_4. The observer saw a 2^0 vertically divided circular field imaged at infinity, against a dark surround.

Circular neutral density wedges were located in the return beam just after the ml assemblies in the path of each of the five bands. Scales were cemented to them and they were turned by the observer through rod and pulley linkages. Where necessary, neutral density filters could be inserted near the wedges, to provide coarse attenuation. Continuous variation of each band could be achieved over a range from 0 to 6 density units.

For initial wavelength calibration, the entrance and exit slits were narrowed to a fraction of a millimetre and a mercury arc or helium Nernst glow tube was placed directly in front of the entrance slit. A highly sensitive photomultiplier tube was placed at the exit slit and the ml tracks were moved, and the micrometer screws were read at the point of maximum response for each spectral line. This procedure produced smooth wavelength calibration curves for each spectrum. The wavelength settings used in the experiment were interpolated on these curves. The wavelengths of the primaries and selected points in the standard spectrum were subsequently checked at frequent intervals using a calibrated spectroscope.

Non-selective energy calibrations were carried out directly using a Farrand-Hornig thermocouple in place of the exit slit. The sensitive element was a 0.75 mm^2 thermal receiver coated with platinum-black. This flake was positioned by a micromanipulator with its centre at the exact position of the narrowed exit slit. Since the width of the exit slit was kept constant at 0.75 mm for all visual observations, the energy measurements were proportional to the radiation received by the eye. The three primary wavelengths, 460 mμ, 520 mμ and 650 mμ, having identical bandwidths with the same three wavelengths in the standard spectrum were measured by visual matches to those standards. The energy measurements were repeated for each new lamp used. The lamps were preseasoned by the factory and were burned only 30% of their expected life in order to minimize blackening. The measurements were checked against some obtained with a photomultiplier photometer, which had been calibrated for relative spectral response by the National Bureau of Standards. The agreement was excellent.

The wedges and filters were calibrated in place using a photomultiplier photometer of good linearity at the eyepiece. Separate calibration curves were obtained for each of the five wedges at each wavelength used in the experiment. The values were transmittance relative to the clear portion of each wedge. The wedges were also in the clear position for all energy measurements.

Stray light was checked for in the standard spectrum by measuring the energy in the 440 mμ band while the collimator lens of the return system, L3″, was covered with a 1 mm thick cobalt filter having negligible transmission above 500 mμ. The average of 10 responses of the thermocouple system was within 0.2% of the product of the measured radiancy of that band without the cobalt filter and the spectrophotometric value for the transmittance of the filter for that wavelength. Further checks were carried out using the Kodak wratten No.12 yellow filter, which has negligible transmission below 500 mμ. With the wedge set for maximum transmission of 440 mμ, and the other bands blocked at the location of the wedges, the dark-adapted eye was unable to detect light in the field. The same results were obtained with the primary side of the instrument, using the 460 mμ primary.

For flicker photometry, an auxiliary light beam, fb, from the ribbon filament source, was admitted to the top half of the instrument and reflected from a beam splitter, bs, located behind the field lens aperture, St, *fig. 1b*, so that it was superimposed on the spectral bands from S″. A variable speed d. c. motor was employed to drive a circular shutter which alternately covered and uncovered the auxiliary light beam and the slit sl''_1 which served the S″ spectrum.

III. PROCEDURE

SIX observers' luminous efficiency functions were obtained by two procedures, equality of brightness setting and flicker photometry. The equality of brightness data were obtained by using the 520 mµ green primary as a standard. The wedge in that beam was adjusted such that the illuminance to the retina equalled 500 trolands, as determined by binocular matching to an extended field of known luminance seen through a 1 mm^2 artificial pupil by the other eye. The colorimeter exit pupil was set to 0.75 mm wide by 2.0 mm high, as used throughout the study.

> The question of bandwidth was a vexing one in this study. It was at first planned to use sufficiently narrow slit widths so that the total bandwidths of the standard bands would nowhere exceed the 10 mµ spectral intervals employed. It was soon discovered that insufficient intensity was thus obtained to keep an equal brightness standard spectrum at high photopic levels.
>
> Therefore a trial experiment was run using an exit slit width of 0.3 mm which met those conditions but produced much lower retinal illuminance. Data for both equality of brightness settings and colour mixture was run on three of the six observers. A comparison of the results with those of the same three observers run at 0.75 mm exit slit (500 trolands) showed no appreciable differences. The study was therefore completed at the latter exit slit width. The total bandwidth ranged from 6.69 mµ for the band centred on 420 mµ to 28.32 mµ for the band centred on 700 mµ. The effective bandwidth, determined by considering the energy to be rectangularly distributed over each band ranges from 4.80 mµ centred on 420 mµ to 20.64 mµ centred on 700 mµ. The bandwidth of the three primaries in these terms was 6.38 mµ centred on 460 mµ, 8.55 mµ centred on 520 mµ and 17.05 mµ centred on 650 mµ.

Following seven minutes of dark adaptation, the observer matched the standard spectrum to the 520 mµ primary at intervals of 10 mµ from 420 - 700 mµ, by adjusting the appropriate wedge. The entire spectrum was run through in different directions each day for four days. The average transmittance of the four wedge settings per wavelength was converted to relative energy. The reciprocals of the relative energies adjusted to

1.00 at the peak of the curve, constituted the relative luminous efficiency function. Each observer also made four flicker photometric matches to the flicker standard (also of 500 trolands illuminance). Relative luminous efficiency functions were derived from these data in the same way.

The same wavelengths of the same spectrum were used for the colour mixture standards as were employed to determine luminous efficiency. The mean wedge and filter settings required in the equal brightness and flicker matches were set in for each wavelength. The observers then matched both the brightness and the chromaticness of these equal brightness standards by manipulating the wedge controls of the three primaries. The resultant wedge and filter transmittancies of the primaries constitute the basic data for the colour mixture functions. Two complete sets of mixture data were obtained for each of the six observers, one matching the standards set equal by brightness matching and one matching those set equal by flicker photometry. Each set consists of the mean of six matches on each standard by each observer. The primaries were randomly set before each match, requiring the observer to adjust all three wedges each time. Six wavelengths were randomly selected for each matching session.

The treatment of the data may be summarized as follows:

(a) The standard spectrum was reduced to equal brightness by determining the radiant flux quantities P_λ required to match a 500 troland standard, by both equality of brightness setting and by flicker photometry. Luminous efficiency was found as:

$$V_\lambda = \frac{Br}{P_\lambda} \tag{1}$$

where brightness, Br, was held constant.

(b) The energy amounts p_r, p_g, p_b, of the three primaries, R, G and B, required to match the equal brightness spectrum were determined by complete match:

$$P_\lambda\ (\lambda) \equiv p_r\ (R) + p_g (G) + p_b (B) \tag{2}$$

(c) Reduced to unit radiant flux, the tristimulus values of the equal energy spectrum in terms of spectral primaries, 460, 520, 650 mμ were obtained:

$$(\lambda) \equiv \frac{p_r}{P_\lambda}(R) + \frac{p_g}{P_\lambda}(G) + \frac{p_b}{P_\lambda}(B) \tag{3}$$

(d) These were converted to luminous quantities for the equal energy colour mixture functions by introducing V_R, V_G and V_B, the luminous efficiency of unit energy amounts of R, G and B.

$$V_\lambda \overset{?}{=} \frac{V_R\, p_r}{P_\lambda}(R) + \frac{V_G\, p_g}{P_\lambda}(G) + \frac{V_B\ p_b}{P_\lambda}(B) \tag{4}$$

If equation (4) is found consistently and within the limits of variability of V_λ, then it can be stated that the additivity law of luminances (Abney's law) is correct for three part metameric matches to spectral lights.

In the following, we shall refer to the sum of the values on the right hand side of equation (4) as the synthetic V_λ or sV_λ and to the equality of brightness matching and flicker photometric results as real V_λ or just V_λ.

IV. RESULTS

TABLES I and *II* show the luminous efficiency of the spectrum for our six observers as determined by equality of brightness matching and by flicker photometry. These values have been set equal to 1.00 at 550 mμ . The mean equal energy colour mixture values and their sums are shown in *Table III.* These are the values computed from equation (4) above. Since V_R, V_G, V_B, were obtained from the mean luminous efficiency functions and $p_r = P_{650}$, $p_g = P_{520}$ and $p_b = P_{460}$ by homochromatic match, the sum of the luminous efficiency weighted mixture functions will be very close to the average luminous efficiency values at these three wavelengths. This agreement simply reflects the exactness of homochromatic photometry in a bipartite field. Had other wavelengths been chosen for the primaries, the points of exact agreement would have been different. *Figs.2* and *3* compare the mean sum of the mixture functions with the average V_λ by the two techniques. There it may be seen that the absolute deviations of the synthetic V_λ based on brightness matching from the equality of brightness V_λ are small except in the yellow through orange regions where the synthetic V_λ is sizably larger than the real V_λ. The agreement between V_λ by flicker photometry and the synthetic V_λ by that method is better, especially over the yellow to orange regions where the brightness matching method showed large discrepancies.

The discrepancies, which are representative of the degree to which the additivity law of luminances breaks down, may also be viewed relative to the magnitude of V_λ at the bottom of *figs.2* and *3*, where the ratios of real V_λ to synthetic V_λ are plotted. In *fig.2* it may be seen that the deviations in the brightness matching experiment are in the neighbourhood of 18-24% from 570 mμ to 630 mμ. Large percent deviations also occur in the far red where the synthetic V_λ underestimates the real V_λ. In the flicker experiment, *fig.3*, the percentage deviations are small over the entire region between the green and red primaries (520 mμ to 650 mμ). The only sizable percentage deviations occur in the violet and blue-green regions.

The amount of discrepancy between the three part synthetic V_λ and the real V_λ by the two methods should more properly be viewed in terms of the variability of V_λ determinations. In *figs.4* and *5* the mean synthetic V_λ, open circles, are plotted together with the individual V_λ curves of the six observers. It should be noted that the real V_λ curves have been pulled together at 550 mμ, but that the mean synthetic V_λ is independent at that wavelength. In *fig.4* for the brightness matching data, it may be seen that

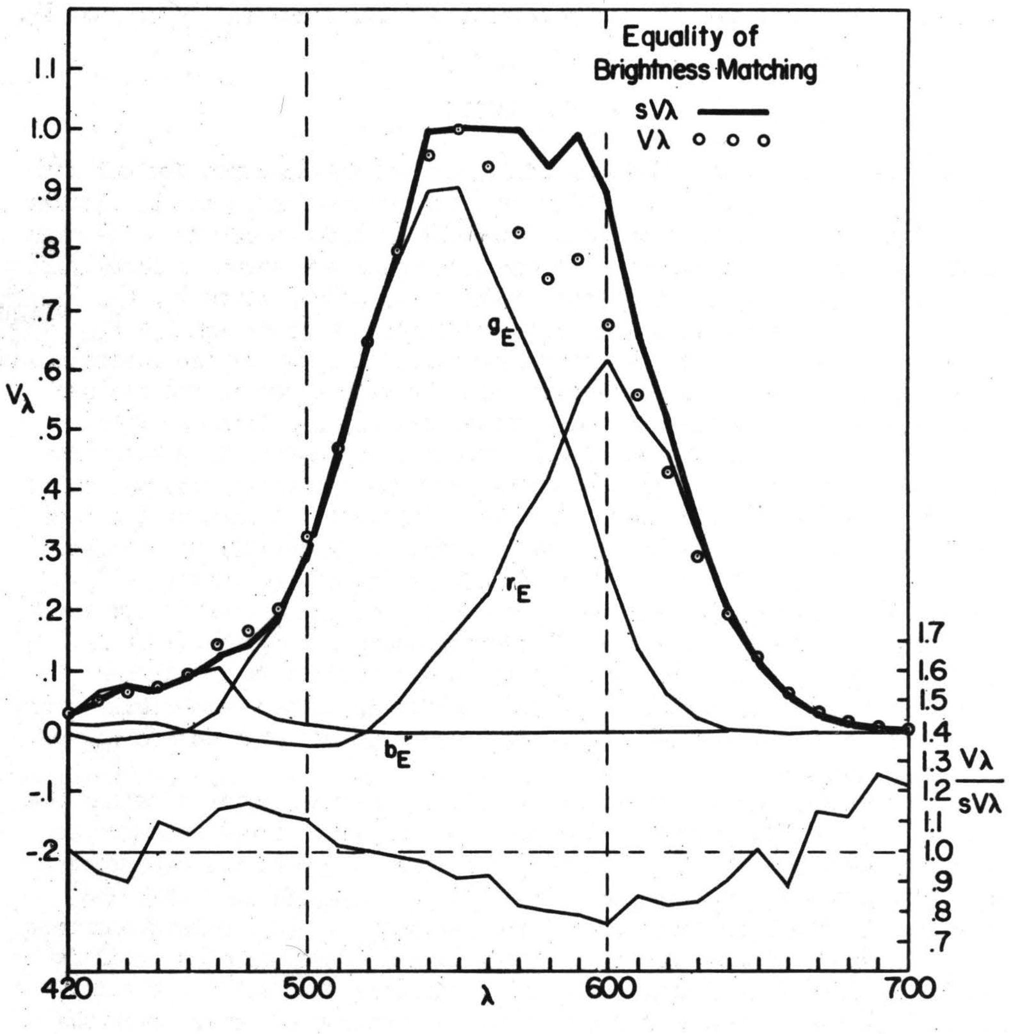

Fig. 2. Mean equal energy colour mixture functions for six observers from the brightness matching experiment, (r_e, g_e, b_e) and their sum, sV_λ, compared with the mean real V_λ by equality of brightness matching. The ratio of real to synthetic V_λ is shown at the bottom.

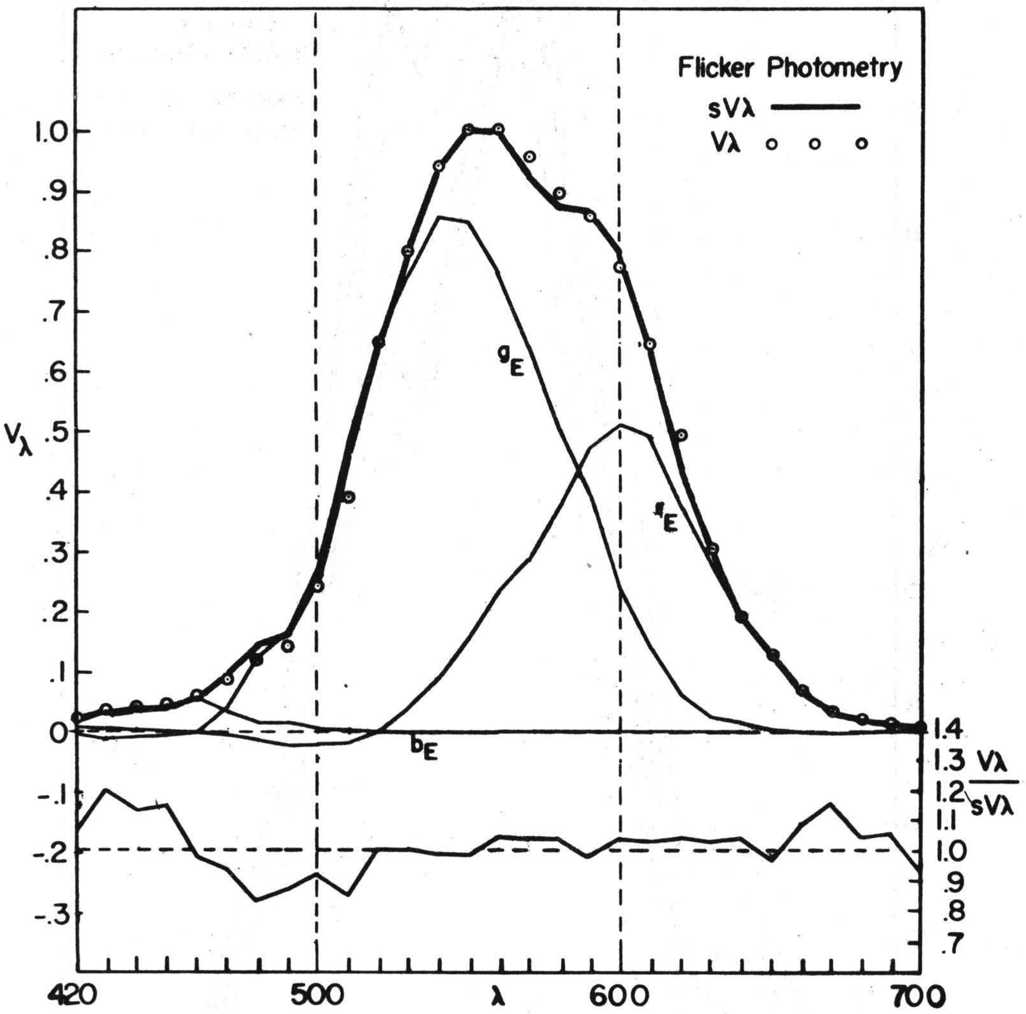

Fig. 3. Mean equal energy colour mixture functions for six observers, from the flicker photometry experiment, (r_e, g_e, b_e) and their sum, sV_λ, compared with the mean real V_λ by flicker photometry. The ratio of real to synthetic V_λ is shown at the bottom.

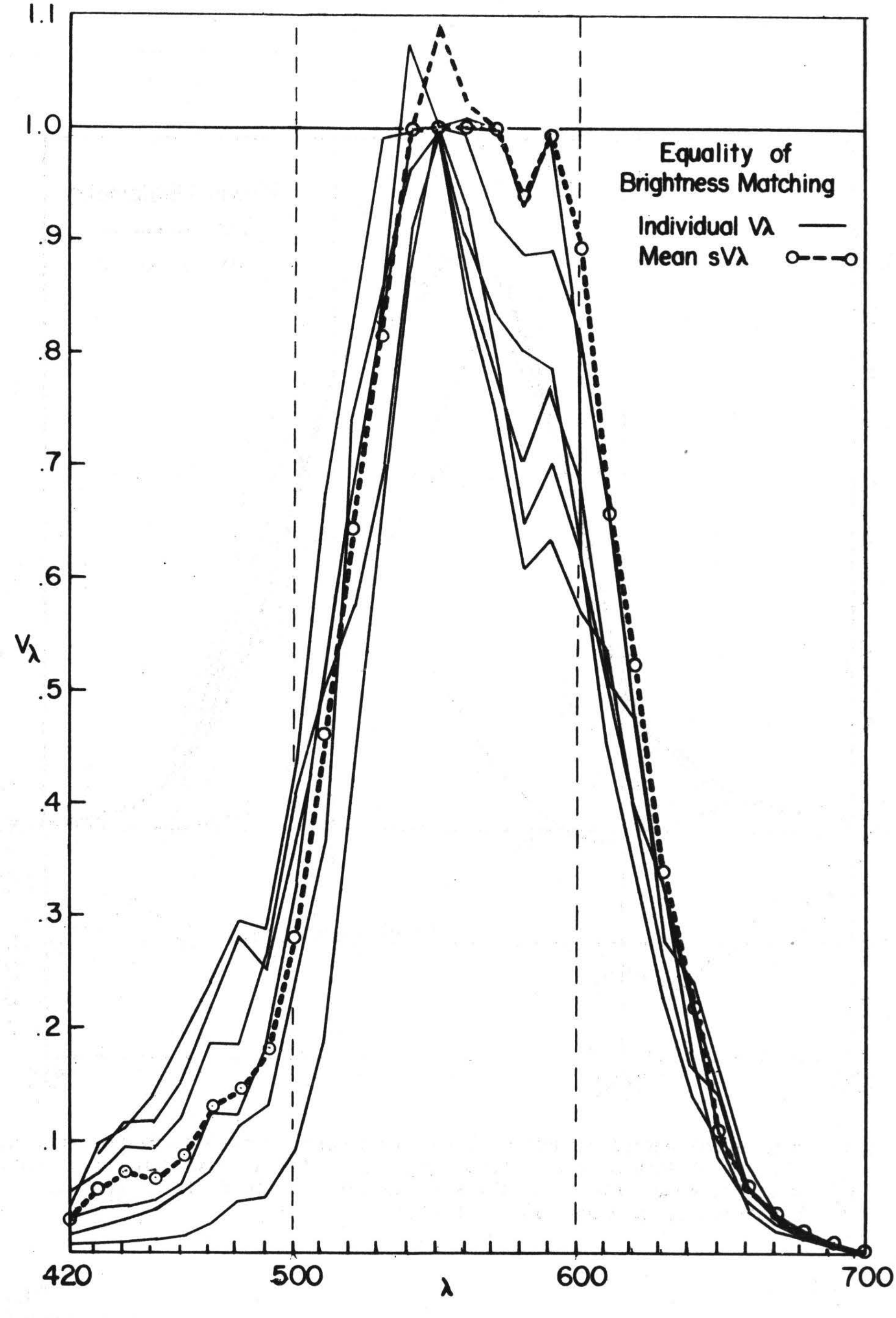

Fig. 4. The mean synthetic V_λ from the brightness matching experiment compared with the six individual observer's real V_λ's by brightness matching.

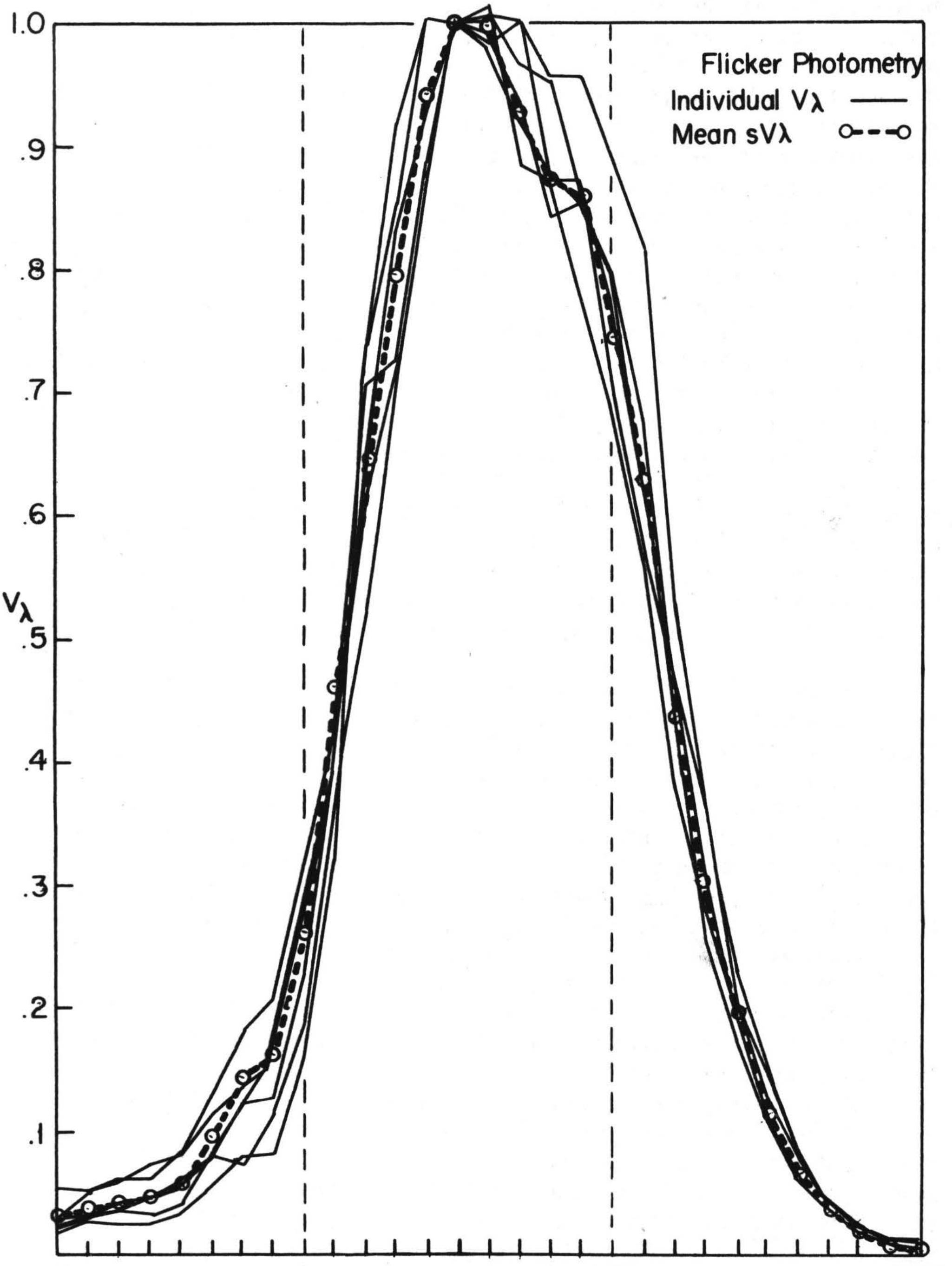

Fig. 5. The mean synthetic V_λ from the flicker photometry experiment compared with the six individual observer's real V_λ's by flicker photometry.

below 540 mμ all the synthetic V_λ values tend towards the centre of the range of real V_λ values. From 550 mμ to 650 mμ the synthetic values either overlie the extreme limit of the range or lie completely outside. Above 650 mμ, they again tend towards the centre of the range. It is important to note in *figs. 4* and *5* that the individual V_λ curves are fairly evenly distributed over the range. A comparison of the synthetic V_λ based on the flicker photometry with the individual flicker V_λ curves, in *fig. 5*, shows that over the entire spectrum, except at 510 mμ, the synthetic V_λ tends towards the centre of the range of real V_λ measures. Thus, on the basis of absolute deviations, percentage deviations and in terms of the variability of real V_λ measures by flicker, the synthetic V_λ based on flicker photometry is a much better approximation to luminous efficiency as determined by that method, than was found for equality of brightness matching.

For practical purposes, we are more interested in obtaining a linear additive combination of the colour mixture results which will predict the results of direct brightness matching. We can take two approaches with these data. We can attempt to find artificial values for V_R, V_G and V_B which will weight the distribution functions such that they sum to better agreement with the real V_λ by brightness matching than was obtained using the experimental values; or, we can explore whether the mixture functions resulting from the flicker experiment, which are in themselves fairly additive to a flicker V_λ, will adequately predict the brightness matching results.

Unfortunately, we cannot combine the distribution functions obtained in the two experiments for they show non-linear differences. While the proportions of the three primaries required in a complete match on the brightness matching spectrum agree well with those obtained for the flicker equated spectrum, *(fig. 6)*, the distribution curves, computed using equation (3), show differences between the two experiments *(fig. 7)*. This simply means that the non-additivities go beyond the luminous efficiency of the primaries. They probably result in part from the difference between the V_λ curves by the two methods *(figs. 2, 3, 4* and *5)*. In any case, we should not combine the two sets of distribution curves in attempting to produce a synthetic V_λ, but must treat them separately.

In *fig. 8* we can now compare three synthetic V_λ curves with the distribution of brightness matching: *(fig. 8a)* the sV_λ based on brightness matching which has already been discussed; *(fig. 8b)* the sV_λ based on flicker photometric values, and *(fig. 8c)* an sV_λ in which the V_R, V_G, V_B were solved for as the three weightings which minimized the squared deviations of the brightness matching mixture functions from the mean brightness matching V_λ. This sV_λ should represent the best possible overall linear fit of those mixture functions to the brightness matching V_λ. It appears from these graphs that the sV_λ based on flicker measures is not as good below 550 mμ, a little poorer over the yellow and orange but closer to the centre of the range in the red than the curve based on

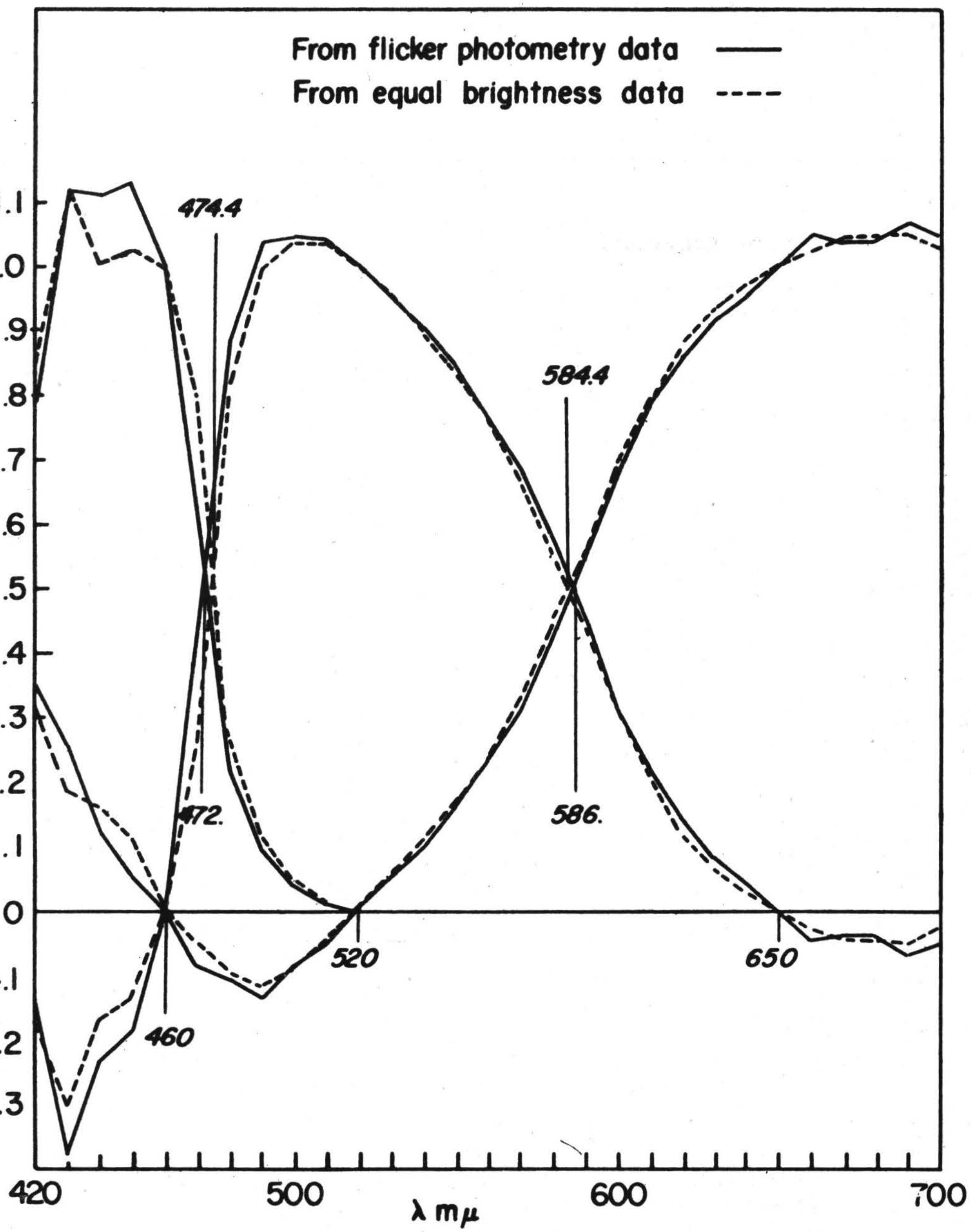

Fig. 6. The proportions of primaries 460, 520 and 650 mμ required to match the spectrum, from the mean brightness matching and flicker photometry equal energy colour mixture functions.

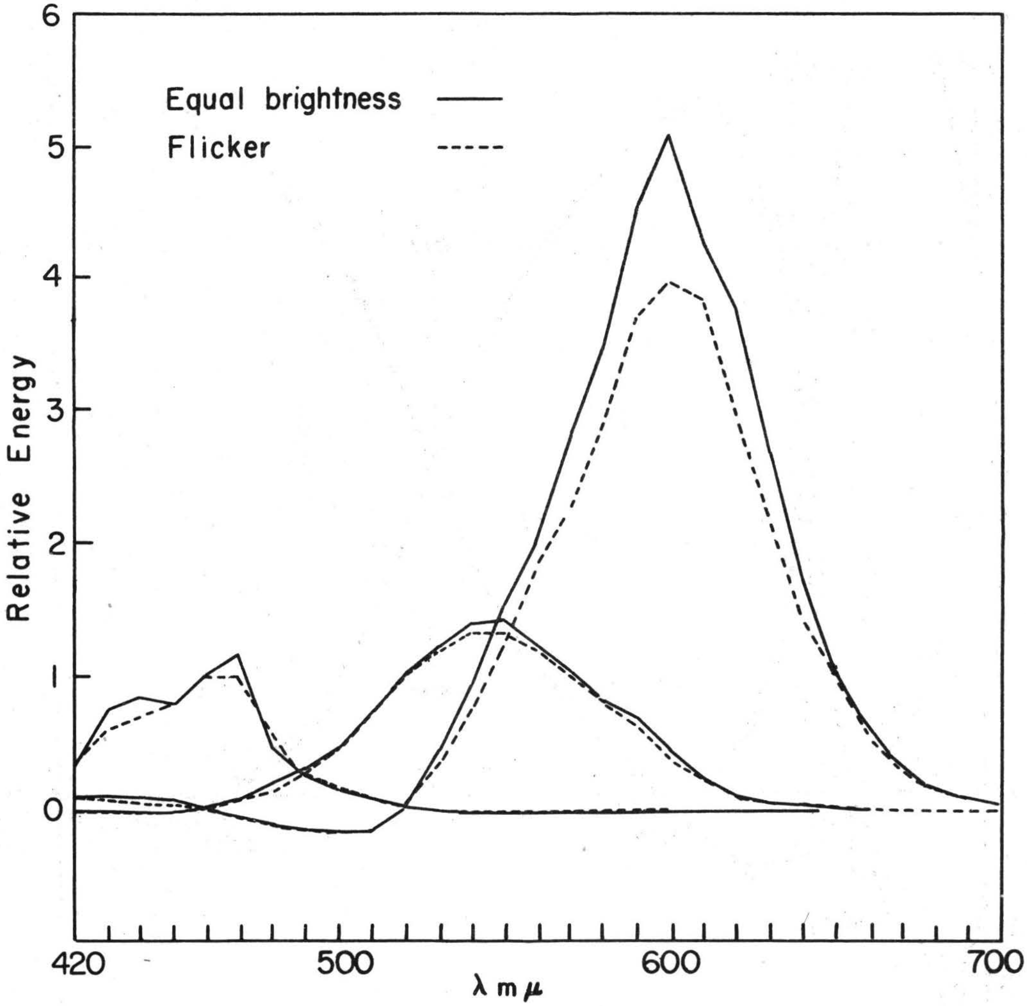

Fig. 7. The equal energy tristimulus values from the brightness matching (solid line) and flicker photometry (broken line) data.

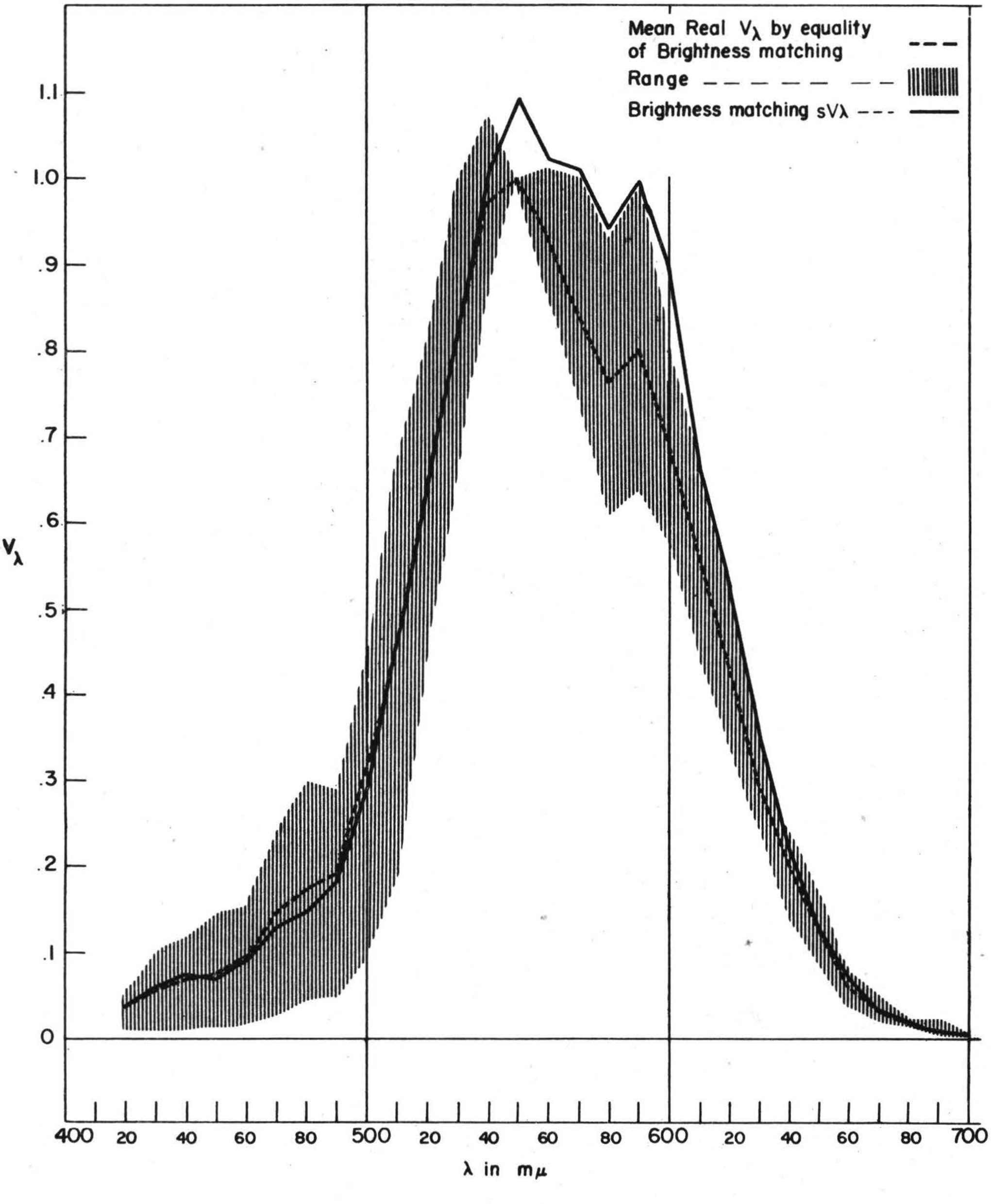

Fig. 8a. Comparison of the mean and range of real V_λ obtained by brightness matching with the mean sV_λ from the brightness matching experiment using the mean experimentally determined brightness matching values for V_R, V_G and V_B.

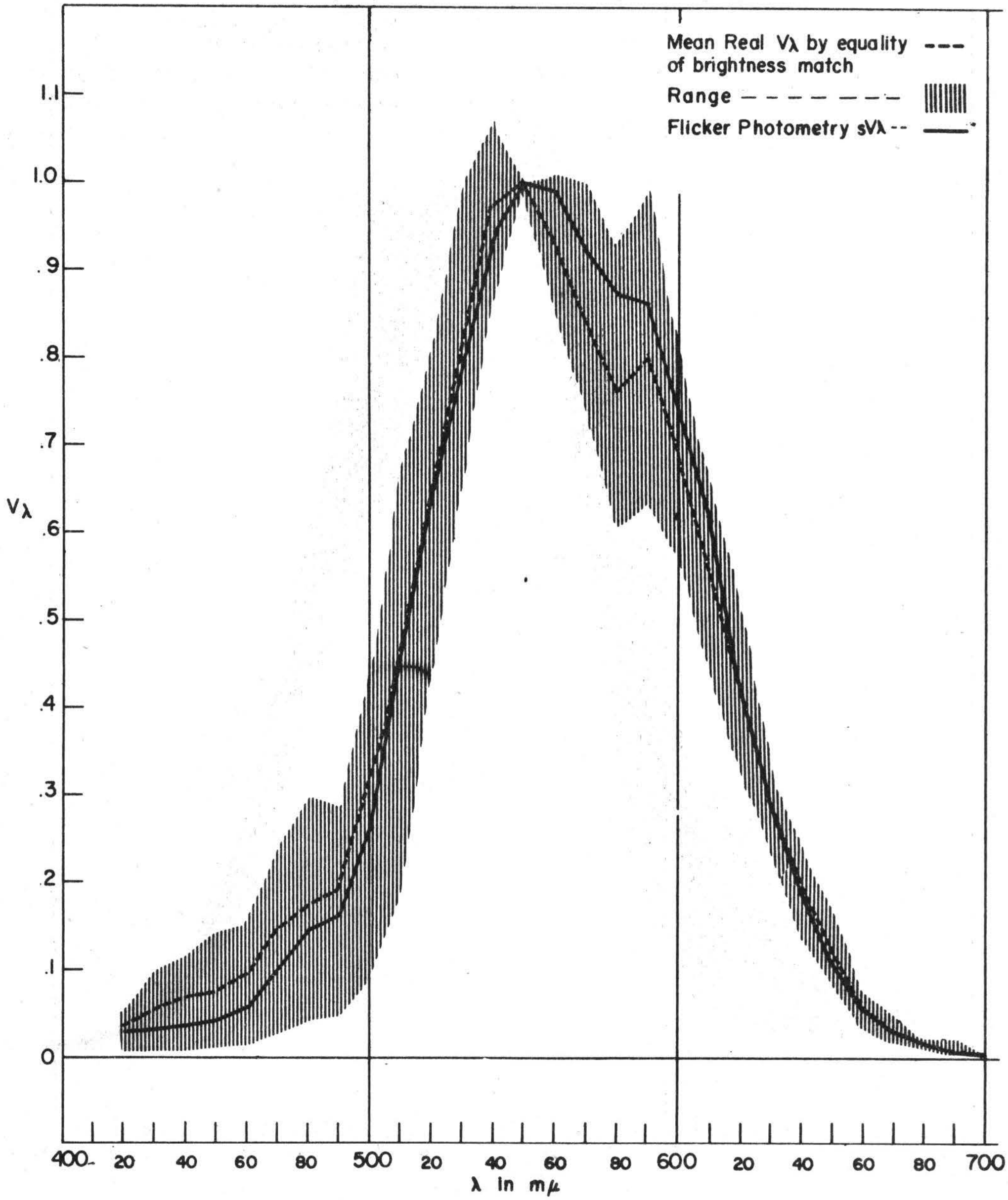

Fig. 8b. Comparison of the mean and range of real V_λ obtained by brightness matching with the mean sV_λ from the flicker photometry experiment using the mean experimentally determined flicker photometry values for V_R, V_G and V_B.

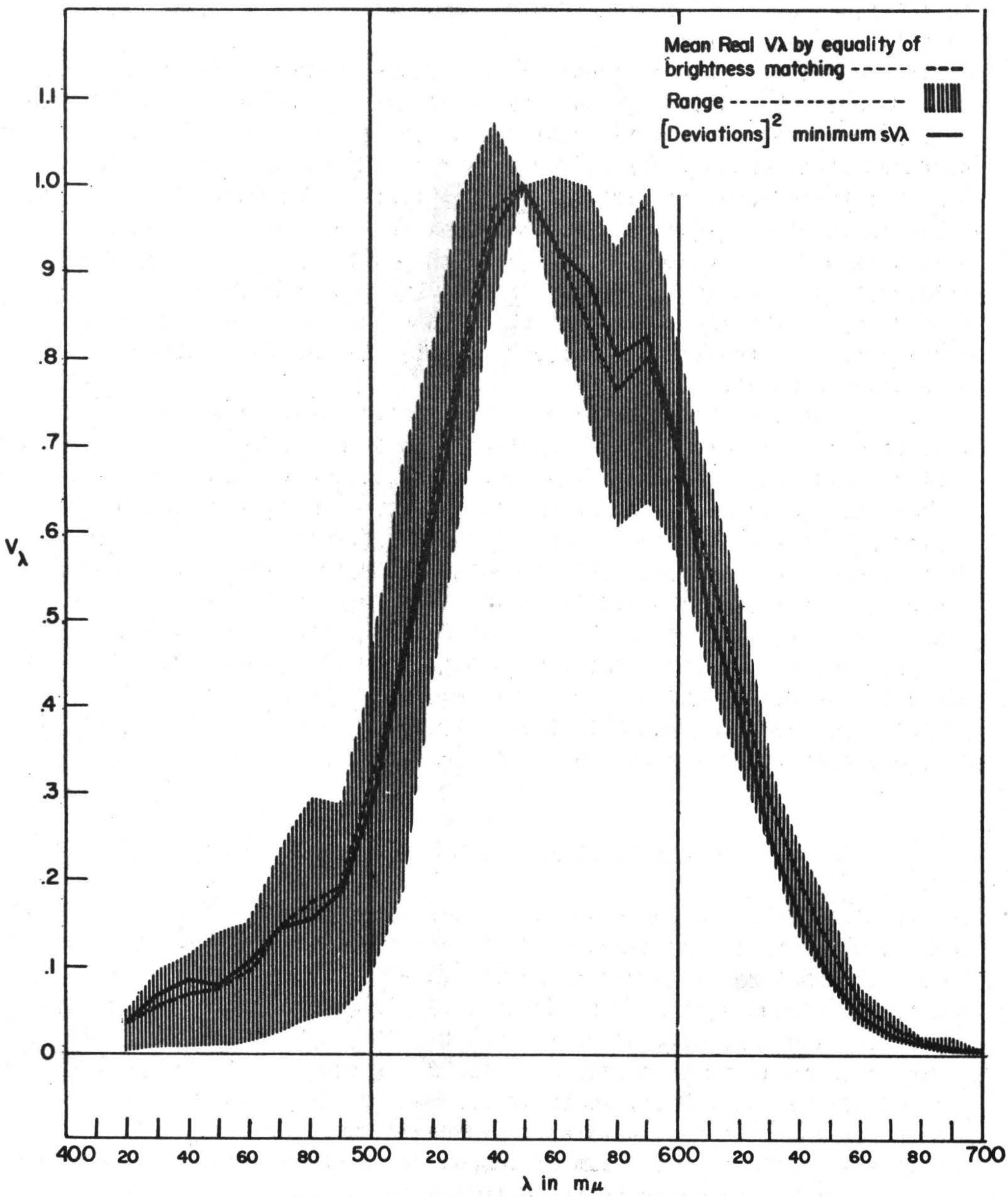

Fig. 8c. Comparison of the mean and range of real V_λ obtained by brightness matching with the mean sV_λ from the brightness matching experiment using values for V_R, V_G and V_B which minimized the squared deviations of the synthetic from the real V_λ.

optimum weightings. The latter curve lies very close to the mean V_λ except in the red, where it nevertheless remains within the range of variability of brightness matching.

The validity of the synthetic V_λ functions which we have considered depends upon the quality of the colour mixture functions which underlie them. *Fig. 9* shows the mean tristimulus coefficients in the W.D.W. system, compared with those of Stiles for the 2° field and also the C.I.E. 1931 observer transformed to our system of primaries. The agreement among the three is excellent above 460 mμ. Below there, both our data and that of Stiles show fair agreement with the C.I.E. on the proportion of the red primary in unit amount of violet, but deviations from the C.I.E. in the proportion of the green and blue required to match violet. Our data deviate more extremely than Stiles' in this regard. This discrepancy will be discussed later.

Our V_λ results, in addition to questions relating to the additivity of luminances, agree with recent findings in showing higher values over the violet region than the 1924 V_λ shows. In addition, it should be noted that each of the individual real V_λ curves by both methods show to some degree an irregularity in the region of 580 mμ, first reported by Sloan *(ref. 13)*. Since these data were gathered at a high photopic luminance, we should no longer entertain the notion that this notch relates to the summation of rod and cone functioning *(ref. 14)*, or that it signifies a separation of green and red response at low light levels only *(ref. 9)*. In addition, the two humps in the violet and blue regions reported by Wright *(ref. 15)*, Ishak *(ref. 16)* and Sperling and Hsia *(ref. 17)* appear in all the individual curves. On the average, the associated notches occur at 450 mμ and 490 mμ.

V. DISCUSSION AND CONCLUSIONS

THE results of this study show that Abney's law does not hold for brightness matching in the presence of full chromatic differences. The results by flicker photometry, however, do not show sufficient deviations from additivity viewed against the variability of V_λ by flicker to lead us to reject the law entirely. It would seem that the 1931 C.I.E. observer was in error in assuming that over a 2° field the results of brightness matching and flicker photometry would agree. Our results are consistent with the hypothesis that flicker photometry evaluates luminance (where total luminous flux is defined as the sum of component heterochromatic luminances); but that brightness matching is contaminated by other factors such as saturation differences, and simultaneous colour contrast, which are variables in determining subjective brightness. In making the judgment that the C.I.E. system is primarily concerned with predicting results by brightness matching, it is necessary to point out that while brightness matching may come close to estimating some subjective brightness situations, it will

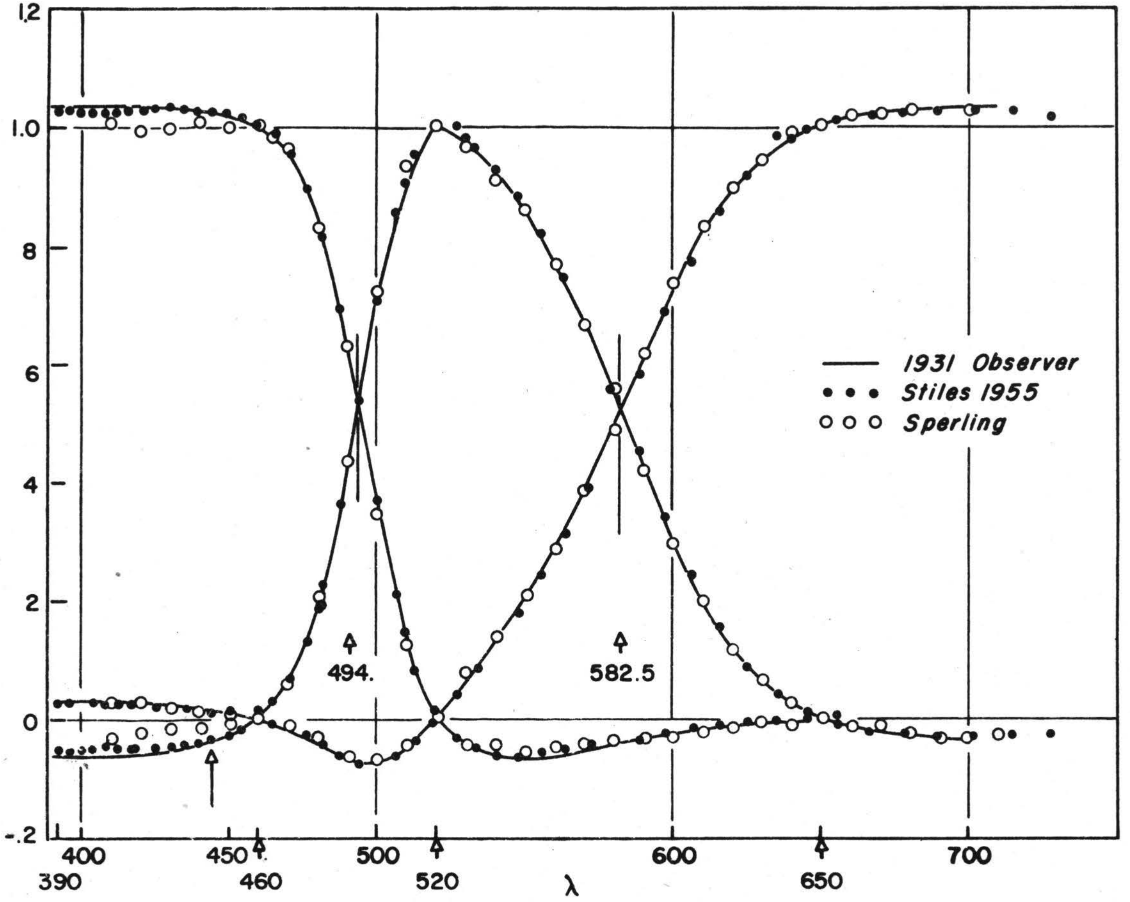

Fig. 9. Comparison of the combined chromaticity co-ordinates for all our data with those from Stiles' 2° preliminary data and the C.I.E. 1931 standard observer, for primaries 460, 520 and 650 mμ in the W.D.W. system.

necessarily fail in that objective for other combinations of variables. It might be better to preserve the present type system on the basis that the sum of the component heterochromatic luminances will predict luminance (as measured by flicker photometry), and will also predict brightness matching within the limits of variability of that method *(see fig. 8b)*. Such a system would not predict brightness matching over most of the spectrum as well as the arbitrarily weighted mixture functions based on brightness matching *(fig. 8c)*, but would have the advantage of preserving luminance as presently defined.

Alternative solutions to the problem, which have not been treated here, are to "divorce" photometry and colorimetry as suggested by MacAdam, using the present or a similar system which predicts luminance and leaving the photometrist to correct the synthetic V_λ of the system to his choice of real V_λ by non-linear correction values. Another such possibility is to introduce non-linear corrections on the basis of the saturation discrimination function. The latter, it seems to us, must await a further study of visual saturation discrimination, and the demonstration that the discrepancies found here are, in fact, entirely accounted for by saturation differences.

The rather large differences between this study and previous ones in the proportions of the blue and green primaries required to match violet, below 460 mμ, are probably related to the very large variability of colour matching in that region. We found that, without considerable practice, our observers would accept very large quantities of negative green in a match, compensating the brightness by adjusting the blue and red wedges. Stiles *(ref. 10)* reports the same difficulty. His solution was to desaturate the match field by introducing green to both sides, resulting in a less saturated colour field than would otherwise have been possible. Wright *(ref. 9)* employed the same technique. We chose, instead, to make all matches on the Maxwell triangle, thus requiring only two colours on each side. This resulted in a maximally saturated field. We also instructed the observers not to look away from the field but to shift the point of fixation from standard to comparison field continually. Previous work had shown that violet desaturates very rapidly with time, requiring less negative green after the first instant, and then remaining rather stable. Our matching procedure would be expected to require less negative green, but to result in more stable responses than would techniques such as Wright's, by which adjustments are made in rapid glances with the observer looking away from the field between adjustments. In addition, there appeared a tendency in some of our observers to decide upon a match towards the minimum of the range of acceptable variation of the green primary. The combination of these factors would appear to explain the observed discrepancies. It seems to us doubtful, in view of these factors, that much emphasis should be placed on the differences in the violet. It is our hope to make a separate detailed study of the time factors involved in matches on violet, also employing a more intense light source in order to go to shorter wavelengths.

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TABLE I. The relative luminous efficiency functions of six observers obtained by equality of brightness matching and their mean.

λ	RMH	WGL	HGS	BMH	SCS	HJO'H	Mean
420	.05348	.01913	-	.00880	.03006	.041	.03040
30	.06831	.02356	.08691	.00926	.03824	.099	.05421
40	.09487	.03097	.11618	.01005	.04039	.111	.06724
50	.09299	.03707	.11764	.01185	.04625	.141	.07446
60	.12324	.05285	.15291	.01591	.06184	.152	.09405
70	.18744	.07485	.21673	.02573	.12419	.240	.14482
80	.18647	.11431	.28254	.04086	.12316	.296	.17389
90	.26772	.13081	.25236	.04763	.18439	.287	.19498
500	.40688	.24190	.36618	.08939	.31703	.441	.31039
10	.50157	.36303	.49054	.18758	.52648	.674	.45720
20	.56631	.74674	.68418	.43567	.67729	.811	.65353
30	.68925	.86404	.81236	.65688	.84661	.992	.81019
40	.91529	.96616	1.00000	.86682	1.07191	.998	.96969
50	1.00000	1.00000	1.00000	1.00000	1.00000	1.000	1.00000
60	.93634	.99414	.92854	.90384	1.01360	.863	.93991
70	.74910	.91450	.81236	.83680	1.00000	.747	.84329
80	.60660	.88616	.64982	.80102	.93704	.708	.76477
90	.63528	.89537	.70273	.78251	.99330	.772	.79686
600	.57133	.81047	.61909	.63307	.79674	.686	.68611
10	.53630	.66806	.44836	.51219	.65636	.510	.55521
20	.38395	.51811	.33764	.47607	.47300	.399	.43129
30	.26295	.27897	.24073	.32573	.30979	.332	.29169
40	.16517	.24266	.13982	.17776	.22711	.237	.19825
50	.13795	.16692	.09654	.08544	.11205	.135	.12231
60	.06014	.07605	.03636	.04605	.06614	.067	.05862
70	.03352	.03432	.01873	.02562	.03669	.050	.03314
80	.01827	.01865	.01436	.01185	.02110	.020	.01737
90	.00908	.02260	.00564	.00576	.00784	.012	.01048
700	.00387	.00478	-	.00282	.00474	.006	.00370

TABLE II. The relative luminous efficiency functions of six observers obtained by flicker photometry and their mean.

λ	RMH	WGL	HGS	BMH	SCS	HJO'H	Mean
410	-	.014	-	.013	-	-	.014
20	.037	.022	.054	.018	.018	.027	.029
30	.051	.034	.053	.026	.026	.037	.038
40	.059	.036	.062	.024	.031	.042	.042
50	.073	.045	.062	.025	.032	.046	.047
60	.081	.056	.081	.034	.041	.060	.059
70	.114	.075	.131	.055	.077	.083	.089
80	.136	.123	.184	.078	.071	.125	.120
90	.164	.126	.207	.079	.115	.159	.142
500	.286	.229	.323	.161	.183	.276	.243
10	.435	.344	.424	.322	.404	.414	.390
20	.613	.688	.706	.725	.519	.644	.649
30	.717	.917	.727	.906	.701	.829	.800
40	.891	1.036	.871	1.036	.878	.928	.940
50	1.000	1.000	1.000	1.000	1.000	1.000	1.000
60	1.104	.983	1.031	.983	1.080	.991	1.029
70	.967	.935	.886	1.018	1.019	.921	.958
80	.952	.841	.871	.879	.956	.879	.896
90	.840	.853	.871	.853	.956	.773	.858
600	.790	.707	.765	.794	.885	.682	.770
10	.674	.592	.608	.610	.818	.558	.643
20	.426	.446	.443	.460	.529	.380	.448
30	.313	.305	.267	.362	.310	.282	.306
40	.197	.193	.175	.209	.234	.177	.198
50	.130	.116	.111	.131	.151	.113	.125
60	.066	.069	.064	.077	.082	.062	.070
70	.035	.038	.033	.042	.041	.031	.037
80	.018	.016	.017	.019	.022	.016	.018
90	.009	.008	.008	.010	.012	.008	.009
700	.004	.004	.004	.005	.012	.003	.005

TABLE III. Mean equal energy mixture functions and their sums (sV_λ), for primaries: 460 mμ, 520 mμ and 650 mμ.

	Brightness Matching				Flicker Photometry			
λ	r_e	g_e	b_e	sV_λ	r_e	g_e	b_e	sV_λ
420	.01101	-.00562	.02889	.03428	.00994	-.00395	.02199	.02799
30	.01094	-.01789	.06527	.05832	.00814	-.01190	.03536	.03160
40	.01202	-.01234	.07462	.07430	.00448	-.00860	.04149	.03737
50	.00794	-.00957	.07049	.06886	.00240	-.00772	.04606	.04074
60	.00000	.00000	.08904	.08904	.00000	.00000	.05936	.05936
70	-.00634	.03264	.10344	.12974	-.00778	.04453	.05928	.09603
80	-.01418	.11962	.04128	.14672	-.01492	.12762	.03182	.14452
90	-.02042	.18001	.02148	.18107	-.02164	.16902	.01576	.16314
500	-.02403	.30008	.01278	.28883	-.02163	.27589	.00974	.26400
10	-.02175	.47621	.00604	.46050	-.02398	.48135	.00478	.46215
20	.00000	.64457	.00000	.64457	.00000	.64900	.00000	.64900
30	.05122	.78320	-.00216	.81559	.04094	.75827	-.00175	.79746
40	.11028	.89932	-.00340	1.00620	.09203	.85365	-.00189	.94379
50	.18592	.90766	-.00312	1.09046	.15618	.84934	-.00248	1.00304
60	.24405	.78121	-.00351	1.02175	.23212	.76285	-.00229	.99268
70	.34256	.67098	-.00277	1.01077	.28877	.63960	-.00220	.92617
80	.42270	.51690	-.00185	.93775	.37193	.50358	-.00158	.87393
90	.55570	.43892	-.00149	.99313	.47493	.39030	-.00137	.86386
600	.61961	.27347	-.00152	.89156	.51068	.23786	-.00163	.74691
10	.52274	.13711	-.00161	.65797	.49164	.13909	-.00134	.62939
20	.46109	.06184	-.00059	.52234	.37471	.06203	-.00094	.43580
30	.32762	.02261	-.00031	.34992	.27801	.02455	-.00040	.30216
40	.21282	.00552	-.00030	.21804	.18561	.00868	-.00022	.19407
50	.12204	.00000	.00000	.12204	.12843	.00000	.00000	.12843
60	.06981	-.00157		.06824	.06678	-.00287		.06391
70	.03186	-.00135		.03051	.03367	-.00121		.03246
80	.01666	-.00074		.01593	.01797	-.00067		.07130
90	.00856	-.00041		.00815	.00908	-.00058		.00850
700	.00427	-.00012		.00415	.00564	-.00026		.00538

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PAPER 11

SOME VISUAL FUNCTIONS OF A UNILATERALLY DICHROMATIC SUBJECT

By C. H. GRAHAM and Y. HSIA

(56753)

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11. SOME VISUAL FUNCTIONS OF A UNILATERALLY DICHROMATIC SUBJECT

By C. H. GRAHAM and Y. HSIA

PROTANOPES, DEUTERANOPES AND NORMAL SUBJECTS

IN 1954 we presented an abstract of a report of research on the luminosity functions of normal subjects as contrasted with those of protanopes and deuteranopes *(ref. 1)*. The data are presented in *fig. 1* which gives the average log luminosity (or log sensitivity) curves for three groups of subjects consisting, respectively, of 7 normal subjects, 5 protanopes and 5 deuteranopes.[2] The basic data of the curves are relative energies required

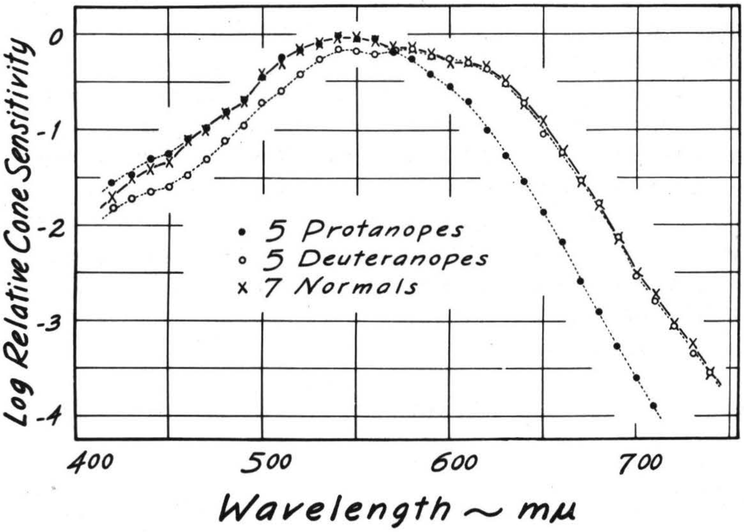

FIG. 1 - The luminosity curves for normal subjects, protanopes and deuteranopes in experiments by Graham and Hsia *(ref. 1)*.

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[1] This work was supported by a contract between the Office of Naval Research and Columbia University and by a grant-in-aid from the Higgins Fund of Columbia University. Reproduction in whole or in part is permitted for any purpose of the United States Government.

[2] The testing of our subjects involved the reading of the Ishihara and Stilling plates and the determination of the proportion of red and green required to match a yellow on the Hecht-Shlaer anomaloscope (1936). In addition, determinations were made, in the case of the dichromats, of the neutral point in the spectrum, that is, the wavelength that is seen as grey. The neutral point determinations were made with a modified Helmholtz colour-mixer *(ref. 11)*. The subjects included 15 men and 2 women (both of the latter having normal colour vision) between the ages of 20 and 35 years.

required for the cones to respond to the spectral lights at the absolute threshold; the logarithms of the reciprocals of these values (i.e., log luminosity values) are here plotted.

The peak of the average normal curve is arbitrarily set at zero (i.e., maximum sensitivity is set at unity). Absolute energies may be calculated by observing that, at 578 mμ, the average normal (interpolated) threshold is 3.5×10^{-8} ergs. This figure amounts to about 10000 quanta. Exposure duration was 4 m/s and the visual angle subtended by the circular stimulus was 42 minutes. These conditions fall within the limits of applicability of the Bunsen-Roscoe reciprocity law.

The average log luminosity curve for five protanopes is represented by filled circles. It is to be observed that the average curve for protanopes shows a greatly decreased sensitivity in the red end of the spectrum as contrasted with the normal subject and deuteranopes. In the blue, the energy requirement for protanopes is similar to that for normal subjects.

The average log luminosity values for the deuteranopes is shown by the open circles. The luminosity for the deuteranope in the green and blue is less than the corresponding luminosity values for the normal subjects. In the red, the values are comparable.

The data of the normal curve are presented by crosses. The curve is a broad function that encompasses the extremes of the colour-blind curves. As contrasted with the normal subject, the protanope has normal luminosity in the blue but shows loss in the red; the deuteranope shows normality in the red but a loss in the green.

COLOUR DISCRIMINATIONS OF A SUBJECT DICHROMATIC IN ONE EYE AND NORMAL IN THE OTHER

OUR data on the three groups of subjects have been enlarged and supported by contributions from another source: a young woman who gave colour-blind discriminations with her left eye and normal discriminations with her right eye *(refs.2,3)*. Her first test results seemed to indicate that she was deuteranopic, but as our observations multiplied, we found that our subject did not give completely typical deuteranopic responses.[3,4] Without going

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[3] We are indebted to Professor Lucy J. Hayner of the Physics Department, Columbia University and Dr. Simon Shlaer, of the University of California, for technical discussions concerning our equipment.

Dr. Gertrude Rand and Miss Catherine Rittler, both of the Laboratory of Ophthalmology, Columbia University College of Physicians and Surgeons, kindly examined our subject and concluded that, although her right eye is normal by all the tests used, including the Nagel anomaloscope, her left eye does not fit a simple category of classical colour defect. We deeply appreciate the cooperation of Dr. Rand and Miss Rittler in the testing of our subject. We are also indebted to Dr. R. L. Pfeiffer of the Ophthalmological Institute Columbia University College of Physicians and Surgeons, for the ophthalmological examination of our subject. No organic disease was found. A correction of -2 dioptres is required for the right eye and -4 for the left. (During the experiment the correction lenses were installed just behind the artificial pupil, on the side away from the subject.)

[4] See p. 11. p283

into detail, it is sufficient to state here that some loss in blue sensitivity seems to be added to the basic loss of green sensitivity in the colour-blind eye of our subject.[5]

Some observations were made with a Helmholtz colour-mixer on the normal and colour-blind eyes of our subject. The Helmholtz colour-mixer is an instrument that makes it possible to match a mixture of two primaries in one half of a visual field against a mixture of two lights in the other half of the field. The mixture in the latter half consists of the remaining primary plus a narrow wavelength band taken from any spectral region.

Due to a number of inherent difficulties, the Helmholtz colour-mixer is not a perfect instrument for performing colour mixture experiments. Despite our recognition of this fact, we made a series of determinations on our subject's normal and colour-blind eyes. We do not wish to labour the significance of our results, but desire, nevertheless, to record the fact that our subject shows trichromatic vision in her normal eye and accepts two-colour matches in her colour-blind eye. For the normal eye, our primaries were the same as Wright's *(refs. 4,5)* 460, 530 and 650 mμ. The data are not sufficiently discriminative to tell us whether or not her trichromatic eye is anomalous, but the evidence from the screening tests tells us that it is not. Her responses on the latter tests and on the anomaloscope were normal. Data obtained on the colour-blind eye show that this eye can match any wavelength of the spectrum with a combination of two primaries, 460 and 650 mμ.

Luminosity curves

The luminosity curves of our subject are shown in *fig. 2*.

The curves for her two eyes show considerable luminosity differences in the blue and green regions of the spectrum. In this region luminosity for the colour-blind eye shows a considerable loss below the level of the normal eye. It may also be seen that our subject's luminosity loss begins at wavelengths quite far into the red. In the red region of the spectrum, both eyes show similar luminosity values; no loss is evident.

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[4] In addition to the tests (footnote 2) used on our other subjects we employed the following on our unilaterally dichromatic subject: Dvorine test (2nd edition), American Optical Company plates, Farnsworth Dichotomous D-15 and Farnsworth-Munsell 100-hue tests.

Dr. Rand and Miss Rittler used the Hardy-Rand-Rittler plates, the two Farnsworth tests, the American Optical Company plates, the Ishihara test, the Rabkin test and the Boström-Kugelberg test on both eyes. The Nagel anomaloscope was also used on both eyes.

[5] For the historical background of work on unilaterally colour-blind subjects, see Judd *(ref.12)*. Judd reports that 37 cases of unilateral colour blindness have been reported. Because of inadequate procedure or background information, only eight have proved useful for theory. The most recent research reported by Judd is that done by Sloan and Wollach *(ref.13)*.

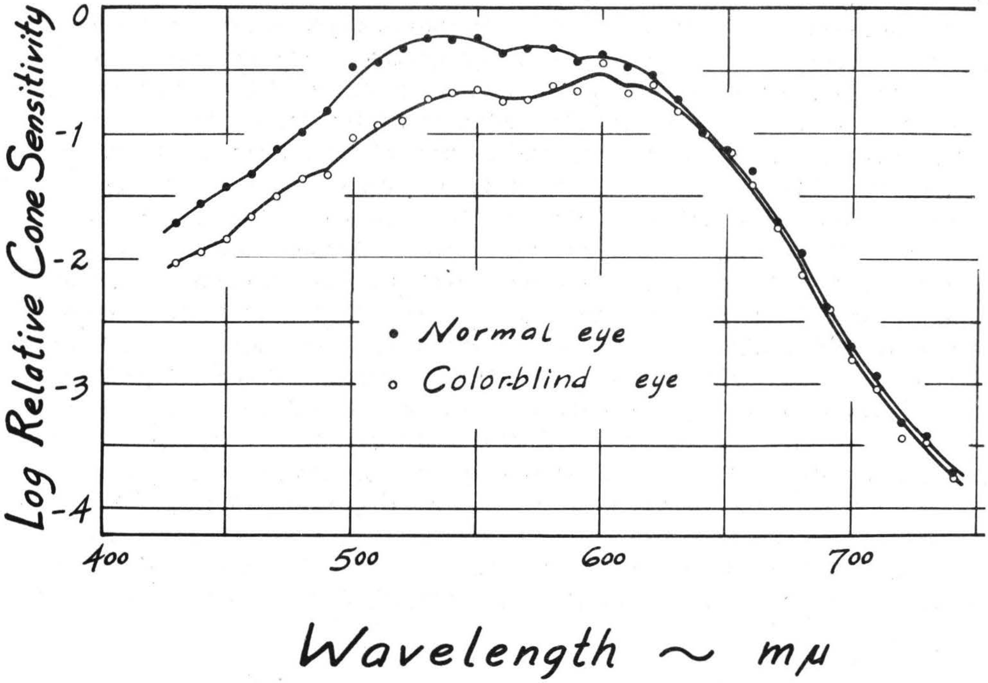

FIG. 2 - The luminosity curves for a unilaterally colour-blind subject. The upper curve is the curve for the normal eye, the lower one the curve for the colour-blind eye. Data of Graham and Hsia *(ref.2)*.

Flicker curves

We have believed it important to find out whether or not the types of luminosity loss that occur at threshold for our subject are maintained at high intensity levels. In order to investigate this problem we, together with Dr. Eda Berger, have measured critical flicker thresholds at various intensities of different colours. The field of view was usually 28 minutes in diameter, but observations were also made with 1-degree and 2-degree fields.

A considerable number of curves in the normal and colour-blind eyes were obtained with a number of colour filters, but the general nature of the result can be demonstrated by a comparison of curves for the blue, green and red regions of the spectrum. The data are shown in *fig. 3*.

The curves for blue light are shown at the top of the graph. In both the normal and colour-blind eyes, flicker fusion increases with intensity, the respective curves flattening out and then dropping at high luminance values. The positions of the curves differ, however. The curve for the

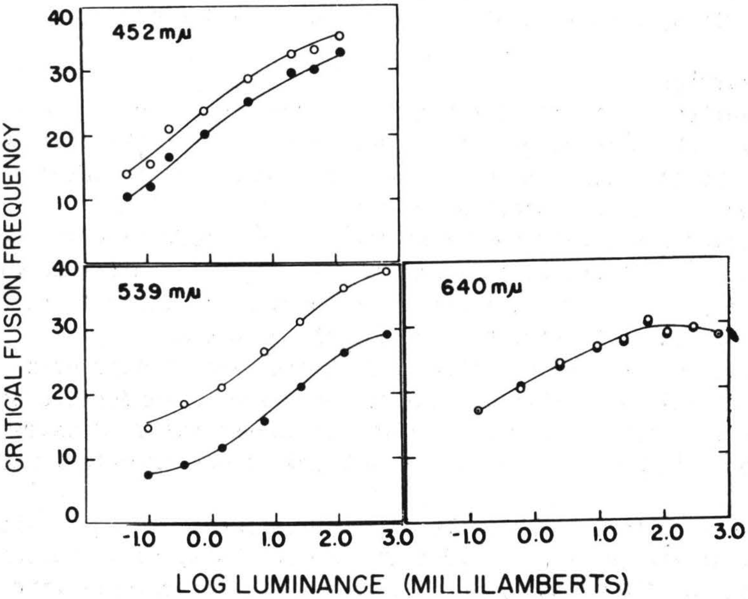

FIG.3 - Critical fusion frequency as a function of luminance for wavelengths in three different regions of the spectrum. In each graph the open circles refer to data for the normal eye, the filled circles to data for the colour-blind eye. Data of Berger, Graham and Hsia (in preparation); see also, Graham, Hsia and Berger *(ref.3)*.

colour-blind eye is displaced downward on the flicker axis compared with the position of the curve for the normal eye.

The curves in the lower left-hand graph represent the data for green light. The displacements of the curve for the colour-blind eye below the position of the curve for the normal eye is striking. In magnitude it is considerably more than was shown for blue light.

In general, the fact of displacement means two things. First, where comparable critical flickers exist on the two curves, a higher intensity is required to produce a given critical flicker in the colour-blind eye than in the normal eye. Second, at any given intensity, fusion frequency is higher in the normal eye than in the colour-blind eye. The latter statement may be especially significant, for it may mean that, if fusion frequency represents available receptor units, then the type of colour blindness represented by our subject is attributable to the loss of green receptors and, to a lesser extent, blue receptors also.

The story for red light is different: the same curve represents the data for the colour-blind eye and the normal eye. No increased intensity requirement or fusion frequency loss for the colour-blind eye is discernible in these data. No luminosity loss exists for red, even at the high intensities giving rise to fusion frequencies near the maximum.

Brightness matches

Another series of experiments bearing on the problem of luminosity loss at intensities above threshold was performed by us in collaboration with Dr. Berger. Specifically, we investigated the binocular brightness matches of our subject in various spectral regions.

The apparatus was so arranged that the subject could view two semicircles filled with light of the same wavelength. One semicircle was seen by one eye and the other by the other eye. They were viewed in such a way that they appeared to be side by side and close together.

The subject matched the semicircles in brightness by appropriate adjustments of intensity. The field of view for each eye subtended 1.8 degrees of visual angle. Eight spectral regions were investigated. Measurements for any colour extended over a luminance range of approximately 2.5 log units.

We shall not go into the details of the experiment. It is sufficient to say that, at all levels of intensity, the intensity of a wavelength band centering on 538 mμ must, when it is seen in the colour-blind eye, be greatly increased if it is to be judged equal in brightness to the corresponding light seen in the normal eye. A blue of 452 mμ requires a medium increase and in the red, the apparent brightnesses of the two colours are the same. Other colours show intermediate degrees of brightness loss.

The results of the present investigation point to the conclusion that the type of colour blindness represented by our subject may be characterized as a loss or inactivation of part of the receptor mechanisms that mediate sensitivity in the green and (probably) blue portions of the spectrum. Furthermore, the data of binocular brightness matching and flicker show that the selective luminosity loss for our subject is not a phenomenon that exists only at cone threshold levels. It exists at all levels of intensity above cone threshold.

DISCUSSION

OUR results on groups of normal subjects, protanopes, deuteranopes, and a unilaterally colour-blind subject have a definite bearing on the question of luminosity loss in dichromats. Our findings on protanopes are the usual ones: protanopes lose luminosity in the red region of the spectrum. Our observations on deuteranopes are in line with some reported by Hecht and Hsia *(ref. 6)*: deuteranopes show a luminosity loss in the green. Our unilaterally dichromatic subject shows a luminosity loss in the green and in the blue over a wide range of intensities.

At the time when Hecht and Hsia reported their experiment the results were not in accord with general presumptions as to the nature of deuteranopia, a condition that was usually interpreted in terms of Leber-Fick theory *(refs. 7,8)*. The interpretation in terms of Leber-Fick theory was in accord with the prevalent idea that deuteranopes did not lose sensitivity as compared with normal subjects *(ref. 9)* but were like protanopes in seeing only yellow throughout the long-wave regions of the spectrum. Leber-Fick theory accounts for protanopia and deuteranopia by the idea that the red and green receptors have developed similar or identical absorption characteristics while their central connections retain their usual pattern of organization. Pitt *(ref. 10)* has suggested that the idea of transformed absorption characteristics applies only to deuteranopia; protanopia, he says, involves a loss system.

Hecht and Hsia concluded on the basis of their experiment that the idea of a loss system was supported by their results. However, they were in a dilemma as to how the notion of loss could account for the colour names reported by unilateral deuteranopes and protanopes. The reason for the dilemma is clear. How could yellow, by trichromatic theory the mixture of red and green, be reported in the absence of either red or green receptors?

The dilemma still remains after a consideration of the present results but it is hoped that our subject will supply data on colour naming that will help resolve the problem. Such data have been obtained, but more will probably be required. Despite the fact that our subject has moved from New York, we believe that arrangements may be made for the further and more definite testing of her eyes. In any case we hope so.

SUMMARY

1. Data have been obtained on three groups of subjects, made up, respectively, of 5 protanopes, 5 deuteranopes and 7 normal subjects. As compared with normal subjects, protanopes show a loss of luminosity in the red. This is the usual finding. In conformity with the results of Hecht and Hsia *(ref. 6)*, deuteranopes are found to show a loss of luminosity in the green.

2. Experiments have been performed on a young woman whose right eye exhibits normal colour vision and whose left eye is dichromatic. The dichromatic eye can match any wavelength in the spectrum with a combination of two primaries, 460 mμ and 650 mμ.

3. Our subject's luminosity function shows a loss of luminosity for the dichromatic eye in the green and blue regions of the spectrum.

4. As shown by data on flicker and brightness matches, the loss of luminosity is maintained at relatively high intensities of illumination.

5. The implications of our findings are considered with reference to the problems of luminosity loss.

ADDENDUM

Colour-Mixture

Last summer, after having made some earlier observations, we had the opportunity, thanks to Commander Farnsworth and Captain Vogel, of the Submarine Base, New London, to examine additional data on the colour-mixture functions of the two eyes of our unilaterally colour-blind subject. The data were obtained by Dr. Sperling. Results will be discussed in a later collaborative report.

The curves in the upper graph of *fig.4* are the colour-mixture data for our subject's normal eye. They are to be contrasted with Wright's well known results on ten normal eyes which are represented by the dashed lines *(refs. 4,5)*. Our primaries are the same as Wright's: 460, 530, and 650 mμ. The colour-mixture function for our subject's normal eye seems, in fact, to be normal.

The data for our subject's colour-blind eye, shown at the bottom of *fig.4* are entirely different from the data of the normal eye. The graph shows that the subject can match any wavelength of the spectrum with a combination of two primaries, 460 mμ and 650 mμ. The open and solid circles indicate two different types of result that were obtained in the short wavelength region depending on the method used. When the test wavelength was matched by the two primaries, the results are as indicated by the solid circles. When the red primary was added to the test wavelength, the results are as given by the open circles. The latter method is the one used by Pitt *(ref.9)*, and it is seen that this result is much closer to Pitt's data, shown by the dashed lines, than are our other results. However, the first method gives results that seem to be more in line with our subject's data on hue discrimination.

Hue Discrimination

A hue discrimination curve was obtained on each eye of our subject. The curves are represented in *fig.5*.

In general, one can say that the curve for the normal eye does not seem to be greatly different from the usual hue discrimination curve obtained on normal individuals. It is quite clear that the normal eye of our subject does not show defective hue discrimination.

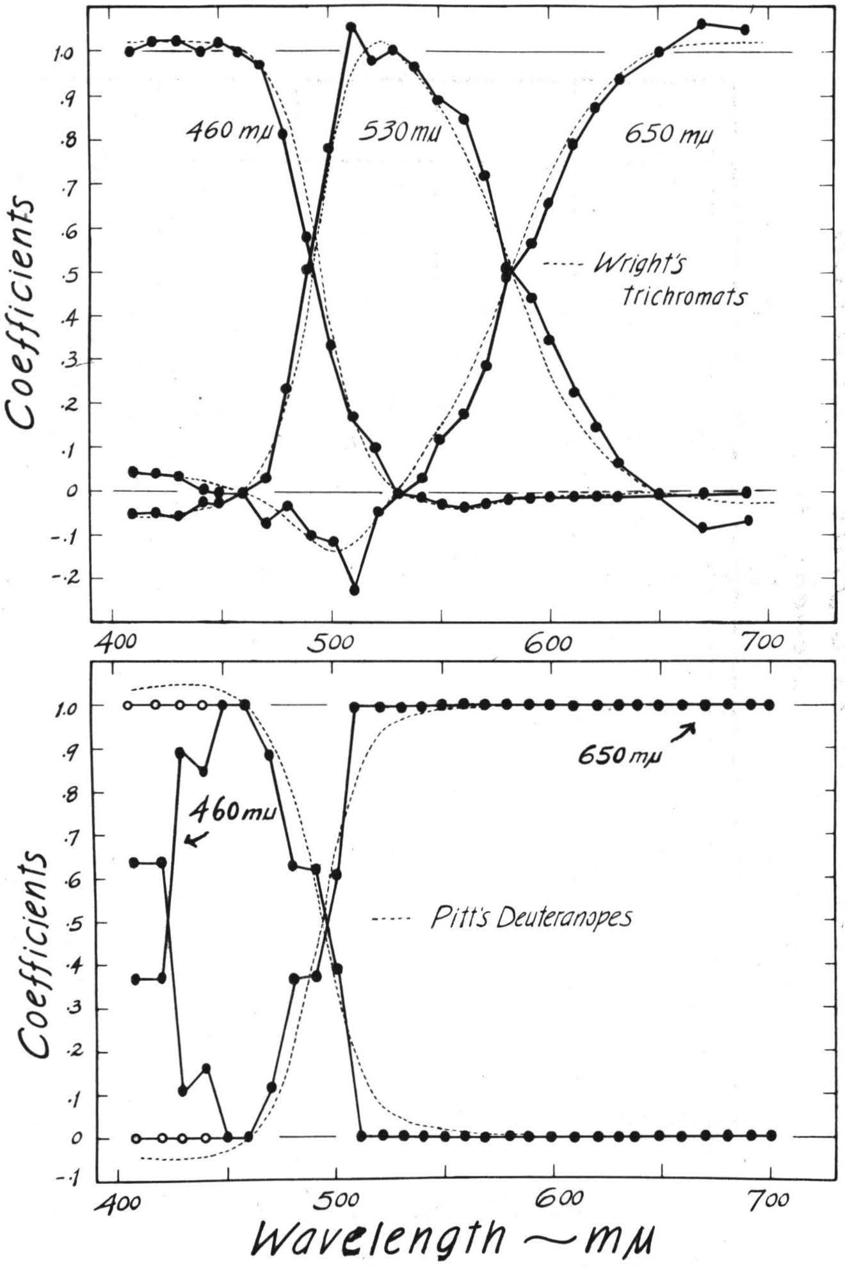

FIG. 4 - Colour-mixture data for the subject's normal eye (upper graph) and for her dichromatic eye (lower graph). The luminous units of primaries for the normal eye fall within Wright's distribution.

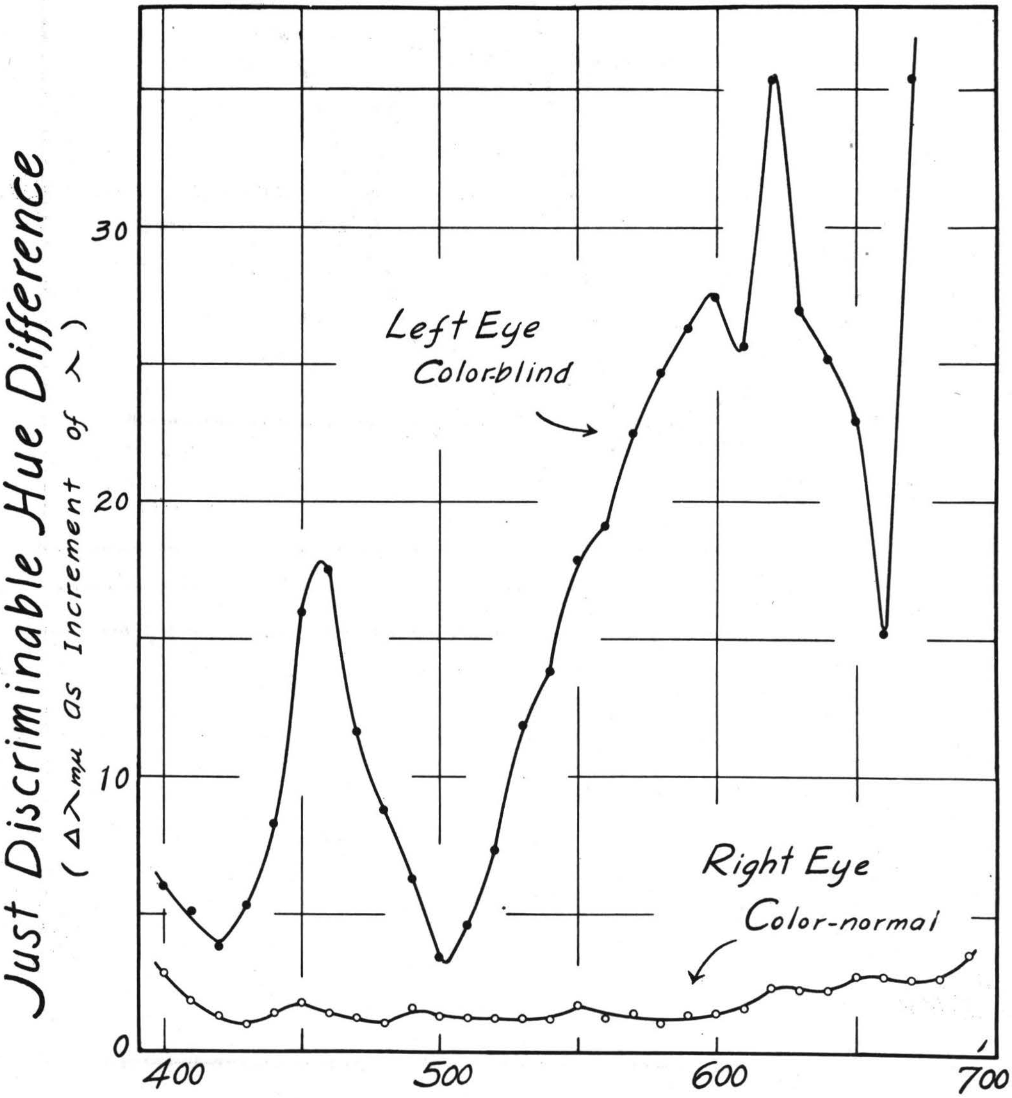

FIG. 5 - Hue discrimination curves for the trichromatic eye (lower curve) and dichromatic eye (upper curve) of our unilaterally colour-blind subject.

The curve for the left eye, the colour-blind eye, is an entirely different function. In the violet, the curve shows rather low values of $\underline{\Delta\lambda}$ with a minimum near 425 mμ; near 450 mμ it shows a rise in $\underline{\Delta\lambda}$. Discrimination improves to a minimum threshold value near 500 mμ in the region of the neutral point; thereafter it rises to high values near 600 mμ. The behaviour of hue discrimination in the spectral region from 500 to 750 mμ seems to be of the sort found in deuteranopes.

The hue discriminations shown in the short wavelengths may be in accord with our subject's colour-mixture data obtained by the two-against-one matches in her colour-blind eye. It is to be remembered that the colour-mixture data in this region of the spectrum indicate that a crossing of curves takes place near 425 mμ. This finding may be in line with the data of hue discrimination which show a minimum near 425 mμ.

Binocular Colour Matches

We should finally like to consider some important data on binocular colour-matching.

The experiment on binocular colour-matching was performed by means of an apparatus, essentially a mirror stereoscope, that was so arranged as to provide slits of colour in the left and right eyes. The subject, in viewing them, could see them side by side, the slit in the left eye being vertical, and the slit in the right eye being horizontal. The slits were so arranged that there was no apparent intersection of the images.

The results are summarized in *fig. 6*. In general it seems that, in her dichromatic eye, the subject matches all wavelengths greater than the neutral point against a wavelength in the normal eye of about 570 mμ. Wavelengths shorter than the neutral point in the dichromatic eye are matched in the normal eye by a blue of about 470 mμ. Thus the two sides of the spectrum below and above the neutral point are seen respectively as a blue equivalent to about 470 mμ and a yellow equivalent to about 570 mμ in the trichromatic eye.

Discussion

What can we say about our results?

We wish to emphasize one result, which we take to be, together with the fact of loss for the dichromatic eye, a major outcome of the experiment. The result centres on the binocular colour-matching of our subject. How can the fact of dichromatic loss be brought into line with this result? How can yellow be seen by a dichromat who shows luminosity loss in the green if, as trichromatic theory demands, yellow is a mixture of green and red?

The account which we shall discuss is indicative of the type of thinking we have engaged in concerning the problem of dichromatic loss and the binocular matching of a unilateral dichromat. We shall here deal only with the seeing of yellow by dichromats.

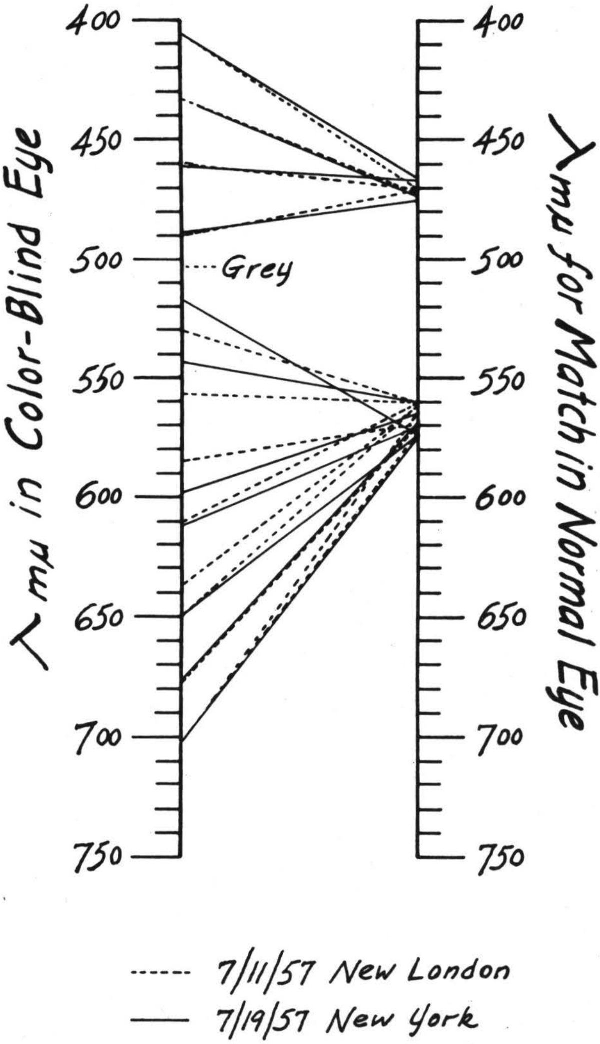

FIG. 6 - The data on binocular matching.

Let it be supposed that in deuteranopia, for example, the red fundamental curve *R* moves toward the short wave part of the spectrum, with of course a change in sensitivity brought about by virtue of the new absorption materials. At the same time, the green fundamental curve *G* moves towards the red. The curves in their new positions must meet at least two requirements. (1) Their constant ratio of ordinates must be such that their luminous fluxes mix to match the single hue, 570 mμ. (The present discussion is non-committal as to primaries and we shall use for purposes of illumination an ordinate ratio of unity). (2) The ordinate values of the transposed curve must sum to give normal luminosity in the range of wavelengths embraced by the curves.

Some of the implications of these suppositions may be analyzed in terms of the König-type fundamentals in the upper graph of *fig. 7*.

For the limiting condition, where the luminosities of the two identical curves add to give normal luminosities, the following device has been used. Twice the ordinate values of the curve designated *Limiting Change* have luminosities equal to the sum of the *R* and *G* ordinates in the normal curves. This amounts to saying that twice the limiting curve has, at any wavelength, an ordinate equal to the ordinate of *R* and *G*. The sum of the two identical ordinates is of course equal to normal luminosity. These superimposed curves, indicated as *Limiting Change* curves, represent a dichromat for whom a central mixture of the identical fundamentals results in seeing yellow throughout the long wave region of the spectrum with *no* accompanying luminosity loss.

As shown in the lower graph, any luminosity loss can be introduced by assuming a curve for deuteranopes or protanopes that lies beneath the *Limiting Change* curve. The respective curves here were plotted in terms of the ratios of (a) protanopic average luminosities to normal and (b) deuteranopic average luminosities to normal (based on our group data of *fig. 1*). Since both curves fall below the *Limiting Change* curve, their doubled luminosity values will show a loss below the summed values for *R* and *G* in the case of normal subjects. Such luminosity curves would result in the seeing of yellow by dichromats in the long-wave region of the spectrum, an expectation that would follow from original uncomplicated Fick-Leber theory. They would, in addition, show the presence of luminosity loss for deuteranopes or protanopes.

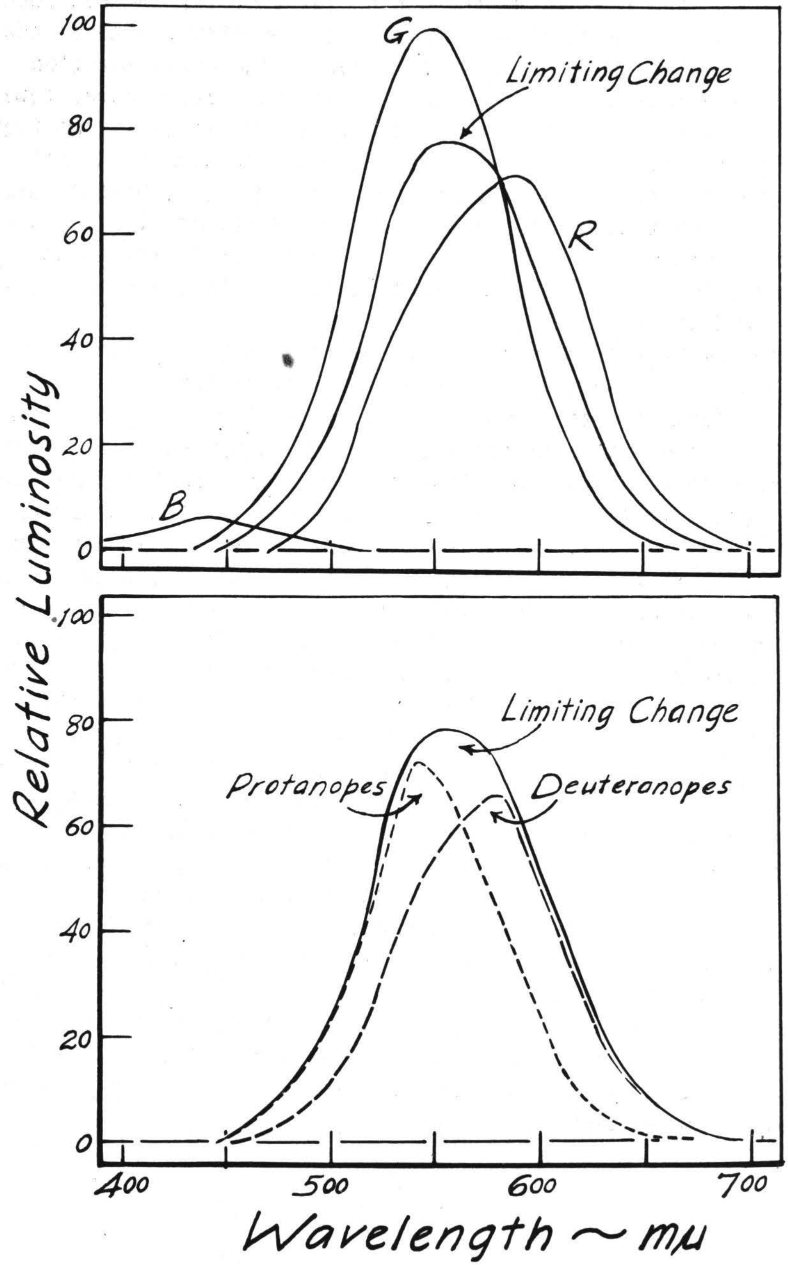

FIG. 7 - Two superimposed curves, indicated as *Limiting Change*, take the place of *R* and *G* in the upper graph. The relation of deuteranopic and protanopic luminosity losses to the *Limiting Change* curve is shown in the lower graph.

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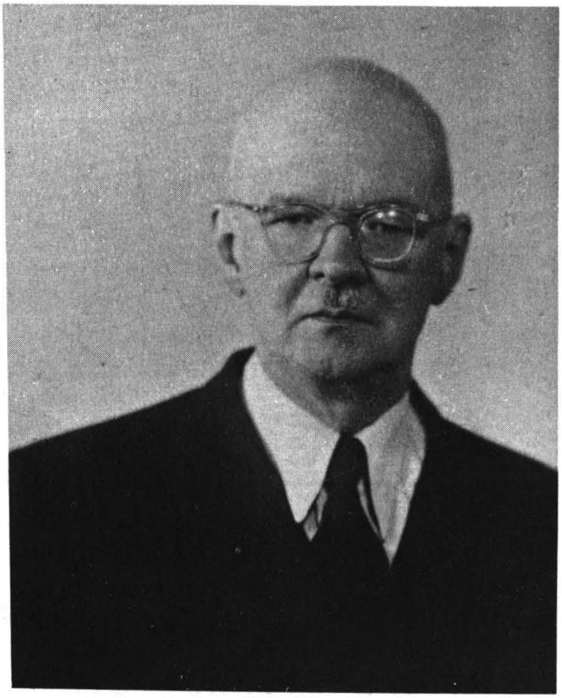
PAPER 30

DETERMINATION OF THE SPECTRAL SENSITIVITY CURVES FOR THE AVERAGE EYE BY THE POSITION OF SINGULAR POINTS IN THE SPECTRUM

By N. T. FEDOROV



N. T. Fedorov, born in 1893, graduated from the Physical Mathematical Faculty of Moscow University in 1915. From 1920–1932, he worked in the Institute of Physics and Biophysics, and from 1932–1935, he was the head of the Optical Laboratory in the Institute for Scientific Research in Photographic Films. Between 1935 and 1942, he was the head of the Laboratory of Physiological Optics of the All-Union Institute of Experimental Medicine. In 1942, he was elected to the Chair of Medical Physics.

30. DETERMINATION OF THE SPECTRAL SENSITIVITY CURVES FOR THE AVERAGE EYE BY THE POSITION OF SINGULAR POINTS IN THE SPECTRUM

(Basic theses)

By N. T. FEDOROV

AN experimental investigation of the fundamental laws of the colour contrast, carried out by us in 1948-49 *(ref. 1)*, has shown the existence in the spectrum of four "singular" points, for which complementary colours coincide with colours of simultaneous contrast, and of two singular points for the purple region *(fig. 1)*.

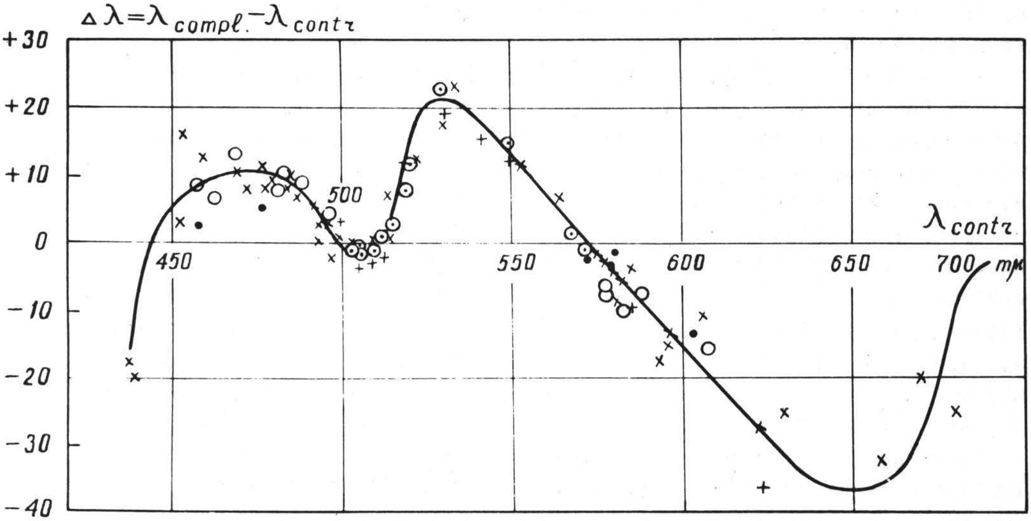


Fig.1. Experimental data for $\Delta\lambda = \lambda_{compl.} - \lambda_{contr.} = f(\lambda_{contr.})$

In addition, it has been indicated by us *(ref. 1)* that similar singular points exist for complementary colours and colours of successive contrast.

Recently (in 1955) a detailed study of the difference between complementary colours and colours of successive contrast was made in the U.S.A. by Wilson and Brocklebank *(ref. 2)*, and these singular points were seen to be located approximately at the same points of the spectrum, where they have been found by us in the phenomena of simultaneous colour contrast. The data of these authors, juxtaposed with ours, are listed in *Table 1*.

TABLE 1

Wavelengths of singular points	Wilson and Brocklebank	Fedorov, Skliarevich, Yuriev, Mashirova
λ_1	445 mμ	438 -446 mμ
λ_2	494.5 mμ	497 -498 mμ
λ_3	511 mμ	510 -511 mμ
λ_4	569 mμ	571.2-571.6 mμ

Considering that these data were obtained independently, by entirely different methods and for different sources of "white" (by us - for the source "B", by Wilson and Brocklebank - for the source "C"), they may be regarded as practically coinciding.

As Helmholtz (*ref.3*), Koenig (*ref.4*), Exner (*ref.5*), Purdy (*ref.6*) and others had already shown, the position of these singular points must correspond to the points of intersection of the fundamental response curves, which characterize the spectral sensitivity of the receptors of the eye and are expressed in the same scale as the white $R = G = B$.

Thus, for instance, for the fundamental response curves R_4, G_4, B_4, according to Judd (*ref.7*), these points of intersection correspond to the wavelengths about 442 mμ, 494 mμ, 511 mμ and 571 mμ, that is they are located very near those found by us. Such a coincidence cannot be accidental and indicates with high probability that the singular points found by us may correspond to the points of intersection of the spectral sensitivity curves of the eye receptors.

These singular points remain unchanged under wide variations of the luminance level of the induction and reaction fields. Moreover, as their experimental determination from the phenomena of simultaneous and successive contrasts is effected by establishing a complete colour match (see *ref.1*), which is done more simply and precisely than the determination of these singular points from the Bezold-Brücke effect or from the effect of hue change in result of chromaticity adaptation, we made use of them for the determination of the fundamental response curves of the normal average eye R, G, B, proceeding from the colour-mixture curves X, Y, Z.

We have plotted seven series of the fundamental response curves, the points of intersection of which lie within the limits of the accuracy of their determination in our experiments.

In *fig.2* three series of these curves are compared, their scale being selected so that the maximum ordinates for G are identical*(*ref.8*).

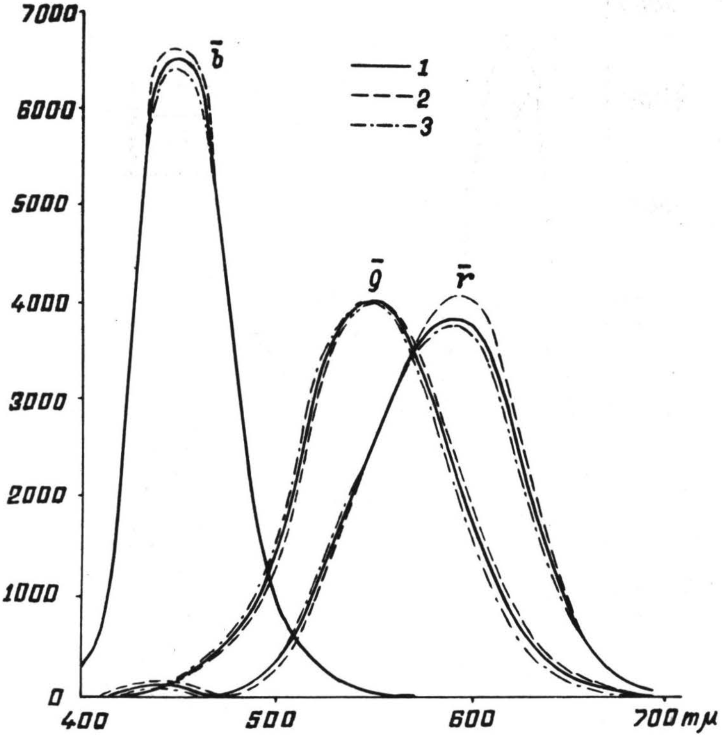


Fig.2. Three series of fundamental response curves
$\bar{r}$ (λ), $\bar{g}$ (λ) and $\bar{b}$ (λ)

In *fig.3* these curves are compared with the known Koenig-Ives (*ref.9*) curves and with the curves obtained by Yustova which are based on the assumption that deuteranopy is connected with the dropping out of the curve $\bar{g}$ (λ) (*ref.10*).

From this comparison it follows that in the case of deuteranopy we have evidently to deal with merging of the curves $\bar{r}$ (λ) and $\bar{g}$ (λ) into one:

$$\frac{\bar{r}(\lambda) + \bar{g}(\lambda)}{2}$$

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* Values of the ordinates for the third set of our curves are given in our paper at the 13th Session of the C.I.E. (*ref.12*).

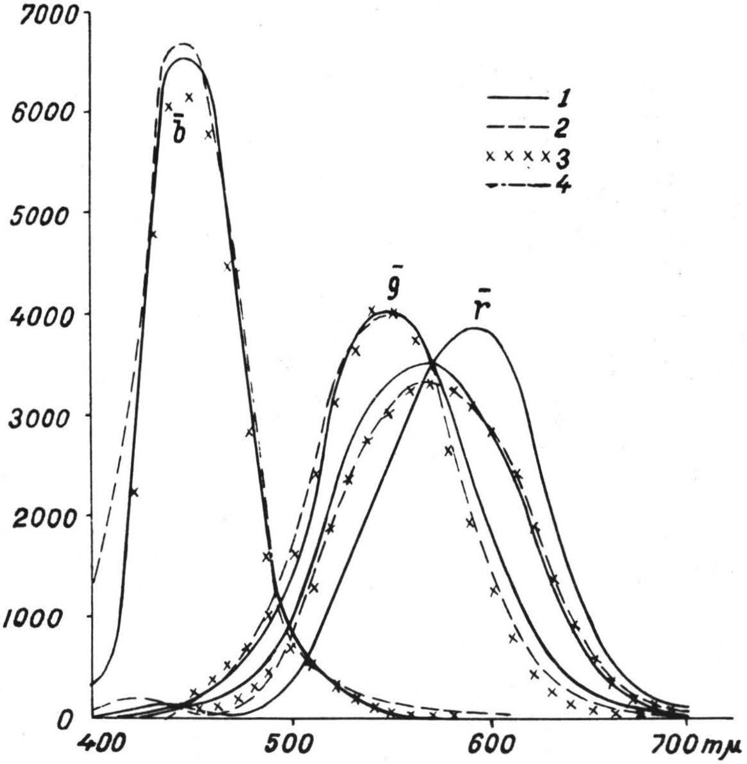

Fig. 3.
1. Our fundamental response curves $\bar{r}$, $\bar{g}$, $\bar{b}$;
2. Fundamental response curves, Judd's variant $\bar{r}4$, $\bar{g}4$, $\bar{b}4$;
3. Yustova's fundamental response curves;
4. $\frac{\bar{r}(\lambda) + \bar{g}(\lambda)}{2}$

This may be connected, for instance, with non-differentiation of the corresponding visual centres or with the fusion of receptors already at the level of the retina. In *fig. 3* the ordinates of the curve (4) represent a halfsum of the ordinates of our curves $\bar{r}$ (λ) and $\bar{g}$ (λ).

Proceeding from the curves *R*, *G*, *B*, plotted by us, we undertook an attempt to check the basic statements of the special colour metric according to Schroedinger (*ref. 11*) and found that the saturation of spectral colours and the hue sensitivity, calculated by Schroedinger's formulas, are in good agreement with experiment up to about 470-490 mμ, where a considerable deviation of the Schroedinger theory from experiment begins. These deviations we explain, in the first place, by the fact that the law of luminance additivity, which is one of the postulates of Schroedinger's theory of the special colour metric, is not valid here. Since the saturation of spectral colours, calculated by Schroedinger's formulas, has a minimum near the point of intersection of the curves $\bar{r}(\lambda)$ and $\bar{g}(\lambda)$ in the blue region of the spectrum, we determined experimentally the saturation of spectral colours for two very experienced colorimetrists and found small but pronounced minima of saturation, beyond the scope of possible errors of observation, in the region 425-430 mμ.

The Schroedinger theory also enabled a peculiar distribution of saturation in the spectrum of dichromats to be explained.

We do not go into greater detail, for a detailed exposition of the results of the verification of the Schroedinger theory was given in our paper at the 13th Session of the C.I.E. (*ref.12*).

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PAPER 31

THE ADDITIVITY OF SPECTRAL HETEROCHROMATIC LUMINANCES IN CONNEXION WITH THE REVISION OF STANDARD SPECTRAL MIXTURE CURVES

By N. T. FEDOROV

N. T. Fedorov, born in 1893, graduated from the Physical Mathematical Faculty of Moscow University in 1915. From 1920-1932, he worked in the Institute of Physics and Biophysics, and from 1932-1935, he was the head of the Optical Laboratory in the Institute for Scientific Research in Photographic Films. Between 1935 and 1942, he was the head of the Laboratory of Physiological Optics of the All-Union Institute of Experimental Medicine. In 1942, he was elected to the Chair of Medical Physics.

31. THE ADDITIVITY OF SPECTRAL HETEROCHROMATIC LUMINANCES IN CONNEXION WITH THE REVISION OF STANDARD SPECTRAL MIXTURE CURVES

By N. T. FEDOROV

SUMMARY

GENERAL results which were obtained in this work may be formulated in the following items:

1. Whereas in the region of the spectrum from 650 to 540 mμ the law of additivity of heterochromatic luminances is strictly established in the blue-violet region of the spectrum (from 490 mμ and lower) departures from additivity were found having the order of magnitude from 5 per cent (for 490 mμ) to 12 per cent and higher (for 430 mμ) of the value of the luminances compared.
2. These departures from additivity for the blue-violet region of the spectrum are observed at a low (10-20 asb) as well as at a high (150-200 asb) level of luminance.
3. For the matching of two mixed colours with different content of blue, a greater luminance should be taken of that mixture which contains more of the blue.

   If arithmetic sums of luminances of the components in the mixtures to be compared are equal, the mixture containing a blue component will appear less bright.
4. Therefore, in the determination of the standard spectral mixtures curves, no operations should be included which infer the validity of the law of additivity of heterochromatic spectral luminances for short wavelengths.

THE ADDITIVITY OF SPECTRAL HETEROCHROMATIC LUMINANCES IN CONNEXION WITH THE REVISION OF STANDARD SPECTRAL MIXTURE CURVES

AN analysis of the literature which is concerned with the additivity of spectral luminances *(ref. 1)* allows the conclusion that, in order to eliminate the influence of saturation and to avoid difficulties of heterochromatic photometry, the verification of additivity of heterochromatic luminances should be done so that the work reduces to the comparison of luminances of colour mixtures, visually identical in hue and saturation, but markedly different in spectral composition (e.g., establishing a colour match: orange + green-blue = green + violet).

It should be emphasized that the existence of departures from additivity beyond the errors of measurement requires a revision of a series of basic conceptions lying at the foundation of photometric and colorimetric measurements, for instance, the standard curve of spectral sensitivity of the eye V_λ, and the spectral colour mixture curves for the 1931 C.I.E. standard observer, which were obtained on the assumption of the validity of the law of additivity of heterochromatic luminances. This thesis was taken into consideration at the 13th Session of the C.I.E. in 1955 *(ref. 2)*.

Thus, the investigation of the problem of additivity of heterochromatic luminances and the estimation of the degree of departure from additivity in certain regions of the spectrum, apart from its importance for the determination of limits of applicability of the special colour-metric of Schrödinger, has a great practical value, as it allows the determination of the limits of applicability of the computation method in practical colorimetry.

The experimental verification of additivity of heterochromatic luminances was accomplished by us, together with V. V. Skliarevich, by matching, in two adjacent photometric fields, two colours identical in hue, saturation and brightness, but each representing in general, a mixture of two different spectral colours. The spectral components of a mixture had therefore to be properly selected and taken in suitable proportions.

If the spectral luminances of the colours to be mixed are known, as well as the proportions in which they are mixed, it is not difficult to calculate the total luminance of the compared mixtures and to test their equality or inequality to luminances obtained by visual colour matching of photometric fields.

The chosen mixtures were obtained and compared by means of a Helmholtz-König-Bechstein colour-mixing apparatus *(fig. 1)*, which was carefully adjusted and calibrated. Without going into details of its description *(ref. 3)* we shall only point out that each of the two semicircular photometric fields of the instrument, which are divided by a vertical line, could be illuminated by light from a corresponding collimator.

By means of two Glan-Thompson prisms and an Abbé birefringent prism, it was possible to obtain in a given photometric field a mixture of any pair of spectral colours in a required proportion and of a proper luminance.

From the birefringent collimator prism the two beams emerge polarized in mutually-perpendicular planes; one of the beams is directed along the collimator axis, the other beam deviates from the axis at a certain angle. The undeviated beam, the wavelength of which depends on the position of the collimator with respect to the dispersing prism, travels along the collimator axis and through the exit slit, to enter the observer's eye. At the exit slit this beam mixes with the deviated beam, going in the same direction, but having a different wavelength, which is determined (at a given position of the collimator with respect to the dispersing prism) by the position of the birefringent prism on the collimator axis. Two Glan-Thompson prisms

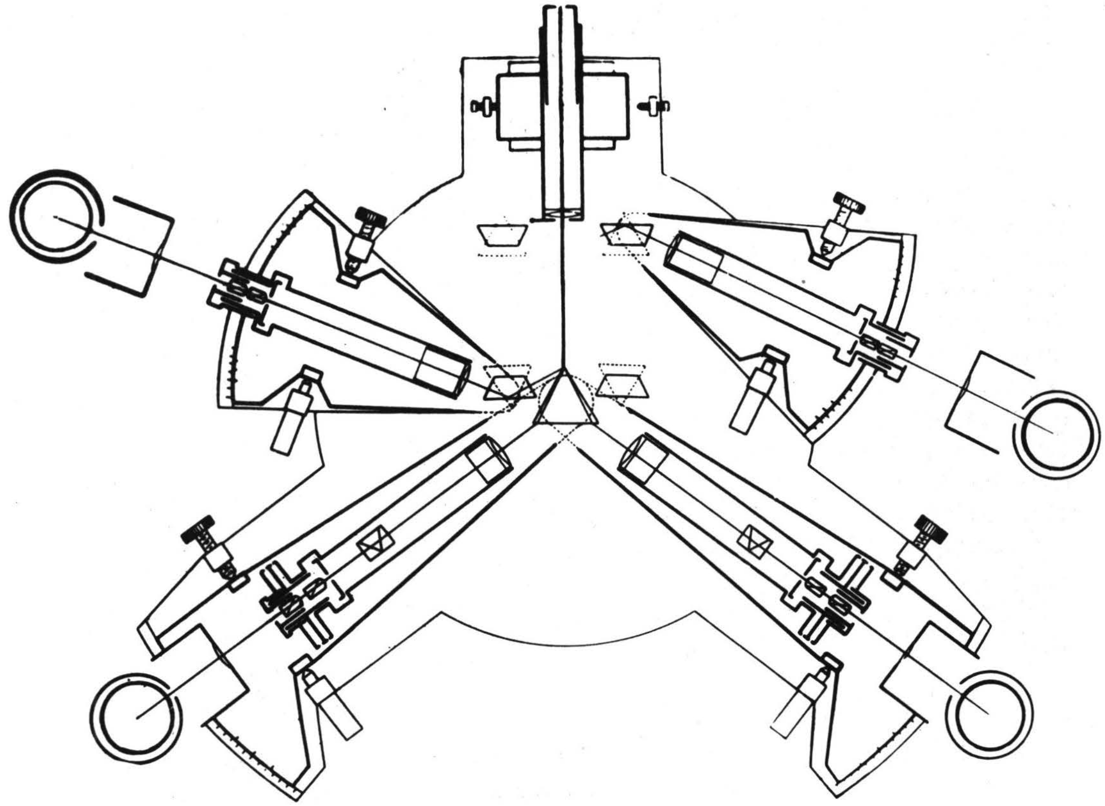

Fig. 1. Diagram of Helmholtz-König-Bechstein colour-mixing apparatus

before the entrance slit enable the total luminance of a mixture and the proportions of its components to be varied.

If α is the angle of rotation of the outer Glan-Thompson prism from the position of extinction of the light transmitted by the system of the two prisms, and β is the angle of rotation of the inner Glan-Thompson prism at which the beam deviated by the birefringent prism becomes extinct, then the luminance of the colour mixture, observed through the exit slit, will be expressed by the formula:

$$L = (L_{I} \mathrm{Sin}^2 \beta + L_{II} \mathrm{Cos}^2 \beta) \mathrm{Sin}^2 \alpha \qquad \ldots\ldots\ldots\ldots (1)$$

where L_{I} and L_{II} are maximum luminances of the components mixed when the Glan-Thompson prisms are parallel. In order to express all luminances in the same relative units it is necessary to know the curve of relative brightness distribution for the beam undeviated by the birefringent prism (at least, for one of the collimators of the instrument). The luminances of all other colours, which enter in a given photometric equation may then be found by comparing them directly with spectral luminances taken from the known curve of brightness distribution $L = f(\lambda)$.

The curve of luminance distribution in the spectrum of the beam undeviated by the birefringent prism for the right basic collimator of the instrument, has been carefully measured for the eyes of N. T. F. and V. V. S. by the "step-by-step" method with $\Delta\lambda = 4$ mμ over the range from 670 mμ to 430 mμ for 300 Watt projection lamps, with a ground glass inserted between them and the exit slit of the collimator, and in addition, by V. V. Skliarevich for his own eye over the range from 630 mμ to 410 mμ for a ribbon filament lamp (16 A, 5.6 V). This was placed so that the image of the incandescent ribbon of the lamp, produced by a specially selected optical system, was lying in the plane occupied in the first stage of the work by the ground glass. The use of ribbon lamps enabled the luminance of the photometric fields to be raised about 30 times, which made feasible the work in the blue-violet region of the spectrum at a field luminance of the order 150-200 apostilbs.

The curve of luminance distribution, obtained for the eye of V. V. S. at high levels of luminance in the blue-violet region, is shown in *fig. 2.*

If the law of luminance additivity is valid, then the following equality should be realized:

$$(L_1 \sin^2 \beta_1 + L_2 \cos^2 \beta_1) \sin^2 \alpha_1 = (L_3 \sin^2 \beta_2 + L_4 \cos^2 \beta_2) \sin^2 \alpha_2;$$

β_1 and β_2, in particular cases, may equal either 0° or 90°.

It has been found that all over the range from the red end of the spectrum to the green, additivity is observed with an accuracy of ± 1.0%, as may be seen from *Tables I(a)* and *I(b)*.

TABLE I (a)

L_{610}	=	$L_{538.5}$	+	L_{641}
L_{594}	=	$L_{538.5}$	+	L_{641}
L_{580}	=	$L_{538.5}$	+	L_{641}
L_{565}	=	$L_{538.5}$	+	L_{641}
L'		L''		

TABLE I(b)

N. F.			V. S.		
L'	L''	ΔL	L'	L''	ΔL
0.337	0.341	+ 0.004	0.353	0.359	+ 0.006
0.380	0.384	+ 0.004	0.380	0.379	- 0.001
0.352	0.344	- 0.008	0.358	0.358	± 0.000
0.293	0.289	- 0.004	0.313	0.314	+ 0.001

NOTE: Since there were errata in the corresponding table of our paper at the 13th Session of the C.I.E. in Zürich *(ref. 4)*, it is here given corrected.

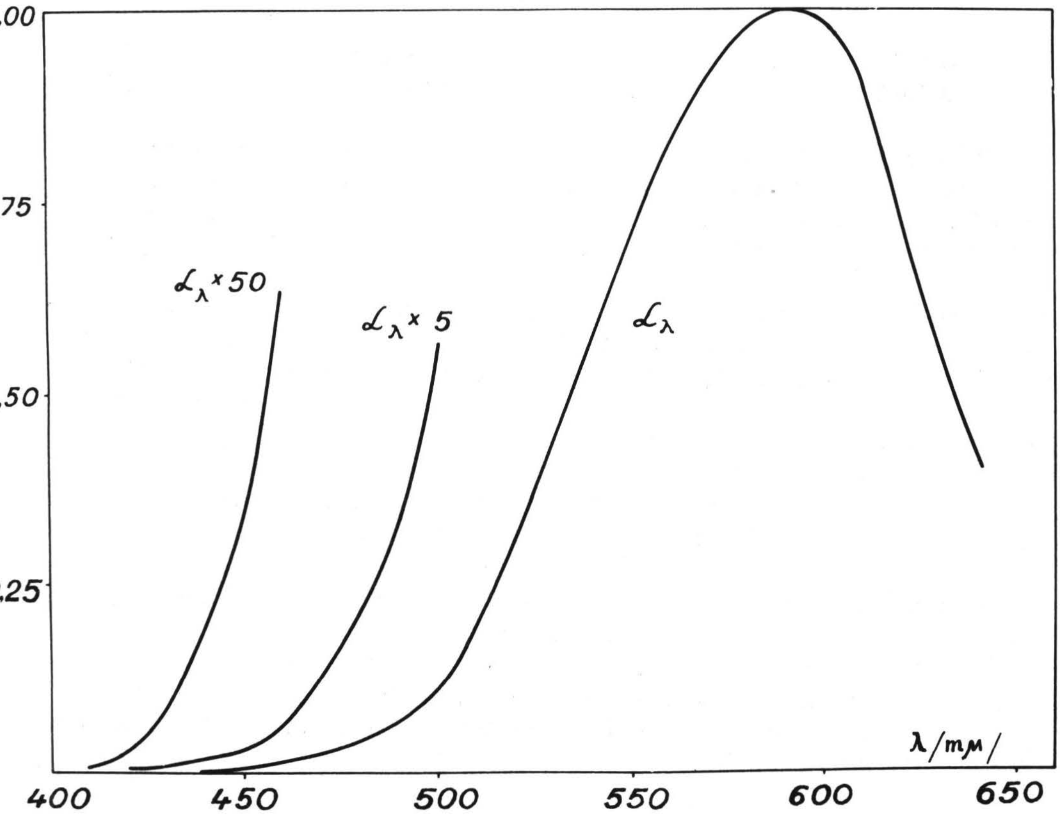

Fig. 2. Luminance distribution in spectrum of the colour-mixing apparatus for the eye of V.V.S.

However, in the blue-violet region of the spectrum the additivity law is broken, as may be seen, for instance, from *Table 2* (other instances are given in our paper at the 13th Session of the C.I.E.).

TABLE 2

$L' = L_{630} + L_{500} = L_{530} + L_{470} = L''$			
L'	L''	$\Delta L/L$	Observer
0.0954	0.1081	7.7 %	V.S.
0.0631	0.0705	11.0 %	N.F.
$L' = L_{530} + L_{494.5} = L'' = L_{573.2} + L_{460}$			
L'	L''	$\Delta L/L$	Observer
0.0777	0.1060	8.1 %	V.S.
0.0880	0.0988	10.4 %	N.F.

In this investigation, however, the luminance of a mixture with $\lambda = 450$ mμ reached only 11 apostilbs. Therefore control observations of the luminance additivity have been undertaken in the blue-violet region of the spectrum at high levels of luminances. This was done in the following way.

First of all, by means of the right basic collimator, which illuminates the left photometric comparison field of the instrument, a mixture of two colours with corresponding wavelenths $\lambda = 500$ mμ and $\lambda = 590$ mμ, was provided in this field. The settings of the right collimator and of its birefringent prism, necessary to produce this mixture, were kept constant during all the measurements in this region of the spectrum. The level of brightness of this field varied but slightly, because the angle of rotation α_2 of the outer Glan-Th. prism of this collimator was always kept constant, and the angle β_2, which determines the proportion of the colour - mixture components when the colour of this field is matched with the colour of the other photometric field, also varied only within $3^0 - 4^0$.

The right photometric field was illuminated through the left collimator successively by the mixtures of the green component ($\lambda = 530$ mμ) and different colours of the blue-violet region of the spectrum ($\lambda = 490$, 470, 450 and 430 mμ) *(fig. 3)*.

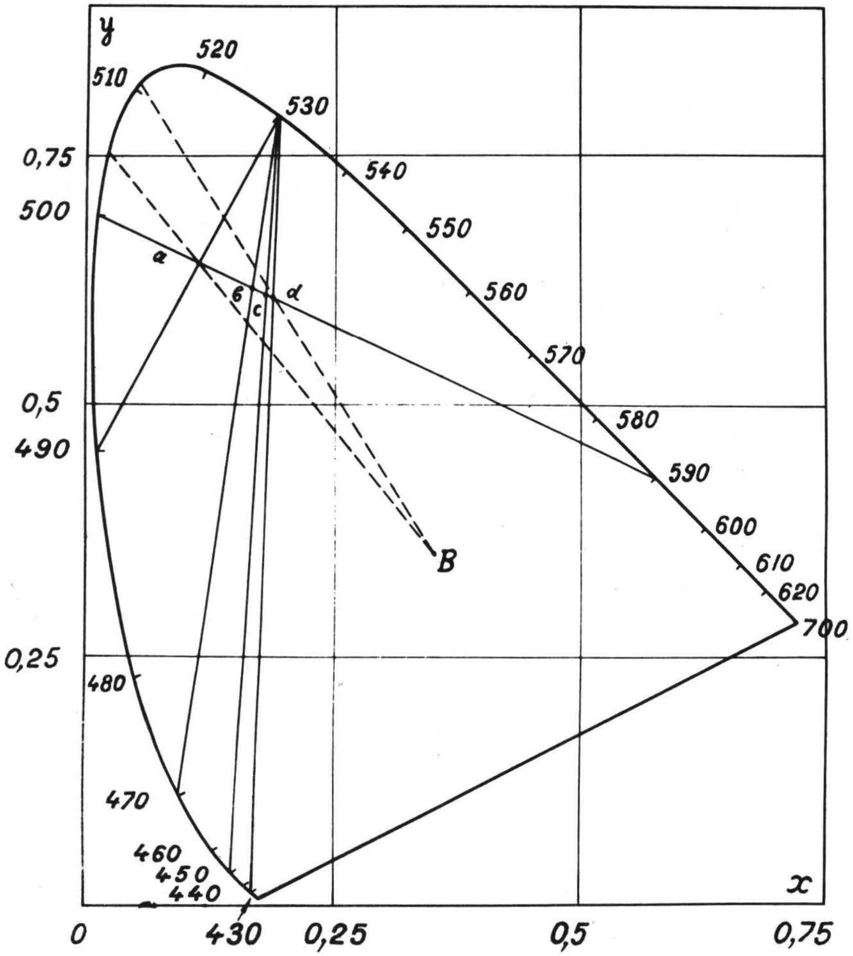

Fig. 3. Mixture diagram.

The luminance of this photometric field remained nearly constant too, for when matched with the left field its luminance was always brought up to the level of luminance of the mixture of the colours with λ = 500 mμ and λ = 590 mμ.

Thus, by varying the angles of rotation α, and β, of the Glan-Thompson prisms in the left basic collimator, and the angle of rotation β of the inner Glan-Thompson prism in the right basic collimator with constant α_2, it was possible to attain the complete matching of both photometric fields, securing at the same time a sufficiently high general level of lightness.

In order to express in the same units the luminance of all components of the mixture, a comparison was made of the spectral luminances of components, which enter into any mixture in the beam of the left basic collimator (for the corresponding position of the birefringent prism), with the luminances of the same wavelengths taken on the curve of luminance distribution for the undeviated beam of the right basic collimator, using a ribbon lamp at $\beta_2 = 0$ and at the initial position of the birefringent prism. Thus, at positions of the left collimator and its birefringent prism found for a given mixture, it was possible to make a comparison of the spectral luminances of the components with the relative spectral luminances of the same wavelengths of the left collimator under the conditions stated above.

When the match is established we have

$$L'_1 = L''_2 \frac{\sin^2\alpha_2}{\sin^2\alpha_1} \qquad \text{...} (2)$$

In a similar way luminances of beam components of the right basic collimator may be compared with the luminances of the same wavelengths taken from the same curve of spectral luminance distribution. In this case the left collimator is used as an intermediate standard.

To test the law of additivity of heterochromatic luminances in the short-wavelength region of the spectrum at high levels of luminance, the following colour equations were used, which include one short-wavelength component:

$$(L_{490} + L_{530})_1 = (L_{500} + L_{590})_2$$

$$(L_{470} + L_{530})_1 = (L_{500} + L_{590})_2$$

$$(L_{450} + L_{530})_1 = (L_{500} + L_{590})_2$$

$$(L_{430} + L_{530})_1 = (L_{500} + L_{590})_2$$

Photometric fields, illuminated by mixtures of chosen colours, were matched by varying the angles of rotation α_1 and β_1, of the polarization prisms of the left collimator and the angle of rotation β_2 of the inner polarization prism of the right collimator; the angle α_2 remained, as a rule, constant and equal to 45^0,

The procedure of full colour matching was repeated not less than ten times for each combination of mixtures, and in addition, for each combination, check observations were made in order to ascertain the reproducibility of the results obtained and their possible discrepancy.

The results of the test of the additivity of heterochromatic luminances in the short-wavelength region of the spectrum are listed in *Table 3*.

TABLE 3

Equations	$L' = (L + L_{530})$ $L = L_{490}, L_{470}, L_{450}, L_{430}$	$L'' = L_{500} + L_{590}$	$\frac{L'-L''}{L''} \cdot 100\%$
$(L_{490} + L_{530})_1$	0.123	0.118	4.2 %
$= (L_{500} + L_{590})_2$	0.129	0.120	7.5 %
			mean 5.9 %
$(L_{470} + L_{530})_1$	0.126	0.116	8.6 %
$= (L_{500} + L_{590})_2$	0.119	0.113	7.5 %
	0.180	0.166	8.4 %
			mean 8.2 %
$(L_{450} + L_{530})_1$	0.112	0.102	9.9 %
$= (L_{500} + L_{590})_2$	0.196	0.175	12.0 %
			mean 11.0 %
$(L_{430} + L_{530})_1$	0.130	0.114	14.0 %
$= (L_{500} + L_{590})_2$	0.143	0.129	10.8 %
			mean 12.4 %

From *Table 3* it may be seen that considerable and systematic departures from additivity of luminances occur in the blue-violet region of the spectrum. These departures from additivity are as high as 5-6% in the blue region of the spectrum and 12-14 % in the violet, and far exceed the limits of the possible measurement errors ($\approx \pm 2\%$), being in agreement with the previous results obtained at low luminances.

Thus, both at low luminance (of the order 10-20 apostilbs) and high luminance (up to 200 apostilbs) of the field a departure from the additivity of heterochromatic luminances takes place in the short-wavelength region of the spectrum, which is characterized by the fact that for two mixtures, visually identical but different in spectral composition, the computed (photometric) luminance is higher than that of the mixture, which contains a short-wavelength component. In other words, although the computed (photometric) luminances of mixtures might be equal, the mixture with a blue (or violet) component would appear visually less bright. For photometric match a greater quantity of a blue (violet) component is necessary.

At the same time, as shown in the first part of the investigation, additivity is practically fully verified (with a precision up to 1.0%) in the red-green regions of the spectrum (from 540 to 650 mμ), which also agrees with the data of some authors (Dresler) *(ref. 1)*.

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PAPER 36

METHODS OF DETERMINATION OF THE CO-ORDINATES OF SPECTRUM COLOURS

By N. I. SPERANSKAYA

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36. METHODS OF DETERMINATION OF THE CO-ORDINATES OF SPECTRUM COLOURS

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ONE of the outstanding problems of colorimetry formulated by the C.I.E. in 1951 was a new determination of the trichromatic co-ordinates of spectrum colours to take the place of data obtained a quarter of a century ago *(refs. 1, 2)*.

Very recently W. S. Stiles *(ref. 3)* reported his investigation, made with the assistance of 10 observers, using 2^0 and 10^0 fields of view*, and accompanied by energy measurements of the radiations. The peculiarity of colorimetric measurements in the case of spectrum colours consists in the appearance, in fields of view of large size, of the so-called "Maxwell's spot", caused by differences in spectral sensitivity of the centre of the retina and of its surrounding area.

This spot hinders colour equalization in both the half-fields observed. Stiles attempted to make measurements disregarding this spot and his data for the 10^0 field were obtained by this method.

In our measurements, which were undertaken before our acquaintance with Stiles' work, some reduction in the intensity of the spot arose from the non-monochromatic character of the colorimeter basic colours and of the desaturating colours as well. However, even this method was still inadequate completely to get rid of the hindrances caused by the spot. Because of the great importance of the problem of a new determination of the co-ordinates of the spectrum colours we have undertaken a special comparison of different methods using 5 observers.

The comparison was made between 2^0 and 10^0 fields and a second 10^0 field with a central zone (approximately 2^0) diaphragmed. A bright fixation point remained in the centre of the zone.

A monochromator was used, on the entrance slit of which the image of the filament of an intense lamp 110 V 500 W, was projected. The objective of the exit tube of the monochromator was projected upon a white plate covered with magnesium oxide. A small, bright, coloured spot obtained on the plate was then projected into the exit pupil of a trichromatic colorimeter of the Demkina type *(ref. 6)*. The variation of the field dimensions was carried out by diaphragms near the photometric cube. The measurements were made from

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* The practice of using a 10^0 field instead of a 2^0 field appeared after Rautian and Lobanova *(ref. 4)* as well as Brown *(ref. 5)* pointed out the increase in the accuracy of colorimetric measurements under these conditions.

400 mμ to 710 mμ inclusive, with intervals of 10 mμ, and in the region 420-480 mμ at each 5 mμ, because of the difficulties in making measurements in this region.

Intervals of 6 - 7 mμ at the spectrum limits and of 1.5 - 2 mμ in the middle were isolated by the exit slit.

Red and blue filters were used at the spectrum ends for eliminating scattered light. Energy measurements were carried out with a selenium photocell which could be mounted in place of the white magnesium plate. The photocell was calibrated by a thermocouple. The voltage across the lamp was varied from 80V to 150V at the spectrum limits because the retinal illumination there fell to a 2 - 3 trolands level.

Colour dilution was carried out by filters, in the main, of the same type as in the colorimeter, and also by purple ones.

Energy was measured twice: before the measurement of every colour and after it. Measurements were performed three times, and mean values derived. Every observer made two complete measurement cycles with a certain time interval, that is six observations in all were obtained for each spectrum colour. Colorimeter scale readings (k, z, c) were reduced to equal energy by dividing by the fraction $m(\lambda)/v(\lambda)$, where $m(\lambda)$ means galvanometer reading and $v(\lambda)$ represents the relative value of photocell spectral sensitivity.

The calculations were made separately for each cycle, the mean value being derived from two cycles. *Fig. 1* shows averaged data for 4 observers,* for each of the fields taken separately. As it is clear from *fig. 1*, there is a systematic discrepancy between the data for a 2° field and those for the two types of 10° field. It should be noted that this discrepancy was of the same character for all the observers, slightly varying in its value only. The most essential difference is observed in the "green" curve $\bar{z}(\lambda)$. Throughout the whole region 460-640 mμ this curve for the 2° field is somewhat displaced with regard to a 10° field towards the longer wavelengths, the displacement being especially large in the blue-green region.

Slight difference in heights of the maxima is also observable. The displacement towards longer wavelengths is observed for the "red" curves $\bar{k}(\lambda)$ as well. For "blue" curves $\bar{c}(\lambda)$ there is, on the contrary, a displacement of the maximum for the 2° field towards the blue region of the spectrum. At times the blue co-ordinate error amounts to 40% in the negative portion of the curve.

The results of the work allow the following conclusions.

1. There is rather an essential difference between the curves for a 2° field and a 10° field of either of the types, a difference also found by Stiles.

2. There are no appreciable differences between the curves for the 10° fields of the two types.

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* We excluded one observer whose "blue" curve *noticeably differed from* the curves of the present group of observers.

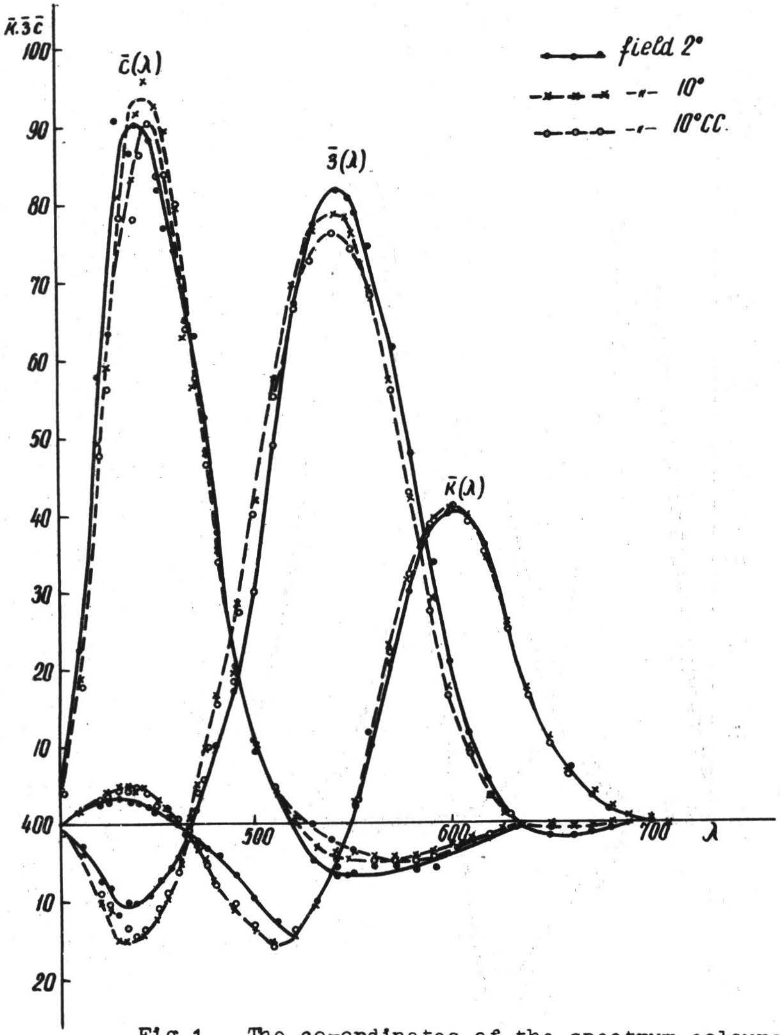

Fig. 1. The co-ordinates of the spectrum colours for 4 observers in the colorimeter system.
N.B. The 10^{0}CC curve refers to data obtained with the 10^{0} field with a central zone diaphragmed.

3. For 10^{0} fields the error of colorimetric measurements is less than for a 2^{0} field.

4. The individual differences between separate observers become apparent to a larger extent, for a 2^{0} field than for 10^{0} fields.

5. The differences between individual observers are equally apparent for both types of 10^{0} field.

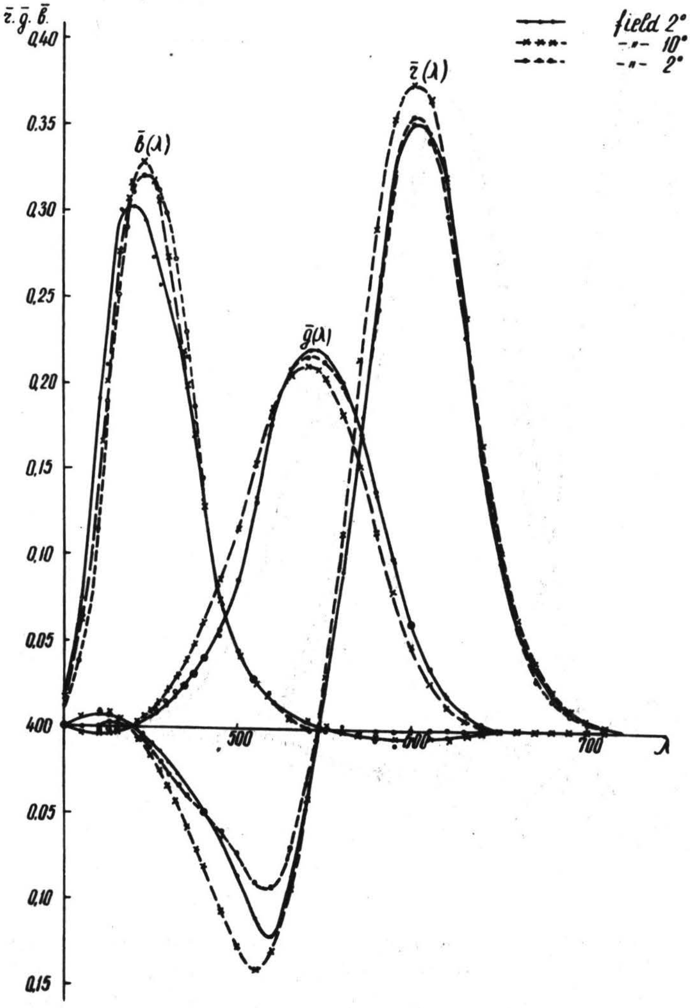

Fig.2. The co-ordinates of the spectrum colours in the 700.0, 546.1, 435.8 mμ system of C.I.E.

Thus, the presence in a 10° field of the "Maxwell's spot", which is rather noticeable for great differences between the spectral compositions of the fields compared, gives rise to the same subjective uncertainty of colorimetric measurements as is caused by the peripheral character of observations made with the centre of the field of view diaphragmed. This makes both the types of 10° field equivalent and preferable in accuracy to a 2° field.

It is of interest to compare (in spite of some simplifying conditions in our experiments) the data for a 2° field with the characteristics of the standard observer of C.I.E. 1931. With this in view, a transformation of the usual kind from the k, z, c system to the C.I.E. System (700.0, 546.1, 435.8 mμ) was undertaken, the co-ordinates of the equal energy source being 1/3, 1/3, 1/3. *Fig.* 2 shows the results of these recalculations together with the data of C.I.E.

In addition, there are plotted in the same figure our curves for a 10° field, recalculated to the same system of basic colours of the C.I.E. The scales of the curves were chosen so that their areas equal those of the C.I.E. curves.

While a sufficiently good similarity of our $\bar{r}(\lambda)$ curves and those of the C.I.E. curves, and also of the $\bar{g}(\lambda)$ curves is observed, there is still a divergence in the negative part of the "red" curve. This could probably be explained by the individual peculiarity of our group of observers. There is a considerable divergence in the course of $\bar{b}(\lambda)$. The shift of the $\bar{b}(\lambda)$ towards the violet end of the spectrum should most probably be explained by the fact that the C.I.E. data are not accurate for this region, maybe because of a lack of accuracy in the corresponding visibility values used for calculation by J. Guild and W. D. Wright. The comparison in the 700.0, 546.1, 435.8 system of the curves for the 2° and 10° fields shows the divergences more strikingly than in the k, z, c system.

In undertaking the determination of spectrum co-ordinates for a great number of observers, a 10° field of view with a diaphragmed 2° central zone should be preferred. Under such conditions the measurements become more suitable for observers who are not specially trained to overcome difficulties connected with the "Maxwell's spot".

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PAPER 29

THE FOUR-DIMENSIONAL COLOUR SPACE OF THE EXTRA-FOVEAL RETINAL AREA OF THE HUMAN EYE

By M. M. BONGARD, M. S. SMIRNOV
and LISELOTTE FRIEDRICH

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29. THE FOUR-DIMENSIONAL COLOUR SPACE OF THE EXTRA-FOVEAL RETINAL AREA OF THE HUMAN EYE

By M. M. BONGARD, M. S. SMIRNOV and LISELOTTE FRIEDRICH

SUMMARY

1. The scotopic receptor of man functions not only at low-intensity brightness levels but also at high-intensity levels of field brightness.
2. The peripheral colour space of man's field of vision is not three-dimensional, but four-dimensional. Three-dimensional equalities for large fields are only approximate, not absolute colorimetric equalities.
3. Three-dimensional equalities for large fields change when field brightness and the state of adaptation of the observer's eye are changed.
4. Four-dimensional equalities are stable. This proves the stability of the photoreceptor spectral sensitivity curves.
5. For practical purposes the application of three-stimulus value curves is quite sufficient. Yet, because of their instability not too much importance should be given to the accuracy of these curves in their standardization.

It is known that colorimetric equalities established by the observer in foveal vision are not accepted by him when observing by means of the extra-foveal part of the retina. Thus, for instance, many authors have observed that Rayleigh's foveal equality is not true for the peripheral field of vision. The reason for this phenomenon is not quite clear. The fact that the equality did not remain valid even for radiations in which light with wavelength less than 540 $m\mu$ was absent, suggested that the phenomenon could not be sufficiently explained by the pigmentation of the macula lutea. But the final solution of this question could be achieved only through an elaborate colorimetric study of the extra-foveal area of the retina.

The routine colorimetric method of matching two adjacent fields is hardly applicable when peripheral vision fields are concerned. The fact is that, because of the low acuity of peripheral vision, the line dividing the two fields is seen indistinctly. This greatly reduces the precision of colour measurements, and at a distance of about 10^0 from the fovea renders them practically impossible.

Therefore we resorted to the colorimetric method of replacement.* In this case the radiations to be matched are presented in succession,

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* Some advantages of the method of replacement were pointed out by Tichodeev.

replacing one another in time, upon the same retinal area. A replacement occurs sufficiently rarely (once in 1-5 sec.), for the principle of the replacement colorimeter not to be confused with that of a flicker photometer.

The experiment is conducted as follows: a combination of primary colours is chosen to match the test colour. After a colour match is obtained the observer at the moments of replacement sees no changes in the field of vision. It appears that when this method is employed the peripheral contrast sensitivity is much higher than when two adjacent fields are compared. The fields employed in the test were of 5^0 diameter with centre removed by 10^0 from the fovea.

The very first tests showed that the colour space of the periphery is not three-dimensional but four-dimensional, i.e. there are four linearly independent colours, whereas any five colours are linearly dependent. This means that on the periphery not three, but four receptors are functioning simultaneously.

Four-stimulus value curves for peripheral spectral colours have been obtained, and are shown in *fig. 1*. *Fig. 1* shows also the curves, theoretically calculated on the presumption that in the periphery the same three receptors function as in the fovea, and a scotopic receptor in addition. A satisfactory coincidence of the theoretical and experimental curves shows that the peripheral colour space is four-dimensional due only to the functioning of the scotopic receptors. (It should be noted that absolute coincidence of the curves is more than should be expected, for the sensitivity curves of the three photopic receptors of the fovea should differ slightly because of the pigmentation of the macula lutea). The four-dimensionality of the peripheral colour space was examined at field brightnesses up to 1000 asb.

As a consequence of the observed activity of the scotopic receptors at high brightness, the usual three-dimensional colorimetric equalities for fields exceeding 1.5^0 should be regarded as approximate, not absolutely precise, equalities. It should be borne in mind that a three-dimensional equality in a large field is to a certain degree aimed at the greatest similarity, but not at a complete identity of stimulation of all the four receptors functioning in these conditions.

As the sensitivity of the scotopic receptors considerably changes with the alteration of field brightness and the state of eye-adaptation, the question arises as to the degree of stability of the three-dimensional equalities.

Tests have been carried out examining the stability of three-dimensional equalities at variable brightness of the fields and of the state of adaptation of the observer's eye. A ring field with an external diameter of 10^0 and internal diameter of 2^0 was employed. (The central part of the field was excluded as the observer, due to the pigmentation of the macula lutea,

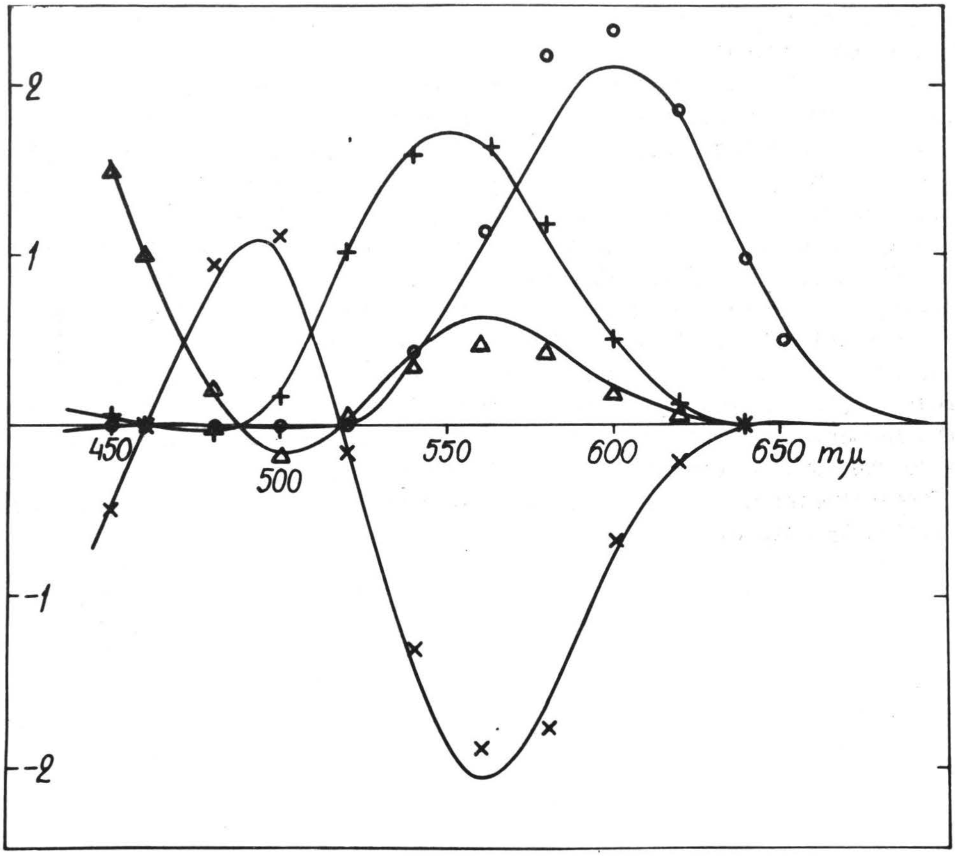

Fig.1. Four-stimulus value curves for man.

——————— Calculation curves.

—o— —x— —+— —Δ— Experimental curves.

would not accept the equality for the fovea and extra-foveal area simultaneously).

It appeared that the observer, having established by the use of three primary colours a certain equality at a certain brightness and certain preliminary adaptation, would not accept it after a change in the state of adaptation or a change in field brightness, (a proportional change of all the radiations involved in the test). To obtain equality in the new conditions it was necessary to change the amount of the primary colours sometimes by 15%.

But the question arises as to whether the inconsistency of the three-dimensional equalities is due to their being only approximate and not exact and precise equalities. Perhaps precise equalities should also change, for instance, because of the changes of the receptor spectral sensitivity curve in adaptation?

To verify this assumption, the stability of the four-dimensional equalities was investigated. The tests showed that observers accepted equalities which they had established by the use of four primary colours after all kinds of changes in the state of adaptation and field brightness. In different experiments the field brightness was changed by 4, 20 and 100 times and adaptation to white, red, yellow, green and blue fields with brightness up to 500 asb. was tested. (The same colorimeter field brightnesses and adapting colours were used as in the tests for examining the stability of three-dimensional equalities). Thus it has been proved that the spectral sensitivity curves for the primary photoreceptors do not change after colour adaptation and alteration of the brightness of the viewed field, while inconsistency of three-dimensional equalities for large fields is due to the functioning of the scotopic receptors.

Three-dimensional equalities for fields of 1.5^0 diameter proved to be sufficiently stable.

DR. W. S. STILES and DR. H. G. SPERLING summarised their papers (7 and 8 respectively). PROF. C. H. GRAHAM presented his paper (11) in its original form together with some new material which has since been included as an Addendum to the paper. PROF. N. T. FEDOROV, in his presentation of papers 30 and 31, said that in connexion with the former paper he would like to demonstrate the arrangement of the compound field of the Helmholtz-König-Bechstein apparatus which made possible the observation and measurement of the colours visible in the phenomenon of simultaneous colour induction. Using a screen D and a fine wire S we could obtain the following compound field, consisting of four parts.

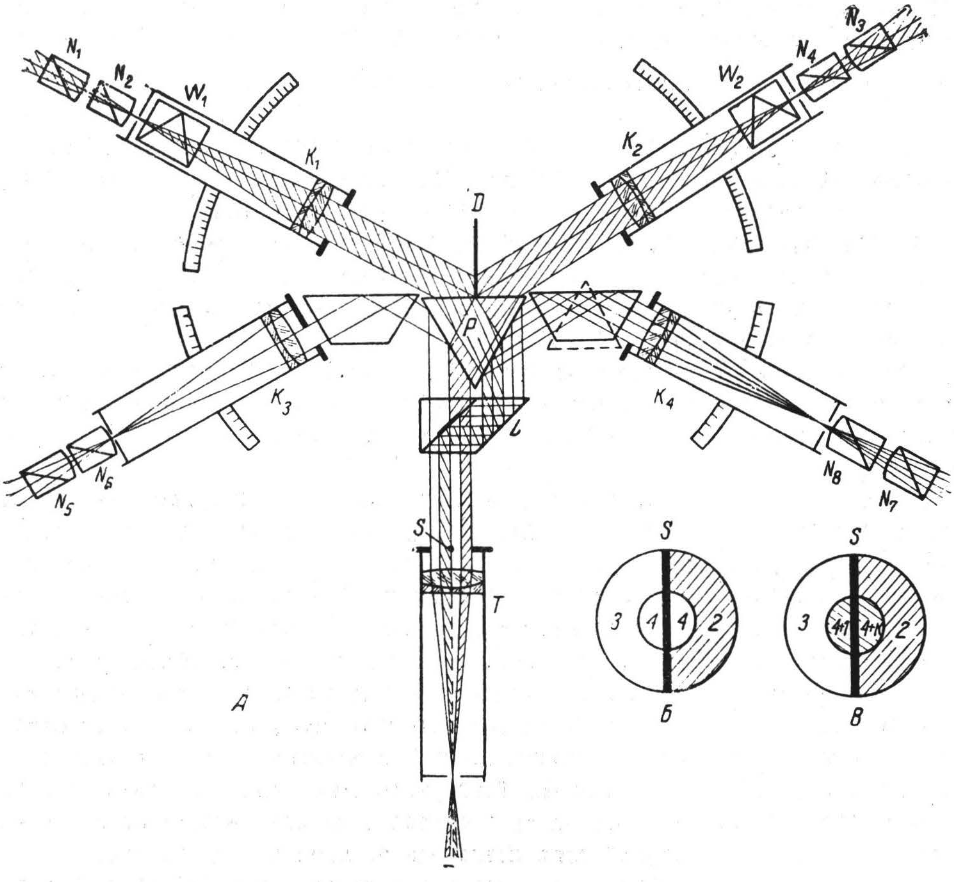

Fig.1.

The field 2, illuminated by the collimator 2, was an inducing one. The right half of the small field 4 was a reacting field. The field 3 was the white field, illuminated by the white light from collimator 3 and having luminosity equal to that of the field 2. (The source of white light was the standard source "*B*"). The colour of the simultaneous induction could be measured by finding the wavelength and quantity of the light to be mixed to the left white field 4, to obtain the colour of the right field 4. In order to find the complementary colours we had to eliminate the screen D and the wire S and to illuminate the large field by the mixture of two spectral colours, which gave colours identical with the colours of the large field.

As pointed out in paper 30, these complementary colours were identical with the contrast colours at only four points of the spectrum and at two points in the region of the purple colours. These particular points corresponded to the six points of the colour locus.

Prof. Fedorov said he would also like to note that the luminosity for protanopes and deuteranopes agreed very well with the curve G (for protanope) and the curve $\frac{R + G}{2}$ (for deuteranope), obtained by him. The two figures (*figs.* *2a* and *2b*) showed the data obtained theoretically by himself and the experimental data of Hecht and Shlaer. Similar results were obtained by Prof. Graham and Dr. Y. Hsia in paper 11 of the Symposium.

THE CHAIRMAN (PROF. W. D. WRIGHT) read in title the papers 36 and 29 by Prof. Speranskaya and by Profs. Bongard, Smirnov and Friedrich, respectively, who were not present. He then called for discussion on all the papers that had been read so far in the Session.

PROF. E. N. YUSTOVA made some general comments on the problem of determining additive sensitivity curves. She wished to point out the importance of the development of the methods of visual colorimetry for the science of metrology.

Of the three methods of colour measurement, i.e., the visual method, the method of calculation and the method of objective colorimetry, the first was the most important and primary. This was because the mixture curves provided the basic standard of colorimetry and the question of the accuracy of their determination was an extremely important one. There were two main problems of measurements in the science of colour: 1. the measurement of the absolute colour co-ordinates by means of mixtures of three primaries (with the help of visual and objective colorimeters), 2. the measurement of small colour differences by comparison with standards (with the help of visual and objective comparators). There were some contradictions in colour measurements. On the one hand as discrepancies in the results of different observers were rather significant there was no need to try to obtain values more accurate than the third digit after the point, when defining chromaticity co-ordinates. On the other hand, because of the very high ability of

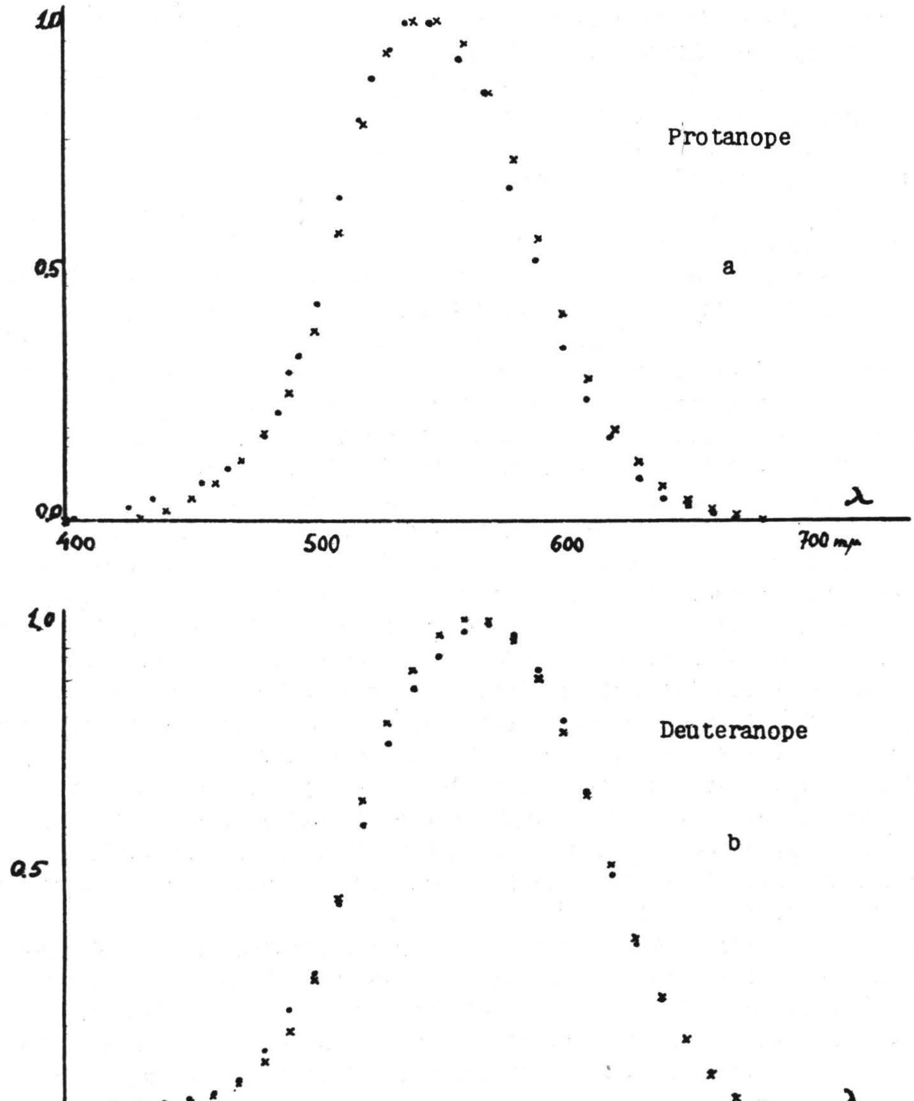

Fig.2. × - our data. (a) $G(\lambda)$; (b) $\dfrac{R(\lambda) + G(\lambda)}{2}$

. - experimental data (S. Hecht and S. Shlaer).
(a) for protanope H.J.;
(b) mean experimental data for two deuteranopes, A.W.G. and S.R.F.

the eye to discriminate colours we needed to achieve measurements of the chromaticity co-ordinates with the accuracy of four digits after the point. The accuracy might be even higher.

The problem of the determination of the mixture curves was the problem of measuring the absolute colour co-ordinates. The accuracy then was limited by the scatter of the readings of separate observers. It was not worth while standardising "the mixture curve" tables with such a great number of digits as was done in the C.I.E. standard of 1931. She Prof. Yustova) considered the accuracy and the repeatability of the results obtained by Dr. Stiles' and Dr. Speranskaya's apparatuses to be good enough. It would be worth while carrying out similar measurements in other countries in order to obtain a more complete knowledge of the peculiarities of vision of different peoples and to define on the basis of comprehensive and versatile statistical material the so-called "normal observer".

COMMANDER D. FARNSWORTH said Dr. Graham's dichromat was a typical deuteranope, apparently in all respects except one, that as you moved past the $\lambda = 460$ mμ point into the violet, a decided desaturation occurred. This raised a fascinating point. What happened in the ordinary dichromat, the typical deuteranope, to the small resurgence in the spectral mixture functions, the little hump of a curve in the blue region? We had always associated this with one of the other main curves and, as Dr. Stiles mentioned in connexion with Dr. Rushton's work, we ought to investigate a little further to see whether we could find another upsurge in the curve for one of these retinal pigments. That was one possibility.

But the thing we ought to observe in this atypical dichromat of Dr. Graham's was that this resurgent curve was still present, the thing we would normally expect to find in all dichromats. But in the typical deuteranope this little curve, which makes for the desaturation as we move past the point of the blue, is apparently not present.

PROF. G. A. FRY referred to the discrepancy between flicker photometry results and direct comparison results. It appeared from Dr. Sperling's work that the non-additivity difficulties were associated with direct comparison data and these might involve simultaneous induction effects with two juxtaposed fields. Had anyone used binocular matching to investigate this?

PROF. W. D. WRIGHT said he hadn't personally, but thought there could be some effect. He would also suggest that apart from induction effects there was the fact that the two retinal areas were in quite different states of adaptation. Prof. Wald had discussed the previous day slow adaptation processes which he attributed to photochemical effects. But there were also large and very rapid adaptations which occurred immediately the two different patches were thrown on the retina. In flicker photometry on the other hand, the two colours were viewed on a patch of retina in the same state of adaptation.

DR. D. L. MACADAM said that in his laboratory Mr. Brenman had just completed a study in which the whole eye was adapted to a uniform field and then for one second only was permitted to make a comparison between two heterochromatic half-fields. The discrepancy under discussion still persisted. Dr. Wright and Dr. MacAdam agreed to pursue later the argument as to what happened to adaptation in the one second intermission of the uniform adapting field.

DR. G. WYSZECKI questioned the statement in Prof. Yurov's paper that the spectral sensitivity of the eye depended on the spectral composition of the radiation perceived. He thought that a definition of brightness would have no meaning if two stimuli could only be equally bright if they had identical spectral compositions. In a paper to the Journal of the Optical Society of America the previous year, he had shown that a brightness or lightness index could be found which was a function of the tristimulus values, and this eliminated the condition that brightness depended entirely on spectral composition as such.

MR. J. GUILD said it was so many years since he was actively associated with this field that he felt no competence to comment on the specific results of recent investigations. There were, however, some matters of general principle which never became out-of-date and which seemed to be relevant to the interpretation of some of the work reported in these papers. In the time at his disposal he could only touch briefly on one matter, namely, the effect on colorimetric and photometric measurements of additivity failure, which arose in different aspects in several of the papers. Some of the references to the C.I.E. standard observer (1931) could be taken to imply that those of us who were responsible for formulating this standard regarded additivity of the luminances and chromaticities associated with visual stimuli as an unquestionable fact and ignored the possible consequences of its failure. This was not so. We were all well aware of the possibility of this failure and of its consequences, and the matter was so much in his (Mr. Guild's) mind that in 1932 he published a long paper dealing with the fundamental principles involved in the application of measurement to visual problems* in which the significance of the quantities evaluated by photometric and colorimetric matching was fully examined. To avoid verbal confusion of anyone who might now read this paper it should be pointed out that the terms *luminance* and *chromaticity* had not been in general use at that time, and that *brightness* (which in its original significance in the English language had objective and not subjective significance) was used for the former, and *colour* or *colour-quality* for the latter. It was shown that the kind of additivity required in order that the numbers obtained from visual matches of heterochromatic luminances or

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*GUILD, J. The Interpretation of Quantitative Data in Visual Problems; Report of a Joint Discussion on Vision held on June 3rd, 1932, by the Physical and Optical Societies; The University Press, Cambridge, England, p.60.

chromas should constitute measurements of these entities, and not merely numerical labels, could be summarised as invariance of the visibility function for the whole of the relevant range of conditions. To construct scales of measurement based on visual matching either for luminance or chromaticity this invariance had to be *postulated,* not because it was believed to be true but because we could not do anything else. If it were not true - or at least sufficiently nearly so for practical purposes over a sufficiently wide range of conditions to be useful - we could not measure these entities. We had to avoid the common fallacy of assuming that every experiment which gave a numerical result was necessarily measuring something. There was no *a priori* reason for assuming that either luminance or chromaticity, or any other magnitude associated with perception, was measurable at all. If we wished to explore the possibility of measuring such things we had first to construct a scale (or scales) of measurement on the hypothesis that the necessary conditions were fulfilled and then to find by experiment the range of circumstances, if any, within which the hypothesis was valid, that was, the range within which the application of arithmetical operations such as addition, multiplication, or integration to the numbers derived from some matches would lead to predictions which were not inconsistent with the results of other matches.

Though not so explicitly stated, all this was implicit in the pioneer papers of H. E. Ives who had been the first worker in this field to show any realisation that general metrological principles had to be considered in connexion with sensory measurements. His many papers on photometry and colorimetry from 1912 to 1923 well repaid re-reading even at the present time, as they laid the foundation of the scientific treatment of these subjects. His work was fresh in the minds of those of us mainly concerned with the investigations on which the 1931 C.I.E. standard observer was based. The experimental work was carried out with a two-degree field for two reasons. The first of those was that the evidence available at that time seemed to show that the additivity which had to be postulated was most likely to be obeyed over a usefully wide range of intensity, at least to a reasonable degree of approximation, with a field of that size. The second reason was that the C.I.E. had recently adopted a standard visibility function based mainly on flicker photometer measurements which, as Ives had shown, implied the use of a small field (and other standard conditions) for visual heterochromatic photometry. It was obviously necessary that a standard observer for colorimetry, which, though established later in time, logically included the photometric standard, should be based on data applicable to the same retinal area.

The decision to derive the spectral distribution functions by combining the experimental spectral chromaticities with the already adopted visibility function was also made for two reasons. One was that it was not easily practicable at that time to make the energy measurements required for a complete self-contained investigation of these functions; but this alone would not have made choice of the C.I.E. visibility function inevitable. There was evidence even then that this function was not satisfactory at either the red or blue extremities of the spectrum and in the paper* in which he (Mr. Guild) described his own work and suggested a standard observer based on the mean of his results and those of W. D. Wright, it was shown that the exclusive use of the C.I.E. function led to erroneous values for the luminosity factors of the primaries of his colorimeter and that the discrepancies were very greatly reduced by using the visibility data of Hyde, Forsythe and Cady at the red end of the spectrum. From the data provided by various previous investigations it would have been easy to construct a visibility function in better accord with the colorimetric results than the C.I.E. function; but the C.I.E. in those days consisted mainly of photometrists most of whom had still to be convinced that a standard observer for colorimetry was sufficiently important to be handled by the Commission at all, and there would have been no chance whatever of securing adoption of a colorimetric standard which involved a visibility function differing from the recently adopted standard. The latter function was therefore incorporated in the spectral distribution functions of the proposed standard observer tables with the knowledge that errors would arise in the luminosity relations of highly saturated colours near the extremities of the spectrum but in the confident belief that the errors would be insignificant in evaluating the properties of the great majority of the products of the colour industries for whom the standard was primarily intended. Since its adoption in 1931 the standard has proved to be sufficiently accurate for a wide range of technical applications. In fact its range of usefulness had far exceeded the expectations of himself and others who had sponsored it with a full knowledge of its possible defects.

This did not mean that a more accurate standard could not be formulated as the result of more modern investigations with the improved technical resources which were now available; and it was his hope, as it was that of his colleagues who had been more directly concerned with the work reported in Dr. Stiles' paper, that this work would provide the necessary data for removing any significant defects of the 1931 standard observer tables which had been definitely established by the experience of users in the last 26 years. If those with whom the decision rested decided that a change was justified there should then be no difficulty in obtaining acceptance of new or modified tables which included a visibility function derived from, and in accordance with, the tristimulus data. This was the only visibility function which had any practical importance in modern technology in which the direct visual comparison of heterochromatic luminances, whether by the

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*GUILD, J. The colorimetric properties of the spectrum, *Proc.Roy.Soc.A.*, 1931, **230**, 149.

flicker or other methods, was being progressively superseded for standardisation purposes by physical methods involving one or more of the "standard observer" functions.

PROF. WRIGHT recalled Mr. Guild's paper to the Optical Convention in 1926 showing that there was support for the view that additivity held within limits.

DR. STILES felt that in speaking of additivity they had to remind themselves that it was of several kinds:

1. Additivity in the sense of obedience to Grassmann's laws when making a complete colour match, and this was the sense in which he had discussed it in his own paper.
2. Additivity in direct comparison heterochromatic brightness matching.
3. Additivity in flicker photometry which there was no *a priori* reason for regarding as brightness comparison at all. In fact, it gave rather similar results to those obtained by direct brightness matching.

They should not mix these various uses of the term. In flicker matching additivity might hold very well and this was probably mainly in mind in 1931, whereas most of the more recent objections related to breakdown of additivity in direct comparison.

PROF. WRIGHT asked whether Ives had not found that direct matching gave broadly the same results as flicker photometry.

DR. STILES thought that was so but that Ives found direct comparison erratic and unsatisfactory for large colour differences and virtually abandoned it in favour of flicker photometry.

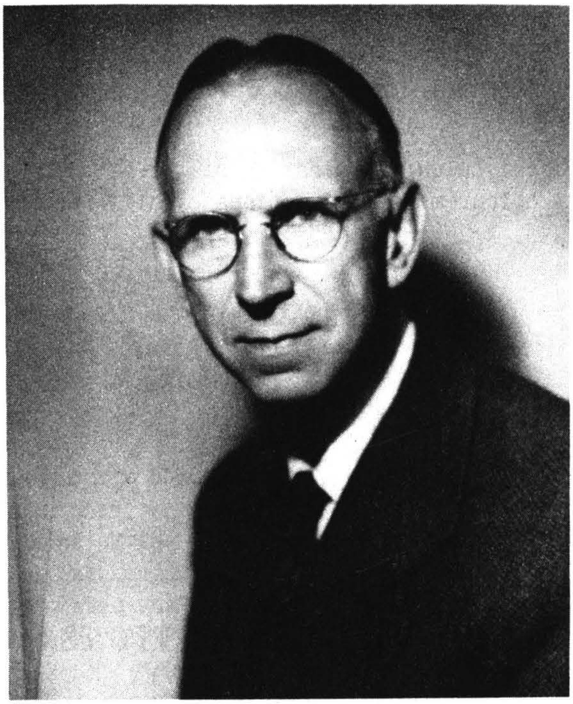
Dr. Stiles went on to ask Professor Fedorov what was the size of the field in which he found non-additivity as reported in his paper (31).

PROF. FEDOROV replied that it was 2^{0}.

PAPER 40

COLOUR-MIXTURE FUNCTIONS: PROGRESS OF FIELD TRIALS

By D. B. JUDD



Deane Brewster Judd, born South Hadley Falls, Massachusetts, U.S.A., November 15, 1900. A.B., Ohio State University 1922; A.M., 1923. Ph.D., Cornell University, 1926. Research Associate in Colorimetry stationed at the National Bureau of Standards, Washington, from the Munsell Research Laboratory, 1926-1927. Associate Physicist and Physicist (Optics) at the National Bureau of Standards, 1927-. Chairman Inter-Society Color Council, 1940-1944. President, Optical Society of America, 1953-1955. President, Munsell Color Foundation, 1942-. American representative on C.I.E. for colorimetry, 1931-1935, 1948-, for artificial daylight 1939. Chairman C.I.E. Working Committee W-1.3.1, Colorimetry, 1955-.

40. COLOUR-MIXTURE FUNCTIONS: PROGRESS OF FIELD TRIALS

By D. B. JUDD

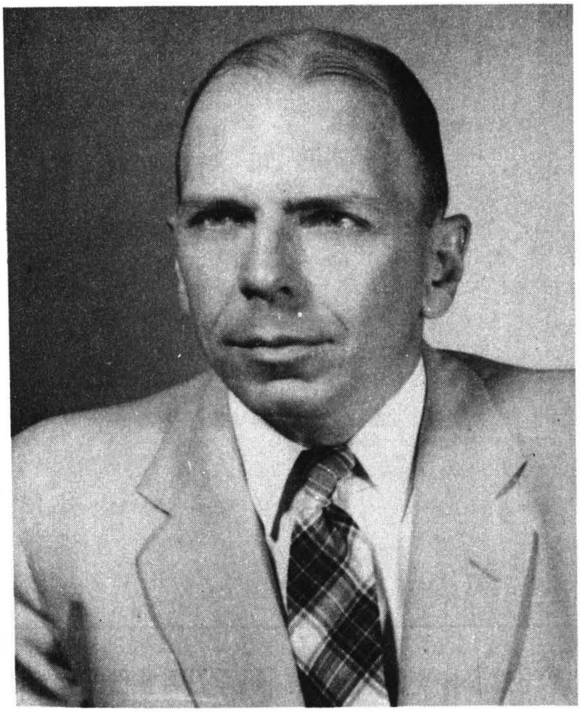
(Only one of the investigations dealt with by Dr. Judd - the one described by Mr. K. L. Kelly in Paper 9 - was ready for publication at the time of the Symposium and in the circumstances Dr. Judd did not submit a formal paper. The following summary of his contribution is taken from the report of the meeting).

DR. Judd explained that his contribution would consist of an examination of the results of three series of field trials of various sets of colour-matching functions - the C.I.E. set, a modified C.I.E. set proposed by himself in 1951, the 2^0 and the 10^0 pilot group sets obtained by the N.P.L. (Stiles and Burch) in 1955. He dealt first with the field trials, carried out at the National Bureau of Standards, on the metameric samples known as the Granville greys. (Paper 9 of the Symposium gives a full report of this work by K. L. Kelly). Dr. Judd then summarised a second investigation by Dr. Cruz and Dr. Juan in which the intersecting sequences of near metameric colour samples known as the Simon textiles had been used. For the 2^0 observations on these samples the C.I.E. functions gave good (the best) prediction and for the 10^0 observations the 10^0 pilot group set gave good prediction. But the interpretation of the results was complicated by differences in the spectrophotometric values for the samples obtained respectively by the Beckman and the General Electric spectrophotometers. With the values given by the latter the good agreement previously noted no longer held. Some further work on the spectrophotometry was required. The third series of trials had been made by the speaker on the rutile and anatase titanium oxide pigments, apparent differences in which had been one reason for re-examining the colour-matching functions. The spectrophotometry had again proved difficult. The observations on the rutile-anatase difference made by 20 observers did not show the same pattern in the colour diagram as he (Dr. Judd) had previously found for 7 observers. It was possible) as suggested by Dr. Stiles, that the big differences in spectral reflectances of anatase and rutile in the extreme violet might by over-weighted visually by much smaller differences at longer wavelengths. Dr. Judd thought that at least some such effect might be coming in for the permanent specimens which were made up as vitreous tiles. If necessary he would revert to titanium oxide pigments rubbed up in oil despite the poor permanence of such specimens. He intended to resolve the present ambiguity on this issue.

PAPER 9

OBSERVER DIFFERENCES IN COLOUR-MIXTURE FUNCTIONS STUDIED BY MEANS OF A PAIR OF METAMERIC GRAYS

By K. L. KELLY



In 1936, Kenneth L. Kelly began his work on colour as a Research Associate for the American Pharmaceutical Association and the United States Pharmacopoeial Convention at the Bureau of Standards in developing a simple, accurate system of colour names for use in the U.S. Pharmacopoeia, the National Formulary, and in general pharmaceutical literature. These colour designations were applied to many of the drug and chemical monographs of these two books of drug standards. This system was revised in 1955 and published with a dictionary of colour names.

He has also done work in colorimetric and spectrophotometric analysis and is now working on suggested revisions of the 1931 standard observer for use in colorimetric specification.

9. OBSERVER DIFFERENCES IN COLOUR-MIXTURE FUNCTIONS STUDIED BY MEANS OF A PAIR OF METAMERIC GRAYS

By K. L. KELLY

I. INTRODUCTION

THE standard observer and co-ordinate system now widely used in the interpretation of spectrophotometric and colorimetric data were recommended in 1931 by the C.I.E. (the Commission Internationale de l'Éclairage) for this purpose so that all subsequent data would be expressed in the same tristimulus system and would therefore be immediately comparable. This system was based on the work done by Guild and by Wright in England and until recently has proved quite satisfactory. However, it was reported to fail in several instances to account correctly for the differences in colour between two samples whose spectrophotometric curves differ in the short wavelength end of the spectrum, especially below 420mμ *(refs. 1,2)*.

These reported failures resulted in much discussion looking toward a possible revision of this standard observer and led Judd to suggest in 1951 a modified set of colour-mixture functions based on the data of Wright and Guild combined with the standard luminosity function modified below 460mμ according to luminous-efficiency data by Gibson-Tyndall, Wald, Weaver, Thomson, and Ishak. Now Dr. W. S. Stiles of the National Physical Laboratory in England has undertaken the first careful direct determination of the colour-mixture functions of average normal observers which the standard observer should represent.

In these discussions, use of a field size larger than the 2^0 field used for the 1931 standard observer has been advanced to accord more closely with viewing conditions in industry. Dr. Stiles is therefore making his measurements with both 2^0 and 10^0 fields and has already reported colour-mixture data for a pilot group of 10 observers whose average age is about 31 years *(ref. 3)*.

In 1949 Walter Granville, then of the Container Corporation of America, painted several metameric gray panels to illustrate the possible effect of angular subtense on colour matching *(ref. 4)*. Two of these were chosen for the work herein reported: a nearly nonselective gray produced by a mixture of white and black pigments called the simplex gray (No. 1), and a selective gray produced by a mixture of yellow, green, purple and white pigments called the complex gray (No. 8). *Fig. 1* and *Table 1* show the spectral directional reflectances of these two grays obtained on the General Electric recording spectrophotometer.

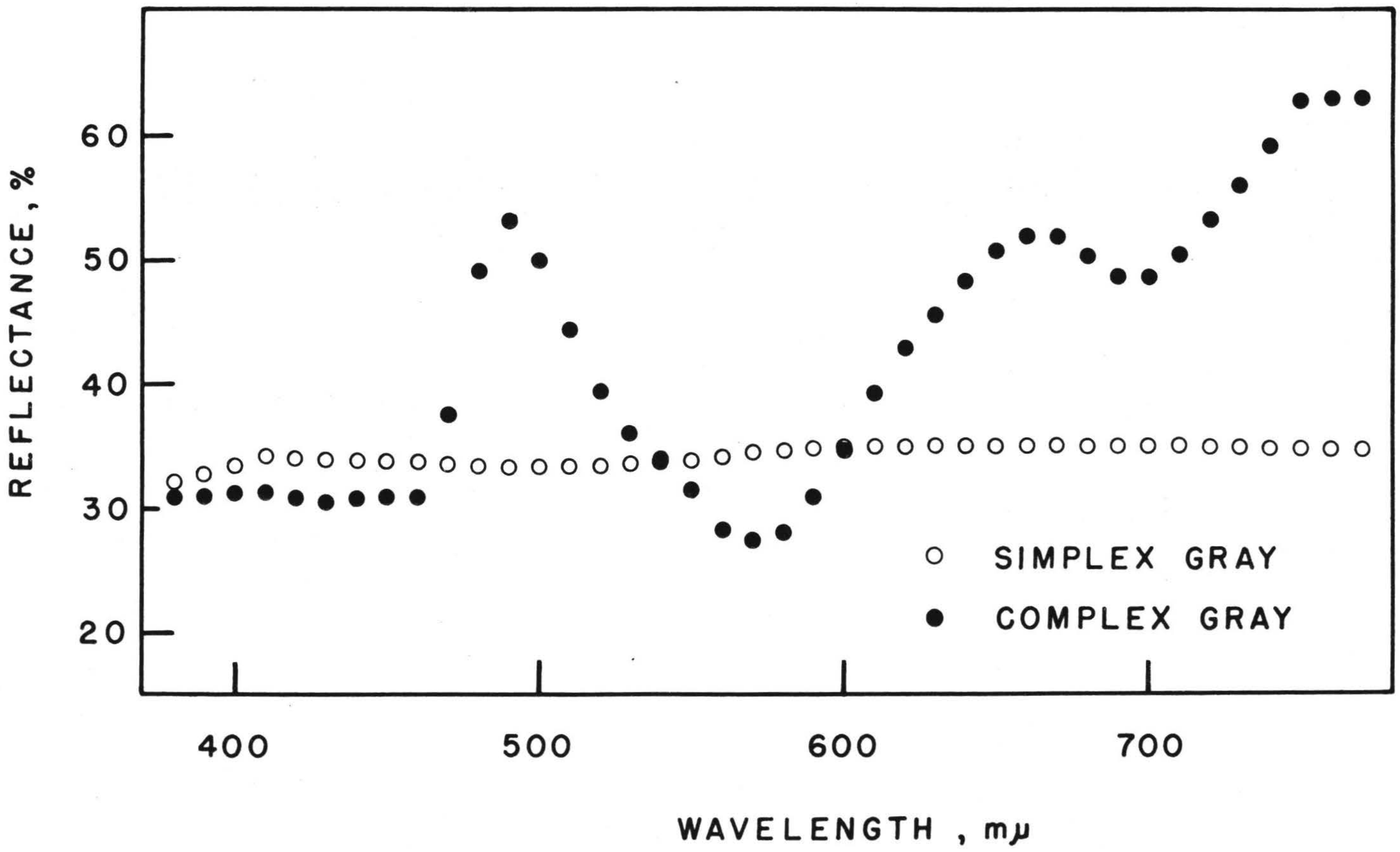


Fig. 1. Spectrophotometric curves of the two metameric Granville grays.

TABLE 1

Spectral directional reflectances of the two Granville grays

Wavelength	Simplex (No. 1)	Complex (No. 8)
380	0·317*	0·305*
90	·324*	·307*
400	·331	·309
10	·338	·310
20	·337	·304
30	·336	·301
40	·335	·305
450	·334	·306
60	·334	·316
70	·332	·373
80	·331	·488
90	·330	·528
500	·330	·497
10	·331	·441
20	·331	·391
30	·332	·357
40	·334	·337
550	·336	·311
60	·338	·289
70	·342	·281
80	·343	·287
90	·345	·306
600	·346	·342
10	·347	·389
20	·347	·425
30	·347	·452
40	·347	·480
650	·347	·504
60	·348	·517
70	·347	·516
80	·347	·500
90	·348	·484
700	·348	·483
10	·348	·502
20	·347	·528
30	·346	·556
40	·346	·589
750	·345	·626
60	·344*	·627*
70	·343*	·627*

*Extrapolated

According to the 1931 standard observer the complex gray will appear greener in daylight (source *C*) than the simplex, and the reverse will be true when source *A* (colour temperature 2854°K, representative of incandescent lamp light) is used. The same effect results from changing the angular subtense of the sample from 10° to 2°; the retinal area stimulated first will include the macula and also a portion of the surrounding retina and second will lie inside the macula only. This change from 10° to 2° will have an effect at least partly analogous to placing a yellow filter (macular pigment) in front of the eye for the 10° condition and thus should have somewhat the same effect as reducing the colour temperature of the source.

The purpose of the present study is to establish a criterion by means of which it will be possible to determine whether any set of colour-mixture functions gives predictions of the character of the colour difference between the Granville grays in accord with observers known to have normal vision by the accepted tests. In particular, this criterion is to be applied to the four sets of colour-mixture functions already mentioned.

II. EXPERIMENTAL METHOD

THE experiment consisted of placing the two gray panels side by side in a nearly vertical position illuminated by C.I.E. source *A* and viewing them through a Davis-Gibson (C.I.E.) *C* filter prepared in February 1955. The spectral transmittances of this filter determined in July 1956 at the start of the observations are given in *Table 2*. The observer was asked to stand at a line which would cause the retinal image of the two panels to subtend a 10° angle and to disregard the Maxwell spot (*ref.* 5) if present. First he was to describe the colour of the simplex gray with respect to that of the complex gray and then, as the operator reduced the voltage on the lamp and thereby its colour temperature, to note when neither panel appeared redder or greener than the other. The voltage of the source at this point was recorded along with the observer's age, sex, and hair and eye colours. Observers not passing the 5th Edition Ishihara Tests for Colour Blindness were eliminated.

The observer was then asked to step back to another line from which the retinal image would subtend an angle of 2° and the experiment was repeated. The combination of source and Davis-Gibson filter resulted in a maximum colour temperature of 6750°K but for those observers requiring a source of higher colour temperature, one or more measured blue daylight glasses (*Table 2*) were used with the Davis-Gibson filter. Therefore, by varying the voltage on the lamp and by using combinations of these filters, colour temperatures could be obtained from 1800°K up and, indeed, it was found that some sources bluer than a source corresponding to infinite colour temperature

TABLE 2

The spectral transmittances and change in reciprocal colour temperature for the daylight filters used

Wave-length mμ	Davis-Gibson "C"	Filter G90A 2.93 mm	Davis-Gibson plus G90A	Filter No. 8331	Davis-Gibson plus No. 8331
380	0.69*	0.751*	0.5182	0.47*	0.32
90	.71	.774*	.5495	.59*	.42
400	.7268	.7967	.5790	.685	.498
10	.7501	.8066	.6050	.712	.534
20	.7475	.7800	.5830	.656	.490
30	.7180	.7585	.5446	.617	.443
40	.6623	.7279	.4821	.555	.368
450	.5836	.6982	.4075	.498	.291
60	.5090	.6647	.3383	.440	.224
70	.4557	.6345	.2891	.388	.177
80	.4056	.6029	.2445	.344	.140
90	.3532	.5777	.2040	.309	.109
500	.2956	.5551	.1641	.280	.0828
10	.2479	.5323	.1320	.254	.0630
20	.2178	.5088	.1108	.228	.0497
30	.2027	.4805	.0974	.199	.0403
40	.1961	.4602	.0902	.179	.0351
550	.1891	.4547	.0860	.172	.0325
60	.1765	.4600	.0812	.176	.0311
70	.1600	.4560	.0730	.171	.0274
80	.1420	.4310	.0612	.150	.0213
90	.1250	.3950	.0494	.119	.0149
600	.1115	.3805	.0424	.109	.0122
10	.1015	.3750	.0381	.103	.0105
20	.0940	.3650	.0343	.094	.0088
30	.0880	.3498	.0308	.083	.0073
40	.0825	.3318	.0274	.072	.0059
650	.0779	.3207	.0250	.066	.0051
60	.0724	.3217	.0233	.068	.0049
70	.0684	.3330	.0228	.072	.0049
80	.0634	.3493	.0221	.078	.0049
90	.0584	.3618	.0211	.083	.0048
700	.0534	.3646	.0195	.082	.0044
10	.0489	.3626	.0177	.080	.0039
20	.0444	.3569	.0158	.076	.0034
30	.0404	.3509	.0142	.073	.0029
40	.0369	.3463	.0128	.068	.0025
750	.0344	.3418	.0118	.067	.0023
60	.033*	.337*	.0111	.066*	.0022
70	.032*	.333*	.0107	.064*	.0020
Δ μrd	202.5	77.9	280.4	187.5	390.0

*Extrapolated

were required. The voltage and filter combinations were noted for each red-green balance point for each observer and the corresponding reciprocal colour temperatures were determined from *Table 3* by subtracting the change in microreciprocal degrees Kelvin (μrd) produced by the filter combination from the reciprocal colour temperature of the bare lamp.

TABLE 3

Colour temperature (θ) in $^{\circ}$K of the lamp, and the reciprocal colour temperature in μrd of the lamp and the lamp with one or more of the daylight filters against voltage on the lamp

Voltage	θ of lamp alone	$10^6/\theta$ of lamp	$10^6/\theta$ of lamp and Davis-Gibson filter	$10^6/\theta$ of lamp and Davis-Gibson with G90A	$10^6/\theta$ of lamp and Davis-Gibson with 8331	$10^6/\theta$ of lamp and Davis-Gibson with G90A and 8331
36.0	1800	556	353	275	166	88
42.7	1900	526	324	246	137	58
49.9	2000	500	298	220	110	32
57.6	2100	476	274	196	86	8
66	2200	455	252	174	65	-13
75	2300	435	233	154	45	-33
84	2400	417	214	137	27	-51
94	2500	400	198	119	10	-68
104	2600	385	182	104	-5	-83
115	2700	370	168	90	-20	-98
126	2800	357	155	77	-33	-111
133	2854	350.4	148	70	-40	-118

III. RED-GREEN BALANCE POINTS FOR THE FOUR COLOUR-MIXTURE FUNCTIONS

THE red-green balance points for the four colour-mixture functions were determined by first computing the chromaticity co-ordinates for the two gray panels for each of the functions for three Planckian sources (277, 221 and 191 μrd), for nine Planckian sources (666, 571, 500, 488, 486, 444, 400, 350, and 308 μrd) combined with the Davis-Gibson "*C*" filter ($\Delta 10^6/\theta$ = 202 μrd), and for the same Planckian sources combined with the double filter Davis-Gibson "*C*" plus G90A ($\Delta 10^6/\theta$ = 280 μrd). These data were plotted on the (x, y)-chromaticity diagram and fall close to the Planckian locus.

It was noted that the direction of the straight line connecting each pair of points as well as the distance between them (indicative of colour difference) varies regularly with the correlated colour temperature of the energy

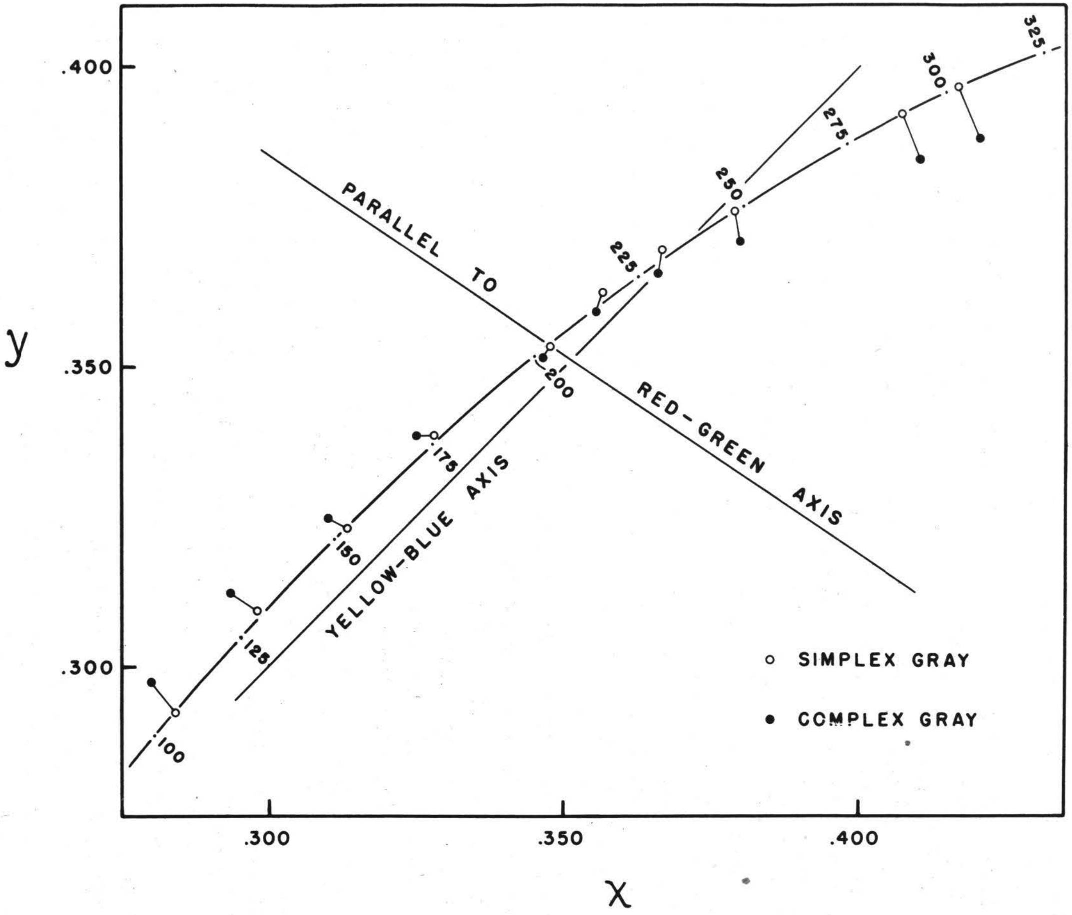


Fig. 2. Illustration of the change in relative position and distance apart of the C.I.E. (x,y)-points of the Granville grays as the colour temperature of the source is changed.

reflected by the simplex gray regardless of whether the computation is based on a Planckian source or on a Planckian source modified by the single or the double filter. *Fig.2* shows the Planckian locus and 10 of the 21 pairs of computed points plotted on the (x,y)-diagram for the C.I.E. standard observer. Note that near 300 µrd the simplex gray is seen by this observer as greener than the complex while at source *C* (148 µrd), the reverse is true.

The red-green balance points for the two Stiles colour-mixture functions were easily determined because they each predicted that under a certain one of the illuminants the two gray panels would be a chromaticity match; that is, the chromaticity points for them were found not to be significantly different. The reciprocal colour temperature of the source satisfying this condition for the Stiles 2^{0} colour-mixture functions is 210 µrd, while that for the Stiles 10^{0} colour-mixture function is 267 µrd.

The other two colour-mixture functions predict that at the red-green balance point there will be a residual yellow-blue difference. Therefore it was necessary to establish some criterion to determine when the red-green difference equals zero. The first criterion used in determining the corresponding reciprocal colour temperature of source was that the line joining the chromaticity points of the two grays be parallel to the yellow-blue axis on the (x,y)-diagram. This axis, having an inclination of 45^{0}, was drawn between the 476 and 578 mµ points on the spectrum locus. These are the proposed centres of the yellow and blue segments of the boundary of real colours on this diagram (*ref. 6*). The corresponding values of reciprocal colour temperature of source satisfying this criterion for the 1931 C.I.E. and 1951 Judd "*i*" colour-mixture functions are 192 and 202 µrd respectively.

The second criterion used in determining the corresponding reciprocal colour temperature of source was that the line joining the chromaticity points of the grays be perpendicular to the red-green axis on a uniform chromaticity scale diagram (*ref. 7*). This axis was drawn from the 508 mµ point on the spectrum locus to the intersection of the line drawn through the 493 mµ point and the "*C*" illuminant point with the line connecting the red and violet ends of the spectrum locus. These are the proposed centres of the red and green segments of the boundary of real colours on the (x,y)-diagram (*ref. 6*).

A perpendicular to this axis was drawn through the "*C*" illuminant point and was found to intersect the spectrum locus at 567 mµ. This perpendicular was transferred to the (x,y)-diagram by drawing it also through the "*C*" illuminant point and the 567 mµ point on the spectrum locus. The angle of inclination of this perpendicular is found to be 66.5^{0} and the corresponding values of the reciprocal colour temperature of source satisfying this criterion for the two colour-mixture functions are 206 and 213 µrd respectively. In *Table 4* are summarized the values of reciprocal colour temperature computed by these criteria to correspond to these balance points for the four sets of colour-mixture functions.

TABLE 4

Values of reciprocal colour temperature of red-green balance points for the four colour-mixture functions by different criteria

Colour-mixture functions	Reciprocal colour temperature of source in µrd yielding red-green balance	
	By chromaticity match	
Stiles' 2°	210	
Stiles' 10°	267	
	Parallel to *Y–B* axis	Perpendicular on UCS triangle to *R–G* axis
1931 C.I.E.	192	206
1951 Judd "*i*"	202	213

IV. RESULTS AND DISCUSSION

TABLE 5 contains the individual data for the 39 observers studied with their descriptions of the two panels and the reciprocal colour temperature of their red-green balance points. Note that observer 39 was able to obtain a match at the 10° position but that it was not possible to run the lamp at a sufficiently high colour temperature for him to obtain a match at the 2° position, presumably due to his very heavy ocular pigmentation.

Since the image of a 2° field falls wholly within the macula, the 2°-field results depend both on the macular and on the lens pigmentations. Likewise, since the image of a 10° field covers the macula and a large region of the surrounding retina of which the area of the macula represents about 10%, the 10°-field results depend only on the lens pigmentation provided the observers disregard the Maxwell spot as instructed. Also the difference between the two may be taken as a measure of the macular pigmentation on the assumption that the spectral sensitivities of the receptors apart from pigmentation are the same throughout the 10° field (*ref. 5*).

The degree of pigmentation of an observer is indicated by his red-green balance point. By this interpretation, Stiles' 10° average observer (balance point at 267 µrd; see *Table 4*) would be the least pigmented of the four.

TABLE 5

Observer initials, age, sex, eye and hair colour, descriptions of colours of gray panels illuminated by source *C* at the 10° and 2° positions, and reciprocal colour temperature of source required by each observer for red-green balance at both positions and the difference between them

Observer		Sex and age	Eye colour	Hair colour	Colour of simplex relative to complex		Reciprocal colour temperature of match point		
					10°	2°	10°	2°	Diff.
1	DBJ	M55	blue	brown	red	green	204	91	113
2	KLK	M45	hazel	brown	red	green	238	103	135
3	GWH	F56	brown	brown	red	green	179	47	132
4	MRF	M27	hazel	d. brown	pink	pink	278	196	82
5	CAD	M44	blue	brown	red	green	196	84	112
6	MMB	F39	blue	d. brown	red	green	213	132	81
7	LEB	M51	d. brown	black	slightly pink	green	213	78	135
8	THP	M41	blue	blond	pink	green	256	149	107
9	IN	M38	blue	red	pink	green	200	110	90
10	CDC	M21	hazel	auburn	pink	green	238	84	154
11	- -	F50	hazel	brown	pink	slightly pink	256	189	67
12	HKH	M39	blue	blond	pink	pink	250	179	71
13	FCB	M62	blue	brown	pink	green	172	118	54
14	RTV	M34	blue	brown	red	green	222	135	87
15	RWC	M46	blue	brown	pink	green	213	71	142
16	WAH	M29	d. brown	black	pink	green	263	182	81
17	GHL	M34	d. brown	black	pink	pink	286	213	73
18	WHA	M47	blue	brown	match	green	189	65	124
19	RLM	F44	brown	d. brown	pink	green	238	159	79
20	WRD	M42	brown	brown	pink	match	227	149	78
21	ECW	M48	brown	brown	pink	green	222	175	47
22	BPG	F37	d. brown	d. brown	lavender	green	227	127	100
23	WBF	F23	brown	brown	pink	pink	256	164	92
24	SAC	F19	brown	brown	lavender	lavender	270	208	62
25	JJ	F29	blue	brown	red	match	278	154	124
26	CAL	F25	hazel	l. brown	lavender	match	250	170	80
27	RPT	M53	blue	brown	match	green	95	-14	109
28	HJK	M52	brown	brown	match	green	170	2	168
29	VIB	F42	d. brown	d. brown	pink	slightly pink	256	175	81
30	KSG	M66	blue	brown	green	green	91	-40	131
31	RD	M68	blue	brown	green	green	130	-28	158
32	WLH	M21	hazel	blond	red	green	233	110	123
33	WFM	M36	blue	blond	red	green	196	29	167
34	PI	F18.5	hazel	brown	pink	pink	278	170	108
35	JCS	M28	green	blond	pink	pink	270	161	109
36	TOT	M38	brown	l. brown	pink	pink	263	192	71
37	RS	M56	blue	brown	red	green	196	84	112
38	JWL	M31	d. brown	black	pink	pink	256	182	74
39	CB	M77	blue	brown	green	green	-118	<-118	-
Averages		41.3					214	< 114	> 100

In *fig. 3*, the reciprocal colour temperature ($10^6/\theta_2$) of the 2^0 red-green balance points for the 39 observers studied (see *Table 5*) are plotted with observer number against observer age. Since no age has been given for the 1931 C.I.E. and 1951 Judd "*i*" colour-mixture functions, the spread in reciprocal colour temperatures required by them for red-green balance as given in *Table 4*, are indicated by a pair of lines which should extend across the graph but have been shown as short sections only to avoid complicating the figure. Stiles gives the average age of his pilot group of 10 observers as 31. It may be seen from *fig.3* that only four of the 39 observers studied seem to have less pigmentation than the 1931 C.I.E. standard observer, only two less than the 1951 Judd "*i*" (both of these on the basis of yellow-blue criterion) and only one less than the Stiles 2^0 colour-mixture functions by this test. It also indicates that the C.I.E. and Judd colour-mixture functions both correspond to very young observers in regard to balance point for the Granville grays.

The rank correlation (Spearman's) between $10^6/\theta_2$ for red-green balance and age of the observers at the 2^0 position is 0.60 with an uncertainty of plus or minus 0.22, the uncertainty being 4.9 times the probable error. Thus it is suggested that there is a poor but significant correlation of lens and macular pigmentations with age. *Fig. 4* shows the correlation of $10^6/\theta_{10}$ with age; the rank correlation is better, being 0.76 plus or minus 0.13. This correlation is attributable to the known increase in lens pigmentation with age. Here again, there are only six observers apparently less pigmented than Stiles' 10^0 colour-mixture functions. From *fig. 5* it will be noted that there is no correlation of macular pigmentation with age, the rank correlation being -0.145 plus or minus 0.61. Also there is no significant correlation of macular or lens pigmentations with either eye colour or hair colour.

From *Table 5* and *figs. 3, 4* and *5* it is possible to classify the observers and the four colour-mixture functions into five groups depending on their amounts of ocular pigmentation. From *Table 5*, it will be seen that certain observers describe the simplex gray as redder than the complex at both the 10^0 and 2^0 positions. These are the young observers and those tentatively considered to have the least ocular pigmentation and constitute group 1. Those in group 2 apparently have a little more pigmentation and so call the simplex redder at the 10^0 position but a match at the 2^0 position. Group 3 comprising the majority of the observers, contains those who call the simplex redder at the 10^0 position and greener at the 2^0 position. With more pigmentation, the observers in group 4 call the simplex a match at the 10^0 position and greener at the 2^0 position. The most heavily pigmented observers, and these contain the oldest observers, call the simplex greener at both positions. Thus these groups are called red-red, red-match, red-green, match-green, and green-green. The same grouping may be made with respect to the colour temperature or reciprocal colour temperature of the

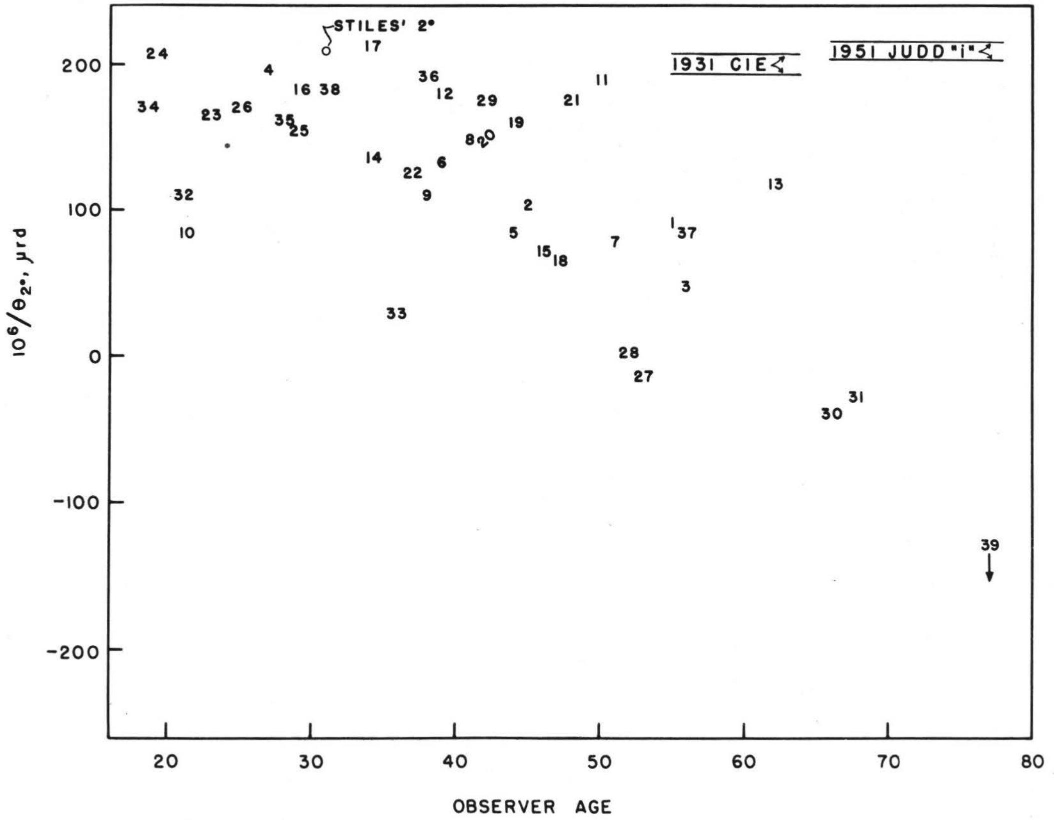


Fig. 3. Reciprocal colour temperature of source required in a 2^0 field by 39 observers to produce red-green balance between the Granville grays plotted against observer age.
Note the tendency of older observers to require lower reciprocal colour temperatures. This tendency suggests that the amount of lens and macular pigmentations combined increases with age.

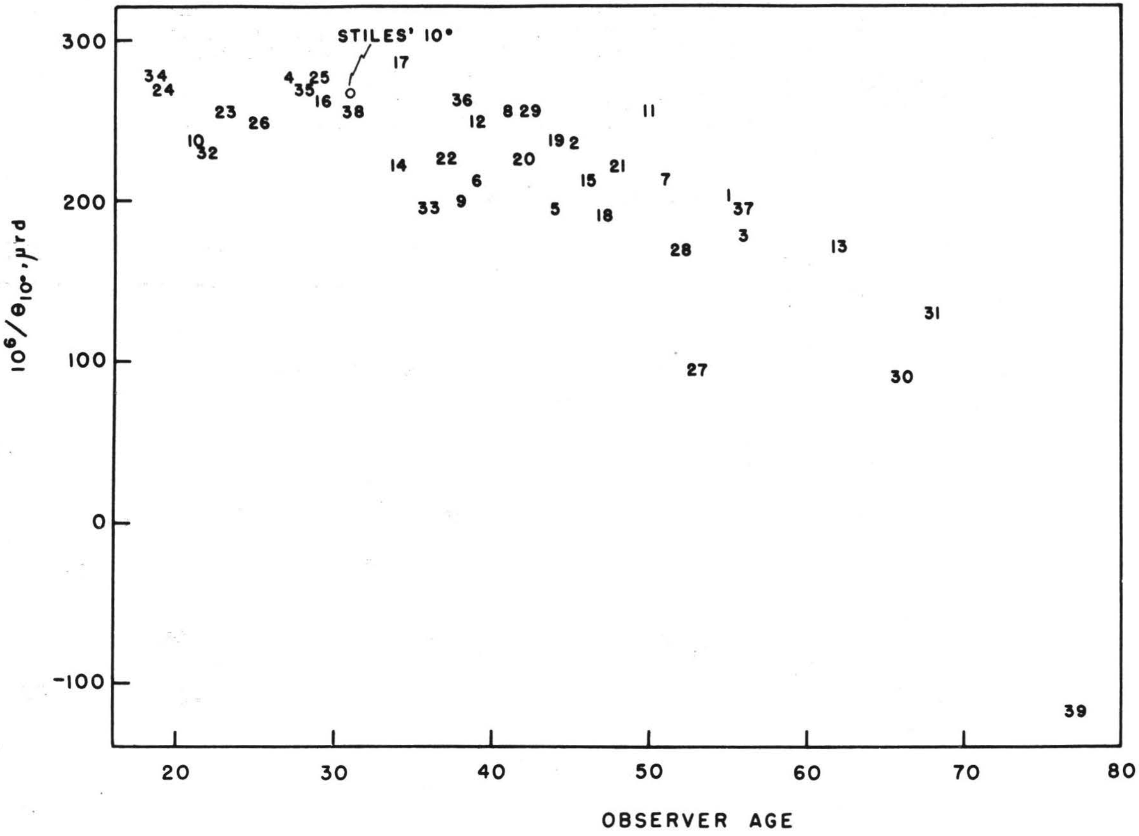


Fig. 4. Reciprocal colour temperature of source required in a 10^0 field by 39 observers to produce red-green balance between the Granville grays plotted against observer age.
Note that older observers require lower reciprocal colour temperatures. This result is ascribed to the known increase in lens pigmentation with age.

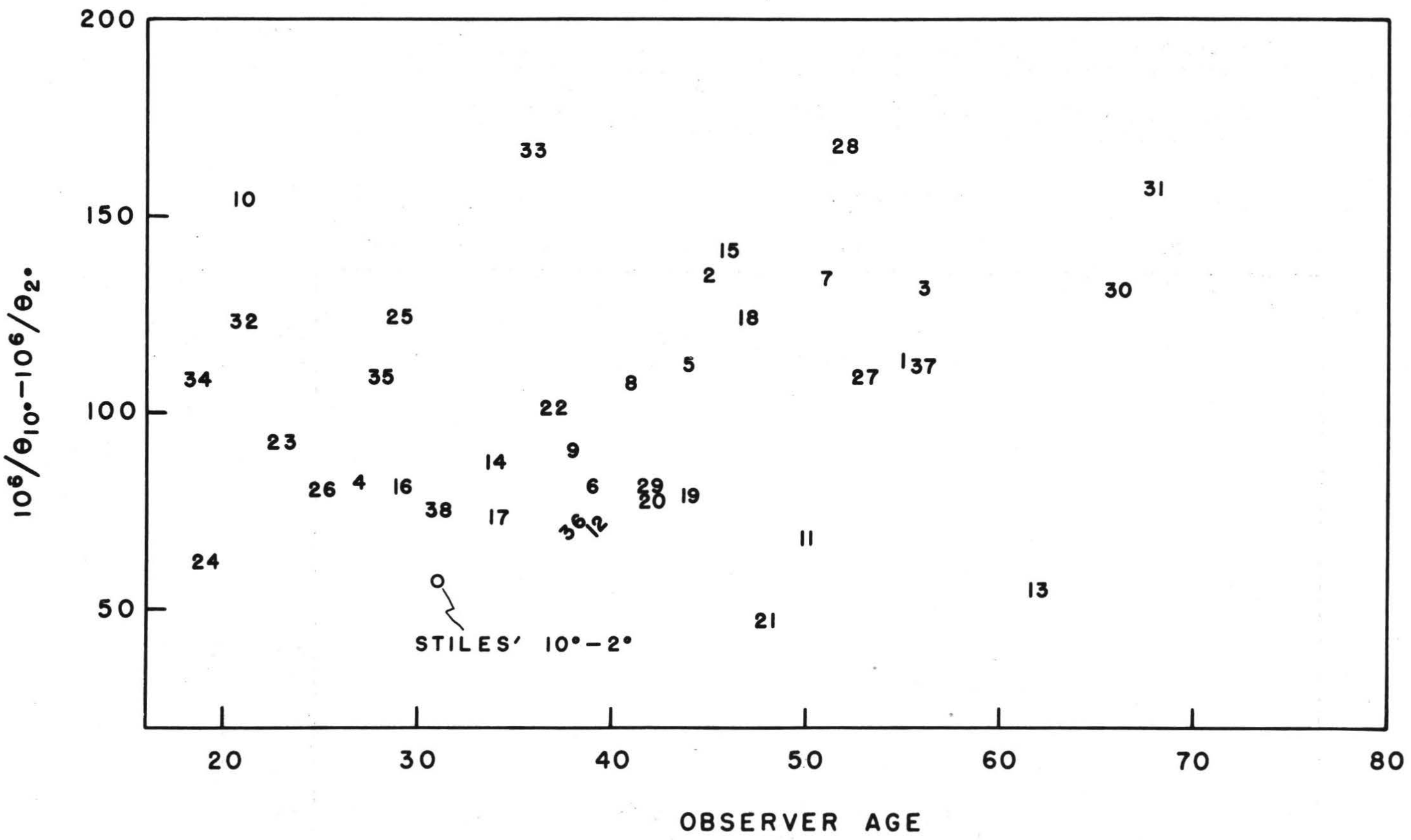


Fig.5. Difference between the reciprocal colour temperatures required for 10°- and 2°- fields by 39 observers to produce red-green balance between the Granville grays. On the assumption that the spectral sensitivities, apart from macular pigmentation, of the receptors are invariant over the whole central 10° of the retina, this difference is a measure of the amount of macular pigmentation. Note that there is no evidence of change in amount of macular pigmentation with age.

red-green balance points at the 10° and 2° positions. The three criteria for the five groups are shown in *Table 6*. According to this classification, all four colour-mixture functions fall into the red-red group of very lightly pigmented observers with the C.I.E. the most heavily pigmented and the Stiles 10° the least.

TABLE 6

Classification of observers into five groups according to indicated ocular pigmentation as shown by their description of the simplex gray relative to the complex gray for source *C*, and alternatively by the colour temperature and reciprocal colour temperature of source required to make neither gray redder or greener than the other

Observer group	Simplex relative to complex		Colour temperature of source at match point	Reciprocal colour temperature of source at match point
	10°	2°		
Red-red	red	red	$\theta_{10^\circ} < \theta_{2^\circ} < 6750^\circ$	$148 < 10^6/\theta_{2^\circ} < 10^6/\theta_{10^\circ}$
red-match	red	match	$\theta_{10^\circ} < 6750^\circ = \theta_{2^\circ}$	$10^6/\theta_{2^\circ} = 148 < 10^6/\theta_{10^\circ}$
red-green	red	green	$\theta_{10^\circ} < 6750^\circ < \theta_{2^\circ}$	$10^6/\theta_{2^\circ} < 148 < 10^6/\theta_{10^\circ}$
match-green	match	green	$\theta_{10^\circ} = 6750^\circ < \theta_{2^\circ}$	$10^6/\theta_{2^\circ} < 148 = 10^6/\theta_{10^\circ}$
green-green	green	green	$6750^\circ < \theta_{10^\circ} < \theta_{2^\circ}$	$10^6/\theta_{2^\circ} < 10^6/\theta_{10^\circ} < 148$

V. CONCLUSIONS

THE reciprocal colour temperature of the source required to produce red-green balance between the Granville grays has been found to vary widely from one observer of normal colour vision to another, and determination of this value of reciprocal colour temperature for any one observer serves to characterize his colour vision in an approximate but useful way. The value of reciprocal colour temperature required in a 10° field for red-green balance of the Granville grays is dependent on the amount of yellow pigmentation in the lens of the observer's eye, and is tentatively taken as a measure of this pigmentation. Similarly, the value of reciprocal colour temperature required in a 2° field for red-green balance of the Granville grays is dependent both on lens pigment and on macular pigment, and the difference in these two values (value for 2° field minus value for 10° field) is tentatively taken as a measure of the macular pigmentation of the observer.

The tentative measure of lens pigmentation afforded by the Granville grays correlates well with the age of the observer, but the tentative measure of macular pigmentation shows no correlation with age. Neither measure shows significant correlation with eye colour or hair colour, but a tendency, significant at about the 15% level of confidence, was found for female observers to have less pigmented eyes than male observers of the same age.

The reciprocal colour temperatures of source required by four sets of colour-mixture functions for red-green balance of the Granville grays have been computed and found to be higher than the average value for 39 actual observers with normal colour vision. Of the three sets of colour-mixture functions intended to refer to 2^0-field observation, the 1931 C.I.E. standard observer agrees best. These data thus afford no basis for supplanting the 1931 C.I.E. standard observer either with the Judd *"i"* colour-mixture functions or the 1955 Stiles 2^0 colour-mixture functions for a pilot group of 10 observers.

With respect to these observations on the Granville grays, the Stiles 1955 data for 2^0 viewing do not fulfil the requirement recommended in 1955 by the C.I.E. *(ref. 8)* that any revision of the "standard observer for colorimetry should represent average normal vision, adjusted as for an observer 30 years of age", but the data for 10^0 viewing are fairly satisfactory in this regard.

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PAPER 38

A MULTI-FILTER METHOD FOR DETERMINING COLOUR-MATCHING FUNCTIONS

(A PRELIMINARY STUDY)

By G. WYSZECKI

Günter Wyszecki was born in Tilsit, Germany, in 1925. He studied mathematics and physics at the Technical University in Berlin-Charlottenburg and there received his Dr.-Ing. degree in 1953. As a Fulbright Scholar he worked with Dr. Judd at the National Bureau of Standards in Washington in 1953/54. In 1954/55 he worked at the Farbforschungs laboratorium of the Bundesanstalt für Materialprüfung in Berlin and since the end of 1955 he has been a member of the Division of Applied Physics of the National Research Council of Canada in Ottawa. His research work includes subjects relating to the measurement of colour, colour order systems, normal and defective colour vision and chromatic adaptation of the eye.

38. A MULTI-FILTER METHOD FOR DETERMINING COLOUR-MATCHING FUNCTIONS

(A preliminary study)

By G. WYSZECKI

THIS paper describes a preliminary study on the determination of colour-matching functions by a method deviating in principle from the method which has been applied until now.

Under the presumption of Grassmann's additivity law in colour matching, tristimulus values (U_i) for any coloured stimulus of given spectral composition $(t_\lambda\, E_\lambda)$ can be computed by the following equations:

$$U_i = \sum_\lambda t_\lambda\, E_\lambda\, \bar{u}_{i,\lambda}\, \Delta\lambda. \qquad i = 1,\ 2,\ 3 \qquad (1)$$

The function t_λ is - for example - the spectral transmittance of a glass filter, E_λ is the spectral energy distribution of the illuminating light source, and $\bar{u}_{i,\lambda}$ are the colour-matching functions which represent the sensitivity of the observer's eye in the matching process. All functions are determined for a wavelength interval $\Delta\lambda$, usually being 10 mμ. The summation is carried out over the visible spectrum.

The determination of the colour-matching functions is traditionally done by measuring tristimulus values of monochromatic stimuli on a visual colorimeter which allows proper desaturation of the test stimuli in order to obtain colour matches throughout the spectrum. In the present study complex stimuli $(t_\lambda\, E_\lambda)$ were used and their tristimulus values (U_i) measured on a visual colorimeter, and then, by means of (1), the colour-matching functions $(\bar{u}_{1,\lambda})$ were computed.

For this purpose the visible spectrum was divided into 10 mμ bands from 390 to 700 mμ providing 32 intervals. The complex stimuli were provided by 115 selected glass filters of different spectral transmittance curves and standard light source *A*. The chromaticities of the 115 filters represented well the colour gamut which is produced by the red-green-blue primaries of the 6-primary Donaldson colorimeter. For each filter *R*, *G*, *B* readings on the colorimeter were obtained for the author's eye. The colour-matching functions are then determined by three sets of 115 simultaneous linear equations with 32 unknowns:

$$
\begin{aligned}
R^{(\alpha)} &= \sum_{\lambda} t_{\lambda}^{(\alpha)} E_{\lambda} \bar{r}_{\lambda} \\
G^{(\alpha)} &= \sum_{\lambda} t_{\lambda}^{(\alpha)} E_{\lambda} \bar{g}_{\lambda} \\
B^{(\alpha)} &= \sum_{\lambda} t_{\lambda}^{(\alpha)} E_{\lambda} \bar{b}_{\lambda}
\end{aligned}
\qquad (2)
$$

α = 1, 2, ... 115 ;
λ = 390 ... 700 mμ, every 10 mμ,
thus providing 32 terms.

The solution is a least-squares solution which was obtained by means of a digital electronic computer. Feeding the solution back into the computer and recomputing $R^{(\alpha)}$, $G^{(\alpha)}$, $B^{(\alpha)}$ values revealed partly large discrepancies relative to the corresponding observed values. The differences between observed and calculated tristimulus values were in fact considerably larger than the observational scatter would allow. This result may be interpreted by a failure of Grassmann's additivity law and/or certain inaccuracies in the mathematical procedure of solving equations (2). Preliminary tests of the latter possibility indicate that probably both the mathematical procedure and Grassmann's law are responsible for the discrepancies found in the analysis. However, at present it cannot be said how much the failure of Grassmann's law has affected the result. Further studies in analysing equations (2) are being made in order to obtain a definite and quantitative answer to the problem. These further studies will also include more observers, different sets of filters and apply various mathematical techniques.

PRESENTATION AND DISCUSSION OF PAPERS 40, 9 AND 38

DR. D. B. JUDD gave an account of the field trials on colour-matching functions carried out by himself and collaborators - papers 40 and 9.

Referring to the difficulties mentioned by Dr. Judd in obtaining a stable medium for titanium pigments, PROF. G. WALD wondered whether mineral oil would not have given more permanent pigment specimens than linseed oil.

DR. D. L. MACADAM reported some observations he had made on the Simon textiles using 19 normal observers. The average for all observers of the separation on the colour diagram of the two samples, one of each type, judged to be in closest match, had been found to have the following values when the computations were made using the various sets of colour-matching functions: 0.011 (C.I.E.), 0.007 (1951 Judd), 0.0068 (N.P.L. 2^0), 0.005 (N.P.L. 10^0).

DR. J. M. BURCH observed that the Kelly field trials for 2^0 and 10^0 showed a considerably larger difference than the predictions from the N.P.L. 2^0 and 10^0 pilot data. This was not so surprising as the conclusion had been reached that the pilot group observers had, on the average, low pigmentation, and the discrepancy with the field tests might be ascribable to an unlucky sampling error.

DR. K. J. MCCREE asked Dr. Judd whether the observers stared at the samples or just glanced. The correlation of the results with age seemed similar for 2^0 and 10^0 fields, but he would expect the 2^0 results to be more affected by macular pigment. DR. JUDD, in reply, said first that his observers always glanced at the specimens. The age dependence of the 2^0 results was attributed to lens pigmentation somewhat scattered by macular pigmentation, for which there seemed no correlation with age. For 10^0 observation the age correlation was similar but less scattered. Dr. Wright had raised the question of the conditions of illumination and view in the Simon textile tests and in the corresponding spectrophotometry. This might be a complicating factor but the specimens were matt-finished textiles. As regards the use of mineral oil for titanium pigments, this was not standard practice but perhaps it would be a good idea.

DR. G. WYSZECKI described his method of deriving colour-matching functions from a series of visual colorimeter (Donaldson) measurements on a large number of accurately spectrophotometered colour filters, the results of which were processed with an electronic computer. He said that the first results by this method gave colour-matching functions rather widely different from those obtained by the spectral stimulus methods. There were computational difficulties but there was some evidence that Grassmann's law was not strictly valid. The work was still in the exploratory stage.

MR. J. W. PERRY mentioned a much simpler procedure he had used which had some points of contact with Dr. Wyszecki's work. In it $\bar{x}$, $\bar{y}$, $\bar{z}$ functions were represented in terms of sets of filter transmission curves. Thus by physical measurements with these filters on any colour the C.I.E. components could be calculated. By altering the coefficients in the calculation, the effective $\bar{x}$, $\bar{y}$, $\bar{z}$ could be changed and the effects studied.

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