its perceived spatial position, without any significant change in the retinal array. This result implies that lateral inhibition at the retina has little to do with everyday perception of lightness. Certainly the available theories that reduce lightness perception to lateral inhibition are in error.

Another important implication also follows. If the perceived lightnesses of surfaces depend on their perceived location in space, depth processing must occur first and be followed by the determination of surface lightness. That is, processing is initiated by a pattern of intensity differences on the retina; then the nervous system uses various depth cues to construct a spatial model to fit the retinal pattern. As this spatial model is completed, lightnesses are assigned to the various surfaces in accord with the coplanar ratio principle.

ALAN L. GILCHRIST*
Institute for Cognitive Studies,
Rutgers University,
Newark, New Jersey 07102

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15. Two features of the adjacency principle suggest ways in which it could be tested against the coplanar ratio principle. (i) According to the adjacency principle, the perceived lightness of a target should move continuously through the gray scale as its apparent position between two inducing fields is varied continuously. The coplanar ratio principle would predict a sharp break in perceived lightness at whatever point in space the target changes its plane of reference. (ii) Gogel and Mershon use adjacency to describe the workings of contrast. The coplanar ratio principle need imply no contrast process. Indeed, a subsequent article will provide further evidence that lightness perception does not involve contrast in any important way (A. L. Gilchrist, in preparation).
16. Mershon (*3*) did provide separate inducing fields for two of the depth planes in which the target appeared. Presumably his results involved less change in lightness than those reported here, because even when his target and inducing fields were coplanar, they were still somewhat separated retinally. In addition, both of his inducing fields had luminances greater than that of the target whereas, in the present studies, the luminance relationship of the target and the surrounding coplanar surfaces reversed for the two different conditions of each experiment.
17. Partially supported by Bell Laboratories, Murray Hill, N.J. 07974. I thank J. Johnston and C. Harris for advice on the writing, D. Dinnerstein for introducing me to lightness perception, H. Wallach for his inspiring example, and especially I. Rock for his formulation of the original problem and for his continued interest in this research.
* Present address: Department of Psychology, State University of New York, Stony Brook 11794.

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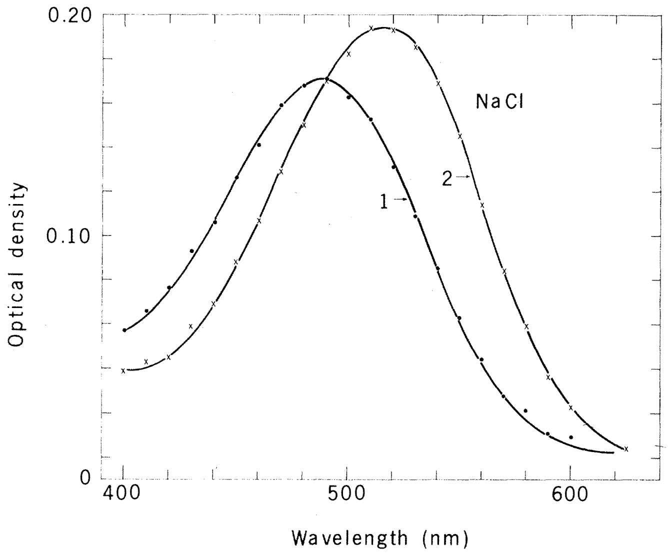
Ionochromic Behavior of Gecko Visual Pigments

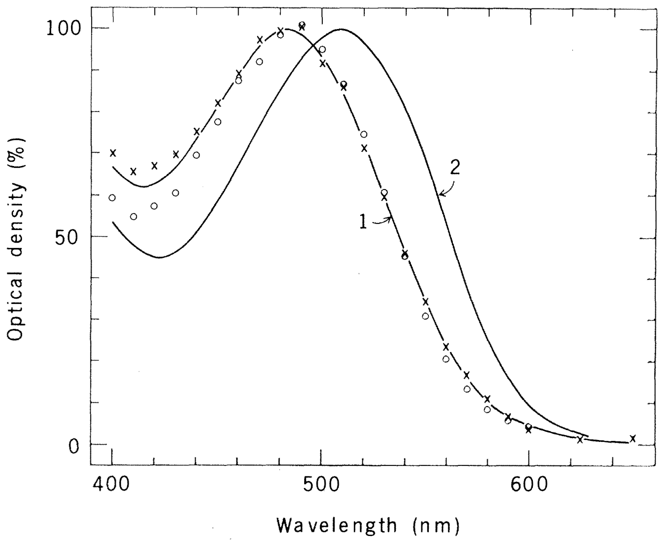
Abstract. *Digitonin extracts of the retina of* Gekko gekko *prepared to minimize the presence of chloride ions show the photopigment to be at about 490 nanometers rather than at 521 nanometers, the position found for the same pigment in situ. The addition of chloride to the extract causes a bathochromic shift in spectral absorbance, the magnitude of the shift being related to the concentration of chloride, within limits. The effect is a specific one, and of all the anions tested only bromide causes a similar bathochromic shift. The nature of the cation is not involved since the same action is produced by the chlorides of sodium, lithium, potassium, rubidium, cesium, calcium, magnesium, beryllium, lanthanum, and choline.*

Proteins that respond selectively or specifically to inorganic ions are of special interest to biological scientists, especially if these proteins are derived from excitable membrane systems. At this time I wish to report the behavior of a visual pigment whose color is altered reversibly by certain specific inorganic anions. This pigment, a retinal-based chromoprotein from the retina of *Gekko gekko*, has a spectral absorbance maximum of 521 nm within the outer segments (*1*). In aqueous digitonin solution the spectral maximum varies according to particular physical and chemical conditions of the extract. One of these conditions is the inorganic ion composition.

The main effect is a chloride response (Fig. 1) that can be elicited in the following manner. The photopigment is extracted out of the visual cells with 1 or 2 percent digitonin made up either in tris-maleate buffer (*p*H 7.2) or in borate buffer (*p*H 8.8). Prior to extraction the isolated retinas are washed once with distilled water and placed in 4 percent potassium alum for 3 to 6 hours at 5°C. The alum-hardened retinas are washed twice with double-distilled water and once with the buffer, and then extracted with 0.1 to 0.2 ml of digitonin per retina. During these operations the temperature is kept close to 5°C. Extracts prepared in this manner never have absorbance maxima (λ_{max}) close to 521 nm, but instead the spectra are located at shorter wavelengths. For the extract represented in curve 1 (Fig. 1) the λ_{max} is at 487 nm, typical for this procedure. The spectral absorbance is not stabilized at this location, for on adding a drop of the same buffer containing NaCl (*2*) the spectrum shifts rapidly toward 521 nm (curve 2, Fig. 1). This shift is quantitatively related to the NaCl concentration, a definite shift appearing with about $4 \times 10^{-5}M$ NaCl and increasing in magnitude up to about $2 \times 10^{-2}M$ (Fig. 2). It seems likely, therefore, that the photopigment extracted in the manner described exists in a deficient state in which its spectral absorbance lies some 25 to 30 nm toward lower wavelengths from the normal 521-state. Addition of NaCl repairs the deficiency but the λ_{max} never shifts beyond 521 nm. While in the hypsochromic, deficient state the pigment is still photosensitive and the shape of the absorbance curve is that of a Dartnall nomogram type. The chloride shift is completed within the time required to add the NaCl and to remeasure the spectral maximum, and is reversed as quickly by diluting the extract with chloride-free buffer. This reversibility is best demonstrated by adding NaCl to a concentration that causes a shift of only 5 to 10 nm and then diluting. In this way the operation is kept within the steep, functional portion of the curve in the inset (Fig. 2).

The chloride effect appears to be independent of the nature of the cation coupled with the chloride, and I have obtained the same shift in the presence of sodium, potassium, lithium, rubidium, cesium, calcium, magnesium, beryllium, cadmium, and lanthanum. Even the organic chloride, choline chloride, produced the same bathochromic shift. Of the anions tested (bromide, phosphate, borate, thiocyanate, nitrate, sulfate, fluoride, and iodide) only bromide elicited the same response as chloride. The effect is anion-specific and the behavior toward the halides suggests some role for ion size. The small fluoride ion is inert as is the large iodide ion, while the intermediate chloride and bromide ions are the active ones (Figs. 1 and 3).





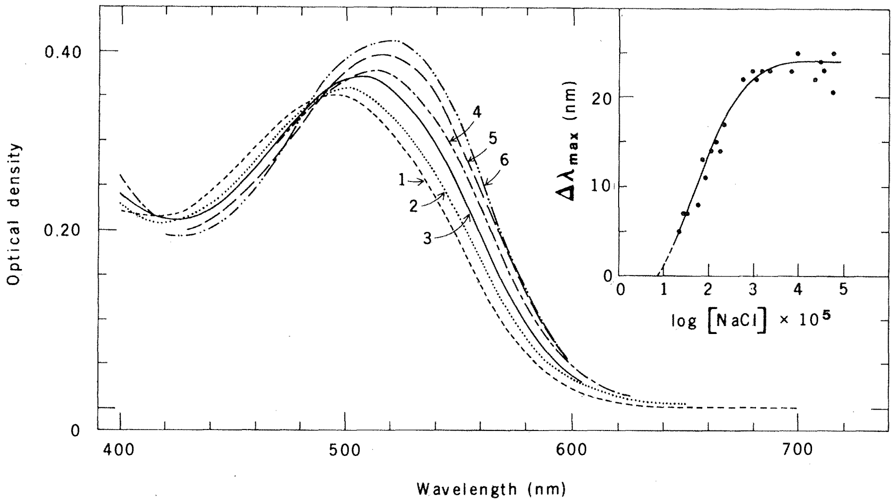


Fig. 1 (top left). The chloride shift. (Curve 1) Spectral absorbance of an extract made up in 1 percent digitonin, with tris-maleate buffer (*p*H 7.2). (Curve 2) Spectral absorbance after adding NaCl to give a concentration of $7.97 \times 10^{-3}M$ NaCl; temperature, 3.6°C. Curve 2 has been corrected for dilution by the addition of NaCl, the dilution factor being computed from weighings made before and after adding the NaCl. Fig. 2 (left). The chloride shift—quantitative aspects. (Curve 1) The pigment in the hypsochromic state. (Curves 2 to 6) After successive additions of NaCl to give $2.70 \times 10^{-4}M$, $8.25 \times 10^{-4}M$, $1.81 \times 10^{-3}M$, $1.04 \times 10^{-2}M$, and $3.75 \times 10^{-1}M$, respectively. Curves 2 to 6 have been corrected for dilutions. (Inset) The bathochromic displacement of the spectral maximum (in nanometers) as a function of log NaCl molarity. The data for the inset are derived from curves 2 to 6 and from other similar experiments. Temperature, 3.6°C. The data for this figure were obtained from extracts made up in borate buffer at *p*H 8.8. Fig. 3 (top right). Specificity of the chloride shift. Curve 1 (solid line), the pigment in the hypsochromic state; curve 1 (X), after the addition of NaF to give $7.40 \times 10^{-3}M$; and curve 1 (open circles), after the addition of NaI to give $7.70 \times 10^{-3}M$. (Curve 2) After the addition of NaBr to give $1.03 \times 10^{-1}M$. All curves have been normalized with maxima at 100 percent. Temperature, 3.3°C. Extract in borate buffer.

In attempting to interpret these results it is easier to eliminate certain ideas than to suggest definite mechanisms that may be involved. The concentrations of chloride and bromide required to produce an effect and the high degree of anion specificity eliminate such factors as ionic strength, lyotropic effects, and chaotropic actions (*3*). Nothing is known about the composition or structure of the gecko 521-pigment or how it may differ from the usual rhodopsins in which I have failed to find a chloride effect. If pigment-521 is like other visual pigments in having 11-*cis* retinal bound to a free amino group of opsin as a protonated Schiff base and if a secondary interaction occurs between the π-electron system of the retinylidene chain and certain negatively charged groups of the opsin (*4*), then the chloride shift can be conceived as an electrostatic action whereby chloride ions, loosely bound at specific anion-binding sites of the opsin, perturb the secondary charge interaction. This perturbation could be a direct influence on the secondary charge field itself or it could be an effect involving a conformational change of the opsin at a region removed from the chromophore.

An interesting feature of this ionochromic action is the magnitude (25 to 30 nm) of the shift, being as large as the natural displacements of many visual pigments occurring in vertebrates. To date I have failed to obtain any comparable behavior with the rhodopsins of frog, fishes, and other vertebrates but I have found it in *Gecko stentor* as well as the Tokay gecko. Recognizing how inaccessible is the chromophore of vertebrate rhodopsins to reagents, Hubbard and Sperling (*4*) turned to squid retinochrome which was found to be reversibly changed in color by such factors as *p*H, aging, and cysteine. Among vertebrate visual pigments that have been extracted the gecko pigments appear to be unusual. In addition to the ionochromic behavior described here, I may mention the reversible thermochromic and thiochromic responses that are already known for these photopigments (*5, 6*). Further studies of these gecko pigments will probably reveal additional interesting properties.

FREDERICK CRESCITELLI
Department of Biology,
University of California,
Los Angeles 90024

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