Monocular and Binocular Aftereffects of Chromatic Adaptation

Abstract. *Supersaturated greens seen after long-wavelength adaptation depend upon contrast from the continuing afterdischarge of bleached "red" receptors in the surround, rather than merely upon inactivation from bleaching of "red" receptors in the test spot area. When test spot and bleach field coincide spatially, supersaturated greens are not seen. Since color mixing but not contrast was found binocularly, color contrast must be a retinal phenomenon.*

It has long been known that after exposure to a light of long-wavelength, of a luminance high enough to bleach away a considerable fraction of the photopigments in the long-wavelength receptors, one sees light from virtually any spectral region as a green which is more intensely saturated than any ordinary monochromatic light (*1*). Furthermore, the spectral sensitivity of the eye during this period corresponds to other estimates of the absorption curve of the middle-wavelength receptor (*2*).

The usual explanation of this is that the long-wavelength receptors have been bleached out, leaving only the response of the middle-wavelength, "green" receptors at all but the shortest wavelengths, whereas ordinarily both "red" and "green" receptors respond to all these wavelengths, in varying proportions. Therefore, not only do all middle wavelengths appear the same color after red adaptation, but they are a supersaturated green because there is no "red" receptor response left to desaturate the "green" receptor response. Our investigation of this phenomenon has led us to conclude that this explanation is false.

With the original intent of measuring the time course of changes in brightness, hue, and saturation resulting from intense adaptation, we set up a haploscopic matching situation. The right eye was bleached with a light of 650-nm wavelength which covered a circular area of 3° visual angle centered on the fovea. Then a semicircular test light was presented to the right eye, and a matching semicircle to the other eye; these together formed a 1° circle when the eyes correctly fixated a tiny line between the semicircles. The wavelength, luminance, and purity of the light to the left eye could be varied to follow the progressive changes in the appearance of test lights of different wavelengths in the bleached right eye. Such a haploscopic matching procedure appeared appropriate because an absence of binocular interaction has been reported in this situation (*3*). It soon became obvious, however, that binocular interaction of a very interesting nature was taking place. After a red bleach, all wavelengths from 480 to 600 nm presented to the bleached eye are seen for varying periods as a supersaturated green, as we have mentioned. We did not expect to be able to match the supersaturation of the green light by manipulating the light in the unbleached eye, but were rather surprised to discover that we could see no green at all with the unbleached eye: those wavelengths around 500 to 510 nm which one would expect to appear green were completely colorless for a period of time. We were later to discover that Fry (*4*) had found much the same sort of binocular interaction earlier in experiments with short wavelength adaptation.

The most attractive explanation of the inability to see green with the unbleached eye after intense long-wavelength adaptation of the other eye would be on the basis of a mixing in the cortex of the "green" signals coming from the test light in the unbleached eye with a continuing afterdischarge of signals from the "red" receptors in the bleached eye. The "red" and "green" signals from the two eyes would then mix and cancel each other (because of the opponent organization of the color vision system) to give an achromatic appearance to the test spot. The presence of such an output from retinal receptors, which continues until all the photopigment is regenerated and which has both perceptual and adaptational effects, has been postulated by Barlow and Sparrock (*5*) as an explanation of the slow recovery of sensitivity in dark adaptation. One does indeed see a continuing red afterimage for a brief time after the offset of the long-wavelength bleaching light; that it is seen only for a brief period although the color changes continue for minutes could be explained on the basis of the afterimage being stabilized on the retina (*5*).

This, however, leaves us in the dilemma of explaining the absence of green seen with the unbleached eye on the basis of continuing "red" signals from the bleached eye, while attributing the supersaturated greens seen by the bleached eye to a complete absence of "red" signals in the bleached eye.

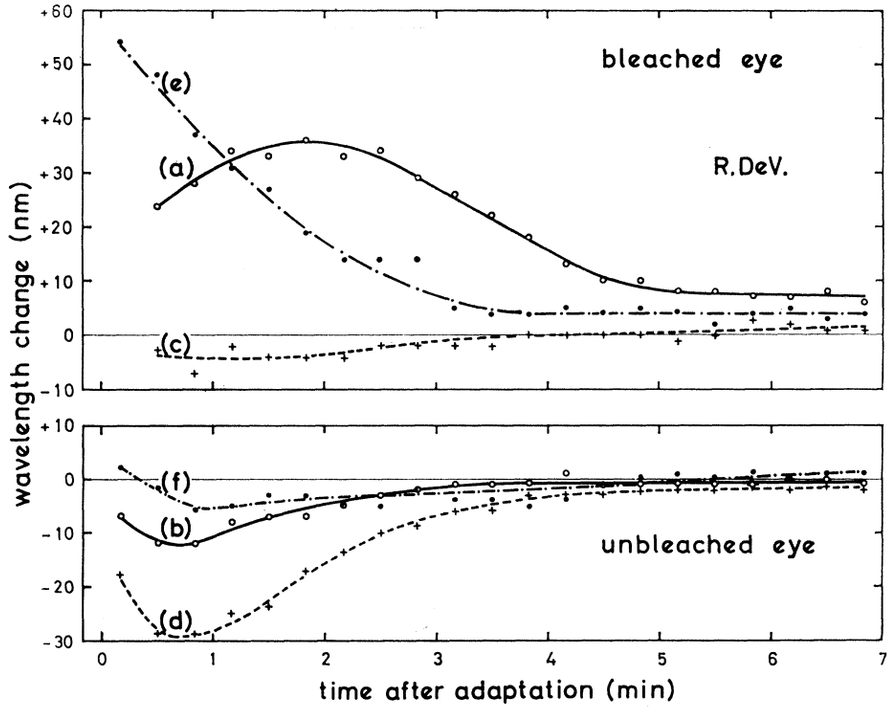

Fig. 1. Change in wavelength from the preadaptation setting to maintain the best yellow color. The various test conditions are described in the text. The initial yellow setting, from which the changes occur, was 576 nm.

The resolution of this dilemma came from the following experiments.

Since haploscopic matching, using the opposite eye as a standard, was clearly invalid, and since both monocular and binocular effects were of interest, the test light was presented to just one eye at a time, and the colors seen with each eye were studied separately by a color-naming procedure. The right eye was bleached with a centrally fixated, circular 3° light of 650 nm at 2.2×10^4 trolands for 2 minutes, presented in Maxwellian view. A monochromatic test light (luminance, 300 trolands) was then presented, again in Maxwellian view, for 5 or more minutes. Several different wavelengths of test lights were used in separate runs, but the results with 510 and 550 nm are most informative. These test lights were presented under four conditions: (i) 1° circular test light to the right eye, centered in the 3° bleached area, (ii) 1° light to the left eye, centered in the binocular field of the bleached area, (iii) 3° light to the right eye, exactly coinciding spatially with the 3° bleached area, and (iv) 3° light to the left eye, coinciding with the bleached area in the binocular field.

Conditions i and ii are essentially the same as those in the initial experiment described above, and the same results were obtained. Lights of 510 and 550 nm were seen by the bleached eye as a highly supersaturated green, whereas the unbleached eye saw 510-nm light as colorless for a time and then highly desaturated, and 550-nm light as shifted far toward the red from its usual greenish-yellow appearance.

The same wavelengths presented to the bleached eye in a test light exactly coinciding with the bleached area, condition iii, produced quite a different effect from the small test spot. Instead of being supersaturated, light of 510 nm was considerably desaturated for a time, and brightness recovered much more slowly; 550-nm light was seen shifted somewhat toward the red, instead of toward the green, and the same slow brightness recovery was noted. The unbleached eye gave much the same results whether the test light was the same size (condition iv) or smaller (condition ii), than the bleached area in the other eye.

The results of the color-naming experiments were confirmed and extended in other experiments in which the observer set a monochromator to the wavelength which made the test spot appear the best yellow. This was done before adaptation, and every 20 seconds after chromatic adaptation. Each setting started from some random wavelength, so the subject had no knowledge of the actual wavelength involved. Each curve presented in Fig. 1 is an average of six such runs on one subject. The results from the other subject are very similar. The experimental conditions were the same as in the color-naming experiment.

In condition i the test spot had to be set at wavelengths as high as 610 nm after adaptation to be seen as yellow with the bleached eye, shorter wavelengths being seen as supersaturated green (see Fig. 1). This shift disappears when the test spot and bleach field coincide spatially (condition iii); in fact, a slight change toward shorter wavelengths is now required to produce yellow. In the case of the unbleached eye, a shift to shorter wavelengths was always necessary to maintain a yellow color, since longer wavelengths appear reddish after long-wavelength adaptation (see Fig. 1, bottom). The relative size of test spot and bleach field make no difference in the direction of the effect in this case.

We believe that these findings can be explained on the following basis. When the long-wavelength adaptation light is turned off, the "red" receptors continue their neural output to some extent until their photopigments are regenerated, as discussed above; this is perhaps shown physiologically in the slow recovery phase of the late receptor potential recorded by Brown *et al.* (*6*). This neural afterdischarge mixes binocularly with the neural response initiated in the unbleached eye to produce colors which are thereby shifted toward the red.

In the bleached eye itself, the continuing afterdischarge of the long wavelength receptors has two somewhat opposite effects: that of desensitizing the "red" receptors in their response to light shined into the eye, and that of a continuing neural output which can mix with the outputs of the other types of receptors. The overall effect in the bleached eye is thus a smaller shift toward red in the color mixture than was the case in the other eye. The anomalous case is when a 1° test spot follows a 3° bleach in the same eye. The supersaturated greens seen in this case seem to be due to simultaneous contrast between the large "red" aftereffect and the small "green spot plus red aftereffect" in the test spot area. The afterdischarge and the new responses of the "red" receptors are eliminated by lateral inhibition from the surrounding "red"-receptor afterdischarge, leaving only the responses of the "green" receptors. The successive contrast produced by blinking (which turns off the test spot but not the aftereffect) doubtless also contributes to the contrast situation.

In short, we propose that far from being produced by an absence of "red" signals from the bleached retina, the supersaturated greens are produced by contrast with the continuing afterdischarge of the surrounding "red" receptors. Additional evidence for this view comes from one other experiment we performed, condition v: an annular bleach of 3° outside diameter and 1° inside diameter was used; the test spot of 1° diameter filled the hole in the annulus and was presented to the bleached eye. The same supersaturated greens were seen here as in condition i, even though the retina in the test spot area had not been bleached at all; the shift in yellow settings was even more extreme initially than in the bleach-plus-contrast condition. Condition vi was the same as v, but with the test spot in the unbleached eye; this adaptation arrangement produced only a slight change in the yellow settings, which could be attributed to stray light. The reason for the different time courses of the effects under the different stimulus conditions is of interest and has yet to be determined.

That contrast colors are not seen when a 1° test spot is presented to the unbleached eye (conditions ii and vi) indicates that whereas color and brightness mixing occur either within the retina or in the binocular cortical areas, color and brightness contrast must be retinal, or at least subcortical, phenomena. Furthermore, the lateral inhibition producing color contrast must be triggered only by the presence of a contour in the bleached eye, since in the absence of a contour the "red" activity in the middle of the bleached area shows no signs of suffering from lateral inhibition, as is indicated by its interaction with the test spot in the other eye.

RUSSELL L. DE VALOIS
Psychology Department,
Indiana University, Bloomington
JAN WALRAVEN
Institute for Perception,
Soesterberg, Netherlands

References and Notes

1. H. Helmholtz, in *Physiological Optics* (1895), vol. 2, pp. 243–45 (J. Southall, Ed.; Dover, New York, 1962); G. Burch, *Phil. Trans. Roy. Soc. London Ser. B* **191**, 1 (1898).
2. G. S. Brindley, *J. Physiol. London* **122**, 332 (1953); P. L. Walraven, A. M. J. van Hout, H. J. Leebeek, *J. Opt. Soc. Am.* **56**, 125 (1966).
3. W. D. Wright, *Researches on Normal and Defective Colour Vision* (Kimpton, London, 1946), pp. 214–216.
4. G. A. Fry, unpublished final reports by Ohio State University Research Foundation (1954).
5. H. B. Barlow and J. M. B. Sparrock, *Science* **144**, 1309 (1964).
6. K. T. Brown, K. Watanabe, M. Murakami, *Cold Spring Harbor Symp. Quant. Biol.* **30**, 457 (1965).

7. This work was performed at the Institute for Perception, Soesterberg, Netherlands, and was supported in part by a NSF senior postdoctoral fellowship to one of us (R.DeV.). We gratefully acknowledge the use of the optical system and laboratory facilities of Dr. P. L. Walraven.

14 October 1966

Mechanism of Antibody Synthesis: Size Differences between Mouse Kappa Chains

Absrtact. *Structural analysis of immunoglobulin light chains has been carried out in an attempt to elucidate the genetic mechanisms involved in antibody synthesis. Analysis of two mouse kappa-chain proteins is almost complete. The differences are localized in one-half of the molecules, and do not reflect the operation of any one mutational mechanism. The peculiar character of the differences is discussed with reference to various theories of antibody formation. The finding that the two proteins differ in size is incompatible with certain proposed theories.*

The nature and extent of variation among immunoglobulin molecules has been studied as a means of testing various hypotheses concerning the mechanism of antibody formation. For this purpose, much attention has been focused on the proteins secreted by plasma-cell tumors, since these proteins can be readily isolated in a homogeneous form. Although these proteins are not functional antibodies made in response to a known antigenic stimulus, they appear to be truly representative of immunoglobulins in terms of their primary amino acid sequences. Most information has become available from structural studies of light-chain subunits of the K antigenic type, which have been obtained from tumors arising in mice and humans (*1–5*).

Such studies have shown that all proteins from within one species contain an extended region of structural identity (*6*, *7*) and that this region comprises approximately the carboxyl-terminal half of the molecule (*3*, this work). The variations are thus limited to the amino-terminal halves of the polypeptide chains.

Hitherto, the differences found in this region could be attributed to the random accumulation of point mutations, and they do not appear to have arisen by any particular genetic mechanism (*1*, *2*, *4*). The most extensive comparison yet made has been that between three human proteins (*2*, *3*), but the data were not sufficient to establish with certainty whether the proteins were of identical size—an important consideration for theories of antibody formation.

In this report we present a comparison between the amino acid sequences of two mouse K-chains [mouse Bence Jones proteins MBJ 41 and MBJ 70 produced by tumor lines MOPC 41 A and MOPC 70 E, respectively (*6*)] and the human K-chain Ag which has been investigated by Titani *et al.* (*2*). Probably MBJ 41 and the human proteins Ag and Cum each contain 214 amino acid residues (*2*, *3*). A slight doubt remains for MBJ 41 in that we have not yet been able to purify the large tryptic peptide containing residues 67 to 103, and hence we cannot assign a definite amino acid to position 87. The size of the human protein Roy (*3*) is not certain, but it probably contains 212 to 214 residues. Protein MBJ 70 has now been found to contain 218 residues, the four additional amino acids being inserted in the region of residues 27 and 28 (Fig. 1).

The evidence for this is quite unambiguous. The tryptic peptide defining residues 25 to 45 of MBJ 41 had the composition $Asp_{3.0}$, $Thr_{1.0}$, $Ser_{3.0}$, $Glu_{4.1}$, $Pro_{0.8}$, $Gly_{1.8}$, $Ala_{1.0}$, $Ilu_{1.8}$, $Leu_{2.1}$, $Lys_{1.1}$, Trp_1, a total of 21 (*8*). The corresponding peptide from MBJ 70 gave the analysis Asp_3, Met_1, $Ser_{3.5}$ $Glu_{4.0}$, $Pro_{3.0}$, $Gly_{2.0}$, $Ala_{1.1}$, $Val_{1.0}$, $Ilu_{1.0}$, $Phe_{1.9}$, $Lys_{2.0}$, Trp_1, a total of 25. The analyses were unchanged by further purification. Fragments were isolated after degradation of the peptides with chymotrypsin (MBJ 41) or subtilisin (MBJ 70) or cyanogen bromide (MBJ 70).

Amino acid compositions and sequences of these fragments were sufficient to establish the structures as shown in Fig. 1. The appropriate data for MBJ 70 are shown in Fig. 2. It should be noted that the -Lys-Pro bond is not susceptible to cleavage by trypsin, and hence the peptide from MBJ 70 contains two basic amino acids. The size difference between the two mouse proteins in this region is of special significance since it could not arise by a translational mechanism involving different readings of a single messenger RNA (*9*).

We have previously reported upon the sequences of the first six residues of a number of mouse and human K-chains (*1*) and established that the same

Table 1. Comparison of related pairs of proteins and genes. The comparison is made by (i) amino acid (AA) substitutions; (ii) the minimum number of nucleotide base changes required to account for these substitutions in terms of point mutations, with the use of the coding assignments of Brimacombe *et al.* (*11*); (iii) classification of base changes as transversions or transitions.

Chains		AA Residues	Changes between related genes: Differences No.	Differences %	Minimum base changes 1	2	3	Transitions	Transversions
				K light chains					
Variable 1–107	Mouse 41	107	42	40	27	12	1	32	17
	Mouse 70	111							
Common 108–214	Mouse	107	45	42	32	13	0	20	28
	Human	107							
				Hemoglobin					
	Human β	146	37	26	28	9	0	28	16
	Human γ	146							