DISCUSSION SECRETARY’S REPORT

J.D. Moreland
Institute of Ophthalmology, London, W.C.1

Wald asked Bridges whether the occurrence of four preferred $\lambda_{\text{max}}$ positions related only to pure $A_2$ pigments or on occasion to $A_1$ pigments as well or to even mixtures. Bridges replied that on many occasions the extracts were mixtures but in all cases analysis was by means of the now standard procedure of partial bleaching. The maxima quoted by Crawford all referred to $A_2$ pigments. Wald asked whether the author would not hesitate a little on finding four maxima in a total of thirty-two species. Bridges replied that he had some hesitation so far as two positions were concerned (543 and 511 nm) for they were based (543 in chub and rudd: Dartnall, Lander and Munz, 1961; 511 in two wrasses: Wald, 1960) on only a few cases, the bulk of his (Bridges) results being centred either at 534 or 524 nm. When the 543, 534, 524 and 511 $A_2$ pigments were found naturally paired with $A_1$ pigments the latter had $\lambda_{\text{max}}$ at 510 (Dartnall et al., 1961), 505 and 499 or 492 nm (Wald, 1960) respectively. In fact, as Dartnall and Lythgoe were later to report, vitamin $A_1$-based pigments of teleost fishes (whether containing vitamin $A_2$-based pigments or not) have $\lambda_{\text{max}}$ grouped at positions 487, 494, 500, 506 and 512 nm, that is at or close to four of these positions.

Pitt, commenting on the inability of rats to form a visual pigment from 4-ketoretinaldehyde, suggested that this, given orally, was rapidly broken down and may have been unable to penetrate as far as the visual cells. Indeed it was remarkable that vitamin A itself was not oxidized very rapidly, suggesting that the body had adequate mechanisms for protecting it. Bridges asked how the absorption maximum 465–470 nm of the artificial visual pigment could be reconciled with the extra double bond conjugated with a polyene chain; the $\lambda_{\text{max}}$ of the normal pigment (without the keto group) being at 497 nm. Pitt replied that the spectroscopic characteristics of 4-ketoretinaldehyde were unexpected. The extra double bond appeared to be chromophorically ineffective for the $\lambda_{\text{max}}$ of 4-ketoretinaldehyde and of
unsubstituted retinaldehyde differed very little. On reduction to the alcohol, however, the double bond did become effective and the $\lambda_{\text{max}}$ occurred at longer wavelengths than for unsubstituted retinol (vitamin A alcohol). Parenthetically, if the Schiff's base was produced in the ternary nitrogen form the $\lambda_{\text{max}}$ was at a longer wavelength than retinaldehyde, but when that was proteinated the $\lambda_{\text{max}}$ moved back to a shorter wavelength because of the introduction of the keto group (Pitt, Collins, Morton and Stok, 1955). Wald suggested that the position of the retinaldehyde absorption maximum (at about 380 nm) was due in part to the polarization of the end carbonyl group ($\ddagger \text{-C=O}$) and to the transfer of charge down the conjugated system promoting a greatly increased resonance and hence a shift of spectrum towards the red. In 4-ketoretinaldehyde two such polarized carbonyls at the two ends of the chain worked against each other tending to neutralize that effect.

Weale pointed out that the data given in the original paper were for difference spectra resulting from 'green' and from 'deep red' bleaches. The 'green' bleach data were not intended to represent an isolation of the chlorolabe pigment; rather, the important feature was the constancy of the $\lambda_{\text{max}}$ of the difference spectra for different intensities of the 'green' bleaching light and its variation for 'red' bleaches (Ripps and Weale, 1963). He emphasized the reproducibility of such results and said that both effects could be satisfactorily explained by assuming either slightly different photosensitivities of erythrolabe and chlorolabe or slightly different densities for each.

Cope remarked that on exposure to light the oxidative phosphor relation in a mixture of extracts of choroidal melanin granules and mitochondria was changed. Answering an interjection by Wald he explained that the direction of the effect depended upon the co-factors present but its magnitude excluded the possibility of experimental error. Furthermore, the reaction was specific to choroidal mitochondria: liver mitochondria were ineffective. In addition, very small amounts of catecholamine hormones changed the radical decay times by up to 50 per cent.

Solid state kinetics seemed appropriate for the radicals since (i) eye melanin consisted of solid particles of an organic polymer, (ii) radical generation and decay occurred in a frozen aqueous suspension of melanin particles, and (iii) this was not abolished after boiling for 15 min, indicating a non-enzymatic process, (iv) the Elovich equation
fitted the radical decay curve: this equation was characteristic of electron transport phenomena at a semi-conductive solid surface (COPE, 1964). Cope thought that free radical generation and decay was due to photo-adsorption of O$_2$ at the surface of the melanin particle with reversible desorption in the dark. This was presumed to be accompanied by reversible electron transfer to dissolved O$_2$ from the unbonded electron pair on a nitrogen atom in a heterocyclic ring of melanin, with stabilization of the remaining unpaired electron by resonance with many equivalent nitrogen atoms throughout the melanin polymer.

SCHOLES illustrated typical sub-threshold discrete potentials recorded intracellularly from the primary receptors of the locust eye. The average height was 1 mV (i.e. about one-fiftieth of the saturated generator potential of the receptor): an effect that appeared only under very dim illumination (SCHOLES, 1964). Since these were discrete potentials they might militate against photoconduction occurring in the response—but the insect and vertebrate eyes were quite different in two ways. First, the membranes along which the photopigment was presumably arranged were continuous with the electrically responsive membrane of the cell in a way that was unlikely in a vertebrate rod cell. Secondly, a disappointing feature was the very long latency of the potentials: in the primary response a short latency was expected. The sub-threshold latencies averaged at about 50 msec whereas recent work by BROWN on the vertebrate receptor potentials indicated virtually no latency. ARDEN pointed out that the latency for insect receptor potentials obtained at low illumination could not be compared with mammalian latencies that were determined using very high intensity lights. His own experiments at low illuminations yielded latencies in mammalian eyes longer than those reported by SCHOLES. SCHOLES agreed but he had supposed that BROWN’s ‘no-latency’ receptor potential did not vary in latency with intensity. He added that the lowest latencies were distributed normally and that his statement was based only on a 1000 trials of dim flashes. It was likely that twice as many trials would yield shorter latencies. The minimum latency actually recorded was about 15 msec.

SAMSONOVA remarked that the EEG had been recorded from the region of the occipital lobes using a unipolar electrode. The light stimulus was presented intermittently with frequencies of 6, 14 or 40 c/s. The exposure time was 50 sec during which period a modified Walter analyser samples the EEG response amplitude for frequencies
arranged in the order 1, 5, 14, 26, 54, 6, 10, 11, 12, 15, 20, 24, 30, 40, 48, 60, 72, 80 and 96 c/s. Results of EEG recordings for one subject and averages for six subjects were shown in which the Purkinje shift could be detected. It was found that the EEG amplitudes for blue and red (440 nm and 660–700 nm) were equal at about 40–50 lux, that is at a higher level than obtained in psycho-physical determinations.

ROSENBERG illustrated the photovoltaic and photoconductive responses of the β-carotene cell (ROSENBERG, HECK and Aziz, 1964). These were thought to be similar to the potentials found in the fish retina (SVAETICHIN and MACNICHOL, 1958). For example, the long wave response (photoconduction) of the β-carotene cell disappeared on removing the applied potential but the short wave response (photovoltaic effect) remained. Similarly, when the glial membrane potential in the fish retina was reduced the ‘red’ potential vanished and only the ‘blue’ response remained.

ARDEN asked ROSENBERG (1) if he wished to compare his results with the ERG or to the S potential (the abstract mentioned both). The former was generated at two different sites and was biologically determined. The SVAETICHIN potentials could only be made smaller by polarization, not reversed, whereas the carotene ‘P III’ potential was reversible; (2) what was the gain of the carotene cell? The gain in a photoreceptor was known to be somewhere between $10^{14}$ and $10^{24}$.

ROSENBERG replied that the source of the receptor generator potential lay in the current flow changes in the receptor outer segment. The exact relation of these changes to the chromatic ‘S’ potentials and the ERG was unknown. The simplest connection appeared to involve passing on such photocurrent changes to the glia and neurones, possibly with amplification. In his opinion such amplification had to be isomorphic. The ‘S’ potentials and ERG might be picked up at different sites, but they had to reflect the shape of the original photocurrent. Gain would occur in the photoconductive process if the transit time of the charges between electrodes was smaller than the lifetime of the charges. In cadmium sulphide crystals a single photon could generate between $10^3$ and $10^4$ carriers but there was no clear evidence at present for a gain mechanism in organic materials. Further gain could occur at other stages (neuronal) of the visual pathway.

FATT thought that the β-carotene cell was inappropriate as a model for colour discrimination by visual receptors. The mechanism by which distinctive types of electrical response could be elicited by light of
different wavelengths could be readily understood if, as seemed likely, the slab of β-carotene used in the experiment was sufficiently thick so that light of \( \lambda \approx 430 \) nm (the maximum of the absorption spectrum) was absorbed almost entirely within a small fraction of the thickness of the slab, while light of longer wavelength, for which the absorption coefficient was less, would have been more uniformly absorbed. Assuming that all absorbed light had the same effect—the creation of charge carriers—the shorter wavelengths would produce most carriers close to the surface on which the light impinged, and as these carriers diffused away (assuming they could not be easily removed by the electrode) the photovoltaic effect would be produced. In contrast, light of longer wavelength would generate carriers more uniformly through the entire thickness of the material; there would be no diffusion gradient and the carriers would be manifest only by photoconduction. It seemed unreasonable to draw an analogy between this system and the processes occurring in the visual receptor. Rod outer segments were thought to absorb only some 10 to 20 per cent of the light passing down their length (probably a smaller fraction for cones) so that there appeared to be little scope for an effect based on absorption being very much greater at the surface which was first reached by the light.

Rosenberg replied that the first experiments were carried out on 100-μ thick carotene cells but that this has been reduced to about 10 μ for cells presently used. For these thinner cells the same effects were observed except that the 'red' response occurred at somewhat shorter wavelengths. However, both cell thickness and voltage were of the same order as in the retina. While there were no experimental data on the absorption of blue light passing through a photoreceptor outer segment, he thought that the laws of absorption would be the same for a photoreceptor as for a β-carotene cell.

Milne said that the survey of cyclorotatory and other involuntary compensatory adjustments in eye position by animals had been initiated after noting how much more successful this appeared to be in certain crustaceans (Milne and Milne, 1961) and cephalopods and cold-blooded vertebrates than in warm-blooded vertebrates—particularly mammals. The phenomenon was apparently not found in insects with mobile heads and in these the relative position of head and body was important in righting reflexes during flight. This suggested that the interpretation of patterns in the visual field was on a more comparable basis among vertebrates, cephalopods and the larger
crustaceans than between crustaceans and insects. Studies had been restricted to ocular movements corresponding to body postures commonly adopted by animals while free to move under natural conditions.

BLAKESLEE, commenting on the merits of spectral lights over Munsell papers in determining the presence of colour discrimination in animals by behavioural methods, said that their experiment was exploratory. It was an attempt to develop an easy method of testing primate colour vision in a zoo setting. The reliability of the method would be tested by comparing the results with those obtained with spectral lights before proceeding to screen a large number of primates.

WALRAVEN, referring to HURVICH's statement in the introductory lecture that there was very little change in the shape of the spectral sensitivity curve for moderate chromatic adaptation despite considerable changes in colour appearance, mentioned BRINDLEY's (1953) results for very intense chromatic adaptation in which the changes in shape were considerable. With such intense adapting light appreciable bleaching of pigment occurred and he had found that BRINDLEY's results were in agreement with suitably chosen linear combinations of Pitt's (1944) fundamental response curves. He had also found that the ratio of sensitivities to 595 and 525 nm during recovery from intense adaptation (595 nm for $5 \times 10^7$ troland. sec) had a 10–15 sec time constant. This was evidence of a neural component in addition to the photochemical component in recovery since the latter had a time constant of a few minutes. He thought that these diverse results could be reconciled in a zone theory of colour perception (WALRAVEN, 1962). This postulated one luminance channel in which the red, green and blue responses were summed; two chromaticness channels, one of which dealt with the red and green responses and the other with the yellow (sum of the red and green) and blue responses: (in which the blue response was multiplied by a chromatic valence factor $a$). Adaptation in the photoreceptors was photochemical but in the chromaticness channels it was neural. JAMESON–HURVICH asked whether the statement that adaptation to strong lights was photochemical but that to moderate lights was neural, was qualitative or had the quantitative laws governing these different mechanisms been worked out and if so, what were they and how did they differ at different levels. WALRAVEN replied that they were qualitative, the existence of separate neural and photochemical components were
presumed on the basis of the different time constants in the recovery
curves found by himself and by Brindley and Rushton. He wished
to stress his view that the neural component of adaptation existed in a
channel peripheral to the point at which it was supposed that the three
receptor responses summed to give the total luminance response.

Gutierrez-Costa said that the linear fractional transform of the
ratio of paired arbitrary spectral responses defined a bimodal function
analogous to those recorded in fish retinae (by MacNichol and
Svaetichin, 1958) and corresponded to a pathway for colour informa-
tion separate from that of luminosity. Two such transforms (for three
receptors) were sufficient to construct a formal chromaticity diagram
for which the metric was presumed linear and in which the loci of
constant hue and the dichromatic (protoproduct and protanopic) con-
fusion loci were defined. Transformation from this formal colour
diagram to the standard CIE diagram was simple but non-linear. He
indicated the freedom with which it was possible to introduce experi-
mental data into the various stages of deductive reasoning to derive the
shapes of the three original spectral responses. By using the CIE (1931)
standard observer luminosity data he found two with maxima at 590,
540 nm and the third duplex with a principal maximum at 540 nm
and a subsidiary probably at 475 nm (not at 500 nm as printed in the
abstract and the rapporteur's report). Further calculations using the
data of MacNichol and Svaetichin (1958) for several species yielded
spectral response functions with maxima at 435, 492, 499, 525, 543,
600 and 630 nm.

Granit, in conclusion, cautioned against drawing analogies between
apparently similar results obtained in widely diverse fields. Method-
ical criticism in each field was likely to be more fruitful. Speaking of the
difficulties of electrophysiological experiments he said that only one
thing was measured—potentials (or conductance of currents).
Conductance changes or remote synaptic actions for a cell with a large
dendritic spread might or might not be reflected at an electrode but
the significance of either event might not always be clear. It was not
possible to speak as though the whole visual world could be represented
by potentials at the retinal level. More centrally miniature potentials
were found occurring in both inhibitory and excitatory directions and
these could interfere without causing any net effect at all. It was
necessary to assume the existence of structures within a neurone which
ensured that the current flows dominantly in one direction in order to
account for the large changes in potential that are found.
REFERENCES


