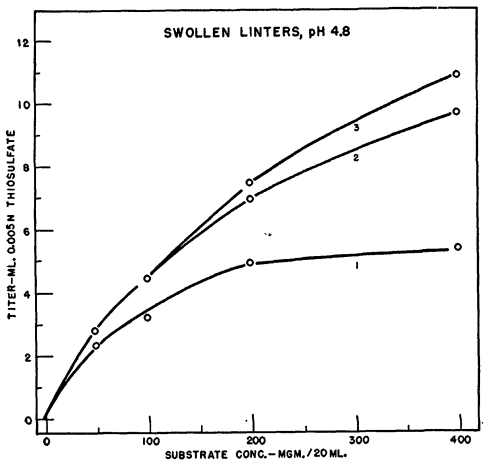

FIG. 2. Effect of substrate concentration on cellulase stimulation by albumin. Enzyme: 66 μg Fraction 80 protein. Albumin: curve 1, 0; curve 2, 56 μg; curve 3, 220 and 440 μg. Buffer: 0.05 *M* acetate. Titer: per 3 ml assay medium.

tion. These effects may explain the present confusion in the literature (*4*) on such properties of crude cellulase preparations as pH optima for different substrates.

Evidence that added protein was partially adsorbed on the substrate was obtained as follows. Before enzyme addition, albumin and substrate (400 mg) were incubated in 19 ml of assay medium for 1 hr and then centrifuged until the volume of the sediment was less than 10% of the total volume. The supernatant solution was replaced by fresh buffer, and the enzyme titer in the resulting medium compared with that in uncentrifuged controls. The titers for precipitated cellulose at pH 5.6 (Table 2) corresponded to about 50% retention of protein by the centrifuged substrate; for swollen linters they were less, corresponding to about 25% retention.

The present data are considered to suggest that cellulase stimulation by protein is due to protein adsorbed on the substrate. A mechanism dependent on adsorption would, as required, be confined to insoluble substrates and be sensitive to low concentrations of added protein and to differences in substrate properties. It also permits a simple interpretation of the effect of increasing the substrate concentration, for this would increase the extent of adsorption. However, no conclusive evidence has yet been obtained to indicate the manner in which adsorbed protein exerts its effect.

TABLE 2

EFFECT ON PROTEIN STIMULATION OF REMOVAL OF ALBUMIN UNADSORBED ON PRECIPITATED CELLULOSE*

Albumin added (μg/20 ml)	Titer: ml 0.005 *N* thiosulfate/ml assay medium	
	Control series (uncentrifuged)	Centrifuged series
450	9.07	8.65
220	8.75	8.17
110	8.03	7.45
55	7.61	6.18
28	6.28	5.86
0	4.63	—

* Enzyme: 68 μg Fraction 80 protein.

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Selective Stimulation of Color Receptors with Alternating Currents

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A sensation of flickering phosphenes is aroused by an alternating current passing through the head. With this sensation as an index, much work has been done to determine threshold strengths of currents as a function of frequencies (*1–5*). Two examples of strength-frequency curves obtained in our laboratory are illustrated in Fig. 1. In these experiments the stimulating

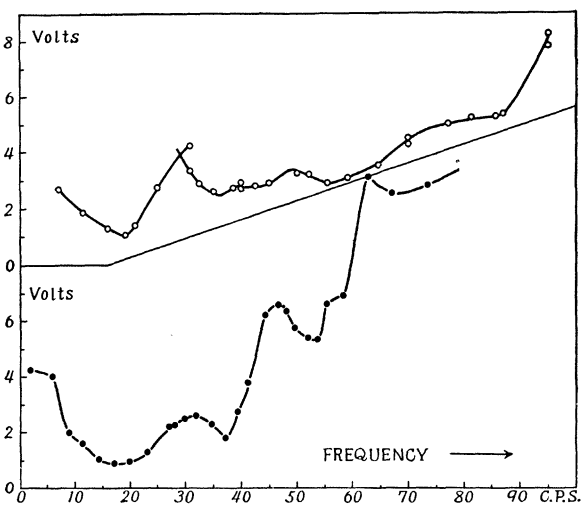

FIG. 1. Strength-frequency curves of moderately light-adapted human eye. Sinusoidal alternating currents and rectangular pulses of varying frequencies were used to obtain upper and lower curves, respectively.

voltage was raised at an approximately constant rate of about 80 mv/sec until the subject perceived the appearance of flicker, and then lowered from a high level sufficient to evoke a sensation of distinct flicker until the subject noticed the disappearance of flicker. The average of both values was taken as the threshold strength.

In another series of experiments a constant voltage

was applied for a period of 0.5 sec instead of increasing or decreasing the voltage during stimulation; starting at sufficiently high level, the voltage was lowered step by step until the subject could no longer distinguish the stimulus in question from one far below the threshold. This second method loses in time, but gains in accuracy, because it is much easier for the subject to distinguish the slightest flicker from the background of intrinsic light of the eye, by virtue of the comparison possible. A strength-frequency curve determined by this method is shown in Fig. 2, in which two sets of data obtained from the same subject on different days are represented by different marks (solid and empty circles).

The minimum at about 20 cps, which is most conspicuous in the curves in Fig. 2, has been observed by all previous investigators, but the other minima, except the one at about 35 cps, have never been noticed, probably because points determined were not numerous enough or the accuracy of measurement was not sufficient to find such minima. Meyer-Schwickerath (*6*) obtained a curve having a single minimum situated at around 20 cps, by using flickering sensations located in the periphery of the retina. He maintained that, based on this fact, the minimum at about 20 cps is concerned with the rod mechanism. This view was supported by Abe (*5*) from the effect of dark adaptation upon this minimum. Taking flickers appearing in the central part of the retina as the index, Meyer-Schwickerath obtained another curve having a single minimum at about 33 cps, which may be identified with the minimum located at about 35 cps in our curves.

The question as to whether these minima of strength-frequency curves are due to different excitabilities of retinal receptors is important and interesting. It seems difficult, however, to answer this question from data of strength-frequency curves alone without resort to any other evidence. In the present investigation, therefore, we approached this question from quite a different angle, making use of the method reported by Motokawa in his previous paper (*7*). The method is based on the fact that, following a brief illumination, the excitability of the dark-adapted eye as tested by a single constant current pulse of 0.1 sec duration recovers along a time course characteristic of the wavelength of the light used for preillumination. Examples of excitability curves obtained by this method are shown in Fig. 3. Each point of these curves was determined by the second method mentioned above; the accuracy of measurement was such that the standard deviation of 10 measurements was 1.3% of the mean value on a skilled subject and 10–15% on untrained subjects.

When white light is used, the excitability reaches a maximum in about 2 sec (broken curves in *C* and *D*, Fig. 3). The maximum of the excitability curve for yellow light lies at about 1.5 sec (*A*), and that for blue light at about 3 sec (*B*). When the eye is exposed to blue light (470 mμ) and then to white light, and the electrical excitability is measured as usual

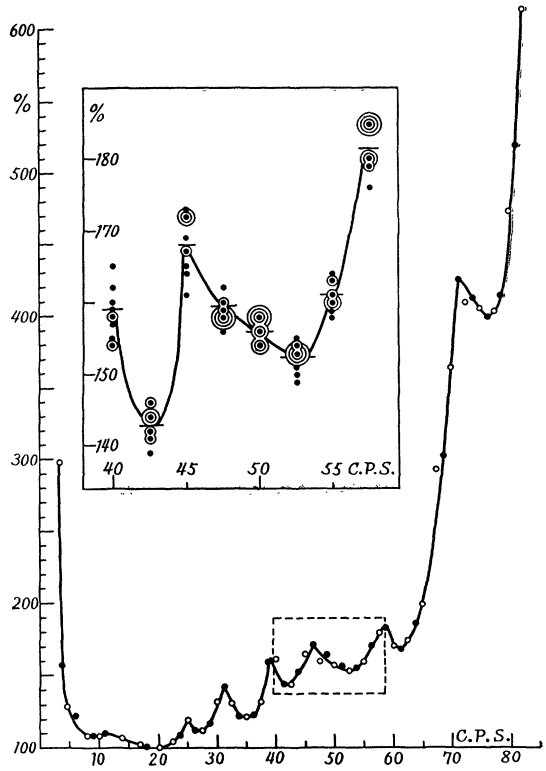

FIG. 2. A strength-frequency curve obtained from a skilled subject. Threshold voltages are expressed in percentage of the threshold at 20 cps. Only a small area of the fovea and parafovea was light-adapted. Intensity of adapting light was five times threshold intensity at fovea. In order to illustrate accuracy of measurement, a part of the curve enclosed by a broken rectangle is represented on a larger scale in the inset, in which 10 values for each frequency of A.C. are plotted, the number of concentric circles indicating how many times the same value was reproduced.

after removal of the white light, then a curve is obtained which resembles neither that for blue nor that for white light, but that for yellow light. The successive stimuli, blue and white, are thus equivalent to yellow light alone. In a similar manner, it can be shown that successive stimuli, yellow and white, are equivalent to blue light (*cf.* *D* and *B*). The curves for successive stimuli, colored and white, are generally much higher than the curve for white light alone, but they cannot be regarded as representing simple summation of the effects of both stimuli, because from this point of view it would be impossible to account for the location of the maximum which corresponds to that of the curve for the complementary color. As a matter of fact, this phenomenon represents the physiological counterpart of the well-known psychological phenomenon, successive color contrast, and is termed "retinal color induction" (*8*).

It was found that an alternating current of 36 cps slightly above threshold could be used as a substitute for the blue light in the experiment on retinal induction stated above; when the white light was preceded by an A.C. of 36 cps lasting for 0.5 sec, we obtained an excitability curve having a maximum at about 1.5 sec,

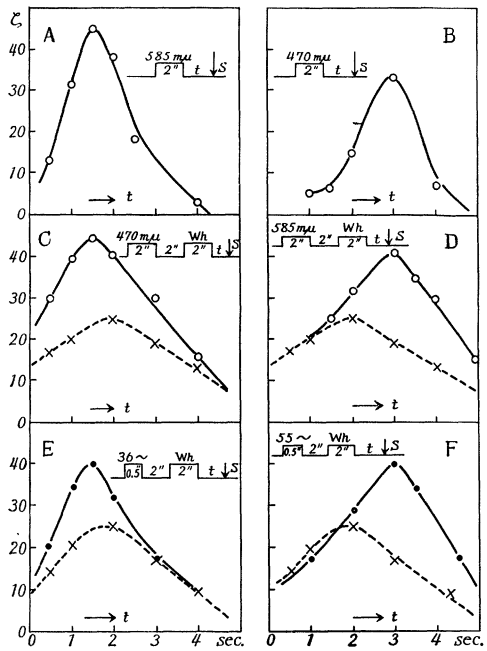

FIG. 3. Retinal induction caused by colored lights and by alternating currents. Ordinates: percentage increases of electrical excitability of the eye over resting level after 2 sec illumination. Abscissas: time in seconds from the end of preillumination. Procedure for experimentation is shown in insets in which *Wh*, *S*, and *t* denote white light, an electric stimulus (that is, a single pulse of 0.1 sec), and the interval between *Wh* and *S*, respectively. *A*, response to yellow light; *B*, response to blue light; *C*, solid curve, response to successive stimulation of yellow and white light; *D*, solid curve, response to successive stimulation of blue and white light; *E*, solid curve, response to successive stimulation of 36 cps A.C. and white light; *F*, solid curve, response to successive stimulation of 55 cps A.C. and white light; *C–F*, broken curves, response to white light.

just like the curve for yellow light (*E*). Similarly, an A.C. of 55 cps could be used as a substitute for the yellow light used in *D* (see *F*). It can be said from these experiments that an A.C. of 36 cps is equivalent to blue light, and an A.C. of 55 cps to yellow light, so far as retinal induction is concerned. From a series of such experiments we could determine functional correspondence between the frequencies of alternating currents and the wavelengths of colored lights. Thus four frequency ranges corresponding to four main colors—red, yellow, green, and blue—were estimated as follows: blue, 33–37 cps; green, 40–45 cps; yellow, 47–55 cps; red, 60–100 cps.

Let us once more survey the strength-frequency curves illustrated in Figs. 1 and 2, taking the result obtained in this experiment into account. In the curve in Fig. 2, there can be distinguished at least six minima other than the one located at about 20 cps, which ought to have no concern with color receptors, if the interpretation stated above is correct. The three minima located at about 35, 42.5, and 52.5 cps must be concerned with blue, green, and yellow receptors, respectively, because these optimal frequencies fall into the three frequency ranges determined above. For the same reason frequencies higher than 60 cps may be correlated to longer wavelengths of spectral lights. In the lower curve in Fig. 1, however, the minimum to be correlated to the green receptors is missing, and in the upper one this minimum is not so distinct as in the curve in Fig. 2. In general the minimum at about 42.5 cps tends to be obliterated. A tentative explanation for this situation may be as follows: Since it is generally much easier to perceive electrically produced flickers in the periphery and in parafoveal regions of the retina than at the fovea, the retinal elements outside the fovea are more likely to be involved in determination of thresholds than those at the fovea. On the other hand, the green receptors are known to be less active outside the fovea than the other kinds of receptors, so that the minimum for the green receptors is generally not so distinct as the minima for the other receptors. The prominence of the minima for the blue and yellow receptors may be interpreted from the same point of view (peripheral dichromatism).

As can be seen clearly in the inset of Fig. 2, the minima of our strength-frequency curves are not artifacts resulting from inaccurate measurements, as might be supposed, but physiological manifestations of the composite nature of the retina, which obviously contains several kinds of receptors characterized by their own time constants. For this reason, a strength-frequency curve may be regarded as a kind of resonance curve, and it is the principle of resonance that provides a means for selective stimulation of color receptors. The relation between the frequencies of alternating currents and the wavelengths of colored lights outlined above can be studied more precisely on the basis of the fact that each minimum deepens specifically under the action of characteristic colored light. The experimental procedure and conditions for this effect are, however, too complicated to be described in the present communication, so that only the result obtained by this method will be summarized: (1) the above-mentioned relation has been fully confirmed, (2) the minima at 77.5, 62.5, and 27.5 cps seen in Fig. 2 have been shown to correspond to red, orange, and violet, respectively.

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