compound, proceeds through the *cyanide-sensitive* terminal respiratory pathway. The other, in which menadione or other electron acceptors can function, does not involve the *cyanide-sensitive* pathway, but rather a diaphorase type flavoprotein-catalyzed *cyanide-insensitive* reaction.

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23 May 1958

Changes in the Perceived Color of Very Bright Stimuli

Abstract. When very intense stimuli in the long-wavelength region of the visual spectrum are viewed continuously, they change in hue from red, through yellow, to green. The relation of the time course of these changes to the intensity of the stimulus is reported.

In general, the perceived color of a visual stimulus is a function of its wavelength composition. It is well established, however, that the color of a stimulus of fixed wavelength varies somewhat as the intensity of the stimulus is changed. An extensive study of this phenomenon, the Bezold-Brüke effect, was reported by Purdy (*1*). He found, for example, that both red and yellow-green stimuli appear yellower at 1000 trolands than at 100 trolands. However, yellow stimuli (at about 575 mμ) were found to be "invariant"—that is, no color changes were observed as intensity was varied.

Our observations have shown that when a very intense stimulus in the long-wavelength end of the spectrum is fixated continuously, it appears red at first but rapidly changes to yellow and then to a deep, rich green. Intense yellow stimuli, at the wavelength found by Purdy to be "invariant," also turn green. Green stimuli may desaturate but do not turn red. Auerbach and Wald (*2*), while studying the effects of very bright stimuli on subsequent dark adaptation, also noted that intense reds turn green.

We have investigated the time course of these color changes at different wavelengths and light intensities. The observer was presented with a Maxwellian view of a bright, uniformly illuminated, circular field, 15 deg in diameter, with a set of cross hairs for fixation. The source was a 17-ca automobile headlight bulb, run at a color temperature of 2800°K from a direct-current power supply. The effective f ratio of the system was 3.0. Heat-absorbing glass protected the eye from excessive infrared radiation. The intensity was varied either by neutral density filters or by a variable on-off ratio flicker vane, run well above fusion frequency. The wavelength composition of the field was controlled by introducing color filters into the collimated part of the optical path.

The subject held three keys. He was instructed to press the first when the stimulus was first turned on, the second when it first turned yellow, and the third when it first turned green (*3*). The keys operated timers, so that the time of each color change was recorded. Each trial consisted of a single presentation of the stimulus at a particular intensity. At least 30 minutes elapsed between trials. For any given color filter, the order of intensity presentations was varied randomly.

In the first experiment a Wrattan No. 29 filter was used. This filter passes all wavelengths longer than about 615 mμ, and none shorter. Four subjects were tested under these conditions, and all showed similar results. The data for one subject are plotted in the upper portion of Fig. 1, which may be interpreted in the following way: At a given intensity —for example, 1×10^5 trolands—the stimulus was judged red for the first 7 seconds, then yellow for 9 seconds, and then green. No further color changes were observed, even though fixation was maintained for as long as 3 minutes. At lower stimulus intensities the color changes occurred later. For this particular filter, the stimulus remained red indefinitely at intensities below about 0.1×10^5 trolands. Each point on these curves is a mean of six judgments for one subject. The between-judgments variability for each of the subjects was surprisingly small.

These color changes are not restricted to broad-band stimulation. The red region of the spectrum, as far out as 640 mμ, was explored with a monochromator of 5-mμ spectral slit width, and similar color changes were found.

The lower portion of Fig. 1 shows a curve for a narrow band-pass interference filter at 5.75 mμ. Since this filter appears yellow initially, only the yellow-to-green curve can be plotted. This wave length is one which Purdy reported to be "invariant"—that is, its hue did not change with intensity. However, the highest intensity employed by Purdy was only 2000 trolands, or 0.02×10^5. At the higher intensities we used, this stimulus turns to a deep, rich green.

Our observations in the green and blue regions of the spectrum failed to reveal changes other than desaturation. Auer-

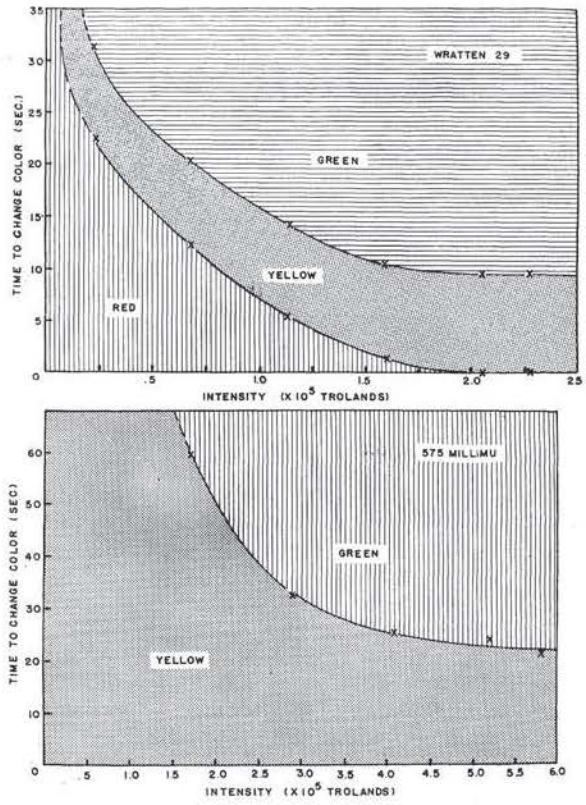

Fig. 1. Time course of changes in hue as a function of the stimulus intensity. The upper plot is for Wratten filter No. 29, the lower, for a narrow band-pass interference filter with a peak at 575 mμ.

bach and Wald (2), however, have noted a blue-to-red change, using a Jena BG12 filter. It may be that the particular wavelength composition of the stimulus is more critical in the blue end of the spectrum. Our failure to confirm their observation may thus be a result of our not having that particular filter available.

The described color changes may be accounted for by photochemical adaptation, if it is assumed that there are at least two photopigments in the human retina, a "red" and a "green," with overlapping absorption spectra, and that the rate of regeneration of the "green" pigment is slightly greater than that of the "red." Equations basically derived from this hypothesis yield good qualitative agreement with our data. The verification of the hypothesis, however, must await the results of more extensive experiments.

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3 July 1958

Low-Temperature Chromatography as a Means for Separating Terpene Hydrocarbons

Recently, a study of the citrus oils was undertaken in this laboratory for the purpose of determining flavor constituents. A gas-chromatographic apparatus was developed for the analysis of terpenes, but it was concluded that preliminary separations were necessary. Kirchner *et al.* (*1*) utilized various forms of silicic-acid chromatography for the separation of terpene hydrocarbons from the oxygenated constituents of citrus oils and for subsequent analysis of the oxygenated fraction. These procedures have been modified and utilized by other workers (*2*). In general, however, silicic-acid chromatography has been unsatisfactory for resolution of terpene hydrocarbons, which may constitute more than 90 percent of a citrus-peel oil.

It was concluded that the gas column would provide an excellent means for monitoring the effluents in a study of liquid-solid chromatographic procedures for separation of the terpenes. Since the results of the above-mentioned workers indicated slight differences in the migratory rates of certain terpene hydrocarbons on silicic acid, columns of various dimensions were prepared for a preliminary study. Synthetic terpene mixtures were then eluted with various organic solvents, and the effluents were analyzed by gas chromatography (as described below) to determine any tendencies toward resolution.

Table 1. Distribution of some terpene hydrocarbons in the effluent from a silicic-acid column.

Compound	Fraction
p-Menthane	3–4
α-Pinene	10–15
β-Pinene	16–21
d-Limonene	34–35

It was observed that results varied with pretreatment of the silicic acid. Silicic acid which was neither washed nor dried did not retain the hydrocarbons to any appreciable extent, and silicic acid which was dried at 105°C and stored over calcium chloride showed only a slight retentive capacity. Increased retention was observed when the silicic acid was washed free of fines (to facilitate packing), dried several hours at 150°C, and used immediately upon removal from the oven. Even after this process, however, the hydrocarbons moved rapidly and displayed only minor differences in migration rates.

On the assumption that migration would be retarded at lower temperatures, experiments were performed in which the columns were operated at the temperature of Dry Ice (−78.5°C). Results indicate that not only are the terpene hydrocarbons retarded at this temperature but that differential retarding occurs. As a result, complete resolution has been obtained in many instances. The example which follows suggests that the technique should be of considerable value in the study of terpenes, and perhaps in the study of hydrocarbons in general.

A special column was constructed for immersion in a vacuum flask, with details as shown in Fig. 1. Silicic acid (Mallinkrodt, 100-mesh) was washed free of fines by the method of Bulen *et al.* (*3*) and dried at 150°C for 48 hours or more. Approximately 5 g of the acid was cooled over calcium chloride (for from 2 to 3 minutes) and added to 50 ml of petroleum ether. A glass-wool plug was tamped into the lower end of the column, the column was half-filled with petroleum ether, and the silicic-acid slurry was added, to give a column 20 cm long. Pressure from a squeeze bulb was applied to the top of the column until no further packing occurred (a sufficient amount of solvent being added to maintain the level above the silicic acid). The column was then suspended in a bath of Dry Ice in acetone, with the level of the coolant well above that of the packing. After 1 to 2 hours, the terpene mixture (consisting of 30 mg each of *p*-menthane, α-pinene, β-pinene, and limonene in 0.5 ml of petroleum ether) was added to the top of the column, and pressure was applied until the solvent dropped to the level of the silicic acid. A glass-wool plug was inserted just above the packing, the solvent assembly was attached, and elution was started with petroleum ether. A flow rate of approximately 3 ml per hour was maintained, and fractions (2.5 ml) were collected manually. After collection of 30 fractions, the petroleum ether was withdrawn from the top of the column with a hypodermic syringe, and a 1:1 mixture of diethyl ether and petroleum ether was added (for elution of the limonene).

Gas chromatography of the fractions was performed with a laboratory-built instrument, in the construction of which a 10-ft column of Apiezon-L or C-22 firebrick, packed in ¼-in. stainless-steel tubing, was utilized. Helium was used as the carrier gas, and samples were injected by means of a Fisher injection assembly. A four-filament conductivity cell (Gow-Mac No. 9285) served as a detector, and the response was recorded on a 10-mv recorder (Varian G-11).

The procedure was as follows: The solvent was evaporated from each fraction at reduced pressure, and the residue

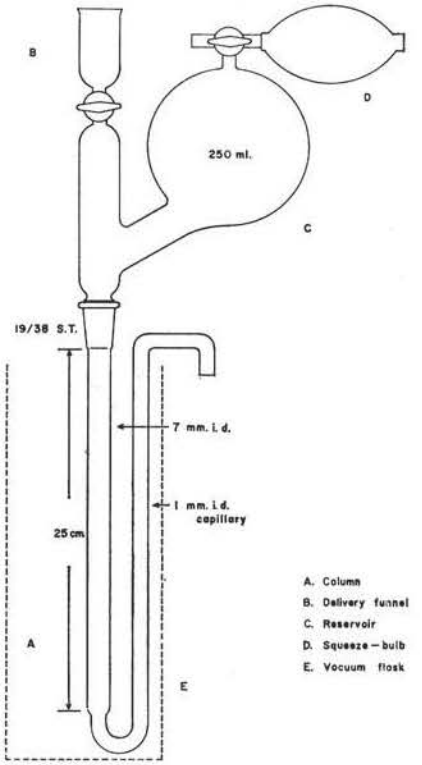

Fig. 1. Assembly for low-temperature chromatography.