Direct Action of Light in Naturally Pigmented Muscle Fibers

I. *Action spectrum for contraction in eel iris sphincter*

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ABSTRACT   Contraction due to light in excised eel irises appears to follow a simple first order law. The action spectrum for contraction has a maximum which agrees with the eel rhodopsin absorption maximum. Inasmuch as rhodopsin is the rod pigment–opsin complex and the iris sphincter pupillae evolves from the pigment epithelium of the retina in the region of the iris, the muscle pigment might be the same as the visual pigment. In the human eye the contraction of the iris sphincter is activated only by light incident on the retina and the pupil diameter varies inversely with the square root of the light intensity. The inverse first power relation observed in the present experiments suggests a more primitive origin for the light reaction in eel irises. Relaxation is a much slower process and can be approximated as the sum of two first order processes.

HISTORICAL INTRODUCTION

In the literature the term *photodynamische Erscheinungen* was originated by Rabb and von Tappeiner around 1900 to describe the killing by sunlight of acridine-sensitized Paramecia and usually refers to deleterious effects produced by light. In its more general sense photodynamic action should embrace all processes in which a light quantum is absorbed by a pigment molecule which, in its excited state, can then initiate oxidation reduction or electron transfer reactions. The distinction between photodynamic action and photochemistry is that the former, while including the latter as the primary event, also includes the subsequent biochemistry. Thus the Hill reaction in photosynthesis, the stimulation of the optic nerve in vision, phototropism, photoperiodism,

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and diurnal variations in bioluminescent organisms should, in the author's opinion, all be classified under the general term photodynamic action.

It is the purpose of this paper to describe some experimental results of the photodynamic action of light in stimulating contraction in excised iris sphincter pupillae of the eel. So far as can be ascertained, this effect, reported by Brown-Séquard in 1847 (1) and by Steinach in 1892 (2) and ascribed by the latter to Friederich Arnold in 1841, is the only case known of a naturally occurring direct muscle stimulation by light. Weale in 1956 (3) reported a partial action spectrum based on threshold sensitivity for *Rana temporaria* using a tungsten lamp and Ilford spectrum filters. In view of the fact that so little work has been done on this effect it seems proper to summarize a portion of the original papers by Steinach.

Steinach reported that he observed this effect in the amphibia *Rana esculenta* and *temporaria*; *Salamandra*; *Bufo*, *Hyla*, and in the fish *Anguilla*, *Esox*, *Perca*, and *Salmo*. However, the eel showed the most intense pupillary reaction in examination of the detached iris. He states that the contraction in detached irises is indistinguishable from that in the irises of the living animal and that the reaction is stimulated in both cases by light incident on the pupillary portion of the iris. He also established that even after complete atropine mydriasis the dilated iris exhibited the same maximal contraction as the non-drugged iris. On the basis of histological examination Steinach concluded that the sphincter pupillae consists of two or three layers of concentrically disposed, spindle-like, pigment-containing smooth muscle fibers. The intervening tissue and stroma of the connecting tissue are stated to be free of pigment. By fixing these pigmented muscle cells in the dark as well as in the light he was able to show direct anatomical proof of the contraction. Localized stimulation by light produced localized contraction with little spreading. Using enucleated eyeballs from *temporaria* and the dispersed spectrum of sunlight with spectral regions identified by the Fraunhofer absorption lines he established the qualitative effects of color as shown in Table I.

Table I is remarkable for two reasons. First it shows the major aspects of the more quantitative action spectrum presented in this paper. Second, it is of historical interest. These experiments were performed years before the first concepts of the discrete absorption of light quanta were developed; years before Paschen in Germany and Pfund and Coblentz in the United States had developed sensitive thermocouples for the measurement of radiant energy. Although Steinach recognized that contraction at any particular wavelength was intensity-dependent he could not have normalized his observations with respect to intensity. His choice of the dispersed solar spectrum as a light source was therefore a fortuitous one. The sun with a surface temperature of 6000K has a maximum energy emission in the 4800 A region, dropping to 80 per cent of this value at 4000 A and 6400 A respectively (4).

With this particular energy distribution relatively little error is introduced in Steinach's original data in terms of a true action spectrum. A different source spectral energy distribution would also explain the divergence in other workers' results referred to by Steinach as to the most effective colors. Other investigators, using lower temperature laboratory flames as light sources, would have had spectral distributions with a predominance of reddish photons relative to blue photons. Thus very careful experimental observations showing yellow as the most effective wavelength region could only await the development of quantum physics and a proper normalization.

TABLE I

Steinach identification	Actual wavelength region	Observed reaction
Red part up to C	$\lambda > 6563$ A	No effect on contraction
$C + \frac{1}{2} CD$	$\lambda \simeq 6200$ A	Barely noticeable constriction only in highly sensitive irises
D	5890 A $\rangle$ Na 5896 A $\int$ doublet	Constriction starts in moderately sensitive irises
$D + \frac{3}{4} DE$	$\lambda \simeq 5400$ A	Rapid constriction begins
Region before F	5400 A $> \lambda > 4861$ A	Maximum constriction
Up to $F + \frac{1}{2} FG$	Up to $\lambda \simeq 4600$ A	Maximum constriction
Blue-violet	$\lambda \gtrsim 4300$ A	Constriction in sensitive irises, strongly intensity-dependent
Violet $(G + \frac{1}{2} GH)$	$\lambda \simeq 4200$ A	No effect

EXPERIMENTAL TECHNIQUES

All the measurements to be reported were performed on excised eel irises, pinned under slight radial tension on beeswax in Ringer's solution. The pins passed through the ciliary portion with the tension adjusted so as to leave the iris sphincter in its normal circular resting state. The circular opening was observed in transmitted red light ($\lambda > 6400$ A) under magnification of 30 times in a dark room. Activating light consisted of a parallel beam incident from above at 45°. The source was a parallel focussed concentrated zirconium arc, adapted with a camera shutter and a filter holder. Narrow band interference filters were used for isolating various spectral ranges. The interference filters, with peak transmissions between 30 and 40 per cent, had half-widths of approximately 10 millimicrons. The combinations of parallel beam and interference filters were calibrated by means of a thermopile and reduced to relative numbers of photons per square centimeter per second. At $t = 0$ the light shutter was opened and the observed changes in diameter of the iris with time were recorded manually on a moving chart. As soon as contraction had ceased the shutter was closed and the observer, who had kept one eye closed during the bright irradiation, now used this dark-adapted eye to observe the relaxation in transmitted red light as a function of time. At the intensities of red light ($\lambda > 6400$ A) required for microscopic observation of the iris the light had no effect either on contraction or on relaxation of the iris. In addition most irises on removal from the eyeball retain a

dense black rear layer of pigment epithelium which serves to protect the iris sphincter pigment from illumination except by light from above. Retention of this layer does not interfere with constriction and in fact makes the iris easier to handle and to pin properly. The iris ciliary portion tends to tear easily when the black pigment epithelium is removed. Irises could be kept for periods of from 24 to 36 hours in the dark at 4°C although this varied greatly from sample to sample. Irises from freshly excised eyeballs showed the best light-dark responses. Response curves as functions of temperature were determined by first storing irises for several hours in the dark at 4°C and then measuring successive responses as the temperature approached room temperature.

RESULTS AND DISCUSSION

The light contraction and dark relaxation effects in eel iris sphincter pupillae can be divided as follows:—

(a) Contraction in Light

Fig. 1 shows the results of irradiating several excised eel irises with different intensities of white light. There are characteristic portions to the contraction curve: an initial lag period and an approximately exponential decrease with a tailing-off to a final diameter dependent upon intensity. One can define the approximate lag period as the time required for the diameter to decrease by one arbitrary division in the eye-piece scale (relative contraction from 1.00 to 0.98) and the approximate contraction rate constant as the first order rate constant of the initial portion of the exponential contraction curve. The total contraction is just $D_o - D_m$ where D_o is the fully dark-relaxed diameter and D_m is the final light-contracted diameter. Fig. 2 shows a plot of the contractions of one particular iris upon irradiation by light of different spectral ranges isolated by interference filters. The regions are identified by the peak transmission wavelengths.

As a better approximation the values logarithm

$$\{1 - [(D_o - D)/(D_o - D_m)]\}$$

can be plotted as functions of t. The entire curve, with the exception of the initial lag portion, fits the equation:

$$C = C_0(\lambda)[1 - e^{-k(\lambda)t}] \tag{1}$$

Here C is the contraction at any time t and $C_o(\lambda)$ is the maximum contraction $(D_o - D_m)$ at any wavelength. From this it can be seen that the approximate contraction rates described in the preceding paragraph are related to the $k(\lambda)$'s of equation (1) by the assumption that $D_m \rightarrow 0$. Fig. 3 shows the reciprocal of the lag times, the contraction rates, and the total contractions

$D_o - D_m$, normalized by the relative number of photons in each spectral range, plotted as functions of wavelength. The horizontal lines indicate the approximate extent of the spectral regions. In any case these action spectra
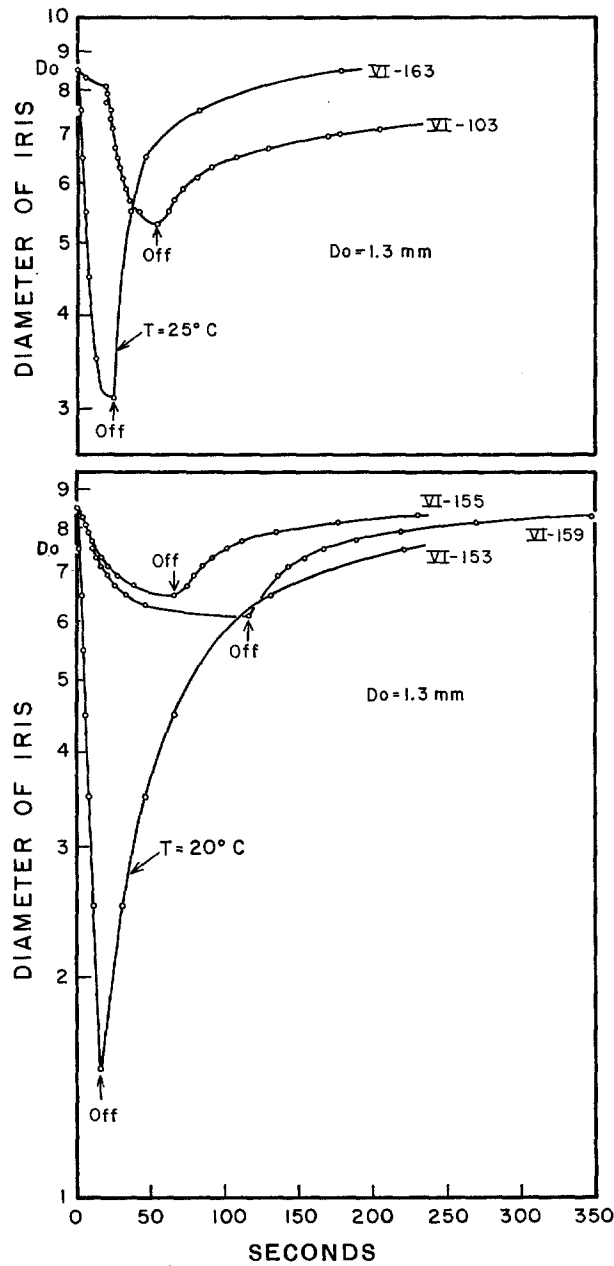

FIGURE 1.   Contraction of excised eel irises for differing incident light intensities showing lag periods and approximately exponential absorption. At the arrow labeled "off" the light was cut off from the iris. The ordinate scale is logarithmic.
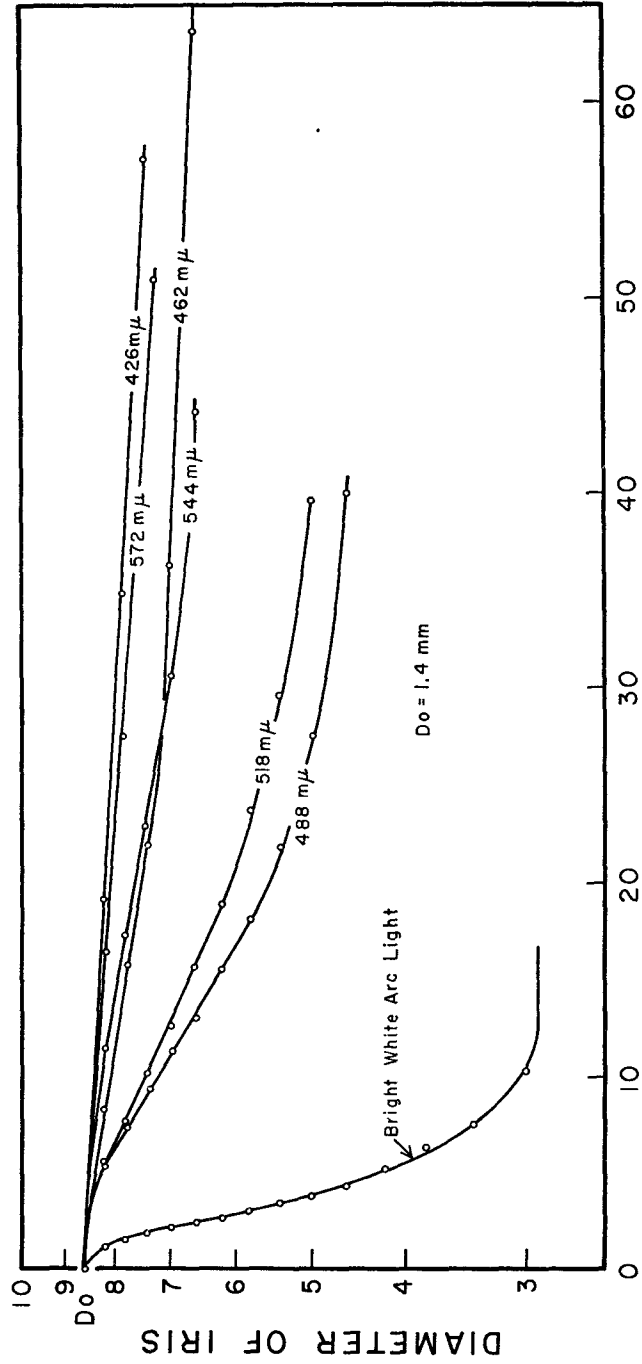
FIGURE 2. Contraction of excised eel iris for various spectral ranges isolated by interference filters. The data are not normalized to incident photon intensity. The ordinate scale is logarithmic.

all show maxima around 500 millimicrons. Rhodopsin, the major eel visual pigment composed of retinene$_1$ + rod opsin has an absorption maximum at 498 mμ (5). Even on the basis of this meager evidence it is interesting to speculate that the effective iris pigment is rhodopsin and that the mechanism of muscle stimulation in the sphincter pupillae is similar to the mechanism of optic nerve stimulation in vision. In view of their common embryonic origin this may not be too unlikely.
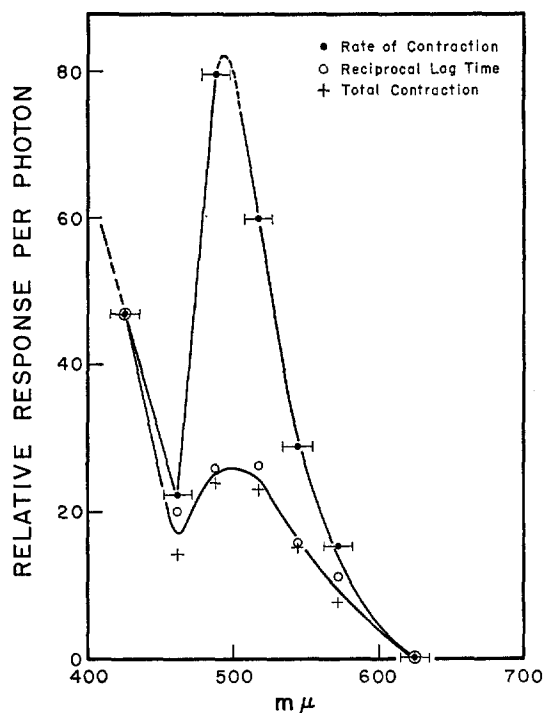

FIGURE 3. Action spectra for various parameters of the light reaction in eel iris. The horizontal extensions of the points represent the range of the spectral regions isolated by the interference filters.

In addition to the maximum at around 500 millimicrons there appears to be a second region of sensitivity below the 400 millimicron region. Unfortunately we were not able at this time to investigate this region more completely. The validity of this second region of sensitivity could imply an ultraviolet-sensitive region similar to that reported by Goldsmith for honeybees (6). However, the positive identification of the pigment or pigments involved is required.

(b) *Contraction in Dark*

The Weber-Fechner relation in biological systems states that the response is a logarithmic function of the stimulus. Therefore in principle an action

spectrum is valid only if it represents the number of quanta per second inci-
dent at any particular wavelength which are required to obtain a fixed
response. In the case of the eel irises it was found that if the iris were sub-
jected to a short flash of light (one-one hundredth to one-tenth second), the
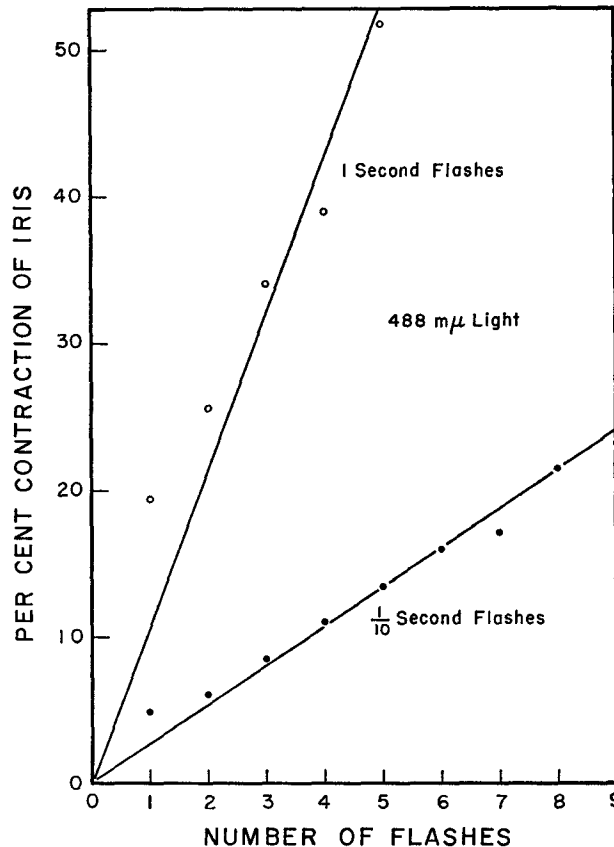iris, while not contracting during the flash, would contract in the dark and

FIGURE 4. Total dark contraction *versus* total light absorbed when light flashes occur
prior to contraction. The flash repetition rate for the 0.1 second flashes was 3 per second
and for the 1 second flashes was essentially 1 per second.

then relax to its original dark diameter. In addition the amount of dark
contraction subsequent to a light flash was proportional to the total light
striking the iris during the flash. This effect is shown in Fig. 4 where the
total dark contraction is plotted as a function of the number of flashes of
light of constant intensity striking the iris. In all cases the light-flashing was
over within the initial lag time. The fact that the responses to the 1 second
flashes are not directly proportional to the responses to the one-tenth second
flashes is due to the use of a different iris. This integration effect and the

observed linearity of response with light flash intensity make it possible to use the total contraction as a measure of the response for different wavelengths, even though the total contractions are different.

Dr. Philip B. Armstrong (7) has pointed out that there still exists the possibility of an intrairidial reflex although thus far he has been unable to demonstrate it. In any case there is no evidence of extraocular innervation.
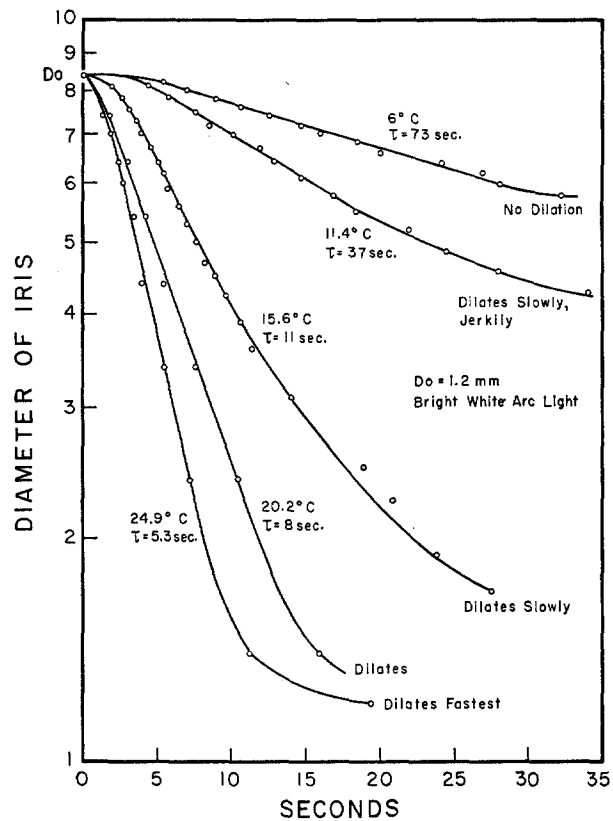

FIGURE 5. Contraction curves of excised eel iris at various temperatures together with a description of the subsequent dilation. The ordinate scale is logarithmic.

(c) Relaxation in Dark

If we plot the relaxation data of Fig. 1 in the form logarithm $(D_o - D)/D_o$ *versus* time the total relaxation can be approximated as the sum of two first order processes; one with a mean lifetime between 20 to 30 seconds and a slower one with a mean lifetime around 120 seconds. These depend somewhat on the condition of the iris and vary strongly with temperature. As might be expected they are independent of the previous stimulating light.

(d) *Effects of Temperature*

In Fig. 5 are shown the contraction curves for one particular iris at various temperatures under constant light intensity. As is noted the relaxation as well as the contraction is strongly temperature-dependent. A plot of the logarithm of the first order rate constant for contraction *versus* reciprocal temperature gave values for the heats of activation of about 26 kcal/mole. While these data show large uncertainties the values are comparable to those for other enzymatic reactions. In the absence of more specific information as to the contraction mechanism, more accurate values for the heat of activation would not be too useful at the present time.

While other workers have reported a photodynamic action in frog muscle (8) the physiological action of the methylene blue–stained muscle has been irreversible and a question therefore arises as to the direct relation of this effect to the reversible contraction described in this paper.

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