VISUAL PURPLE

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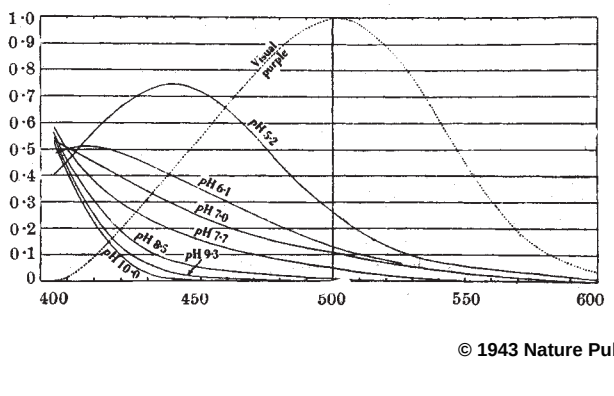
WHEN the late Dr. R. J. Lythgoe, of University College, London, about 1935, took up research on visual purple—the photosensitive substance of the retinal rods—our knowledge in this field had advanced but little since the beginning of this century. To a large extent his own precise and well-planned work, partly carried out in fruitful collaboration with Dr. D. F. Goodeve as an expert on photochemistry, is responsible for the clarity that now makes it possible to write about this subject with feelings of satisfaction. As very few British physiologists have taken an active experimental interest in the subject of vision, the work of Lythgoe has not received the recognition that it deserves, and so, perhaps, it is fitting that an appreciation should come from a colleague abroad. Though all Lythgoe's contributions to the problems of sight were stamped with the same hall-mark of quality, no better tribute can be paid to his memory than to indicate in a brief review the place and significance of his work on visual purple against the background of past and present contributions to the same subject—one particularly dear to him. I can only quote a selected number of papers in the list of references. From these papers and an earlier review (Granit, *Documenta Ophthalm.*, **1**, 7; 1938) those interested in the subject will easily find the rest of the references. Lythgoe's own summary (*Brit. J. Ophthalm.*, **24**, 21; 1940) is of particular interest as a final statement of his matured views on the subjects of visual purple and dark adaptation.

The old work of König (1894) and his pupils and that of Trendelenburg (1904) had already suggested rather definitely that the human scotopic luminosity curve, which pictures the spectral distribution of sensitivity of the dark-adapted (scotopic) eye, was determined by the spectral absorption curve of visual purple. But the precise form of this absorption curve was still the subject of discussion. Kühne had shown about 1880 that visual purple was bleached by light to a yellow photoproduct (visual yellow) and, if he was right, as others afterwards denied, the yellow substance could be expected to interfere with the measurements of visual purple absorption at the shorter wave-lengths. However, the most recent evidence (Nakashima, 1929, Hosoya, 1935, Chase, 1936) supported Kühne's view and indicated that the cause of older differences of opinion lay in the varying degrees of acidity of the solutions.

Clearly it was necessary to study the spectral absorption curves for visual purple extracts of a very high degree of purity and to follow up this work by analysing the absorptive properties of the various photoproducts found in the course of the bleaching of solutions containing this extremely light-sensitive substance. With characteristic thoroughness and technical perfection Lythgoe embarked upon this programme of research. In the accompanying figure is shown Lythgoe's (1937) absorption curve for visual purple with its maximum at 0·502 μ. Illumination destroys this substance, leading to formation of a photoproduct (left in illustration) that shifts its spectral locus with the degree of acidity of the solution, being yellow in moderately acid solutions and colourless in alkaline solutions, because its maximum is then in the invisible ultra-violet. For this reason Lythgoe called this photoproduct "indicator yellow". This substance is very insensitive to light, and can scarcely play a part in perception except as a filter. This work was soon confirmed by others (Saito, 1938, Chase, 1938, Wald, 1938) but has never been surpassed in accuracy. Krause and Sidwell (1938) have followed the changes farther out into the ultra-violet region.



Engaged in studying the breakdown of visual purple under the influence of light, Lythgoe (1937) made the important discovery that the first step in this process was the formation of an orange intermediate photoproduct which was immediately transformed to indicator yellow, unless the solution before illumination had been cooled in ice. On account of its colour and instability to heat he called this substance "transient orange". The difficult task of measuring the absorption curve for transient orange was successfully tackled by Lythgoe and Quilliam (1938), who found it to possess a maximum around 0·470 μ. This substance may play a part in vision. Thus, as the first result of Lythgoe's work, the old inheritance of concepts with no definite physical meaning such as 'visual yellow' and 'visual white' were replaced by concepts corresponding to substances characterized by their absorption curves and certain other properties such as stability to heat, light, acidity, etc. Kühne's 'visual yellow' embraced both indicator yellow and transient orange. If substances other than these two are formed in the course of bleaching or spontaneous breakdown of visual purple or its yellow photoproduct, as claimed by Wald (1938), they have not yet been demonstrated in a convincing manner. Lythgoe himself was unable to find them.

The discovery by the Danish workers, Fridericia and Holm (1925), confirmed by Tansley (1931) with a more accurate method, that vitamin A deficiency leads to delayed regeneration of visual purple and to nightblindness, inspired several workers to study the vitamin A content of the retina. It was found (Haurowitz, 1933, v. Euler and collaborators, 1933–35, Krause, 1937–38, Wald, 1935–37) that vitamin A and carotene occurred in the pigment layer, but that visual purple was identical with neither substance. However, Wald made the significant discovery that the yellow photoproduct obtained by illumination, or disruption of the visual purple molecule by other means, could easily be extracted by organic solvents and then had the chemical property of a carotenoid. Wald called it 'retinene'. His claim that the final colourless product of bleaching *in vivo* is vitamin A has been opposed by Krause and Sidwell (1938). At any rate, vitamin A is not formed during the bleaching of visual purple in solution. The closely related visual violet, found in certain fishes (Köttgen and Abelsdorff, 1896, Bayliss, Lythgoe and Tansley, 1936) and possessing an absorption spectrum shifted

some 0·030 μ towards the red, breaks down in a similar manner, forming a russet-coloured product, another carotenoid, retinene$_2$ (Wald, 1939). It is an interesting fact that retinæ possessing visual violet contain vitamin A_2 instead of the ordinary vitamin A_1. No doubt, therefore, the A-vitamins are of importance for the formation of the chromophoric group of the visual purple molecule even though it may not be possible at present to describe the nature of this relationship. Lönnberg (1935–37) has isolated large amounts of another carotenoid, xanthophyll, from the retina, and Lythgoe (1940) undoubtedly was right in pointing out that some essential link in our knowledge is missing as long as this finding has not been accounted for.

All old and recent work (Hecht, Krause, Lythgoe, Wald) shows visual purple to be a protein. It has a high molecular weight and is precipitated by water-soluble organic solvents. It shows cataphoretic properties with an iso-electric point (Broda, Goodeve, Lythgoe and Victor, 1939), has the ultra-violet absorption spectrum of proteins (Krause, Goodeve, Lythgoe), and the kinetics of its thermal decomposition, studied by Lythgoe and Quilliam (1939), is consistent with the same view. We may regard visual purple as a 'chromoprotein' in which the chromophoric group is Wald's carotenoid retinene. The molecular weight of that part of the chromoprotein which contains one chromophore is called its 'carrier weight'. Broda, Goodeve and Lythgoe (1940) found the carrier weight to be somewhat below 26·500, a value not far from Svedberg's fundamental protein unit (about 17·600). Putting the molecular weight, determined by Hecht and Pickels (1938), equal to 270·000 and dividing by the carrier weight 26·500, gives the number of chromophores attached to each molecule, which accordingly is about 10. The chromoprotein occupies some 5–10 per cent of the volume of the outer limb of the rod. From different points of view both Lythgoe (Bayliss, Lythgoe and Tansley, 1936) and I arrived at the conclusion that the photosensitive substance is localized on the surface of the rods.

According to Lythgoe (1940), then, light acts on the chromoprotein by loosening the bond between protein and chromophore and so renders the latter more soluble in organic solvents. Thus retinene becomes extractable. Indicator yellow is probably still a conjugated protein, apparently identical with Wald's retinene-protein. Goodeve and Lythgoe think it probable that the essential chemical change caused by the excited visual purple molecule is one of hydrolysis.

Goodeve and Lythgoe in collaboration with Dartnall and Schneider have published several important contributions to the photochemical aspects of the problem. They have determined the quantum efficiency of visual purple (equal to or not far below unity), followed the time course of bleaching, proved that Lythgoe's absorption curve exactly reproduces photosensitivity, determined the molecular extinction coefficient ($2·3 \times 10^4$ for wave-length 0·560 μ), and studied the photosensitivity in the ultra-violet. In an aphakic eye (eye without lens) the sensitivity to light was found to reach far out into the ultra-violet (0·300 μ), following there, too, the curve for the photosensitivity of visual purple.

As is well known, visual purple regenerates in the dark, even in solution (Kühne, 1878), though under such conditions to a relatively limited degree (Hecht, Lythgoe). It has been shown by Zewi (1939, 1940) that there are two kinds of regenerative processes in the living retina, one of which is dependent upon the oxygen supply. The other takes place also in a nitrogen atmosphere. A most interesting point is that the rate of regeneration is favoured by previous illumination of the retina (Bauer, 1911, Zewi, 1939) or even *in vitro* by illumination of solutions (Chase, 1937). But if the animals are illuminated with strong light for a *brief* period there may first be a period of absolute standstill of the regenerative processes (Zewi). Now, to quote Lythgoe (1940): "It seems possible that we have two methods for the regeneration of visual purple. Regeneration from its breakdown products almost certainly involves the addition of energy to the system, and this energy can be provided either by the absorption of light or by a chemical process needing oxygen". The vitamins and other carotenoids are probably involved in relatively complex catalysed reactions which so far are unknown. The regeneration from the orange or yellow photoproducts probably consists in a strengthening of the bond between the protein and its chromophore. Lythgoe found regeneration from transient orange to be particularly fast, and made the remarkable statement that the chromoprotein, regenerated from this stage, had an absorption band and other properties which did not quite agree with those of the parent visual purple. He suggested that cone substances may be formed in this manner. Elsewhere (1941, 1943) I have pointed out that the electrophysiological evidence also supports the view that cone substances are closely related to visual purple.

At this stage Lythgoe's work was interrupted by his untimely death, at a moment when he planned collaboration with a biochemist to elucidate the chemical structure of the photosensitive substances in the retina. A particularly fascinating line of research thus awaited Lythgoe just when he had to lay down his tools. How admirably they were used I hope to have indicated by this attempt to interpret a consistent and harmonious development of experiments and ideas, hand in hand.

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PHYSIOLOGY OF COLOUR VISION

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IN a previous article in NATURE[1], attention was directed to the possibility that the physiological basis for colour vision might lie in the relative responses of the rods and the cones. A curve was plotted to show the relationship between wave-length and the summation of the rod and cone responses at each wave-length. The characteristics of this curve suggested that it had some affinity with the well-known colour triangle. Further analysis of this phenomenon may not be without interest and also provide answers to certain questions raised by Prof. H. Hartridge in his recent communication[2].