appeared on this medium were isolated. Those calluses which were not able to maintain a normal rate of growth on unsupplemented medium were tested on a number of different nutritional supplements in medium 2, as outlined by Holliday (*7*), to determine their auxotrophic requirement.

Over 1.75×10^6 haploid cells were examined. Of the 119 calluses that were isolated only six proved to be auxotrophic (Table 2). There is little or no restriction on the auxotrophic types which can be recovered since auxotrophs for nucleic acid bases, vitamins, and amino acids have been observed. All six auxotrophic calluses retained their haploid chromosomal constitution. Plants were differentiated from four of the mutant calluses.

It is significant that so few mutants were isolated and that these mutants all proved to be leaky; for they continued to grow slowly on unsupplemented medium. This is in contradistinction to similar work with ferns (*3*). One explanation may be that the selection procedure is not able to detect nonleaky auxotrophs. This could occur because either the low plating efficiency or one of the experimental parameters is not permitting auxotrophs to survive selection. Mutant single cells might be killed by the long starvation period designed only to inhibit their growth. However, this explanation is partially excluded by further experiments with only a 6-hour starvation period. In these experiments no increase in the number of auxotrophic or nonauxotrophic cells surviving the treatment with BUdR and near-visible light was observed. Another explanation may be that nonleaky mutants are not being induced.

Because *N. tabacum* is an allopolyploid, the haploid cell may actually contain two copies of essential genes. The low number and leaky quality of the auxotrophic mutants could be due to a lack of functional diploidization of the *N. tabacum* genome, and thus a lack of functional haploidization in the somatic haploid cell. Although Smith (*8*) noted that most morphological mutants of *N. tabacum* act as if the species is a functional diploid, this explanation may not be valid for loci involved with the metabolism of essential nutrients. Clausen and Cameron (*9*) noted that the morphological characters hairy filaments and yellow burley were each determined by duplicate factors belonging to different parental genomes of the allopolyploid *N. tabacum*. This evidence argues that the functional diploidization of the *N. tabacum* genome is not complete. The auxotrophic mutant characteristics appear best explained by the assumption of incomplete diploidization. Thus, there may be more than one functional copy of metabolically important genes in the haploid genome of *N. tabacum*.

The data on the growth of the auxotrophic mutants further implies a physiological differentiation between the two functional copies of a duplicated gene. If the two copies are identical, then a mutation in one of them would not be expected to appear as a mutant, inasmuch as a low, residual amount of enzymatic activity will still permit wild-type amounts of growth in other eukaryotes. A mutation in one of a pair of duplicate genes should not reduce the normal growth rate by more than half. Such a reduction is found only with auxotroph number 82. The remaining five auxotrophs show much slower rates of growth. This phenomenon could be explained by assuming either that the two gene copies are subject to different regulatory controls or that the copies are differentiated so that they operate in different pools or compartments within a cell.

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Trichromatic Mechanisms in Single Cortical Neurons

Abstract. *By chromatic adaptation, all three cone mechanisms of rhesus monkey vision can be identified in single neurons of striate cortex. This trichromatic interaction occurs in cells sensitive to color and indicates that striate cortical cells tend to be more wavelength discriminating than cells at lower stages of the primate visual system.*

Color vision in man (*1*) and in certain primates (*2*) is trichromatic because only three variables are required to produce all color sensations. This trichromacy depends upon the photopigment molecules in the outer segments of cone receptor cells. There are three types of cones, each of which absorbs wavelengths of light differently; one is more sensitive in the blue, another in the green, and a third near the red region of the visible spectrum (*3*). The outputs of these three channels are analyzed by the remainder of the visual system to produce a variety of color impressions.

Knowledge of the processing of color information in the primate central nervous system is confined mostly to the retina (*4, 5*) and the lateral geniculate nucleus (*6, 7*). Less is known about color vision in other areas of the visual nervous system such as the superior colliculus (*8*) and the cerebral cortex (*9, 10*).

In the retina and lateral geniculate nucleus of many vertebrates there are two major types of neurons, the on-center and the off-center cells. Although both types receive excitatory and inhibitory signals, the spatial distributions of these excitatory and inhibitory mechanisms differ over their receptive fields (*11*). An on-center cell receives relatively more excitation and an off-center cell relatively more inhibition in the center of its receptive field. On-center cells are excited and off-center cells are inhibited when light is turned on in the center of their receptive fields; the converse can occur when the light is turned off.

Animals with color vision have cells in which the spectral characteristics of these excitatory and inhibitory processes also differ; that is, a cell is excited by one wavelength and inhibited by another (*4–7, 12*). Such cells are called spectrally opponent, and they are usually on- or off-center cells. Spectrally opponent cells seem to represent an essential step in color discrimination by the vertebrate central nervous system.

In the rhesus monkey, whose color vision is similar to man's, such spectrally opponent cells are abundant in the

retina (*5*) and in the lateral geniculate nucleus (*6, 7*). These cells receive signals from at least two of the three cone mechanisms present in the monkey's retina, but they rarely receive signals from all three. Cone mechanisms sensitive to red and green (*5–7*), green and blue (*5, 7*), and blue and red (*6*) have all been found to be paired antagonistically in single retinal and geniculate cells of the monkey. In 1961 De Valois and Jones reported that green-on, purple-off cells occurred predominantly in layers 3 and 4 of the monkey's lateral geniculate nucleus, but there was no indication in this early work that a rod input to these cells had been eliminated. Although such cells should be relatively easy to recognize, no subsequent investigations of the monkey's retina and lateral geniculate nucleus have discovered them, which suggests that trichromatic interaction may be an unusual event at this point in the primate visual system.

This report demonstrates that trichromatic interaction is much more common in single cells in the monkey's striate cortex. The nature of this interaction reveals something about how color information is transferred from the retina through the lateral geniculate nucleus to visual cortex.

Responses of single cells in striate cortex of rhesus monkeys anesthetized with Nembutal are recorded with glass micropipettes filled with 3*M* potassium chloride; the electrodes have resistances ranging from 10 to 100 megohms. The electrodes are inserted into the cortex in a closed chamber to minimize vascular pulsations (*13*) and, sometimes, to allow the recording of intracellular responses. Only one eye is stimulated. The central retina, including the fovea and optic disk of the eye, is viewed through a modified fundus camera and visual stimulator (*5*). Eye movements of approximately ¼° can be detected. Light in two independent beams is obtained from a well-regulated 1000-watt high-pressure xenon arc lamp. One beam is a test light; the other is used for chromatic adaptation. The test stimulus is a stationary row of three spots (each measuring 0.015 mm²) with their centers separated by 0.7 mm as measured photographically on the retina. This row can be oriented in different directions around the central spot in attempts to elicit maximum responses from cortical cells (*10*). The row's overall position on the retina is determined by using the middle of the three spots to locate the retinal region producing the strongest response. The energy and wavelength of these light spots can be changed by monochromatic interference and neutral-density filters. The energy of these stimuli have been measured by a calibrated thermopile and galvanometer. The test stimuli are 200-msec pulses of light presented every 1 or 2 seconds. Stimulation begins approximately 1 minute after any change in the adapting field. Action spectra are obtained for individual cells. An action spectrum is defined as the energy required to produce the same criterion response from a cell at a number of different wavelengths. In this case the criterion was a threshold response at 15 different wavelengths in the presence of chromatic adapting lights. One of three wavelength bands is used in the adapting beam. Each is obtained with Corning sharp-cut glass filters 2408 (red), 3482 (yellow), and 5543 (blue). The energies of these adapting lights in $\log_{10}$ quanta sec^{-1} mm^{-2} of retina are approximately 11.2, 11.5, and 11, respectively. These energies have been found to be sufficient to raise rod thresholds above cone thresholds and to isolate and identify three cone mechanisms in the retina of rhesus monkeys (*5*).

Table 1. Cone mechanisms [blue (B), green (G), and red (R) sensitive] identified in single color-sensitive cortical cells by selective chromatic adaptation.

No. of cells	On response	Off response
5	R	G
2	G	R
3	R	B
3	G	B
1	B	G
6	R	G, B
2	R, B	G
2	G	R, B
3	G, B	R

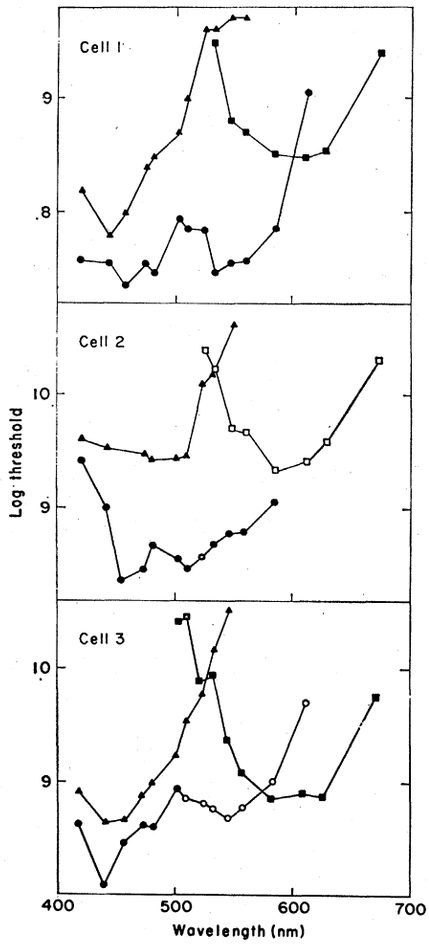

Fig. 1. Thresholds in $\log_{10}$ relative quanta for eliciting responses from three cells in monkey's striate cortex at different wavelengths ($nm = 10^{-9}$ m) in the presence of red (circles), yellow (triangles), and blue (squares) adapting light. The filled symbols signify an "on" response; the unfilled symbols signify an "off" response at threshold.

Cells have been studied from no deeper than 2 mm below the surface of striate cortex contralateral to the eye being stimulated. Their receptive fields are located in the nasal parafovea and perifovea, are eliptical in shape, and do not cross over into temporal retina. The area of cortex studied is determined by dissection after an experiment.

Figure 1 shows the relationship between the logarithm of the relative energy on a quantal basis necessary to elicit threshold responses from three different cortical cells in the presence of each of the chromatic adapting lights.

Cell 1 has its lowest threshold in the red region of the spectrum in the presence of the blue adapting light; it has its lowest threshold in the blue region in the presence of the yellow adapting light; and it has two minima, one in the blue and another in the green, in the presence of the red adapting light. This cell is excited whenever a light of any wavelength goes on within its receptive field, and in this function three cone mechanisms appear to cooperate. Such a cell has not been classified as sensitive to color, although the possibility that it contributes to the sensation of white cannot be ruled out.

Cell 2 discharges when the light is turned off in the presence of the blue adapting light, and the action spectrum of the cone mechanism that produces this effect has its lowest threshold in the red region of the spectrum. It discharges when the light is turned on in the presence of the yellow adapting

light, and the mechanism responsible for this effect has its lowest threshold in the blue region of the spectrum. In the presence of the red adapting light, the cell also discharges when the light is turned on, but in this case its action spectrum has two minima, one in the blue and another in the green region of the spectrum. This cell is considered to be sensitive to color, being excited by blue- and green-sensitive cones when the light goes on and by red-sensitive cones when the light goes off; it must respond best when cyan enters just after red leaves its receptive field.

Cell 3 discharges when the light goes on in the presence of either the blue or the yellow adapting light, but the cone mechanism mediating the discharges in the presence of the former adapting light has its lowest threshold in the red, whereas the mechanism mediating the discharges in the presence of the latter adapting light has its lowest threshold in the blue region of the spectrum. In the presence of the red adapting light, discharges are obtained whenever blue light goes on or green light goes off. This cell is considered to be sensitive to color, being excited by blue- and red-sensitive cones when light goes on and by green-sensitive cones when light goes off; it must respond best when magenta enters just after green leaves its receptive field.

Figure 2 shows suprathreshold responses of the magenta-on, green-off cell (Fig. 1, cell 3) to stimuli from different regions of the spectrum in the presence of the red, blue, and yellow adapting lights. Figure 2 demonstrates that it can sometimes be difficult to detect whether a cortical cell is sensitive to color by simply comparing its suprathreshold responses to monochromatic stimuli. On the yellow background the cell responds to all wavelengths with no obvious color selectivity. On the blue background it appears to be most sensitive to a narrow band of wavelengths in the red region of the spectrum. On the red background its color selectivity becomes more apparent since it is strongly excited by blue light at "on" and by green light at "off," but even this gives an incomplete picture of its color sensitivity. A more complete way of identifying the cone mechanisms that interact on such cells is to examine their action spectra at threshold on at least three adapting fields with stimuli that permit separation of "on" from "off" responses.

Table 1 shows the distribution of cone mechanisms identified in this manner

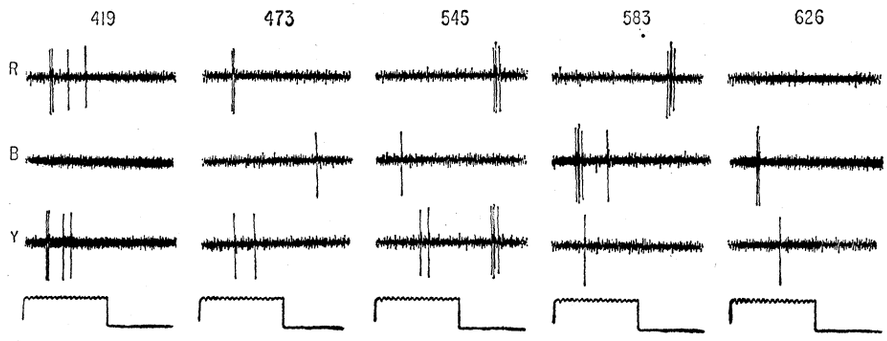

Fig. 2. Suprathreshold responses of a magenta-on, green-off cell in the monkey's striate cortex at five different wavelengths (numbers in nanometers) in the presence of a red (*R*), blue (*B*), and yellow (*Y*) adapting light. The lowest row is the response of a photocell showing the time course of the light stimulus (duration, 200 msec). The amplitude of the cell's responses is approximately 1 mv. Positivity is upward.

in 27 color-sensitive cortical cells. Of these 27 cells, 13 cells receive signals from three different cone mechanisms. Therefore, in striate cortex three cone mechanisms can interact on a single neuron, and this triplex interaction occurs in cells that are sensitive to color.

It is interesting to consider how trichromatic sensitive cortical cells can be built up from retinal and geniculate cells. An input from only two spectrally opponent on- or off-center geniculate cells could produce triplex opponent color responses in the cortex if both of the cone mechanisms commonly paired antagonistically on a geniculate cell could excite a cortical cell. Color-sensitive cortical cells could also receive signals from both on- and off-center cells. This possibility receives some support from occasional intracellular responses from such cells; these responses show that there are transient depolarizing potentials at the "on" and the "off" phase of the light stimulus and that this "off" response is not due to a post-inhibitory rebound.

The blue-sensitive cone mechanism seems to be transmitted from the rhesus monkey's retina by only one class of ganglion cells, which show opponent color responses and which may belong to the midget ganglion cell system described anatomically by Polyak (*5*). These cells are either on- or off-center, and they characteristically mediate only one cone mechanism in the center of their receptive field. Retinal ganglion cells that do not show opponent color responses are larger cells and are not so common near the fovea; although they handle signals both from rods and from red- and green-sensitive cones, they do not seem to receive an input from blue-sensitive cones (*5*). Action spectra of spectrally nonopponent cells in the monkey's lateral geniculate nucleus have also not revealed an obvious response from blue-sensitive cones (*6*, *7*), but experiments designed to specifically isolate the blue-sensitive cone mechanism have not been done. On the other hand, the blue-sensitive cone mechanism has frequently been found in spectrally opponent geniculate cells. The presence of a blue-sensitive cone input in spectrally nonopponent cortical cells raises the question of what lower-order neurons conduct these signals to the cortex. It is possible that some subcortical spectrally nonopponent cells do receive an input from blue-sensitive cones, but they have not yet been discovered. It is also possible that spectrally opponent geniculate cells affect cortical cells that do not exhibit spectrally opponent behavior. This could happen if the antagonistic surround mechanism of some spectrally opponent geniculate cells had little or no influence on some cortical cells.

Knowledge that color vision is trichromatic is based on color matching experiments obtained in human subjects in the steady state (*1*). Trichromatic color sensitive cortical cells show only transient responses at either the "on" or the "off" phase of a light stimulus, and they show no response in the steady state. These seemingly paradoxical results can be reconciled if one considers that human observers may only detect color at the boundaries of objects, where transient changes are being continuously produced by eye movements; it is at this point that a color-sensitive cortical cell can respond either when a particular color enters or a contrasting color leaves its receptive field.

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Mediation of Immunity to Tumor Isografts in Mice by Heterologous Ribonucleic Acid

Abstract. *The growth of tumor isografts in inbred mice is inhibited by intraperitoneal injections of syngeneic spleen incubated, in vitro, with ribonucleic acid extracted from guinea pigs immunized with the same mouse tumor. This inhibition is partially tumor-specific. Treatment with ribonuclease abolishes the response.*

Ribonucleic acid (RNA) extracted from lymphoid tissues of immunized animals can transform normal lymphoid cells to immunoreactive cells (*1*). Mannick and Egdahl (*2*) and Sabbadini and Sehon (*3*) transferred allograft immunity with RNA extracted from the regional lymph nodes or spleens (or both) of animals in which skin allografts were being actively rejected. Recipients of spleen cells that had been incubated with this RNA rejected specific skin grafts in an accelerated fashion. Similar results have been obtained in our laboratory (*4*). Rigby (*5*) has prolonged the survival of mice bearing Ehrlich ascites tumors by administering syngeneic spleen cells previously incubated with RNA from the spleens of mice immunized with this tumor. Cohen *et al.* (*6*) suggested that the conversion of normal lymphoid cells to immunoreactive cells by RNA from lymphoid organs of immunized animals ("immune" RNA) was at least partially strain-specific within a single species. We have observed a decreased incidence in the growth of tumor isografts in inbred mice after administration of syngeneic spleen cells incubated with heterologous RNA preparations—that is, RNA extracted from the lymphoid tissues of guinea pigs immunized with the mouse tumor to be treated.

A benzpyrene-induced fibrosarcoma, designated BP-4, carried in C3H/FB (mammary tumor agent free) mice was used to immunize Hartley guinea pigs. Each pig received 0.5 ml of a concentrated tumor cell suspension in complete Freund's adjuvant in each foot pad. An intraperitoneal injection, without adjuvant, was also given. After 10 to 14 days, the spleens and the axillary, popliteal, and inguinal lymph nodes (sites of antigen processing) were excised and immediately frozen in Dry Ice. RNA was extracted (*4*), washed, dissolved in Earle's balanced salt solution (BSS) to a concentration of 400 to 1000 μg/ml, and made 0.7*M* with respect to sucrose. Such preparations contained, per milliliter, 75 to 150 μg of DNA and 65 to 100 μg of protein (*7*).

Cell suspensions were prepared from the spleens of normal C3H/HeN mice by passage through No. 40 stainless steel mesh in medium 199 and filtration through No. 80 stainless steel mesh. The cells (10^7 to 10^8 per milliliter) were then incubated in the RNA solutions at 37°C for 20 minutes in a shaking water bath. They were washed in BSS and counted, and the concentration was adjusted to 1 to 2×10^8 viable cells (by trypan blue exclusion) per milliliter. Normal spleen cells were also incubated with RNA extracted from guinea pigs immunized with a mixture of normal C3H lung, liver, kidney, and spleen cells. As a control, RNA was prepared from the lymphoid tissues of pigs immunized with Freund's adjuvant only, and RNA extracted from pigs immunized with BP-4 was treated with ribonuclease (20 μg/ml) (*8*) for 15 minutes at 37°C prior to incubation with spleen cells.

Normal C3H mice were divided into groups of approximately 30. Each mouse was given, on two successive days, intraperitoneal injections of 5 to 10×10^7 spleen cells that had been

Table 1. Development of BP-4 tumor isografts in C3H mice after subcutaneous injection of 10^4 tumor cells. Experimental mice also received intraperitoneal injections of syngeneic spleen cells incubated with indicated RNA preparations. Results are combined from three separate experiments.

Groups	No. developing tumors/ total No. in group
Recipients of spleen cells incubated with RNA from guinea pigs immunized with:	
BP-4 tumor cells	19/58 (32.7%) $P < .01$*
Normal C3H lung, liver, kidney, and spleen	26/49 (53%) $.2 < P < .3$*
BP-4 tumor cells (ribonuclease)†	35/54 (65%)
Freund's adjuvant only	36/46 (78%)
Controls	
Challenge controls (no spleen cells)	89/150 (59.3%)
Spleen cells not incubated with RNA	16/27 (59%)

* P value by χ^2 with Yates's correction for this group when compared to control groups. † These RNA preparations were treated with ribonuclease prior to incubation with spleen cells.