# The Photoactivated Relaxation of Smooth Muscle of Rabbit Aorta

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ABSTRACT Smooth muscle of strips of rabbit aorta, placed in a state of active tonic contraction by addition of a stimulating drug, relaxes during exposure to light. The relaxation is reversible. The extent of relaxation produced by a standard exposure depends on the preexposure level of active contraction but not on the nature of the stimulating drug used to produce contraction. With strips brought to an intermediate level of contraction, the degree of relaxation (steady state levels) is a rectangular hyperbolic function of radiation intensity. The kinetics of the relaxation process during irradiation and the recovery process following irradiation are consistent with the hypothesis that the primary photoactivated material initiates a reaction or reactions leading to a product which inhibits some process involved in the production of active contraction. The photorelaxation does not require the presence of oxygen. It is potentiated by reducing the temperature of the aortic strip. The action spectrum of the photorelaxation shows relatively low effectiveness at wavelengths above 450 mμ. The effectiveness increases markedly and progressively as the wavelength is lowered below 450 mμ, reaching a peak at 310 mμ. A deep trough occurs at 280 mμ. However, both peak and trough probably result from internal filtering due to absorption by proteins in the aortic strip. It is surmised that if a correction could be made for this internal filtering, the action spectrum would rise continuously down to wavelengths at least as low as 250 mμ.

Several years ago the finding was made that contracted strips of rabbit thoracic aorta undergo relaxation when exposed to light of sufficient intensity. In two preliminary reports (Furchgott *et al.*, 1955; Furchgott, 1955) it was pointed out that this photoactivated response differed from others previously reported for smooth muscle (Adler, 1919; Azuma and Hill, 1926; Azuma, 1927; Supniewski, 1927; Blum, 1941) in the following respects: (*a*) it could be produced by visible and near ultraviolet radiation without the addition of a photosensitizing agent; (*b*) it occurred in the absence as well as in the presence of oxygen; (*c*) the response was a reversible relaxation

rather than a contraction, and was not associated with any injury to muscle function. The present paper is a more detailed report on some of the characteristics of this photoreaction, including the action spectrum associated with it.

## MATERIALS AND METHODS

Helically cut strips of the descending thoracic aorta of the rabbit were prepared and mounted for recording as previously described (Furchgott and Bhadrakom, 1953; Furchgott, 1955, 1960). The strips used were generally 2 to 3 mm. wide, 2 to 3 cm. long (unstretched), and about 0.3 to 0.4 mm. thick. Previous histological studies on such strips have shown that the smooth muscle fibers, which are circularly arranged in the wall of the aorta, are oriented at approximately 15 to 25° relative to the longitudinal axis of the cut strips. The strips were mounted in cylindrical glass or quartz muscle chambers of 20 ml. working volume in a thermoregulated bath (either a large rectangular glass-walled bath as previously described, or a smaller lucite bath described below). Unless otherwise indicated, the temperature of the bath was maintained at 37° ± 0.1°C. In the present experiments care was taken to mount each strip so that it was not twisted and had its intimal surface facing the radiation source. The solution used was Krebs-bicarbonate solution (Umbreit *et al.*, 1949) aerated with 95 per cent $O_2$–5 per cent $CO_2$ (to give a pH of 7.4), and containing 0.01 M glucose.

General illumination in the laboratory was kept at an intensity which had no detectable relaxing effect on the aortic strips. Usually overhead, fluorescent lights could be used.

Recordings were made either under isotonic or isometric conditions. In the former case an ink-writing lever was used, exerting a tension of 4 gm. on the strip and giving a tenfold amplification. In the latter case, an RCA transducer tube (No. 5734) was used in a bridge circuit connected to a Sanborn D.C. amplifier (model 150–1000), which fed the amplified signal into a Sanborn recorder (model 150-101). The aortic strip, through a connecting thread, pulled downward on a stiff wire exerting an upward tension against a small rod projecting from the pin of the transducer tube. Over the range of tension usually employed (0 to 10 gm.), displacement of the recording galvanometer was directly proportional to change in tension. The resting tension of aortic strips used with isometric recording was usually about 2 gm.

Both with isotonic and isometric recording testing was not begun for a period of 2 to 3 hours following the suspension of an aortic strip in the muscle chamber. This period was sufficient for essentially complete stretching to an equilibrium length under isotonic conditions, or for fall of tension to an equilibrium value under isometric conditions, and also for development of full sensitivity of the strips to various stimulating drugs (Furchgott and Bhadrakom, 1953). After the initial equilibration period, sustained contractions were induced by addition of selected drugs, and the effects of radiation on such contractions were studied. The following stimulating drugs were used: l-epinephrine bitartrate, l-norepinephrine bitartrate, l-phenylephrine hydrochloride, histamine acid phosphate, acetylcholine bromide, and 5-

hydroxytryptamine creatinine sulfate. When using epinephrine or norepinephrine to induce tone, disodium ethylenediamine tetraacetic acid (EDTA) was added to the muscle chamber in a final concentration of $10^{-5}$ to inhibit metal-catalyzed oxidation of the catechol amines (Furchgott, 1955, 1960). In all experiments reported here on the determination of action spectra and on the determination of light intensity-response curves, phenylephrine was the drug used to induce contraction. This drug was selected because of all the drugs used it was best able to maintain an essentially constant level of contraction in the aortic strips for the long periods of time required in the experiments referred to. All drug concentrations are expressed in terms of the salts used on a weight per volume basis (grams of drug per milliliter of solution).

For determination of the action spectrum of the photoactivated relaxation a Bausch and Lomb monochromator (model 33-86-45 with a grating of 600 grooves per mm. blazed to give maximum efficiency in the ultraviolet region) was used with an air-cooled xenon arc (Hanovia lamp type 510C1, 150 watts) as a source. The collecting lens at the exit slit of the monochromator was removed and replaced by an upright cylindrical lens, consisting of a quartz tube (18 mm. outside diameter) filled with distilled water, the center of which was about 40 mm. beyond the exit slit and about 33 mm. in front of the aortic strip. This lens, by condensing the image of the exit slit horizontally on the strip, while still permitting it to diverge vertically, produced a fairly even illumination of the whole strip when the exit slit of the monochromator was set at the standard width of 3 mm. The strip itself was mounted in a quartz muscle chamber which was positioned immediately behind a circular quartz window at one end of a rectangular thermoregulated bath, constructed of lucite and of approximately 1 gallon capacity.

Relative intensities of radiation at different monochromator settings over the range of 270 to 700 m$\mu$ were determined with an Eppley surface type thermopile placed 70 mm. beyond the exit slit of the monochromator, with no lens intervening. The thermopile output was measured by using a Leeds and Northrup galvanometer (model 2284B) in a circuit with an Eppley microvolt comparator (Eppley bulletin No. 4, 1956). Both the entrance and exit slits of the monochromator were set at 3 mm., giving a beam of 10 m$\mu$ band width. Since fluctuations in intensity of the xenon arc sometimes occurred in a random manner (see section on determination of action spectrum), care was taken to make the determination of relative intensities at different wavelengths during a period of time when the arc intensity was stable—as indicated by a constant intensity at one "reference" wavelength which was checked at intervals throughout the period. For purposes of converting relative intensities to absolute intensities, the thermopile was calibrated with a standard lamp from the National Bureau of Standards. Relative intensities of the xenon source at wave lengths below 270 m$\mu$, which were too weak for accurate thermopile readings, were determined with a photomultiplier tube (Photovolt tube B tube with quartz diffusing plate, connected to Photovolt recorder, model 520 M). A curve for the relative sensitivity of the tube as a function of wavelength was obtained over the range of 434 to 234 m$\mu$ by comparing tube output and thermopile output obtained on irradiation with various lines from a mercury arc (Bausch and Lomb model 33-86-42-01) over this range. The data obtained for relative intensities at different monochromator

settings, which were used in the present work for obtaining "corrected" action spectra, are presented in Table I.

When wavelengths above 490 mμ were being tested in action spectrum experiments, a No. 3389 Corning filter (cut-off between 420 and 410 mμ) was inserted behind the exit slit to prevent any radiation of wavelengths below 420 mμ (resulting from the second order spectrum or scattering) from contaminating the radiation of higher wavelengths.

TABLE I

RELATIVE INTENSITIES AT DIFFERENT WAVELENGTHS OBTAINED WITH XENON ARC AND GRATING MONOCHROMATOR

| Wavelength setting of monochromator | Relative intensity* | Wavelength setting of monochromator | Relative intensity* | Wavelength setting of monochromator | Relative intensity* |
|---|---|---|---|---|---|
| mμ | | mμ | | mμ | |
| 240 | 0.0098 | 370 | 1.302 | 500 | 2.624 |
| 250 | 0.0152 | 380 | 1.501 | 510 | 2.624 |
| 260 | 0.0398 | 390 | 1.709 | 520 | 2.709 |
| 270 | 0.0604 | 400 | 1.917 | 530 | 2.689 |
| 280 | 0.1000 | 410 | 2.020 | 540 | 2.709 |
| 290 | 0.1750 | 420 | 2.125 | 550 | 2.709 |
| 300 | 0.2542 | 430 | 2.167 | 560 | 2.709 |
| 310 | 0.3583 | 440 | 2.417 | 570 | 2.653 |
| 320 | 0.5038 | 450 | 2.750 | 580 | 2.653 |
| 330 | 0.6540 | 460 | 2.957 | 600 | 2.541 |
| 340 | 0.8328 | 470 | 3.707 | 625 | 2.541 |
| 350 | 1.000 | 480 | 3.039 | 650 | 2.457 |
| 360 | 1.169 | 490 | 2.852 | 675 | 2.291 |

* Monochromator slit widths were set for 10 mμ band width. All intensities are given relative to that at 350 mμ. Thermopile was used for measurements from 270 to 675 mμ, and photomultiplier was used for measurements below 270 mμ. For details of procedure see text.

To vary the intensity of radiation falling on the strip at any one wavelength the width of the entrance slit of the monochromator was varied from 0.2 mm. up to 3 mm. while the exit slit was kept at a width of 3 mm. A curve for relative intensity as a function of entrance slit width was obtained from determinations made with the photomultiplier tube, placed at an appropriate distance from the exit slit, as a receiver. Corrections for any fluctuation in xenon arc intensity during such determinations were made on the basis of checks on the intensity at the standard entrance slit width of 3 mm. immediately following each test at a smaller slit width. The curve obtained at a setting of 350 mμ was essentially the same as those obtained at settings of 300, 400, and 450 mμ. This curve, which showed a 27.6-fold increase in intensity over the range of entrance slit widths from 0.2 to 3 mm., was used for converting slit widths to relative intensities.

## RESULTS

### *Relationship of Active Tone and Relaxation by Light*

Aortic strips which have first been placed in a state of sustained (tonic) active contraction[1] by addition of a stimulating drug will relax when exposed to sufficiently intense radiation from appropriate sources (*e.g.*, tungsten filament, mercury arc, xenon arc, and direct bright sky light). Records of relaxation due to radiation from different sources are shown in Figs. 1, 3, and 4. The relaxation is reversible as evidenced by the fact that the contraction returns to essentially the preirradiation level during the postirradiation period.

The extent of relaxation of a strip on exposure to light is dependent on the level of active contraction exhibited by the strip prior to illumination, and is independent of the nature of the stimulating drug used to produce the active contraction. In several experiments in which contractions of approximately equal magnitude were produced successively in a single strip by addition of different drugs—including epinephrine, norepinephrine, phenylephrine, histamine, acetylcholine, and 5-hydroxytryptamine—the degree of relaxation produced by a standard exposure to light (fixed intensity for a fixed time) was essentially the same regardless of the stimulating drug used. The records from one such experiment in which the contractions were produced by appropriate concentrations of epinephrine, histamine, and phenylephrine, respectively, are shown in Fig. 1B.

With most strips brought to a steady level of contraction with a given stimulating drug and then subjected to a series of standard exposures spaced at about 10 minute intervals, the degree of relaxation with each exposure

[1] Active contraction (or active tone) is defined here as a shortening under isotonic conditions or an increase in tension under isometric conditions which can be antagonized by potent relaxing agents (*e.g.*, $NaNO_2$ and glyceryl trinitrate) or by anoxia. If the contraction is low to moderate in degree (about 10 to 20 per cent of the maximum obtainable with a high dose of a stimulating drug such as epinephrine or phenylephrine), it can be antagonized completely by potent relaxing agents, and almost completely by anoxia; however, as the degree of contraction is increased toward the maximum, the antagonism by relaxing agents or anoxia becomes less effective (Furchgott and Bhadrakom, 1953; Furchgott, 1955).

On the basis of this definition, contractions produced by all stimulating drugs used in this work (excluding KCl) would be classified as active. Low to moderate contractions produced with excess KCl (enough to raise $K^+$ in the medium about two to four times) would appear to be only partly of the active type, since they are only partly suppressed by relaxing agents or anoxia. And finally, low to moderate contractions produced by increasing the osmolarity of the medium with excess NaCl would not be of the active type at all, since they are unaltered by relaxing agents or anoxia.

Aortic strips in the absence of added stimulating agents usually appear to be almost free of spontaneous active contraction, since potent relaxing agents or anoxia usually produce almost negligible relaxation in such strips.

was essentially constant as long as the level of contraction following recovery from each exposure was essentially constant. However, with some strips, especially those under isometric conditions, there was a tendency for the degree of relaxation to increase progressively with each exposure at the beginning of a series of standard exposures, and only to become essentially constant after about the fourth to sixth exposure in the series.

Aortic strips which have not been placed in tonic contraction by addition

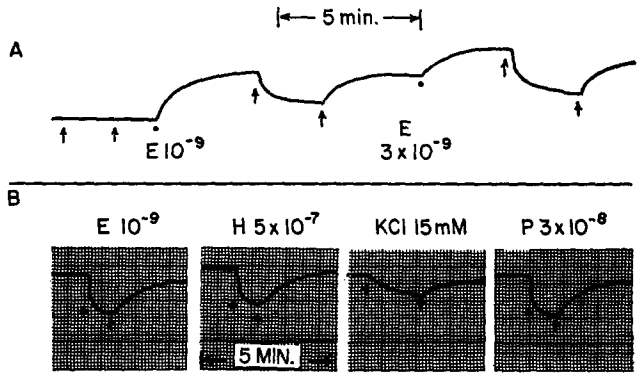


FIGURE 1. Examples of isotonic relaxation of aortic strips on exposure to light. Pairs of arrows bracket period of exposure. A, relaxation on illumination with mineralite long wave ultraviolet lamp placed a few inches from muscle chamber. Note that no relaxation by light occurred before contraction had been produced by addition of epinephrine (*E*). B, relaxation of aortic strip brought to approximately the same level of tonic contraction by addition of three different stimulating drugs and by excess KCl. Illumination was with tungsten microscope lamp. After testing effect of light on contraction produced by one stimulating agent, muscle chamber was flushed, and strip was allowed to relax to essentially resting length (base line on record) before addition of next stimulating agent. Note that degree of relaxation was approximately the same when contraction was produced by epinephrine (*E*), histamine (*H*), or phenylephrine (*P*); but considerably smaller when it was produced by excess KCl.

of stimulating agents usually exhibit either no relaxation or barely detectable relaxation even when exposed to very intense illumination (as shown in the first part of the record in Fig. 1A). This finding is not unexpected, since these strips usually exhibit negligible spontaneous active contraction in the absence of stimulating agents,[1] and therefore the relaxing action of light, like that of chemical relaxing agents, cannot be demonstrated because of the lack of active contraction to be antagonized. It should be noted, however, that when appreciable active contraction does develop spontaneously, as will occasionally happen in a large series of experiments (Furchgott and Bhadrakom, 1953; Furchgott, 1955), the relaxing action of light can then be readily demonstrated in the absence of added stimulating drugs. Indeed, the accidental discovery of the photoactivated relaxation was made on strips

which were by chance intermittently exposed to the illumination of bright sky light after the spontaneous development of a low level of active contraction.

Fig. 2 shows the results of an experiment in which an aortic strip was brought to different levels of tonic contraction under isometric conditions and at each level was exposed to 3 minutes of radiation from the monochromator set at 350 mμ with 10 mμ band width. Different levels of contraction were obtained by successively increasing the concentration of phenylephrine in the muscle chamber until essentially maximal contraction was obtained at a concentration of $10^{-5}$. After each addition of the drug, time

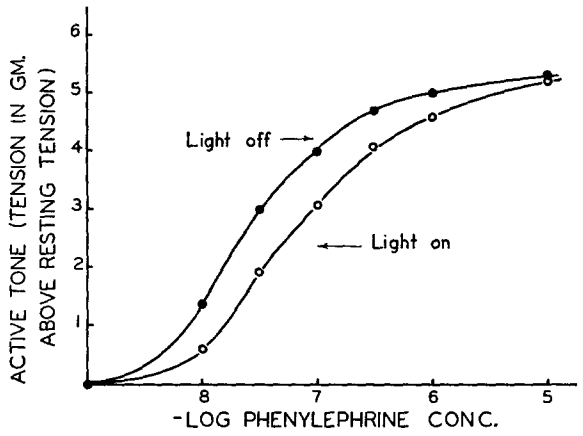


FIGURE 2. Relaxing effect of radiation on an aortic strip brought to different levels of contraction with phenylephrine. Difference between points on two curves at given concentration of phenylephrine indicates extent of relaxation produced by 3 minute exposure to 350 mμ radiation from xenon arc. For details see text.

was allowed for the contraction to attain an almost steady level, the effect of radiation was then tested, and finally the contraction was allowed to recover to an almost steady level before the next addition of drug. In the tests carried out at the higher levels of contraction, tension during postirradiation recovery did not quite return to the preirradiation level. This was not unexpected, since under isometric conditions, even in the absence of irradiation, high levels of tension produced by high concentrations of stimulating drugs are not perfectly maintained and for a while undergo a gradual decline. Therefore, in the tests at higher levels of tension, an interpolation was made between the level prior to irradiation and that following postirradiation recovery in order to estimate that level which would have existed in the absence of irradiation at the time of the termination of the exposure to radiation.

From Fig. 2 it can be seen that the percentage fall on irradiation was greatest when the level of contraction was low, and that it diminished steadily

as the level approached a maximum. On the other hand, the greatest absolute fall on irradiation occurred at intermediate levels of contraction. Results showing a similar relationship between the extent of relaxation on irradiation and the level of active contraction prior to irradiation have also been obtained with aortic strips under isotonic conditions, using phenylephrine, epinephrine, and histamine as stimulating drugs.

In the experiments of Figs. 1 and 2, irradiation of strips at low to moderate levels of contraction reduced the level by over 50 per cent, but fell considerably short of completely suppressing it; *i.e.*, of producing complete relaxation. In the experiment of Fig. 3 more intense radiation, namely that from an unfiltered Bausch and Lomb mercury arc (No. 33-86-42-01) at a

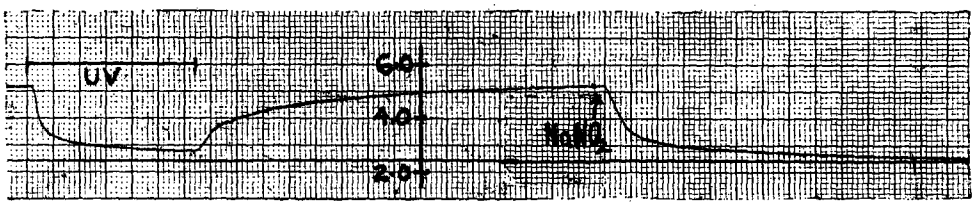


FIGURE 3. Almost complete relaxation from moderate level of contraction on exposure to very intense radiation. Contraction was produced with $3 \times 10^{-8}$ phenylephrine. Base line at 2.4 gm. indicates resting tension before addition of drug. Radiation source was Bausch and Lomb mercury arc (No. 33-86-42-01) at a distance of about 10 cm. from aortic strip. Time between heavy vertical lines is 20 seconds. After strip had recovered from the photoactivated relaxation $3 \times 10^{-4}$ $NaNO_2$ was added to muscle chamber.

distance of about 10 cm., was tested on a strip which had been brought to a moderate level of contraction with phenylephrine. In this case there was almost complete relaxation. For comparison there is included in the figure a record of relaxation of the same strip by $NaNO_2$ at a concentration previously found capable of causing complete relaxation from low levels of contraction. It will be noted that even though the relaxation by this agent slightly exceeded that obtained with radiation from the mercury arc, the initial rate of relaxation with radiation was significantly faster.

### *Effect of Light on Contractions Produced by Excess KCl or NaCl*

It will be noted in Fig. 1B that the degree of relaxation on irradiation was definitely less when contraction of the aortic strip had been produced by addition of excess KCl than when it had been produced by a stimulating drug. This is not surprising in view of the earlier finding that "KCl contractions" are also more resistant to the actions of chemical relaxing agents,

such as $NaNO_2$ and isoproterenol, than are drug-induced contractions (Furchgott and Bhadrakom, 1953). A possible explanation of the greater resistance of KCl contractions to light and chemical relaxants is that the total contraction produced by excess KCl results partly from active contraction and partly from some alteration in the physical properties of the aortic strip; and that the latter alteration, unlike the active contraction, cannot be antagonized by either light or chemical relaxants.[1]

An even more extreme case of resistance to relaxation is encountered with contractions produced by increasing the osmolarity of the Krebs solution bathing the strips by 20 to 50 per cent with excess NaCl. In the face of such contractions, neither intense radiation nor high concentrations of $NaNO_2$ produce any detectable relaxation; which leads to the surmise that these contractions in hypertonic solution must be completely due to alterations in physical properties of the strips, and not at all to active contraction.[1]

*Kinetics of Relaxation and Recovery*

On exposure of an aortic strip to radiation, there is a measurable delay between the beginning of the exposure and the onset of relaxation. This delay or latent period, is about 1.5 seconds under conditions of isometric recordings (Fig. 4C), and about 5 seconds under those of isotonic recording. (This greater delay with isotonic recording may be due in part to friction between the lever and recording paper and the lower sensitivity with this type of recording.) If exposure to radiation of fixed intensity is maintained for a sufficient length of time to produce a steady state level of relaxation, the relaxation process shows three distinct phases: an initial slow phase following the latent period and lasting about 5 seconds; an intermediate fast phase lasting about 30 seconds and accounting for about 60 to 80 per cent of the relaxation; and a final slow phase lasting from about 1 to 3 minutes (Fig. 4). Those strips which require the longest periods to relax to a steady state level on irradiation also require the longest periods to relax to the level of resting tone after washout of a stimulating drug (Furchgott and Bhadrakom, 1953). After termination of a radiation exposure of sufficient duration to produce a steady state level of relaxation, there is a delay of about 1.5 seconds before the occurrence of the first detectable increase in contraction at the beginning of the recovery process. The recovery process also shows a short initial slow phase, an intermediate fast phase, and a final slow phase (Fig. 4). The fast phase, which is not so fast as the fast phase of the relaxation process, accounts for about 50 per cent of the total recovery and usually is about 30 to 50 seconds in duration. The final slow phase of recovery usually lasts from 3 to 6 minutes and its time course closely resembles that of the final slow phase of a contraction following the addition of a stimulating drug (Furchgott and Bhadrakom, 1953).

On exposure of strips to radiation for periods of 5 to 30 seconds, the relaxation process continues for a short period after the termination of the exposure, and then is followed by a three phase recovery process with characteristics

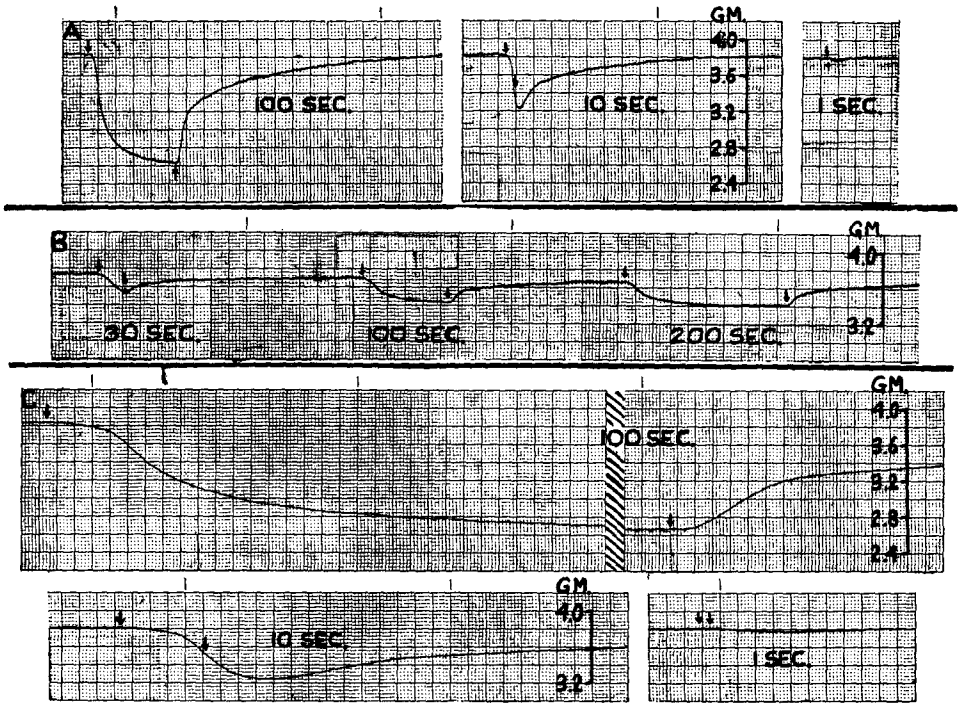


FIGURE 4. Effects of variations in duration and intensity of radiation on the relaxation and recovery of an aortic strip. Initial resting tension of strip was 2 gm. and contraction was produced with $3 \times 10^{-8}$ phenylephrine (prior to records shown). Radiation was from xenon source passing through monochromator set at 350 mμ. Arrows mark beginning and end of each exposure (durations indicated on records). In records A and B recording paper speed was one large box per 20 seconds, and in record C, one large box per 2 seconds.

A, recordings at slow paper speed of responses to exposures of 100, 10, and 1 second at high radiation intensity (entrance and exit slit widths of monochromator set at 3 mm.).

B, recordings at slow paper speed of responses to exposures of 30, 100, and 200 seconds at low radiation intensity (entrance slit width set at 0.5 mm.).

C, recordings at fast paper speed of responses to exposures of 100, 10, and 1 second at high intensity. Each recording in this series immediately followed the corresponding recording at slow paper speed in series A.

like those for the recovery process after longer exposures. With exposures of 10 seconds duration as much as one-third to one-half of the total relaxation occurs after the termination of the exposure (Fig. 4). Since the latent period between the beginning of an exposure and the first detectable relaxation is

about 1.5 seconds (isometric recording), it is possible with intense radiation exposures of 1 second or less to obtain small relaxation effects occurring entirely after the termination of the exposure.

*Relaxation as a Function of Radiation Intensity*

In a number of experiments with isometric recording, an aortic strip which had first been brought to an intermediate level of contraction with a stimulating drug (usually phenylephrine) was subjected to a series of exposures of different intensities. In such experiments the monochromator was generally set at 350 mμ, and the intensity was varied by varying the width of the en-

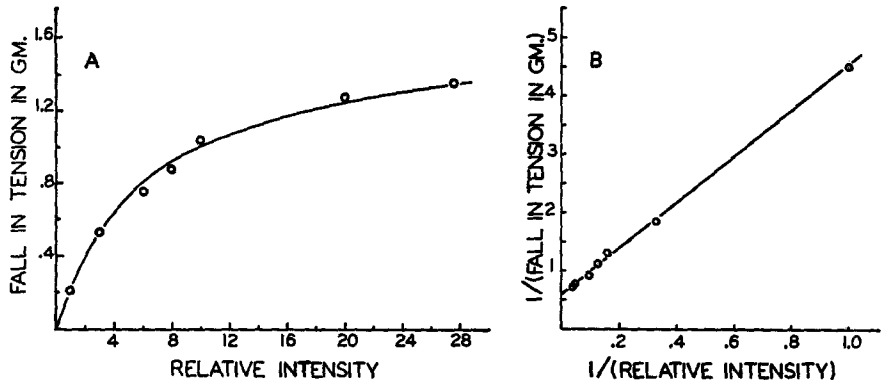


FIGURE 5. A, relaxation as a function of intensity of radiation. Aortic strip was under isometric conditions. Resting tension was 2.2 gm. and increment in tension after tonic contraction with phenylephrine was 2.3 gm. Radiation was from xenon arc with monochromator set at 350 mμ. Relative intensity of 1 is approximately equivalent to 10 μw/cm². B, reciprocal of relaxation as a function of the reciprocal of radiation intensity (same data as in A).

trance slit (see Methods). In the usual procedure each exposure was of sufficient duration (usually 2 to 3 minutes) to permit attainment of an essentially steady state level of relaxation, and essentially complete recovery was permitted before the next exposure. An alternate procedure used in some experiments was to progressively increase or decrease the intensity of radiation in a stepwise fashion, allowing 2 to 3 minutes for exposure at each intensity, and allowing recovery only after completing the series of exposures. The results obtained with either procedure showed that relaxation at low levels of intensity was almost directly proportional to intensity; but at higher levels the increment of relaxation per increment of intensity became less and less as the relaxation appeared to approach a maximum asymptotically.

The results of one typical experiment are plotted in Fig. 5A. In this experiment, as well as in others like it, the data closely fitted a hyperbolic curve

given by the equation:

$$R = \frac{R_m I}{K + I}, \tag{1}$$

in which $R$ is the relaxation in grams, $I$ is the intensity of the incident radiation, $K$ is a constant with dimensions of intensity, and $R_m$ is a constant representing the "maximal relaxation possible" in grams which is approached when $I$ greatly exceeds $K$.

Since equation (1) on rearrangement gives,

$$\frac{1}{R} = \frac{1}{R_m} + \frac{K}{R_m I} \tag{2}$$

a reciprocal plot of the data obtained in "intensity-relaxation" experiments could be fitted by a straight line whose extrapolated ordinate intercept and slope could be used for determining $R_m$ and $K$. Such a reciprocal plot is shown in Fig. 5B. In general it was found that in experiments in which a strip was brought to a level of moderate contraction with phenylephrine (25 to 50 per cent of the maximal contraction which this drug can produce at high concentrations), the calculated maximal fall in tension with radiation, represented by $R_m$, was insufficient to overcome completely the rise in tension of the moderate contraction. Thus, in the experiment of Fig. 5, $R_m$ was found to be 1.7 gm., which was about 25 per cent less than the rise in tension of 2.3 gm. produced by the phenylephrine present. Occasionally, however, in experiments in which strips were brought only to a level of low contraction with a stimulating drug, $R_m$ was found to exceed the rise in tension associated with the contraction. It should be noted that prior to our study of relaxation as a function of intensity using the monochromator and isometric recording, preliminary experiments had been carried out using a tungsten lamp and isotonic recording. Different relative intensities on the aortic strip were obtained by varying the distance of the filament from the strip. The results of these experiments with polychromatic light also showed relaxation to be a hyperbolic function of intensity.

*Relaxation by Light under Anaerobic Conditions*

Under anaerobic conditions, produced by rapidly bubbling 95 per cent $N_2$–5 per cent $CO_2$ through the Krebs-bicarbonate solution in the muscle chamber in place of the usual 95 per cent $O_2$–5 per cent $CO_2$, relaxation of aortic strips by radiation can still be obtained. Indeed, if a strip is brought to the same level of contraction, first under aerobic conditions and then under anaerobic conditions (using a suitable higher concentration of stimulating

drug anaerobically to counteract the relaxing effect of the anoxia itself[1]), the degree of relaxation on exposure to a fixed radiation source is about as great, or slightly greater under anaerobic conditions and the relaxation process follows much the same time course under both conditions. These similarities are apparent in the typical experiment shown in Fig. 6A. However, as is apparent in this figure, the recovery process following termination of the radiation exposure under anaerobic conditions is slower than under aerobic conditions. In fact, under anaerobic conditions it is usually not possible to distinguish two distinct major phases of recovery (initial fast and final slow), as under aerobic conditions.[2]

If an aortic strip is brought to the same level of contraction under aerobic conditions before and after an intervening period of anoxia of 1 hour, the relaxing response of light after the period of anoxia is distinctly greater than that before the period of anoxia.

*Relaxation by Light at Reduced Temperature*

Progressive reduction of temperature over the range from 40° to 20° produces a progressive lengthening of an aortic strip under isotonic conditions, or progressive diminution of its tension under isometric conditions. This effect of temperature occurs in the absence of active contraction (no stimulating drugs added), is reversible, and is apparently due to a decrease of elasticity of the strip with decrease in temperature. Under isotonic conditions (4 gm. tension on a strip of about 1 mm.$^2$ cross-section) there is about a 2 per cent lengthening per 10° fall in temperature. To obtain the same degree of active contraction at 25° as at 37° about three times as much stimulating drug is required at the lower temperature.

If a strip is brought to about the same level of contraction both at 37° and at 25° by appropriate concentrations of a stimulating drug, such as phenylephrine, and is tested at both temperatures with a standard 1 minute exposure to light, the degree of relaxation is always somewhat greater at 25° (Fig. 6B). At this lower temperature both the time course of relaxation during exposure and the time course of recovery after exposure are much slower than at 37°, with the latter time course showing a greater relative slowing than the former.

[2] Frequently under conditions of anaerobiosis or low temperature, recovery may appear to be incomplete, since the highest level of contraction attained during prolonged recovery may be lower than the level existing just prior to exposure to radiation. However, this apparent incomplete recovery results from the tendency of strips under such conditions to lose active tone gradually whether or not they are exposed to radiation. If a closely matched control strip from the same aorta is mounted in the same muscle bath as the experimental strip and is subjected to exactly the same treatments as the latter except for exposures to radiation, changes in the contraction height of the control strip may be used to make corrections for changes in the experimental strip not due to radiation. Such corrections show that apparent incomplete recovery is due to spontaneous loss of active contraction and not to some irreversible depressing action of the radiation.

With strips requiring about 4 minutes for complete recovery of active tone following irradiation at 37°, about 15 minutes is required for complete recovery at 25°.[2]

*Action Spectrum for Photoactivated Relaxation*

To obtain the action spectrum of the photoreaction an aortic strip under isometric conditions was first brought to a steady level of moderate contraction (about 2 gm. tension above a resting tension of about 2 gm.) with phenylephrine, and was then subjected to several exposures, ranging from high to

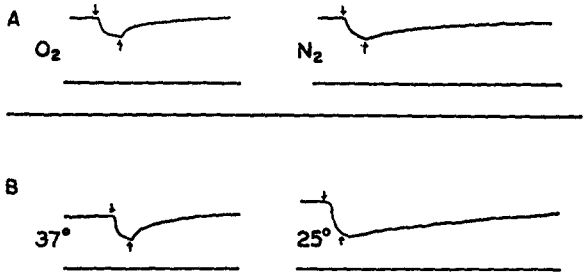


FIGURE 6. A, effect of anoxia on response of aortic strip to radiation. Isotonic recording. Tonic contraction was produced with $2 \times 10^{-8}$ phenylephrine in presence of oxygen ($O_2$) and with $10^{-7}$ phenylephrine in the absence of oxygen ($N_2$). In both tests radiation was from tungsten source (focussed microscope lamp) for a period of 1 minute. Arrows indicate beginning and end of exposure; line at base indicates level of resting length before addition of phenylephrine. B, effect of temperature on response of aortic strip to radiation. Isotonic recording. Tonic contraction was produced with $10^{-8}$ phenylephrine at 37° and $2 \times 10^{-8}$ phenylephrine at 25°. Radiation was the same as in A.

low intensity, from the monochromator set at the reference wavelength of 350 mμ. The results of these exposures were used to estimate the approximate maximal possible relaxation ($R_m$), as described earlier, and a small standard intensity (obtained with a small standard entrance slit width) was selected which gave only about 10 per cent of the maximal possible relaxation. After several exposures to ascertain the constancy of response to this small standard intensity at 350 mμ, exposures were successively made at other wavelengths. Over the range from about 300 to 420 mμ the intensity was adjusted at each wavelength tested so that the extent of relaxation was rather close to that obtained with the standard intensity at 350 mμ. At wavelengths below and above this range the full intensity obtainable with both entrance and exit slits set at 3 mm. was used, since the degree of relaxation even with such a setting was less than that at 350 mμ with its standard intensity. By limiting the degree of relaxation at all wavelengths to less than about 10 per cent of the estimated $R_m$, each response fell within the approximately linear portion

of the intensity-relaxation curve, and thus could be directly compared with the response at the reference wavelength of 350 mμ in the construction of an action spectrum.

The duration of exposure at each wavelength was sufficiently long (usually 2 to 4 minutes) to allow an essentially steady state level of relaxation to develop. At the end of each exposure at a given wavelength in the range from 250 to 390 mμ, the aortic strip was immediately reexposed to 350 mμ radiation at the standard intensity for about 2 minutes more before it was allowed to recover in the absence of radiation (Fig. 7). (In some experiments the strip was exposed to 350 mμ radiation before as well as after exposure at another wavelength.) By comparing the response at a given wavelength with the

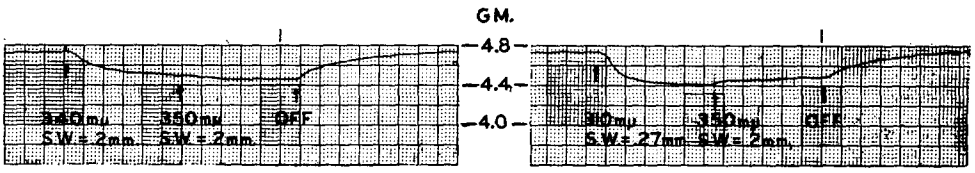


FIGURE 7. Typical records from action spectrum experiment, comparing sensitivity at other wavelengths (340 and 310 mμ shown) with that at reference wavelength of 350 mμ. Source was xenon arc. Recording was isometric. Resting tension before contraction was 2.0 gm. Phenylephrine ($2 \times 10^{-8}$) had been added about 120 minutes before first record shown and had produced a tonic contraction which was well maintained at about 2.7 gm. above the resting tension. Distance between heavier vertical lines represents 20 seconds. Under arrow marking the beginning of each exposure is shown the monochromator setting and the entrance slit width (s.w.) used. The relative intensities used at 350, 340, and 310 mμ (calculated from data on intensity as a function of wavelength and of slit width) were 1.0, 0.833, and 0.717 respectively.

individual 350 mμ response immediately following it, it was possible to minimize errors due to variations in xenon arc intensity or sensitivity of the aortic strip during the rather prolonged period of testing required in action spectrum experiments. In the region above 390 mμ, in which the action spectrum falls off markedly, tests at the reference wavelength of 350 mμ were usually introduced for comparison only after every third or fourth test at other wavelengths. In each experiment for determination of the action spectrum the sensitivity in response per unit of quantal flux at any wavelength, λ, relative to the sensitivity at 350 mμ taken as unity, was calculated as follows:—

$$\frac{\text{Relaxation in gm. at } \lambda}{(\text{Intensity used at } \lambda)(\lambda \text{ in m}\mu)} \div \frac{\text{Relaxation in gm. at 350 m}\mu}{(\text{Intensity used at 350 m}\mu)(350)}$$

Points representing the mean results of six experiments are plotted in Fig. 8. Going from high to low wavelengths, the curve fitted to these points,

although showing a steady gradual rise, stays at a very low level from 700 to about 450 mμ. It begins to rise much more rapidly as it approaches and goes into the near ultraviolet, reaches a peak at 310 mμ, descends steeply to a deep trough at 280 mμ, and is sharply rising again at 250 mμ (the shortest wavelength from the xenon arc with sufficient energy for testing). In five of the six individual experiments, the main peak occurred at 310 mμ, and in one at 320 mμ. It will be noted that the curve fitted to the mean points shows an upward bulge (but no distinct peak) around 350 mμ.

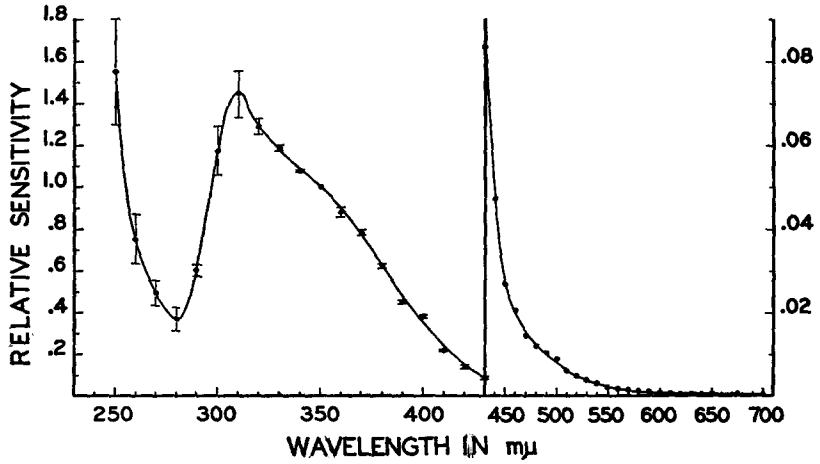


FIGURE 8. Action spectrum of photoactivated relaxation of the rabbit aortic strip. Relative sensitivity at any wavelength is relaxation per unit quantal flux relative to that obtained at the reference wavelength of 350 mμ. Vertical bars through points below 430 mμ indicate standard errors of means. See text for details.

## DISCUSSION

Alterations in the contractile activity of vertebrate smooth muscle on exposure to ultraviolet or visible radiation have occasionally been reported (Adler, 1919; Harris, 1925; Azuma and Hill, 1926; Azuma, 1927; Supniewski, 1927; Blum, 1941). However, all previous investigators except Harris found it necessary either to use short-wave ultraviolet radiation or to add a photosensitizing agent such as a porphyrin or a fluorescent dye (*e.g.*, eosin) before using visible or long-wave ultraviolet radiation. In addition, the photoreaction reported by them was almost always a stimulation rather than an inhibition of contractile activity; and if inhibition did occur, it appeared to be due to irreversible damage to the smooth muscle, since the contractile activity did not return to the control level on cessation of radiation. Harris did report that visible light in the absence of added photosensitizing agent inhibited activity of strips of frog stomach, but Azuma and Hill were unable to confirm this.

The photoactivated relaxation of smooth muscle of rabbit aorta described in this paper is the first clear cut example of a reversible alteration in contractile activity of verterbrate smooth muscle by visible and near ultraviolet radiation without the use of an added photosensitizing agent. The aortic strip must contain some endogenous photosensitive material which is activated by the radiation, and this material in the activated state somehow leads to inhibition of some reaction necessary for the production of active contraction. It should be pointed out, however, that this inhibition can be overcome if the stimulus for the production of the active contraction is sufficiently intense. This is evident in experiments such as that represented in Fig. 2, in which radiation of high intensity gave marked relaxation at low and intermediate levels of contraction but gave barely detectable relaxation in the face of maximal contraction produced by very high concentrations of a potent stimulating drug such as phenylephrine or epinephrine. The relationship between the level of contraction and the degree of relaxation by intense radiation is very similar to the previously reported relationship between the level of contraction and the degree of relaxation produced by the chemical relaxing agent $NaNO_2$ (Furchgott and Bhadrakom, 1953).

The results of the kinetic studies do not favor the concept that the relaxation is due to a direct reaction of radiation on any material which is a component in a reaction directly involved in the production of active contraction (that is, a reaction which is a direct link in the series of reactions from initial stimulus to final alteration in state of contractile proteins which is recorded as contraction). If there were such a direct action, one would expect a much shorter latent period between onset of irradiation and onset of relaxation than actually found, and also a much quicker termination of the relaxation process following the termination of a short radiation exposure than actually found (Fig. 4C).[3] One hypothesis, of several considered, which is more consistent with the kinetic data is that the primary photoactivated material initiates a chemical reaction, or series of reactions, which yield a product which inhibits some reaction directly involved in the production of active contraction. The latent period of over 1 second at the beginning of a radiation exposure may reflect the time necessary for the accumulation of sufficient product to exert a detectable inhibitory effect; while the continued development of relaxation for several seconds after termination of a short exposure may indicate that the reaction or reactions initiated by the primary photoactivated material are still yielding additional inhibitory product even though the photoactivated material itself has decayed. Extending this hypothesis to the results obtained in long exposures of sufficient duration for at-

[3] This conclusion is based on the likely assumption that the primary photosensitive material which is activated when it absorbs radiation, has an extremely short lifetime (of a much smaller order of magnitude than 1 second) in the activated state (Reid, 1956).

tainment of a steady state of relaxation, it is proposed that the concentration of inhibitory product is increasing during the rapid phase of the relaxation process, and that it is decreasing during the rapid phase of the postirradiation recovery process. However, the slow final phases of both the relaxation and recovery processes may not be so much due to continuing changes in the concentration of an inhibitory product as to a gradual adjustment of certain physical components of the aortic strip to a level of tension and length in dynamic equilibrium with the level of activation of contraction resulting from the preceding change in concentration of the inhibitory product.

A decrease in temperature slows down both the relaxation process during exposure to radiation and the recovery process after termination of radiation, with the latter process being slowed somewhat more than the former. Anoxia does not alter the time course of the relaxation process significantly, but it does slow down the fast phase of the recovery process following termination of radiation. This effect of anoxia may indicate that the rate of reversal of the photoactivated inhibition is dependent on the rate of energy metabolism of the aortic strip; or it may simply reflect a change in rate due to the presence of products of anaerobic metabolism such as lactic acid.

While on the subject of relaxation in the absence of oxygen, it is well to point out that many photodynamic effects on animal tissues have been found to be dependent on the presence of oxygen, and the conclusion has been drawn that these effects result from photoactivated oxidations (see Blum, 1941, for review of this topic). In view of our finding that the photoactivated relaxation of aortic strips is as great, if not greater, under nitrogen than under oxygen, it is possible that this relaxation may be the end result of a photoactivated reduction.

The finding that the degree of relaxation is a rectangular hyperbolic function of the radiation intensity may indicate that some step subsequent to the primary photoactivation is approaching "saturation" as the intensity is increased. As a first approach let us assume that the following reactions occur:

$$h\nu + A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} A^* \tag{3}$$

in which $h\nu$ is a quantum of light absorbed by the photosensitive molecule $A$ to give the excited molecule $A^*$, $k_1$ is proportional to the absorption coefficient of $A$ at the wavelength used, and $k_{-1}$ is the rate constant for decay of the excited state; and

$$B \underset{k_{-2}}{\overset{A^* k_2}{\rightleftarrows}} B' \tag{4}$$

in which molecule $B$ is converted to a product $B'$ in a reaction which is activated by $A^*$, $k_2$ is the rate constant for the conversion, and $k_{-2}$ is the rate constant for the reconversion of $B'$ to $B$ by a relatively slow thermal reaction. If the radiation at the wavelength used is absorbed only to a small extent in the thin aortic strip, and if the concentration $(A^*)$ is insignificant compared to $(A)$, so that $(A_t)$, the total concentration of $(A)$ and $(A^*)$, is essentially equal to $(A)$, then at the steady state, assuming first order kinetics,

$$(A^*) = (k_1/k_{-1})I(A_t) \tag{5}$$

in which $I$ is the intensity of radiation. Also at the steady state, assuming first order kinetics,

$$(B') = (k_2/k_{-2})(A^*)(B). \tag{6}$$

Now, if one designates the total concentration of $(B)$ and $(B')$ as $(B_t)$, and substitutes for $(A^*)$ from equation (5), one can obtain

$$(B') = \frac{(B_t)I}{\{k_{-2}k_{-1}/k_2k_1(A_t)\} + I}. \tag{7}$$

Finally, if one assumes that relaxation, $R$, is proportional to $(B')$, and the maximal possible relaxation, $R_m$, is obtained only when $(B')$ is essentially equal to $(B_t)$, then equation (7) gives

$$R = \frac{R_m I}{K + I} \tag{8}$$

in which $K$ is substituted for the multiple constant term in the demonimator.[4] This type of equation was shown earlier in this paper to satisfactorily fit the data from experiments on relaxation as a function of intensity. However, it should be stressed that the assumptions on which equation (7) was derived are as yet unproven, and it is quite probable that a more complex kinetic system gives rise to the hyperbolic relationship between relaxation and intensity.

By determining the action spectrum of the photoactivated relaxation, it was hoped that some information might be obtained concerning the chemical nature of the photosensitive material.[5] Since an action spectrum may be ex-

[4] It should be pointed out that the assumption that relaxation is proportional to the concentration of $B'$ does not necessitate that $B'$ itself be a substance which inhibits some step directly involved in the production of active contraction. Such a substance might be the end product of further reactions initiated by the presence of $B'$.

[5] In the preliminary notes on the relaxation of aortic strips by light (Furchgott *et al.*, 1955; Furchgott, 1955), an action spectrum peak near 360 mμ was reported. However, this early work was performed with a tungsten source and a lucite muscle chamber, and the final action spectrum was not properly corrected for intensity as a function of wavelength.

pected to resemble closely the absorption spectrum of the photosensitive material, our present action spectrum makes it very unlikely that the photosensitive material is any one of a number of substances which one might initially suspect (for example, porphyrins, flavins, pyridine nucleotides, etc.). When we first obtained an action spectrum peak at around 310 mμ, we thought that the photosensitive substance might be a material with an absorption peak at that wavelength. However, this peak now appears not to be the result of a true absorption maximum of the photosensitive material, but rather appears to be an "artefact" resulting from the absorption of protein in the aortic strip. Since aortic strips have a thickness of about 0.4 mm. there will be marked absorption by the proteins of such strips in the region between 300 and 260 mμ, with a maximal absorption near 280 mμ. Such absorption would greatly decrease the intensity of radiation in this region reaching the photosensitive material in the inner layers of the strip relative to the incident intensity on the surface of the strip. Since the incident intensity as a function of wavelength was used in constructing the action spectrum, it may be concluded that both the peak at 310 mμ and the trough at 280 mμ are the result of "internal filtering" by the protein in the muscle strip. If this is so, then it is likely that an action spectrum corrected for protein absorption in the 280 mμ region would continue to rise steadily as one continued from 310 mμ to shorter wavelengths in the ultraviolet. It is premature to speculate extensively on what type of material might give such a corrected action spectrum. However, it is conceivable that a complex of a metal, such as iron, with a protein, might be a likely type of material.

Finally, it should be noted that the rabbit aorta is not the only smooth muscle preparation on which we have been able to obtain photoactivated relaxation. The same phenomenon has been observed with strips of cat aorta, rat aorta and dog carotid artery. In addition, we have been able to produce relaxation of strips of stomach and intestinal smooth muscle of rabbit and cat with light when such preparations are suspended in a Krebs solution containing appropriate concentrations of sodium nitrite (Furchgott, data to be published).

The work reported in this paper was supported by a research grant from the Life Insurance Medical Research Fund.
*Received for publication, July 21, 1960.*

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