THE TIME-RELATIONS OF THE PHOTO-ELECTRIC CHANGES PRODUCED IN THE EYEBALL OF THE FROG BY MEANS OF COLOURED LIGHT.

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In a communication published in a recent number of this *Journal* I described the characters of the photo-electric effects which are produced in the excised eyeball of the frog when this is illuminated by white light[1]. In that communication I also referred to the occurrence of effects similar in type to those produced by illumination, which are produced when the illumination suddenly ceases.

It was further pointed out that whilst the two photo-electric responses, that due to illumination and that following the cessation of illumination, were similar as regards type and general character, they differed in regard to certain features of their time-relations. The analyses of these time-relations appeared to show that differences, admittedly small and therefore not wholly free from suspicion, were always observed; the differences between the time-relations of the two effects, particularly the periods of delay, were, however, sufficiently constant to suggest that the two responses should be considered as distinct. The conclusions thus arrived at involved the assumption that the frog's eye possessed the capacity of giving both an excitatory response to light and an excitatory response to darkness.

In my further experiments with coloured light I have been able to obtain more convincing evidence of this twofold capacity of the eye, and in addition I have ascertained other features which appear to be characteristic of the response of the eye to different varieties of coloured light. As far as I am aware no such investigation as that described in the present communication has been hitherto undertaken, for although a number of observers have determined the galvanometric effect when the eye is stimulated by coloured light, these observations are, from the nature of the recording instrument, incapable of revealing the time-

[1] This *Journal*, xxix. pp. 388 to 410. 1903.

relations of the changes in the eye which produce the galvanometric deflections[1].

The capillary electrometer used in the Oxford Laboratory has, however, given excellent results in regard to the time-relation of the excitatory change in eyeballs subjected to varieties of coloured light; these results bring out so many points of interest that it seems desirable, without waiting until the whole investigation is completed, to place on record the chief features which appear to characterise the time-relations of the eye responses under these conditions.

The Methods used in the present Research. Two different methods have been employed for varying the character of the light; the first consisted of screens of such materials that light of a definite spectral colour was alone filtered through them to the eyeball; the second method consisted in a special arrangement whereby the spectrum itself was obtained and the eyeball placed so as to be illuminated by any desired portion of this spectrum. Both methods present certain advantages and certain defects. The coloured filters are limited as regards variety, since it was found impossible to make any definite colour-screens other than red and violet ones; on the other hand the filter method can be made extremely exact as regards both the colour used and the elimination of all illumination except that due to the light which is transmitted through the screen. The spectrum method has obvious advantages as regards range and variety of hue, it is however always attended by the risk of overlapping regions and by the necessity of adopting special precautions against false light, etc. In many ways the two methods supplement one another, and in the present research the employment of the two has proved of the utmost service.

In order to obtain records of the photo-electric effects in the excised eyeball of the frog, the apparatus described in my previous communication was at first used, but later this was replaced by one of a more permanent and satisfactory character. A long black box, 4½ feet long, 16 inches wide and 15 inches deep, contains the chamber for the eyeball and various accessory apparatus. Upon one of the narrow ends of the box is fitted a specially constructed slit; this slit admitted of very fine adjustment and was guarded by two falling zinc shutters; the fall of the first shutter allowed light to traverse the

[1] Dewar and McKendrick, *Trans. R. S. Edinburgh*, 1873; Kühne and Steiner, *Untersuch. a. d. Physiol. Institut*, Heidelberg, 3 and 4, 1880, 1881. Waller, *Phil. Trans.* 193 B., London, 1900.

slit, the fall of the second shutter stopped the passage of the light. The box was so placed that the slit could be directed towards a source of light; the light used was that of an electric arc placed at a distance of about 5 feet, and was condensed on the fine slit by means of an appropriate lens of either glass or quartz. A portion of the light which traversed the slit and thus entered the box was reflected back and emerged from a hole carefully guarded so as to prevent the entrance of other extraneous light; this reflected beam, as in the method employed in my previous experiments, and described in my previous paper, was so deflected by appropriate mirrors and prisms as to reach the photographic chamber through which the recording sensitive plate was moved by Burch's special piston-recorder. On this moving plate were thus cast, (1) the projected image of the mercurial meniscus of the sensitive capillary electrometer, (2) the beam of light which was allowed by the movement of the shutters to traverse the slit and thus illuminate the eyeball, (3) the shadow of a tuning-fork arm vibrating 5 times per second. Each record thus contains in the middle the curve of displacement of the meniscus, beneath which is a dark band, indicating the commencement, duration and cessation of the stimulating light, whilst above it are time vibrations, each full wave being 0·2 second. The moving plate holder in its passage opened two keys which formed part of two electro-magnetic circuits; the armature of each electro-magnet was one of the shutters guarding the slit, hence when the moving plate caused the successive opening of the keys, the slit was first opened and then shut. It will be obvious that by shifting the two keys any desired duration of illumination can be obtained, and that either the onset or the cessation of the resulting illumination can be arranged so that it occurs when the plate has reached any desired position.

There is little to be said as to the preparation, which consisted in all cases of the freshly excised eyeball of R. temporaria. It was arranged in a moist chamber between two non-polarisable electrodes, one of which made contact with the posterior surface (fundus), the other by a kaolin ring with the circular margin of the cornea; the visual axis was thus left uncovered and was arranged so as to coincide with a small aperture in the cover of the eye chamber; this eye chamber was placed so that the light which entered the box should fall on the aperture.

As regards the mode of experiment it was usual to first observe and record photographically the amount and direction of any resting eyeball currents; as a general rule the resting difference was such that a current traversed the eyeball from fundus to cornea, but occasionally

reversed differences were observed. The response of the eyeball to a brief illumination was then observed in order to ascertain whether the preparation was a satisfactory one, and if so, then a series of successive records was made. In all the observations and the records of stimulation effects due to illumination the response of the eye showed itself as a difference of potential causing a current to flow through the eyeball from the fundus to the cornea; whenever a response occurred on the cessation of illumination the response was of a similar type. This was the case whether the illumination was that of either white or any variety of coloured light. A large number of records have been made; many of these for various reasons were not suitable for exact analysis, but a very considerable number afforded excellent material for the purpose and from these could be deduced the time-relations of the photo-electric changes.

Special arrangements were provided in the case of those experiments carried out by spectrum light; these will be described later in connection with the spectrum results.

Illumination through red and violet filters.

A large number of experiments were made by allowing the mixed light of the electric arc to pass through red and violet filters which were situated in front of the chamber containing the excised eyeball.

Various filters were tried but only two were found to be of a thoroughly satisfactory character as regards colour; these two were, (1) a glass trough 6 cms. in width with ruby-glass on its face and containing a strong solution of bichromate of potassium, and (2) a glass trough 3 cms. wide containing a strong solution of ammonio-sulphate of copper. In order to test the filters the transmitted light was examined spectroscopically by means of a large Hilger spectroscope; the solutions were made of such strength and the troughs of such thickness that with the red filter, only the red end of the spectrum up to a point short of the D line was visible, whilst with the blue-violet filter the whole of the red and green and the greater part of the blue-green border were completely cut off. Attempts to make a monochromatic green filter failed, hence the experiments were limited to the photo-electric reactions produced respectively by an undoubted red and an undoubted blue-violet light, and this part of the research is therefore limited to comparing the

photo-electric responses occurring under these conditions with those produced by white (mixed) light[1].

It is impossible to attach any absolute value to the relative amounts of the photo-electric changes produced by the light through the two filters since several essential data such as the photometric value of the total light and the luminosity of the two colours could not be accurately determined; in this respect the spectrum experiments are more valuable. On the other hand there is always in spectrum experiments the possibility of overlapping areas and of false illumination from other portions of the transmitted light, whereas with the filters it is at any rate certain that only light of a definite quality as regards colour (viz. red and blue-violet respectively) reached the eyeball; upon this last point I desire to lay special stress. Although the records of the various magnitudes of photo-electric effect produced by the different lights traversing the filters cannot be considered as sufficiently absolute to serve as a foundation for a scheme of reaction capacities, they have a relative value. Thus the reaction to light coming through the red filter appears to be of a pronounced character, whilst that to light coming through the blue-violet filter is distinctly feebler; and since this relationship is borne out by the spectrum experiments we are at least justified in concluding that the capacity of the eyeball to respond photo-electrically to red light is greater than its capacity to respond to blue-violet light. It has already been stated that the photo-electric changes produced by red and by violet light, as indicated by the recorded displacements of the mercurial meniscus of the capillary electrometer, resemble the changes which are produced by the action of mixed light as regards their general type. Thus at the *onset* of the illumination a sudden alteration always occurred in the potential difference between the two contacts, and this was always of such a character as to indicate a current through the eyeball from the fundus to the cornea; this "illumination response" was produced by either of the colours or by white. Whilst the illumination persisted this initial change continued to develope until it attained a maximum, after which it generally tended to subside; when the illumination ceased a second increase in this difference of potential occurred, this being what I now term the "dark response." The capillary records thus present the same general appearance as those which were reproduced in my previous paper already

[1] The amount of blue coming through the blue-violet filter was so small that the light may be considered as "violet" although it is described here as "blue-violet" since this is its exact character.

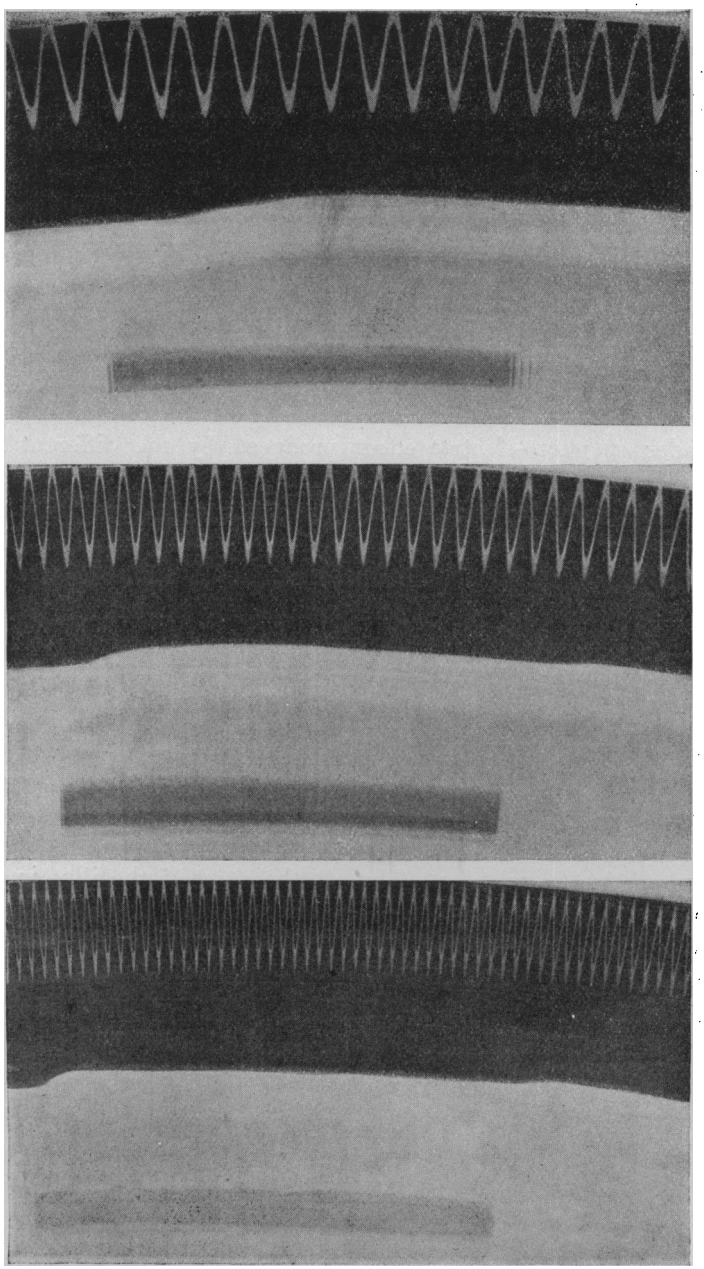

Fig. 1. The three photographic records are to be read from left to right; the time vibrations are those of a fork vibrating 5 D. V. in one second; the onset, duration, and cessation of the stimulating light are indicated by the dark band. The displacement of the capillary meniscus shows in each case the photo-electric response, the eyeball being illuminated by red light.

referred to. It seems unnecessary to give a number of examples of such records since their real value is only realised after subjection to appropriate and careful analysis, by which means the time-relations of the varying E.M.F. which they indicate can be determined. In order, however, to furnish examples of the materials submitted to analysis facsimile reproductions of electrometer records are reproduced in Figs. 1 and 2. The records shown in Fig. 1 were obtained by illuminating the eye through the red filter for a given period; the onset, duration, and cessation of the illumination are recorded by the dark strip below each record. The time vibrations at the upper part of the upper experimental record are those of a fork vibrating 5 times in a second. It will be noticed that the recording surface moved most rapidly in the case of the upper experimental record and most slowly in that of the lowest one. It will be noticed further that the duration of the illumination is 2 seconds in the upper record, 4 seconds in the middle record, and about $7\frac{1}{2}$ seconds in the lowest record. The response at the onset and during the persistence of the red light, indicated by rise in the level of the meniscus, is well marked in all three records; the response on the cessation of the illumination is distinct in the case of the longest illumination ($7\frac{1}{2}$ seconds) which gave the lower record, and is also indicated in the case of the illumination for 4 seconds which gave the middle record.

In Fig. 2 are shown two records of the maximum electrical responses obtained with the blue-violet filter, the ones selected being some of the largest ones which I possess: the majority of the records show less conspicuous responses to this form of illumination.

It will be noticed that the recording surface moved most rapidly in the case of the upper record. In the upper record the duration of illumination was $6\frac{1}{2}$ seconds, and although there is a distinct "illumination response" indicated by a rise in the level of the meniscus, there is no terminal or "dark response" following the cessation of the illumination. The illumination itself is shown by the lower dark band. In the case of the lower record, obtained on a more slowly moving plate, the duration of illumination was about 10 seconds, there is a distinct "illumination response" indicated by a rise in the level of the meniscus, and a second terminal or "dark response" occurs after the cessation of the light.

A number of records similar to those here reproduced have been subjected to analysis. From these analyses a selection has been made of such as seemed typical, and these have been plotted as curves of

which the ordinates represent the calculated difference of potential between the contacts, whilst the abscissæ indicate the observed time after the commencement of the illumination to which the calculated

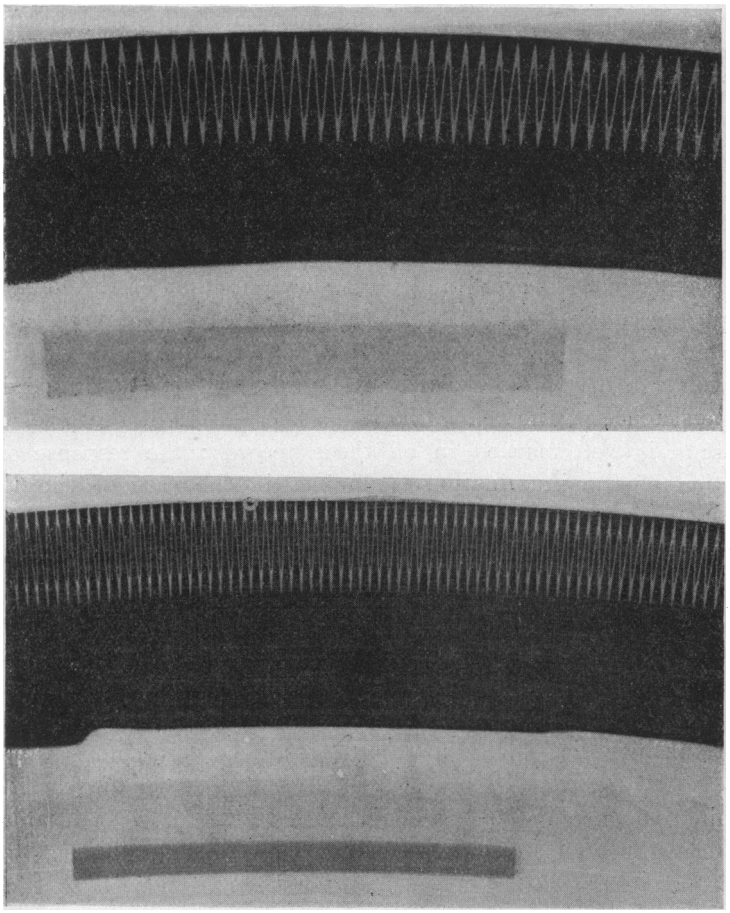

Fig. 2. The two records given in this figure show two of the largest of the responses which were obtained by illuminating the eyeball with violet light.

difference of potential refers. In Fig. 3 are shown three such plotted curves. They represent the photo-electric changes produced on the frog's eyeball by illumination through colourless, red and violet filters respectively.

It will be noticed that the change evoked by the illumination

through the red filter and displayed in the middle curve is very pronounced, being quite as marked as that evoked by the white light

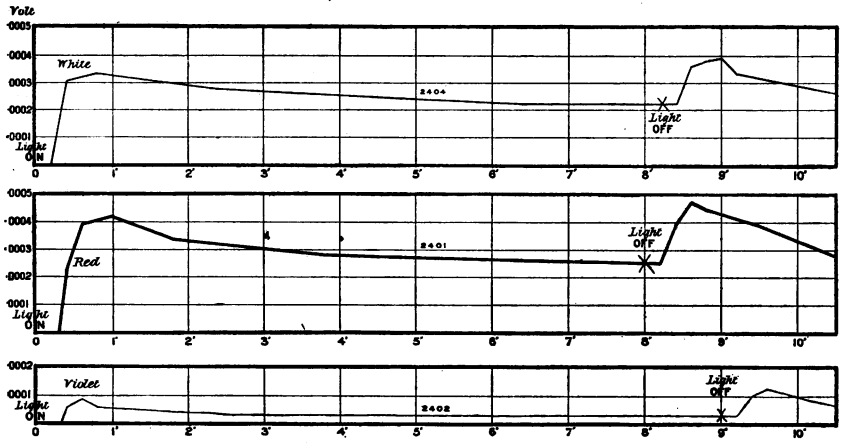

Fig. 3. Plotted curves constructed from the analyses of three typical electrometer records. The illumination began in each case at the left-hand edge marked zero, and lasted until the point marked on each curve by a cross. Abscissæ indicate time after the commencement of the illumination; ordinates the E.M.F. of the change in ten thousandths of a volt. The illumination in the case of the upper record was white light, in that of the middle one red light, in that of the lowest one violet light.

through the colourless filter shown in the upper curve. It will be further noticed that the cessation of both the white and the red illumination is followed by a "dark response" which is superimposed on the residue of the illumination effect; I propose throughout this communication to call the terminal effect "the dark response," in contradistinction to the initial or "light response" evoked by the actual illumination.

Initial and terminal changes of the same type (*i.e.* "light responses" and "dark responses") are shown in the lowest curve when the eyeball was illuminated by violet light and when this illumination ceased, but they are obviously of much smaller amount than those evoked by either the white or the red light. In the figures the duration of the illumination is indicated as follows; it commences at the zero upon the left-hand side of each curve and ceases at the point marked with a cross upon each curve; the duration of the white and red light was therefore in these instances about 8 seconds, that of the violet light about 9 seconds.

The occurrence of an excitatory effect, or "dark response," on the cessation of the illumination by either red or violet light is, as in the

case of white light, dependent upon there having been a sufficient period of illumination. The period for which the coloured light must last in order that sudden darkness may evoke a further excitatory response is much longer when the violet light of low luminosity is the source of the illumination than when the red light is used. It follows that a duration of illumination can always be found which gives on its cessation marked "dark responses" provided the light used is white or red, but which gives no "dark response" if the light has been violet. In Fig. 4 are shown plotted curves of eye responses in illustration of this point.

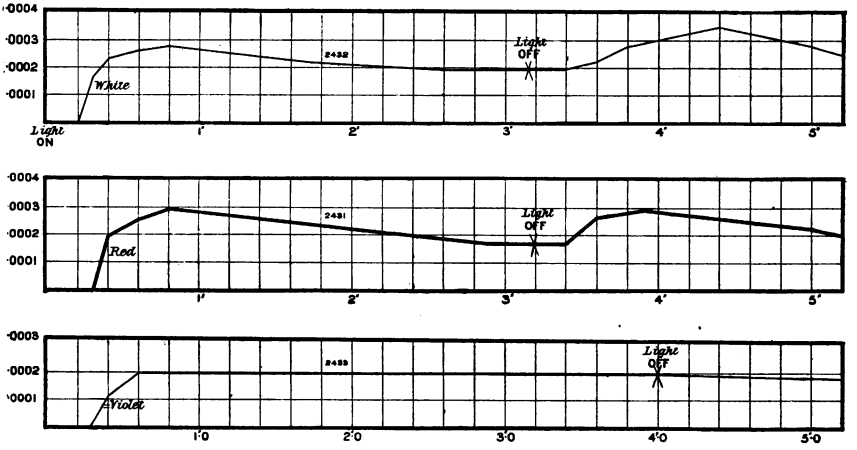

Fig. 4. Plotted curves constructed from records of photo-electric responses. The records were made on a faster travelling surface than those of Fig. 3. The duration of the illumination is shown on the record, it commenced at 0 and ended at the point marked with a cross on each curve. There is no terminal response on the lowest curve, which shows the effect of violet illumination.

It will be noticed on comparing the three curves given in the figure that in the case of white and red light (the upper and middle curves) each of which lasted for 3 seconds, there is a "dark response" following the cessation which occurred at the point marked by a cross. On the other hand in the case of violet illumination, shown in the lowest curve, there is no "dark response" although the light lasted for 4 seconds.

An examination of a large number of such records shows that the minimal duration obtained in the case of white light after which darkness evoked a "dark response" was 1·5 seconds, that of red light 2·5 seconds, whilst in the case of blue-violet light, a dark response was only once observed after a duration of 6·5 seconds illumination, and

was not generally obtained unless the duration was increased to from 8 to 9 seconds. This is an interesting feature of the responses to the two coloured lights; but the most important characteristics are those connected with the period of delay. A different period of delay for the two responses is brought out by all the records, but is especially noticeable in those taken on more rapidly moving plates. A number of such records have been made so as to include the start of the "light response" and of the "dark response" respectively. These will now be referred to separately.

THE PERIOD OF DELAY OF THE RESPONSES TO RED AND VIOLET LIGHT.

The results are illustrated by the curves shown in Fig. 5, which give analyses of typical records.

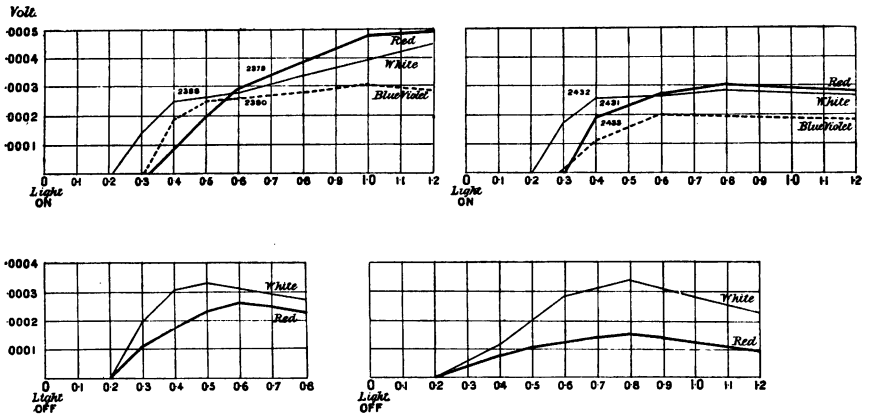

Fig. 5. Plotted curves constructed from records obtained on fast moving plates. The two upper ones show the start of the illumination response with white, red, and violet illumination respectively; the two lower ones show the start of the terminal response produced when the light ceases.

In this figure each of the upper groups contains three curves, representing respectively the start of the responses to white, red, and blue-violet illumination. It will be noticed that the period of delay is shortest in the case of the response to white light, being 0·2 second, that it is longest in the case of red light, being over 0·3 second, and that as regards blue-violet light it is intermediate in duration, being somewhat less than 0·3 second.

The increased delay in the response to red light is evidently not due to the smaller size of the effect, since in one of the groups of curves the red response is seen to be actually more pronounced than that evoked by white light, and yet its delay is half as long again as that of the white light response.

Moreover, the blue-violet response is smaller in magnitude than the red response, yet its delay is less than that evoked by the more potent red light. These differences are corroborated by the results to be referred to later, obtained by the use of the spectrum.

A further point of interest is connected with the period of delay of the "dark response" which follows the cessation of the coloured illumination. In order to obtain distinct terminal responses of this type a previous illumination of from 12 to 15 seconds was allowed to affect the eye and the travelling plate then shut off the light. The analyses of two sets of dark responses following white and red illumination respectively are shown in the lower part of Fig. 5. The moment at which the light ceased is coincident with the point on the left hand marked zero. It will be seen that the "dark response" commences after the same period of delay, namely 0·2 second, whether the light was white or red, and hence these differ from the "light responses" which are always perceptibly longer with red than with white illumination.

These characters of the period of delay for the "light" and for the "dark responses" appear to me suggestive indications as to the reactive capacity of the eye: I have therefore grouped in the following table the delay which occurred in such records as from their nature admitted of accurate measurement. The results fully confirm the conclusions derived from the experiments already described, and it will be seen later that the work carried out by the help of the spectrum affords results which show the same features.

A glance at the table shows the extraordinary constancy of the "dark response" whatever the preceding illumination; it shows also the invariably more prolonged delay of the "red response" and the more varying duration of the "blue-violet response" where these are compared with the delay of the response to the white light through the clear filter.

The evidence furnished by the records thus demonstrates that whereas the time-relations of the "light response" vary with the quality of the stimulating light, those of the response occurring on the cessation of the light are as regards period of delay extremely constant. It is thus most improbable that the latter can be regarded as in any

Period of Delay of the Photo-electric Response of Frog's Eyeball at 15° C.

		Red filter		Blue-violet filter		Clear white filter	
		Illum. response	Dark response	Illum. response	Dark response	Illum. response	Dark response
Delay	1	0·28″	0·20″	0·23″	0·20″	0·22″	0·18″
,,	2	0·30	0·20	0·30	0·20	0·18	0·18
,,	3	0·32	0·20	0·22	0·20	0·20	0·23
,,	4	0·30	0·20	0·25		0·20	0·18
,,	5	0·28	0·20	0·28		0·20	0·20
,,	6	0·30	0·20	0·22		0·18	0·20
,,	7	0·28	0·20	0·20		0·23	0·20
,,	8	0·28	0·20	0·32		0·20	0·20
,,	9	0·28	0·20	0·30			
,,	10	0·28	0·20	0·30			
,,	11	0·30	0·20	0·28			
,,	12	0·28	0·20	0·26			
,,	13	0·33	0·20				
,,	14	0·30					

sense a rebound from the condition produced by the illumination. Why should it possess this constancy of delay? The only satisfactory explanation appears to me to be that the physical condition which acts as the stimulus is unaltered in character under all these varying conditions and thus possesses uniform physical characters. Whatever the quality of the previous illumination its cessation causes one constant physical condition, namely, that of darkness; and if we assume that this sudden darkness is the stimulus then the response evoked by its occurrence should always show the same delay. The conclusion thus reached involves, as a consequence, the assumption that the retinal excitable structures possess the capacity of developing the excitatory state whether they are acted upon by sudden light or by sudden darkness. As far as the photo-electric indications of this state are concerned, it is evident that the reaction is of the same fundamental type in the two conditions, but the finer details of the one condition, that of the light response, are obviously distinguished from those of the other condition, that of the "dark response;" hence there must be two distinct substances, one reacting to light, the other reacting to darkness.

A further question of very great interest is related to the varieties of "light responses." The problem as to the number of fundamentally distinct colour reactions has been approached from many different sides, but as far as I am aware it has not hitherto been approached from the experimental point of view now under consideration. It is not to be

expected that experiments with only two colour filters can give extended information as to this. Nevertheless the results are very suggestive, especially since the further results obtained with the spectrum extend them so as to cover the whole range of coloured light.

It is therefore worth considering how far the photo-electric responses obtained with undoubted red and undoubted blue-violet illumination differ in regard to their characteristic features. In the first place it is well to bear in mind that in one respect all illumination responses are alike. The response is always of the same electrical sign. There is no photo-electric evidence of changes evoked by retinal stimulation of a fundamentally opposed type. Since in all the numerous records obtained from the frog's eyeball, the change if present has the same sign, there is no shadow of photo-electric support for retinal excitatory states evoked by light, based some on katabolic (dissociative) and others on anabolic (associative) processes.

There are however distinct differences of detail between the red and the blue-violet responses; these fall under three categories, (*a*) differences in the period of delay, (*b*) differences in the rate of development, (*c*) differences in the magnitude of the photo-electric effect.

As regards the first of these it will be noticed that whereas the period of delay with red and blue-violet light is distinctly longer than for white light, that for red is the most constant and the most prolonged. It appears from experiments with the spectrum that the presence of blue light tends to shorten the blue-violet reaction time. No constant difference in the rate of development of the response to the two varieties of light could be made out, but as a rule the smaller violet response attains its maximum more quickly than the larger red one (see Fig. 6). Finally a constant and distinct difference is always found in the magnitude of the effect which each variety evokes. It is surprising what large photo-electric responses may be produced in favourable specimens by comparatively dull red light; indeed it often happens that the response thus evoked equals or even exceeds in magnitude the response evoked by white. In illustration of this last point attention is drawn to the analysed curves shown in Fig. 6.

In this three groups of curves are given; they are analyses of records made under special precautions as regards condition of eyeball, temperature, previous exposure, and steadiness of illuminating source. In the upper group the response to red illumination reaches a maximum of nearly ·0005 volt, whilst that to blue-violet only attains one of ·0002 volt and then begins to decline. A similar difference in the value of

the two responses is shown in the left-hand lower group of curves. The right-hand lower group affords a striking illustration of the not infre-

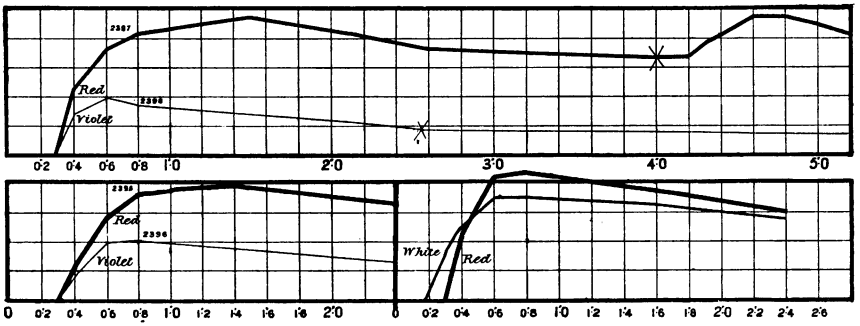

Fig. 6. Plotted curves constructed from photographic records to show the comparative size and rate of development of the response to red and the response to violet light. The right-hand lower pair of curves give an instance of a red response exceeding in magnitude that previously obtained in the same eyeball by white.

quent observation that the response to red light can attain a maximum which actually exceeds that of any response obtainable in the same eye by white; the white light used was obtained by replacing the red filter by a trough filled with water and narrowing the slit. The relationship between the magnitude of the photo-electric effect and the quality of the stimulating light has formed the subject of investigation by all those investigators who have used galvanometric methods for the detection of the photo-electric response. It is however not surprising that their results are somewhat conflicting, since the method employed involved what, as compared with my own experiments, must be considered as a prolonged exposure to light (10 seconds in Waller's experiments) whilst the galvanometric effect is undoubtedly the expression of a summed result, in which the several phases of development, persistence, and decline of any response cannot be followed.

Dewar and McKendrick consider that the results alter in magnitude with the luminosity of the quality of the light, and it will be seen from the observations to be described in the next section of this paper that this view is supported by the magnitude of the initial response to light as determined from the electrometer records[1]. Holmgren found that both the red and blue ends of the spectrum were less potent stimuli than the green-yellow regions[2]. Waller considers that red is the

[1] Dewar and McKendrick. *Transactions Roy. Soc. Edin.*, 1873, p. 141. Dewar, *Proc. Roy. Institution*, 7, p. 360. 1876.

[2] Holmgren. *Untersuch. a. d. Physiol. Institut*, Heidelberg. 1882.

least potent, green and yellow the most, and that any colour evokes responses which are much smaller in magnitude than those produced by white light[1]. These discrepancies are probably related to the summation of total effect previously referred to. The electrometer method affords information as to the greatest magnitude of E.M.F., reached by the response during any part of the illumination. If this maximum subsides in one case and persists in another, then obviously the former might produce a smaller total galvanometric deflection than the latter. It may even happen that, since the magnitude of the galvanometric deflection is determined by the duration quite as much as by the intensity of any electrical current, an effect attaining a higher E.M.F. but which somewhat rapidly subsides, would give galvanometrically a smaller deflection than one which persisted throughout at a lower level of E.M.F. It appears that this is the case in regard to red and white light, the response to the latter persisting when that to the former has begun to decline, and hence the results described in the present communication must not be taken as contradicting the experimental facts as observed by recording the galvanometric deflections. As regards interpretation however there is I think little doubt that any conclusions as to the stimulating efficiency of different colours can only be based on the comparison of such records as will show the maximum change attained in any given instance. It is in this respect that the present research differs fundamentally from those conducted by means of the galvanometer, and therefore appears to me to indicate the possibility of obtaining the necessary data from which to make a true comparative chart of the relative stimulating efficiency of different illuminations. As far as the results at present described furnish such data it would appear that red is a potent stimulus, the change it evokes attaining a maximum little inferior to that of the change evoked by white and in some instances actually greater, whilst blue-violet is a feeble stimulus and evokes a change which is always inferior as regards its maximum development to that evoked by red. This point is illustrated in the table given later on (see p. 25).

THE RESPONSE OF THE EYEBALL TO THE LIGHT OF DIFFERENT REGIONS OF THE SPECTRUM.

The spectrum used for these experiments was that obtained by means of a grating set upon a biconvex lens having a principal focal

[1] Waller. *Phil. Trans.* Vol. 193 B., pp. 136, 137.

length of 18 cms.; this was kindly placed at my disposal by Mr G. J. Burch, who also assisted me in fitting it up for the purpose of the present research. It was fixed on a stand in the large black box previously referred to and at such a distance behind the adjustable slit as was found to be most suitable. It produced a series of extremely vivid spectra; that of the second order being the sharpest was arranged to fall on the aperture in the lid of the dark moist chamber containing the eyeball. The light of the arc lamp was focussed on the slit by means of a quartz lens of 15 cm. focal length, and the spectrum focussed at 62 cms. from the grating showed the well-known fluorescent bands in the ultra-violet region when it illuminated appropriate substances such as solutions of fluorescine, quinine, paraffin oil, etc. The fluorescent bands could also be seen when the spectrum was thrown on any ordinary white paper, on the skin of the hand, the cornea, and either the sclerotic or the lens of the eyeball; the lens of the eyeball gave particularly obvious fluorescent effects and shone out like an opal in the dark ultra-violet region. The moist chamber containing the eyeball was covered by a special cover pierced by a small aperture immediately over the subjacent preparation which was arranged so as to face the aperture; the cover consisted of a close-fitting hinged lid which could be raised or lowered as desired. The chamber was fixed on a stand which could be shifted by means of a screw adjustment along the arc of a circle of 62 cms. radius, the centre of which coincided with the fixed position of the grating. By this means the chamber could be shifted so that the aperture should lie in any desired region of the projected spectrum. The undeviated light which was transmitted through the centre of the grating was intercepted by a special mirror fixed in the large black box and reflected out by a special channel; this was by means of appropriate mirrors and prisms allowed to reach the sensitive plate which recorded the movements of the meniscus of the capillary electrometer. In addition to the above apparatus, the large box contained a further arrangement for determining and varying the amount of light passing through the grating. A white screen could by means of a special lever be interposed at any moment in the path of the undeviated beam; the white screen when illuminated by the beam was so situated that it could be viewed through a tube fixed in the lid of the large external box; the tube contained a double prism photometer on the Lummer-Brodhun principle. The value of the light passing through the grating was by this means compared with that of a standard candle placed at a fixed distance 76 cms. from the photometer outside the box; as the

two illuminations were of different quality it was found necessary to view them both through red glass; it was ascertained that by this means inequality in the two sources of light could be readily detected. In practice the standard candle was fixed permanently at a given distance (76 cms.); by means of the slit, which admitted of very fine adjustment as regards size, the amount of light passing through the grating could be always adjusted until it corresponded in photometric value with that of the candle.

A serious difficulty in connexion with such spectrum experiments arises from the unavoidable general illumination of the interior of the large black box; this was largely got rid of by fixing in appropriate positions black tubes for the various beams and wooden screens to guard the eye chamber. It was however necessary to constantly keep in mind the possibility of error due to false light and for this purpose to carry out control experiments of various kinds. The control most generally adopted was of the following character.

The preparation being placed in the moist chamber and this inserted into the box, the whole box was closed by a wooden lid and it was arranged that the aperture leading to the eyeball should lie in the dark portion of the spectrum below the visible red end. An experiment was now carried out, the light passing through the slit by the release and fall of the shutters. In this case there was the usual general illumination of the large black box, but the eyeball gave in only a few instances quite slight and hardly recognisable indications of any photo-electric response; in a large number of instances it gave no response at all. It thus followed that the various means employed for preventing extraneous light from reaching the aperture leading into the eye chamber were adequate and that the only light reaching the eye was that of the region of the spectrum which was focussed upon it.

As regards the general results obtained when the eyeball was so situated as to be illuminated by various portions of the visible spectrum, I have never failed to obtain responses, although these, as was to be expected, differed in magnitude in different preparations.

The behaviour of the eyeball to the ultra-violet portion of the spectrum is of some little interest, as feeble responses of variable extent and capricious as to their production are seen in several of my photographic records. I believe that these responses are largely related to the production in the eye media of fluorescence since they are especially prominent when the eyeball lies in regions which cause the lens to fluoresce: it is also possible that the retina may fluoresce

under these conditions. Further observations on this subject are now in progress but one or two general points in connexion with the subject may be appropriately mentioned here. There is little doubt that this region does not evoke the obvious and characteristic responses which are produced by the bright portions of the spectrum. Helmholtz drew attention to the fact that with sunlight even the ultra-violet region is faintly visible provided that the bright part of the spectrum is completely masked; he leaves it undecided whether this ultra-violet visibility is the direct action of the ultra-violet light or is produced through the causation of fluorescence[1]. Garten used the ultra-violet rays for his extremely beautiful records of the pupillary reactions in man[2], his plan of experiment being to keep the eye in the ultra-violet region and so obtain photographs of the pupil without an illumination which would cause a reaction; when a bright light illuminated the eye the pupil constricted and when the light ceased it dilated, both events being recorded on the continuous photographic plate which was affected by the reflected ultra-violet rays. It thus appears that the pupillary response to ultra-violet light is so inconsiderable as to be negligible, the pupil acting normally to the onset and the cessation of visible illumination even when kept continuously in the ultra-violet region.

The capricious character of the small photo-electric responses which I have obtained from the frog's eyeball when placed in the ultra-violet region has led me to doubt whether these can be considered as evoked by the ultra-violet light at all. It must be remembered that with the grating method the different orders of successive spectra tend to tail off into one another. In order therefore to obtain additional evidence of a reliable character I have employed in addition to the spectrum the filter described by Professor Wood[3]. This consists of thin dense cobalt glass coated on one side with a thin gelatine film lightly stained with nitroso-dimethyl-aniline and covered by a thin piece of Chance's signal-green glass.

The filter was placed behind the aperture leading into the moist chamber. It was ascertained that no visible light traversed the filter, but that when solutions of fluorescine, quinine, etc., were placed in the path of the filtered light they showed quite obvious fluorescence, which was at its maximum in the ultra-violet region but extended into violet and green portions of the spectrum, now invisible, in the case of

[1] Helmholtz. *Physiol. Optik*, 2 Aufl. pp. 279, 304.

[2] Garten. *Archiv f. d. ges. Physiol.* LXVIII. pp. 68 to 94. 1897.

[3] R. W. Wood. *Astro-Physical Journal*, XVII. p. 133. 1903.

fluorescine as far as the *D* line. Wood's photographs and observations show that the filter allows actinic rays and rays capable of causing fluorescence to pass, but cuts off others. I have obtained under these conditions no trace of any photo-electric response in the frog's eyeball and pending future experimental enquiry am inclined to believe that the eyeball response is not evoked by light which is not recognised as visibly bright. The fact that a sensitive photographic plate is affected by the ultra-violet light coming through the filter whilst the eyeball remains unaffected is a striking demonstration of the fundamental difference between the chemical nature of the changes in the eyeball reacting substances and in such substances as are used in photography.

It is noteworthy that except in the doubtful case of such ultra-violet regions as cause fluorescence, the photo-electric response of the frog's eyeball fails when the ether vibrations which reach it are not those which can give rise in our own eyes to changes resulting in visual sensation, thus demonstrating that the response is not due to those rays which are either solely thermal or solely actinic, but is evoked by rays of the quality necessary to produce visual effects in the case of our own eyes.

The similarity in range between the photo-electric response of the frog's eyeball and the visual limits of our own eyes is a point of some importance. I am well aware of the gulf which divides the two classes of phenomena, for we are dealing in the responses of the frog's eyeball with photo-electric effects in an animal whose visual sensations must from their nature remain always unknown, whilst as regards ourselves it is extremely improbable that the photo-electric responses in our own eyes can be ever ascertained with any precision. In spite of this gulf the parallelism of range in the two classes of phenomena is so suggestive that it seems very desirable that other aspects of the two classes of phenomena should be investigated in such a manner as to allow of their comparison; such aspects might well comprise those of luminosity, rapid stimulation, fatigue, contrast, etc. Some of these I hope to attempt in future experiments. As far as the present investigation has proceeded it has been confined to the determination of the time-relations of the photo-electric response evoked by submitting the eyeball for a known period to illumination by definite regions of the visible spectrum, and the results like those previously described, which were obtained with colour filters, show certain constant and definite characteristics. These are especially related to the duration of the period of delay of the response, and the maximal E.M.F. to which it attains.

In order to indicate the character of the results some different experiments will be described in a little detail.

EXP. (1). The frog's eyeball was excised and placed in the dark moist chamber so that it should be, when desired, illuminated by such light as reached it through the small aperture in the lid of the chamber, placed directly over the cornea of the eyeball.

The aperture was about 8 millimetres in diameter and could be arranged so that it should lie in any desired part of the spectrum cast by the grating whenever the light was permitted by the fall of the shutters to traverse the shutter and reach the chamber; the particular spectrum used was that of the second order of the various grating spectra.

The eyeball having been kept for 15 minutes in the dark was exposed by the travelling of the photographic plate-carrier and the consequent fall of the slit shutters to the red end of the spectrum for 1·6 seconds; a distinct response occurred, the analysis of the electrometer curve being shown in that one of the upper curves of Fig. 7 which is marked by the word "Red." The response begins after a delay of 0·275 second, it reaches a maximum of ·00045 volt at 0·8 second.

A period of five minutes' darkness was allowed and then the experiment

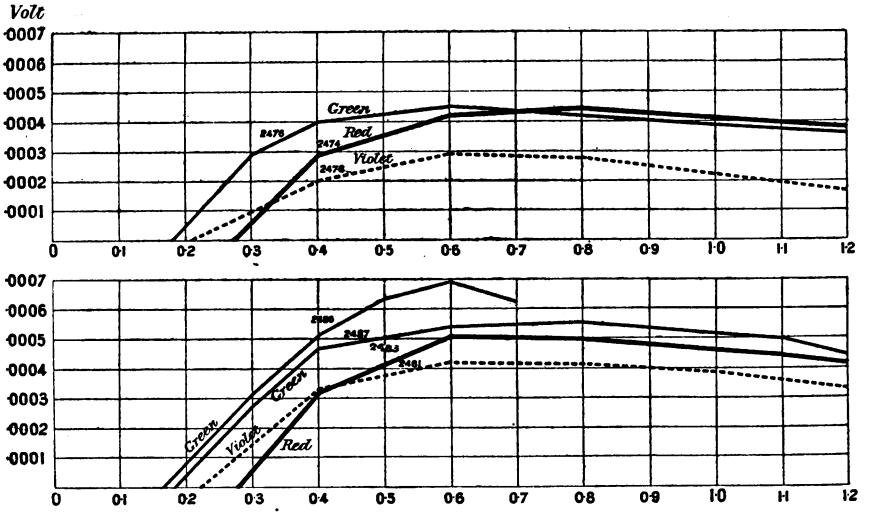

Fig. 7. Plotted curves constructed from electrometer records of eyeball responses to the light from the red, green, and violet regions of the spectrum. The upper records are selected as fairly typical of the majority of such records; the lower ones are selected to show the most pronounced responses obtained by this means. The duration of the illumination is not shown but is indicated in the description given in the text; it lasted longer than the scale admits.

was repeated with similar results. During a further period of 5 minutes' darkness the position of the chamber was shifted by a special adjusting screw which could be worked outside the external large dark box; it was ascertained that the shift would bring the aperture over the eye into the green region of the spectrum. The eyeball was then exposed by the passage of the pendulum and fall of the shutters guarding the shutter to this green region of the spectrum for 2·7 seconds. The analysis of the resulting response is shown in the curve marked *green* in the upper set of Fig. 7. It begins after a period of delay of 0·18 second and reaches a maximum of ·00045 volt at 0·6 second. After an interval of 5 minutes' darkness this was repeated with similar results. In the next dark interval of 5 minutes the chamber was shifted so that the aperture should be in the blue-violet region of the spectrum, and then exposed for 2 seconds to this light. The analysis of the resulting effect is shown in the upper curve marked *violet*. It begins after a period of delay of 0·22 second, and attains a maximum of ·0003 volt at 0·6 second.

The quantity of light traversing the slit was carefully adjusted so as in all cases to be equal, so far as could be ascertained by the photometric comparison on Lummer-Brodhun's principle, with the light of the standard candle situated at a distance of 76 cms.

The experiment shows that as regards magnitude of response the shorter red and the longer green illuminations were equally effective stimuli, whilst the longer blue-violet was perceptibly less effective; it also shows definitely that the period of delay of the red response was the longest, that of the green response the shortest, whilst that of the blue-violet was longer than the green one but distinctly shorter than the red one. It is noteworthy that the delay of the green response is quite as short as that of responses produced by white light which were described by me in my previous paper in this *Journal*.

Exp. (2). The eyeball was arranged as described in experiment (1), but in this case the successive order of illuminations was altered, the first effect being now produced by the light of the violet end of the spectrum. The violet light lasted for 2·2 seconds and an analysis of the response is given in the curve marked *violet* in the lower group of Fig. 7. The response began after a period of delay of 0·22 second and reached the exceptionally high maximum of ·0004 volt at 0·6 second, the largest as yet observed. After a period of darkness of 6 minutes and the appropriate shifting of the chamber the eye was illuminated by the red region of the spectrum: the resulting response is shown in the curve marked *red* in the lower group of Fig. 7. This began after a period of delay of 0·28 second, and reached a maximum of ·0005 volt at 0·6 second. After a similar period of darkness and appropriate shifting

of the chamber the eye was illuminated by a green light of quite short duration, 0·5 second; the analysis of the response is shown in the earliest and largest of the curves marked *green*, in the lower group of Fig. 7. It is remarkable for its short latency, only 0·16 second, and for its large magnitude which in spite of the short duration of the light reached a maximum of ·0007 volt at 0·6 second. The green illumination was repeated 6 minutes later, and in this case lasted for 2·3 seconds, it evoked the response of which the analysis is shown in the second of the curves marked *green* in the lower group of Fig. 7. It began after a delay of 0·18 second and reached a maximum of ·00055 volt at 0·8 second.

Exp. (3). In this experiment an eyeball was illuminated for longer periods of time than in the preceding. The order was as follows: first red for 6 seconds, then violet for 6 seconds, then green for 5·5 seconds, and finally red again for 5·5 seconds, periods of 6 minutes' darkness elapsing between each of the successive illuminations. All the illuminations evoked responses, but in addition the cessation of the illumination was followed in the case of the red and green light by terminal "dark responses." The periods of delay of the illumination responses were 0·3 in the case of red, 0·28 in the case of violet, 0·21 in the case of green, and finally 0·3 again in the case of red. The periods of delay of the terminal or "dark responses" were as follows; on the cessation of the red illumination 0·18 and 0·2 second, on the cessation of the green illumination 0·18 second.

Exp. (4). In this experiment there were the same 6 minute intervals of darkness between the successive illuminations, but the duration of the period of illumination was more prolonged, being 8 seconds, whilst the order was varied, the green illumination being the first, then the violet, and finally the red.

Responses were obtained in each of these regions of the spectrum; the periods of delay were as follows; with the green light 0·18 second, with violet light 0·26 second; with red light 0·26 second. The longer duration of the illumination allowed of its being sufficient to ensure that its cessation should evoke "dark responses"; those following the cessation of the green and red light were very distinct and in each case the period of delay was 0·18 second, the dark response following the violet light was feeble and its starting point is a little uncertain on the record but the period of delay is certainly not more than 0·2 second.

Exp. (5). The order of the successive illuminations in this experiment was first red, then green, and finally violet, the duration of illumination being in each case 5·5 seconds and the periods of darkness 8 to 10 minutes. The responses obtained admitted of very accurate measurement, the response to red commenced after a delay of 0·28 second, that to green after a delay of 0·2

second, and that to violet after a delay of 0·26 second. Feeble responses occurred on the cessation of both the red and green illumination, these "dark responses" both commencing after a delay of 0·2 second; there was no dark response on the cessation of the violet illumination.

In the foregoing five experiments it will be noted that the order has been varied very considerably, the succession being (1) first red, then green, then violet; (2) first violet, then red, then green; (3) first red, then violet, then green; (4) first green, then violet, then red; (5) first red, then green, then violet. All the red responses are characterised by their long periods of delay varying from a minimal duration of 0·26 second to a maximal of 0·3 second. The green responses are all characterised by a short period of delay varying from a minimal duration of 0·16 second to a maximal of 0·2 second; the violet responses show a delay which is intermediate as regards duration between those just referred to and shows more variability, having a minimal duration of 0·22 second and a maximal of 0·28 second; these results are not perceptibly affected by the order of the successive illuminations. Further in spite of the differences observed in the delay of the illumination responses to red, green, and violet light, the "dark responses" occurring when the illumination ceases are all characterised by a uniform short delay varying between 0·18 and 0·2 seconds.

Exp. (6). Various attempts were made to compare the responses produced by the light from other regions of the spectrum. It was found that on either side of the green region the response always tended to resemble in its time-relations that evoked by the green light, *i.e.* it showed a shortened period of delay and an increased magnitude of effect. In the following experiment the illuminations were first red, then that of the D line (*i.e.* red and green mixture), then green, and finally blue-green. The red evoked a large response with a characteristic long delay of 0·275 second and reaching a maximum of ·0005 volt; the D line neighbourhood evoked a larger response, having a shorter delay of 0·2 second and attaining in 0·8 second a maximum of ·00055 volt; the green evoked a characteristic response, with a short delay of 0·18 second and a maximum of ·0006 volt; the blue-green evoked one with a delay of 0·2, this was of smaller magnitude, attaining a maximum of ·0004 volt at 0·6 second.

It thus appears that on each side of the green region in the so-called yellow region and in the blue region, effects are produced which might be explicable as due to the undoubted shading off of the green portion of the spectrum.

The results here brought forward confirm those previously described obtained with coloured filters, as regards the long delay of the effect which characterises the response to red light and also the high maximum which such a response can reach; they show that the response to the violet end of the spectrum resembles that obtained with the blue-violet filter in the comparatively low maximum to which it attains and in the circumstance that the response has a delay shorter than is obtained with red light. In addition to this confirmation the spectrum experiments bring forward evidence as to the stimulating efficiency of the middle green region; the response obtained by this illumination resembling both in its short delay and its magnitude that evoked by white light. It is possible that the very remarkable similarity in the delay, which is from 0·16 to 0·2 second in the case of both green and white light, may be a coincidence, but it is more probable that it is based upon the identity of the exciting agent in the two cases; in other words, it is the green components in mixed white light, using the term in its widest sense, which initiate the quick response which this light evokes. It might be thought that the luminosity of the green portion of the spectrum is the chief agent in producing this more rapid response; but this can hardly be the case since the red region which has a high luminosity although it evokes a response of considerable magnitude, is especially characterised by always producing one with a long period of delay.

There are considerable differences as to the maximum of the response produced by any given light in different samples of eyeball, but the following table indicates the maximum attained in a number of different specimens.

Maximal E.M.F. of Photo-electric Response.

Red light	Green light	Violet light
·00035 volt	·00059 volt	·00025 volt
00045	00049	00030
00035	00045	00018
00040	00049	00020
00038	00054	00025
00034	00055	00024
00042	00054	00023
00050	00070	00040
00030		00027
00040		00012
00033		00020
00032		00011
00030		00030
00052		00036

The red response varies between ·00052 and ·0003, it averages ·00038 volt; the green response varies between ·00067 and ·00043, it averages ·00054 volt; the violet response varies between ·00040 (an exceptionally large one) and ·0001, it averages ·00024 volt.

In considering all the evidence afforded by the records of the responses of the eyeball of the frog to coloured light, it will be noticed (1) that green light evokes a response characterised by comparatively short latency, and that this reaches the highest maximum; (2) that red light evokes a response of the same sign but characterised by much longer latency, and that whilst it also reaches a high maximum, it falls a little short of the green effect; (3) that violet light evokes a response of the same sign characterised by a latency shorter than that caused by red but longer than that caused by green, and especially characterised by its smaller amount. It will be further noticed that the cessation of adequate illumination causes a response having the same sign as the illumination one but characterised by a constant short latency.

The inference to be drawn from these facts as to the time-relations of the responses appears to be that the frog's eye always reacts to stimulation by developing excitatory processes of the same fundamental type, but that having regard to the time-relations of these processes we are justified in assuming at least four different varieties of change; (*a*) the change produced by red illumination; (*b*) the change produced by green illumination; (*c*) the change produced by violet illumination; (*d*) the change produced by sudden darkness. These four different reactions have the same general character but are distinguished either by the duration of the period of delay or by the extent of the process as judged by the maximal E.M.F. developed.

If it is permitted to associate these results with the well-known theories of colour vision, then it is evident that the Young-Helmholtz theory which assumes three primary colour reacting substances, red, green, violet, is in remarkable accordance with the data just put forward. In one respect only are the photo-electric responses not in accord with that theory, for although the responses appear to show that there is a substance capable of being stimulated by darkness there is no such fourth reacting substance postulated in the theory. On the other hand this is the only fact which is in any sense applicable to the rival theory advanced by Hering, and even this is not in accordance with the assumption of Hering that the sensation of black although caused by stimulation is brought about by a change of opposite chemical type to that produced by white. The photo-electric responses being all

of the same fundamental type are not in harmony with the assumption that there are three reacting substances, a red-green one, a blue-yellow one, and a white-black one, and that the process is of opposite sign (dissociation or association) in each member, a process of one sign occurring with red, blue, or white, whilst that of the opposite sign occurs with green, yellow, or black.

The evidence furnished by the electrometer records confirms in this respect the conclusions arrived at by Waller with reference to the character of the retinal currents of the frog's eye excited by light. In the account which he gives of the galvanometric effects produced by coloured light he states, "No complementary or antagonistic influence as regards retinal response is to be detected in any of these experiments. All colours seem to act in the same direction more or less powerfully according as they are more or less luminous[1]."

So far therefore as the photo-electric responses in the frog's eye can be justifiably utilised in connexion with the subject of colour vision, it would appear that these are in remarkable harmony with the three colour theory of Helmholtz as modified by Maxwell, whilst they are in direct conflict with the theory advanced by Hering.

Burch[2] concludes from the results of an elaborate investigation into the phenomena of artificial colour-blindness that there are four primary colour sensations, red, green, blue, and violet. It is quite possible that with sunlight, some evidence of a specific photo-electric response to blue may be forthcoming; there is no such distinct evidence in the case of the light from an electric arc such as was employed in the present research, but as this light is deficient as regards blue when compared with sunlight, the negative character of the results at present obtained cannot be regarded as conclusive.

The method opens up several fruitful lines for future investigation, the most fundamental of these is the relation between the responses which are produced by monochromatic light of different known luminosities. If satisfactory results can be obtained, and the question of submaximal and maximal stimulating efficiency thus determined, then it will be possible to apply a drastic test for the purpose of ascertaining how far the red, green, and violet responses are due to changes in different retinal substances. This test is the photo-electric effect caused by simultaneous, successive, and superimposed illumination by these different colours. If the substances which are affected by red, green,

[1] Waller. *Phil. Trans.* 193 B. 1900, p. 137.
[2] Burch. *Phil. Trans.* 191 B. 1899, p. 32.

and violet light are distinct, then successive maximal responses evoked by these primary colours should be superimposed each with its proper period of delay, whilst the fatigue produced by prolonged illumination with one colour should not seriously affect the capacity of the eye to respond to another. These two investigations I hope to be able to carry out after the necessary modifications in the existing apparatus have been made.

Summary of results and conclusions.

1. Photo-electric responses giving analysable records with the capillary electrometer are obtained when the excised eyeball of the frog is subjected to the influence of coloured light, whether this light is obtained by coloured filters or by using definite regions of the spectrum.

2. Such photo-electric responses fail or become extremely feeble if the eyeball is placed in the infra-red or ultra-violet regions of the spectrum; in the latter case an uncertain factor is involved, that of fluorescence, which needs further investigation.

3. The range of light vibrations within which the frog's eyeball gives definite photo-electric responses, corresponds very closely to the range of vision in the case of our own colour sensations.

4. The records obtained by the capillary electrometer afford data from which the time-relations of the response to any given colour can be determined.

5. All the responses are of the same general type, whether they are evoked by white or by coloured light.

6. An additional response is obtained when the light is suddenly replaced by darkness, this is of the same fundamental type as the illumination response.

7. There is no evidence of any excitatory process except those of the fundamental type; this type shows itself electrically by a difference of potential between the fundus and the cornea of such a character that a current flows through the eyeball from the former to the latter.

8. The time-relations of the responses evoked by the various coloured lights and by darkness are not identical. The obvious differences in this respect are sufficiently distinct as to suggest four responses independent as regards their causation.

9. The four distinct responses are the following:

(a) The response to red light characterised by a long latency of nearly $\frac{3}{10}$ second and by its attaining to a considerable maximum averaging about ·0004 volt.

(*b*) The response to green light characterised by the same short latency as that found in the response to white light, *i.e.* less than $\frac{2}{10}$ second; it is also characterised by its magnitude, the maximum reached averaging over ·0005 volt.

(*c*) The response to violet light characterised by a latency longer than that of the green response but distinctly shorter than that of the red one ($\frac{25}{100}$ second). It is also characterised by its low intensity, the maximum reached averaging only ·00024 volt.

(*d*) The response to sudden darkness, characterised by a remarkably constant latency of not more than $\frac{2}{10}$ second, whatever the character or quality of the previous illumination; this response is dependent for its production upon the change from previous illumination to the condition of darkness and varies in magnitude with the duration and luminosity of the previous light. It is most readily obtained if the previous light has been white, is easily obtained if this has been green or red, but is only obtained after violet illumination when this has lasted for some time, generally 8 seconds or more.

10. The results appear to be in accordance with the theory of Young-Helmholtz as modified by Maxwell, which assumes three distinct colour reactions, viz. red, green, and violet. In addition they appear to indicate that the eye reacts to sudden darkness.