Physiology of the Retina and the Visual Pathway

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PREFACE

The theme of this book can be shortly stated: "How does the human visual pathway work?" Very little mention will be found in it of the elegant investigations that have been made of the eyes of invertebrates. Necessarily, much of the evidence considered comes from vertebrates other than man; but the primary interest remains human, and discussion of the comparative physiology of the vertebrate eye is introduced only where it is needed in assessing the extent to which facts or inferences established for lower vertebrates are likely to be valid for man.

The first three chapters describe what can be learned of the function of the retina and visual pathway from purely objective experiments of the kind familiar in non-sensory branches of physiology. Most of these observations are electrophysiological and biochemical. No special section is devoted to the relevant anatomy, but anatomical evidence on questions of function is frequently introduced. The last four chapters consider the results of sensory experiments, that is experiments in which an essential part of the result is the report by a human subject of his own sensations. Historically, these are as old and as respected a source of speculations about the functioning of the eye as are the objective observations, but it is not often that they have been used as rigorously in argument, and a whole chapter (though a short one) is devoted to a general discussion of the problem of drawing physiological conclusions from sensory experiments.

This is a tightly written book, very full of information and for this reason not easy to read from end to end consecutively. To make the browser's task easier, I have provided it with an analytical list of contents, full indexes, and abundant internal cross-references in the text. Its vocabulary should not be difficult; time and trouble have been spent in keeping as small as possible the number of technical words special to visual physiology that are used.

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I

THE PHOTOCHEMISTRY OF THE RETINA

The photolabile pigments of the retina

An organ which is to respond to light and signal its presence and pattern to the brain must contain a pigment, that is a substance which absorbs light, for light cannot influence matter unless it is absorbed by it. The only exception to this rule, the pressure exerted on a mirror by light reflected from it, is quantitatively minute, too small by many orders of magnitude to be of physiological significance. We shall therefore expect to find, in any cell capable of transmitting a signal to other cells under the influence of light, a pigment which absorbs light of that range of wavelengths which can stimulate the cell. There is good reason to believe, as we shall see in Chapter V, that the light-sensitive structures of the vertebrate retina are the rods and cones; and in the rods of a large number of species and the cones of one, appropriate pigments have been found.

It was H. Müller who in 1851 first described the reddish colour of the outer segments of the rods of many vertebrate eyes. The observation was neglected, and was made again by Boll in 1876. Boll noted that the colour disappeared rapidly from the retina after it had been removed from the animal, and described the sequence of changes between the initial red and the final absence of colour. He found that if the animal had been exposed to bright light before killing, no redness was to be seen in the retina. The importance of these observations was quickly appreciated by Kühne, who in a series of papers (Kühne, 1878*a*, *b*, 1882*a*, *b*; Ewald and Kühne, 1878*a*, *b*, *c*; Ayres and Kühne, 1882) showed that a purplish-red pigment which he called "Sehpurpur" or "Rhodopsin" could be extracted from the retinae of a wide range of vertebrates with a solution of bile salt, and that the colour, both of the retinae and of the pigment in solution, was stable in the dark but rapidly bleached by light of those wavelengths that it absorbed. He also made many experiments on the chemical properties of the pigment and its regeneration after bleaching both in the retina and in solution.

There can be little doubt that, for those rods that contain it,

rhodopsin is the pigment by which light has to be absorbed to cause transmission of a signal to the neural layers of the retina. In man and in a number of other species possessing rhodopsin, the spectral sensitivity curve of the eye in the fully dark-adapted state has been compared with the absorption spectrum of rhodopsin, and a very close agreement found. In three fresh-water fish, whose retinae contain instead of rhodopsin a different pigment, porphyropsin, the spectral sensitivity of the dark-adapted eye, determined from the reaction of the intact fish to a moving pattern of light and dark bands (Grundfest, 1932), or from the electroretinogram or the action potentials of single retinal ganglion cells (Granit, 1941*b*), has been found to agree well with that of porphyropsin.

Pigments different from rhodopsin, but resembling it in being rapidly bleached by light, have been extracted from the retinae of a number of vertebrates. Most of these pigments are known or presumed to be derived from the outer segments of rods, but there is one, iodopsin, extracted from separated fragments of rods and cones from the retinae of poultry (Wald, 1937; Wald, Brown and Smith, 1955), which is very probably a cone pigment. Attempts to extract photolabile pigments from retinae which entirely lack rods have so far been unsuccessful; but Rushton has provided clear objective evidence (see p. 35) for the presence of at least two photolabile pigments in the rod-free foveal part of the living human retinae, and these are almost certainly cone pigments.

There is no necessary reason why the pigment which allows a rod or cone to be influenced by light should be detectably decomposed by light. It could resemble a sensitizer in a photographic emulsion; the absorbing molecule passes the energy of an absorbed quantum immediately to a molecule of some other substance, and itself returns to the state in which it was before the quantum was absorbed, so that by ordinary criteria it is photostable. No photostable pigment likely to be concerned in the detection of light has yet been extracted from the retina of any vertebrate. It may be that all vertebrate receptive pigments are photolabile; but this is by no means certain, for the methods that have been used to investigate retinal pigments have mostly been specially designed for the detection of photolabile pigments. In some invertebrate receptors, pigments which seem to be photostable have been found. Kühne (1878*c*) extracted one from the crayfish, *Astacus fluviatilis*, Krukenberg (1878) several from a number of cephalopods, and

Granit (1947) one from the house-fly. These, however, may not be receptive pigments, and may even not be truly photostable. Hubbard and St George (1958) have shown that the rhodopsin-like pigment of the squid, which was earlier thought to be photostable, is in fact converted by light to a different substance with almost the same absorption spectrum; earlier investigators assumed because the absorption spectrum remained the same, that no chemical change had occurred.

The great majority of the investigations that have been made of the chemical properties of visual pigments have used rhodopsin extracted from the retinae of frogs or cattle. The rhodopsins of frogs and cattle differ slightly in their absorption spectra, the maxima of which lie at 505 and 497 mμ respectively; but in their other properties they, and the rhodopsins of other species, appear to be closely similar. In the two sections which follow, rhodopsin will be taken as a typical receptive pigment, and its properties in solution and within the rod described in detail.

The chemistry of rhodopsin in solution

Rhodopsin is ordinarily extracted from a suspension of the outer limbs of rods, separated from other retinal tissue by centrifugation, by means of an aqueous solution of a detergent, that is a substance whose molecules or ions are hydrophilic at one end and hydrophobic at the other. The most extensively used extracting agent is digitonin, a glucoside which has the advantage of being colourless; but there are now many other satisfactory agents (Bridges, 1957). It is uncertain whether rhodopsin is soluble in water in the absence of a detergent; the observation of Broda, Goodeve and Lythgoe (1940), that it is precipitated from digitonin extracts if the digitonin is removed from them by dialysis, suggests that it is probably insoluble.

Chemical nature of rhodopsin

Wald (1938) bleached solutions of rhodopsin with light, mixed them with an equal volume of cold acetone, and extracted the resulting precipitate with light petroleum containing a trace of acetone or ethyl alcohol. He found that the extract contained the pale yellow carotenoid *retinene*. Subsequently Morton and Goodwin (1944) proved that retinene is the aldehyde of vitamin A. These observations provide the simplest of the several pieces of

evidence which together show beyond reasonable doubt that one part of the molecule of rhodopsin is very closely related to retinene. The stereo-isomeric configuration of this carotenoid part of the molecule and the way in which it is joined to the non-carotenoid part will be considered later.

It has long been generally supposed that the non-carotenoid part of the molecule of rhodopsin is a protein. Rhodopsin has a high molecular weight (see below), is bleached by many chemical agents which denature proteins, moves cataphoretically in an electric field with speed and direction depending on the pH, the isoelectric point being 4.47 (Broda and Victor, 1940), and is thermolabile, the kinetics of its thermal decomposition being similar to those of proteins (Lythgoe and Quilliam, 1938*a*, Hubbard, 1958). Solutions of it are bleached by proteolytic enzymes (Ayres, 1882, Radding and Wald, 1958), and precipitates of it, made by removing by dialysis the substances of low molecular weight from digitonin extracts of frog's rods, contain about 12 per cent of nitrogen, an amount not very different from that characteristic of proteins (Broda, Goodeve and Lythgoe, 1940). Its ultraviolet absorption spectrum shows a strong band at 278 mμ, similar to that found in proteins which contain tyrosine and tryptophan (Collins, Love and Morton, 1952*a*). This evidence leaves little doubt that the main part of the rhodopsin molecule is protein, but does not exclude the possibility that there may be some other constituent in addition to the protein and retinene. We shall see later that after retinene has been removed from rhodopsin, the residue can combine again with retinene, provided that the latter is in the correct stereo-isomeric form, to re-form rhodopsin. This residue, capable of combining with retinene, is known as opsin; the name does not prejudge the question of whether it is wholly or only partly protein in nature. The recent experiments of Krinsky (1958*a*) indicate that it may be a lipoprotein.

Molecular weight

Aqueous solutions containing rhodopsin and digitonin sediment in the ultracentrifuge as if they contained a homogeneous population of particles of molecular weight about 270,000 (Hecht and Pickels, 1938). All the rhodopsin and nearly all the digitonin sediments with these particles. They are thus presumably micelles containing molecules of both substances. Hubbard

(1954*b*) measured the extinction coefficient at wavelength 500 mμ of solutions containing a known weight (and therefore a known number) of micelles. By using the data of Wald and Brown (1953) for the extinction coefficient of rhodopsin at this wavelength per mole of retinene liberated on bleaching, she was able to infer that each micelle contains only one labile retinene group. There must therefore be only one rhodopsin molecule in each micelle and one labile retinene group in each molecule. Hubbard also measured the nitrogen content of her solutions. Digitonin contains no nitrogen, and the amount of nitrogen in the solutions was such that, if it were all contributed by a typical protein containing 15 per cent of nitrogen, the molecular weight of protein in each micelle would be 40,000. Thus the molecular weight of rhodopsin must be 40,000 if the micelles contain no other nitrogenous substance extracted from rods together with the rhodopsin, and if rhodopsin contains 15 per cent of nitrogen. If Broda, Goodeve and Lythgoe's value of 12 per cent nitrogen is assumed, the estimated molecular weight becomes 50,000. On the other hand, if some nitrogen-containing compound other than rhodopsin is extracted from the rods by digitonin, and enters the micelles together with rhodopsin, the true value may be less than 40,000.

Krinsky (1958*a*) has recently obtained solutions of rhodopsin containing a much smaller amount of nitrogen, corresponding to a protein molecular weight of only 18,000. His estimate for the total molecular weight, however, is 32,000, for he finds that the solutions contain a large amount of phospholipid, which by pre-treatment of the rods with alum and fat solvents or with a bacterial phospholipase can be reduced, but never below 20 moles per mole of rhodopsin; from this he argues that opsin is a lipoprotein containing about 20 phospholipid residues per molecule.

The effect of light on solutions of rhodopsin

If a solution of rhodopsin, buffered to pH 9.3, is exposed to bright white light at room temperature for a few minutes, its purplish-red colour is found to be bleached to a very pale yellow. If the solution is then made acid, the colour alters reversibly to a deep yellow; a substance which acts as a pH indicator is present. This substance was named "indicator yellow" by Lythgoe (1937). In alkaline solution the colour is quite stable for several hours, and acidification at the end of several hours produces the same depth