STUDIES IN LUMINESCENCE. II.

By Edward L. Nichols and Ernest Merritt.

A Spectro-photometric Study of Fluorescent Solutions Belonging to Lommel's First Class.[1]

THE law expressed by Stokes[2] in his memoir entitled "The change of Refrangibility of Light" to the effect that in fluorescence the fluorescent light is always of greater wave-length than the exciting light has been called in question by Lommel, who pointed out that for certain fluorescent bodies there is an unmistakable overlapping of the regions in the spectrum occupied by the exciting light and by the fluorescence which it produces. Lommel made the further very important statement that for this class of substances the character and composition of the fluorescence spectrum is independent of the wave-length of the exciting light. Lommel's results in so far as they had to do with the non-validity of Stokes's law were confirmed by Hagenbach.[3] A few years later Lubarsch[4] published measurements in confirmation of Stokes's law. A later paper by Lommel,[5] in which he described the fluorescence of the so-called chameleon colors, led Hagenbach to new experiments, in the course of which he discovered what he believed to be a source of error in his former measurements, and he reaffirmed the law of Stokes for all such substances. In 1877 Brauner[6] obtained results in confirmation of Lommel's view. In 1879, Lubarsch[7] published further experiments on fluorescence, this time in favor of Lommel's results. Lamansky[8] in 1879 described meas-

[1] Read at the St. Louis meeting of American Physical Society, Dec. 29, 1903, under the title "The Spectrometric Study of Fluorescence."

[2] Stokes, Phil. Transactions 1852, p. 463.

[3] Hagenbach, Poggendorff's Annalen, 146, pp. 65, 232, 373, 508.

[4] Lubarsch, Poggendorff's Ann., 153, p. 428, 1874.

[5] Lommel, Poggendorff's Ann., 159, p. 514, 1876.

[6] Brauner, Wiener Anzeiger, 19. p. 178, 1877.

[7] Lubarsch, Wied. Ann., 6, p. 248.

[8] Lamansky, Comptes Rendus, 88, p. 1192, 1879.

urements in confirmation of Stokes's law. In a still later paper Hagenbach[1] returned to the defence of Stokes's law as against Lommel[2] and Lubarsch,[3] who in the meantime had published further articles dealing with his objections and criticizing Lamansky's method. Wesendonck[4] in 1885 made observations with the sun's spectrum, using two concave mirrors and a prism, in the course of which he obtained very conclusive evidence that the fluorescence of naphthalin-roth extended to wave-lengths shorter than that of the exciting light. In 1886, Stenger[5] took the question up at length. He found that whether he used Hagenbach's method of illuminating the free surface, Lommel's method of grazing incidence through the side of a flask, or Lubarsch's fluorescent eye-piece, his measurements confirmed Lommel as to the invalidity of Stokes's law but not as to the independence of the fluorescent spectrum from the character of the exciting light. He also made experiments in colaboration with Hagenbach, who was finally converted to the same view.

It is our purpose in this paper to describe results obtained by the application of the spectrophotometer to the measurement of the fluorescence spectrum of those substances concerning the fluorescence of which, especially with reference to the validity of Stokes's law, the long-continued discussion already described arose. No attempt apparently has been made to apply the spectrophotometer to the study of fluorescence; yet it is obviously possible to determine both the limits and the maximum of a spectral region for which a curve of intensities can be plotted with far greater accuracy than by the method hitherto pursued by all observers, *i. e.*, that of attempting to set the cross-hair in the eye-piece of a spectroscope in the region of greatest brightness, or at the point where the spectrum ceases to be visible. Experimenters have perhaps been deterred from the use of the spectrophotometer because of the faintness of fluorescence spectra. It is true that fluorescent light from many substances is so weak as to preclude all measurements of its spectrum; but it is

[1] Hagenbach, Wied. Ann., 18, p. 45.

[2] Lommel, Wied. Ann., 8, p. 244, 1879.

[3] Lubarsch, Wied. Ann., 9, p. 665, 1890; also Wied. Ann., 11, p. 68, 1880.

[4] Wesendonck, Wied. Ann., 26, p. 521, 1885.

[5] Stenger, Wied. Ann., 28, p. 201.

also true, as we have found in the course of the experiments to be described, that settings can be made in cases where the brightness of the spectrum is far below that necessary to arouse the sense of color and where the presence of light can be detected only after prolonged shielding of the eye. The use of the cross-hair in such cases is out of the question, for the field is much too dim to render it visible, while every attempt to illuminate it from the side would flood the eye-piece with light sufficient to quench that under observation.

The instrument used in most of our observations was the spectrophotometer of Lummer and Brodhun. In order to secure the greatest possible sensitiveness to weak fields of view the ocular lenses in the eye-piece were used, the eye being focussed upon the aperture in the eye-piece and not upon the face of the prism. By means of metal screens attached to the collimator slits of the

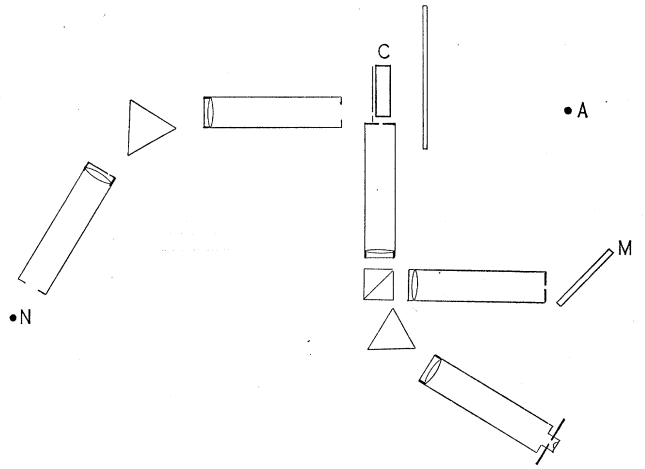

Fig. 1.

instrument the length of slit was regulated so as to avoid overlapping of the spectral images and to give two contiguous spectra in the field of view. One loses by this method the advantage of the method of contrast, but the sensitiveness of the instrument for low intensities is greatly increased. Since in most cases it was desired to employ monochromatic light for the excitation of fluorescence, the spectrophotometer was employed in connection with a large spectrometer as shown in Fig. 1. The eye-piece and slit of

the spectrometer were removed, and in place of the latter a Nernst filament (N) was mounted vertically. This filament afforded a nearly linear source of light, giving a sufficiently powerful continuous spectrum. The filament was attached to the arm carrying the collimator tube so as to move with the latter and to remain in the vertical plane passing through its axis. The observing telescope was clamped, and different portions of the spectrum were brought into the field as desired by movements of the collimator tube. The liquid, the fluorescence of which was to be studied, was placed in a rectangular cell (C) upon one face of which a real image of the spectrum was focussed. This face of the cell was provided with a metal screen having a vertical opening 1 mm. wide through which the light used for exciting fluorescence could pass. This opening (see Fig. 2) was so placed that the exciting light entering the cell

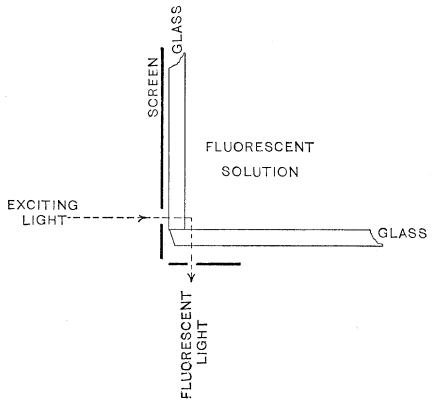

Fig. 2.

would be parallel to the adjacent face of the cell and as near to the same as practicable. In adjusting the metal screen one edge of the slit or opening was made to exactly cover the glass forming this face of the cell, as shown in the figure. The fluorescence spectrum was observed from a direction at right angles to the path of the exciting beam, and in order to bring the brightest fluorescence regions of the liquid into the field the vertical plane of the collimator of the spectrophotometer was adjusted so as to bring into the field of view the layer of liquid lying next to the face of the cell through which the exciting light entered. By this arrangement, the width of the

opening through which the exciting light entered the cell and the width of the slit of the spectrophotometer each being 1 mm., the average depth of liquid within which fluorescence was produced was approximately 0.55 mm. and the average distance which the fluorescent light passed through the liquid before leaving the cell was also 0.55 mm.

The source *A* of the comparison spectrum (Fig. 1) was an acetylene flame, the light from which was reflected diffusely from the face of the block of magnesium carbonate (*M*) mounted at an angle of 45° at the end of the collimator slit. Measurements were made by varying the width of the slit until the two regions of the spectrum under observation were equally bright.

The substances specified by Lommel as belonging to his first class, in which it is possible to excite fluorescence by means of light of wave-length longer than that of a portion of the fluorescence spectrum, and in which the distribution of intensities in the fluorescence spectrum is independent of the character of the exciting light, are *naphthalin-roth*, *eosin*, and *chlorophyll*. To this list Stenger added the substance *fluorescein*. The last-named substance, on account of its intense fluorescence and the location of the fluorescent band in the middle of the spectrum, in the regions of the highest luminosity, was selected for detailed study for the purpose of testing the conclusions reached by Lommel and the other investigators mentioned in the opening paragraphs of this paper.

Ten cubic centimeters of alcohol were saturated with fluorescein at room temperature and the solution was filtered. To 40 c.c. of distilled water one drop of a normal solution of sodium carbonate Na_2CO_3 was added. Two parts of the concentrated alcoholic solution were then mixed with 100 parts of the water thus rendered alkaline. The fluorescence spectrum of this solution, excited by the undispersed rays of the acetylene flame, was first measured; the distribution of intensities being compared, as in nearly all our subsequent experiments, with the spectrum of the light diffusely reflected from the surface of the the block of magnesium carbonate shown in Fig. 1. The absorption spectrum of the solution was then taken, the transmission through the cell, which had a thickness of 1.1 cm., being measured by means of the spectrophotometer.

The source of the transmitted light was a second similar block of magnesium carbonate illuminated by the same acetylene flame that served for the comparison spectrum.

Much stress having been laid by some of the previous observers upon the influence of stray light, the following measurements were made. The cell was filled with distilled water, set up precisely in the position in which it had been placed in the study of the fluorescence spectrum, and similarly illuminated by means of the acetylene flame. No measurable stray light was found, but an exceedingly weak fluorescence spectrum due to the glass walls of the cell was detected. Since the maximum of the fluorescence spectrum of the glass was found to lie further to the violet than the fluorescence spectrum of the fluoresceïn, and since moreover it was of scarcely measurable intensity, it was not deemed necessary to take further cognizance of these sources of error.

To determine the fluorescence spectrum of the solution when excited by monochromatic light, a mercury arc-light of the Lummer pattern was set up in front of the slit of the spectrometer. It was found that the violet lines from the spectrum of this arc produced fluorescence corresponding, as regards the position of the maximum and the general form, with the curve previously obtained by means of the light of the acetylene flame, but that the green line ($\lambda = .575$) excited no fluorescence. This latter result was to be expected since this light lies altogether outside of the absorption band of the solution in a region for which almost complete transparency exists. The spectrum of the acetylene flame was subsequently tried as an exciting source, but it was too weak to give easily measurable intensities of fluorescence. The slit of the spectrometer was then removed and a Nernst filament was mounted in the axis of the collimator tube as described in the specification of the apparatus and shown in Fig. 1. This filament was found to be of abundant brilliancy. It was maintained at constant brightness by means of a variable resistance, which was adjusted whenever the fluctuations of an ammeter placed in the electric circuit indicated it to be necessary.

Measurements of the fluorescence spectrum of the solution were made by means of the spectrum of the Nernst filament using as exciting light three nearly monochromatic regions of wave-length

$\lambda = .518\ \mu$ to $.536\ \mu$, $\lambda = .487\ \mu$ to $.502\ \mu$ and $\lambda = .460\ \mu$ to $.471\ \mu$. The curves thus obtained are plotted in Fig. 3, together with the

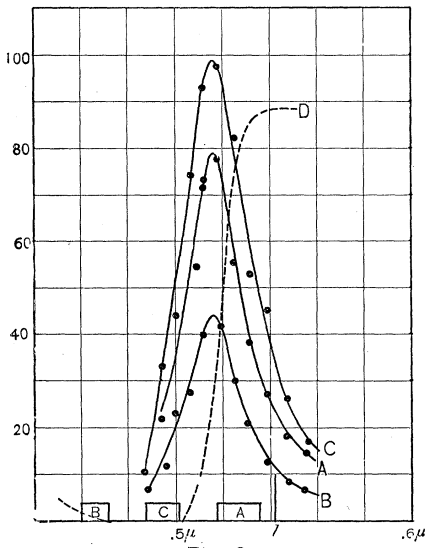

Fig. 3.

Fluorescein. Fluorescence spectra obtained when the exciting light lies in different regions of the spectrum. For curve *A*, the exciting light was confined to the region marked *A* on the horizontal axis, etc. Vertical scales arbitrary.

curve of transmission for the solution. It will be seen from this figure that the maximum of intensity of the fluorescence spectrum in these three cases lies in the same region at $.517\ \mu$ and that there is no evidence of any shifting of the fluorescence spectrum with the wave-length of the exciting light. It is obvious, moreover, that not only is it possible in the case of this solution to obtain fluorescence of refrangibility less than that of the exciting light, but that in the case of the curve marked *A* the maximum of the fluorescence spectrum is of shorter wave-length than the shortest wave-length used in excitation. These curves likewise agree fully in character and as regards the position of their maximum, with that for the fluorescence spectrum of the same solution when excited by the undispersed light of the acetylene flame. (Not shown in the figure.)

These curves for the fluorescence spectrum do not correspond precisely with the typical curve, meaning by that term the curve representing the distribution of intensities in the fluorescence spectrum of the surface layer of the fluorescing liquid. It is possible, however, in the case of a non-turbid medium, to compute from the observed curve the approximate form of the typical curve. Fig. 4 shows

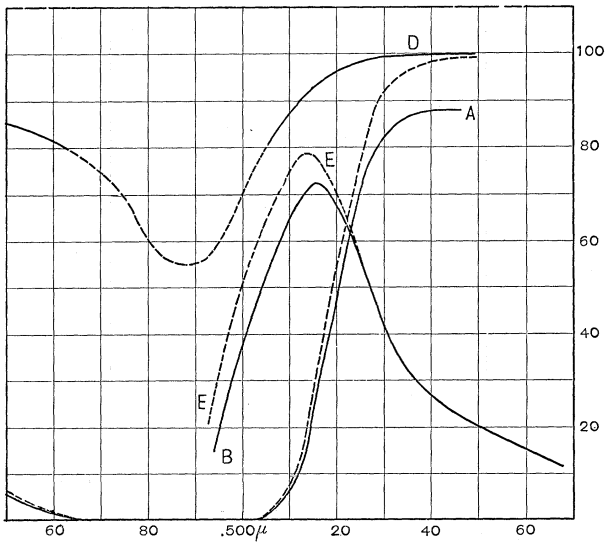

Fig. 4.

Fluorescein: typical fluorescence spectrum (E E).

graphically the result of such a computation.[1] Curve A gives the transmission of a glass cell containing a layer 1.1 cm. in thickness of the fluorescein solution. The dotted curve of similar form gives the transmission corrected for losses in the cell when filled with distilled water. From this by the well-known law of variation of absorption with the thickness, the curve D is found for a layer 0.055 cm. in thickness, which is the estimated mean distance through the solution which the fluorescent light passes before entering the slit of the spectrophotometer. The curve B is the observed curve of the distribution of the intensities in the fluorescence spectrum, and from this was computed the curve E, which represents, as nearly as the accuracy of the data will allow, the typical curve for this

[1] In this figure and in Figs. 5, 6, 7 and 8 the scale of wave-lengths has been doubled.

substance, corrected for absorption. It will be seen that in the case of this solution, under the conditions of the measurements, the absorption of the fluorescent light by the solution produces only a slight shifting of the maximum toward the shorter wave-lengths. When the fluorescent light passes through a considerable layer of the solution the effects of absorption are much more marked and there is a decided change of color. When a thin layer of the solution of fluoresceïn is viewed by reflected light its color is green, whereas the fluorescence of a mass of the liquid appears decidedly yellowish. This change is shown graphically in the curves of Fig. 5. In this figure *A* is the transmission curve

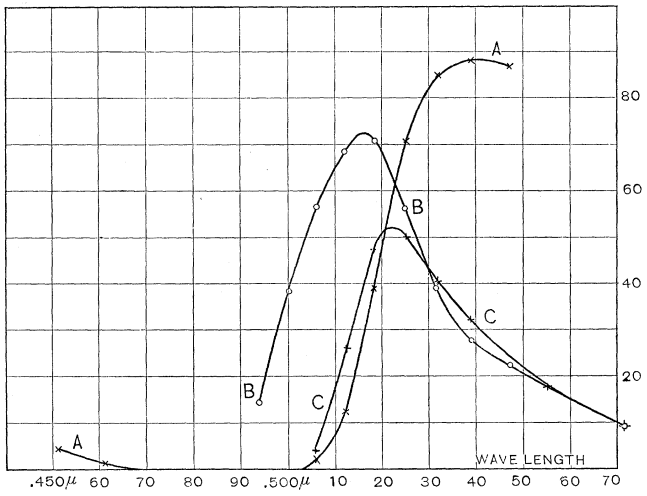

Fig. 5.

Fluoresceïn. Effect of absorption upon the fluorescence spectrum.

of the solution; *B* is the observed curve of fluorescence when the slit through which the excited light enters the cell is placed so that the fluorescent light passes through .055 cm. of the solution before exit; and *C* is the fluorescence curve when the slit is shifted to such a position that the fluorescent light passes through 1 cm. of the solution. It will be seen that the maximum is shifted from .516 μ to .522 μ, and that while the two curves are nearly coincident on the side towards the red the values on the other side of the curve fall off very rapidly as the result of the increased absorption. The

boundary of the fluorescence spectrum towards the violet in the one case would lie at about wave-length .505 μ, whereas in the thinner layer it would be visible to at least .490 μ. It is obvious that the color of the fluorescence in the latter case will contain a great excess of green.

The effect of diluting the fluorescent solution is similar to that of diminishing the distance through which the light passes. The results of observation upon a solution of fluoresceïn, diluted until the intensity of the fluorescence spectrum was diminished as far as would permit of satisfactory readings, are shown in Fig. 6. The

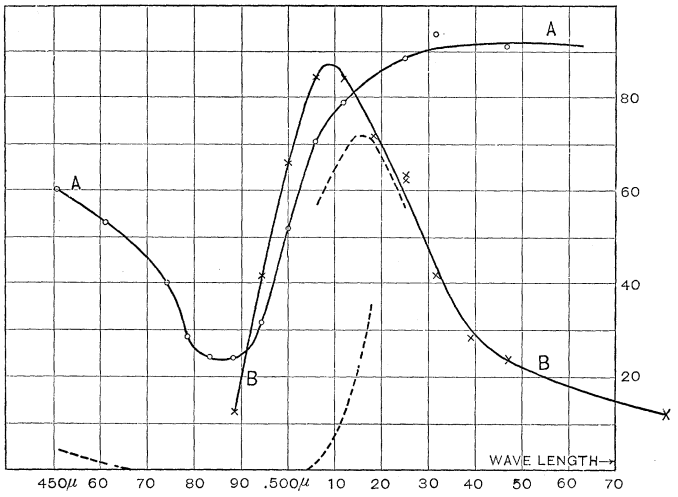

Fig. 6.

Fluoresceïn. Effect of dilution upon the fluorescence spectrum.

curve AA represents the transmission of the cell filled with the dilute solution and BB is the distribution curve of its fluorescence spectrum. The dotted lines show the corresponding transmission curve and a portion of the fluorescence curve for the solution before dilution. It will be noted that in this case, as in the case of the comparison of thick and thin layers of a given solution, the curves are coincident toward the red, but that the dilute solution has its maximum shifted towards the green ; also that the ordinates on this side of the curve show an increase indicative of the change of color, which, as is well known, is always observed as the result of diluting the fluorescent solutions of this substance.

Although, as has been shown in Figs. 4 and 5, the modifications produced by absorption in the curves of the fluorescence spectrum may be very marked, the effect of absorption diminishes rapidly with dilution of the solution. If we apply the correction for absorption, for example, to the curve for the dilute solution in Fig. 6 we find as is indicated in Fig. 7 that the change is insignificant. In

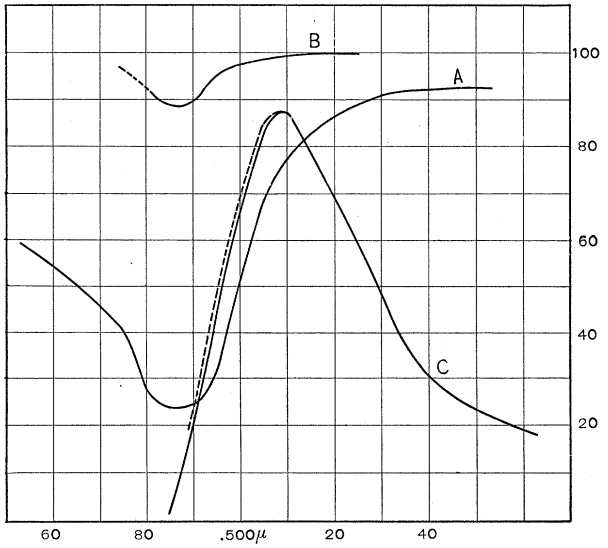

Fig. 7.

Fluoresceïn. Typical fluorescence spectrum for dilute solution.

this figure *C* is the observed curve for the fluorescence of the dilute solution, *A* the transmission curve of a layer 1.1 cm. in thickness, and *B* the computed curve for the transmission of the mean layer of liquid through which the fluorescent light has to pass. The correction for this absorption is indicated by means of the dotted line.

It having been established that the fluorescence of bodies of this class is independent, as regards the distribution of intensities, of the wave-length of the exciting source, it would be of interest to inquire whether the fluorescent energy for a given wave-length of the fluorescence spectrum varies with the wave-length of the exciting light, the energy of which is constant, or whether it depends only upon the energy. The rigorous determination of these relations

involves a knowledge of the distribution of energy in the spectrum of the exciting source, a difficult matter to determine with accuracy for the shorter wave-lengths of the visible spectrum. The curve shown in Fig. 8 may, however, be of some interest in this connec-

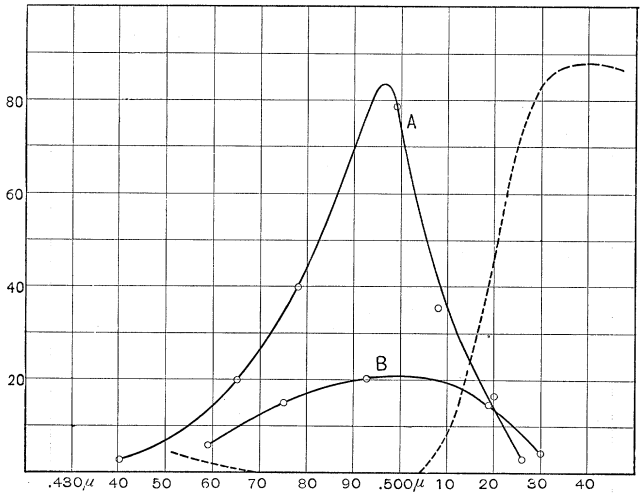

Fig. 8.

Fluoresceïn. Intensity of fluorescence as a function of wave length of exciting light. A refers to Nernst glower as source, B to acetylene flame.

tion. It represents the intensity of fluorescence, taken at the maximum of the fluorescence spectrum of the solution of fluoresceïn, as a function of the wave-length of the exciting light. Curve *A* was taken with the Nernst filament as a source, curve *B* with the acetylene flame. The dotted line shows the absorption band for a layer of the solution 1.1 cm. thick. It will be seen that fluorescence begins approximately at the wave-length at which the solution seems to be transparent, and that the maximum lies well within the absorption band but is shifted to the red. The longer wave-lengths within the band are more effective on account of their greater energy. The difference in the form of the curves *A* and *B* is probably ascribable to the different distributions of energy in the spectra of the sources of light employed.

In addition to the measurements on fluoresceïn the positions of the fluorescence spectra of solutions of eosin and naphthalin-roth

were determined by the method already described, and the transmission curves of the solutions were taken. Dilute solutions in alcohol were made, that of the naphthalin-roth being about $\frac{1}{200}$ saturated. The results obtained with these solutions, which are shown in Figs. 9 and 10, afford additional corroboration of the statements of Lommel. They are indeed in every respect analogous to those obtained with fluoresceïn and lead to the same conclusions. Each solution was excited by three distinct regions of the spectrum, one lying as far toward the red as practicable, one towards the blue and one at an intermediate wave-length. Curves showing the dis-

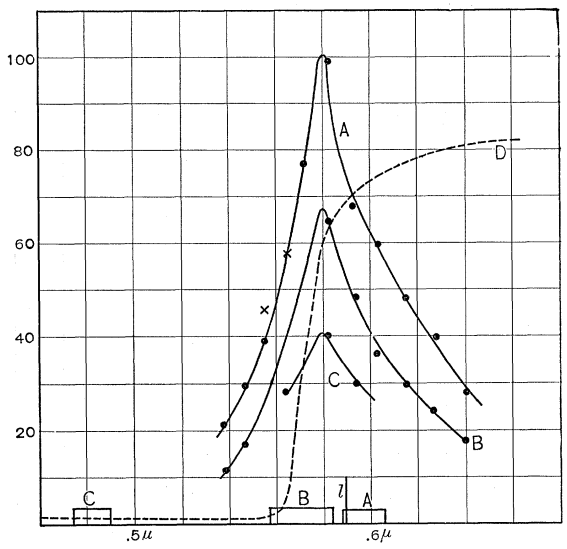

Fig. 9.

Eosin. Fluorescence spectra observed when the exciting light lies in different regions of the spectrum. Curve *A* was obtained when the exciting light was confined to the region marked *A* on the axis of wave-lengths, etc. Vertical scales arbitrary.

tribution of intensities in the three spectra thus produced are shown in the figures and it will be noted that, as in the corresponding curves for fluoresceïn, the position of the maximum is entirely independent of the wave-length of the exciting light and that the general character of the curve remains unchanged. In the case of these two solutions, as in that of fluoresceïn, it was possible to obtain a measurable amount of fluorescence by the use of light of

greater wave-length than that of the maximum of the fluorescence spectrum. The form of the fluorescence curve is very similar for these three substances but each has its own place in the spectrum. The maximum for fluorescein (Fig. 3) is at .517 μ, that for eosin at .580 μ and that for naphthalin-roth at .594 μ. The position of the maximum of these three curves with reference to the absorption band appears to vary with the different substances. The maximum for eosin coincides approximately with the infra-edge of the absorption band; that for fluorescein lies slightly (about .05 μ) towards the violet, while the maximum for naphthalin-roth is much further displaced towards the short wave-lengths.

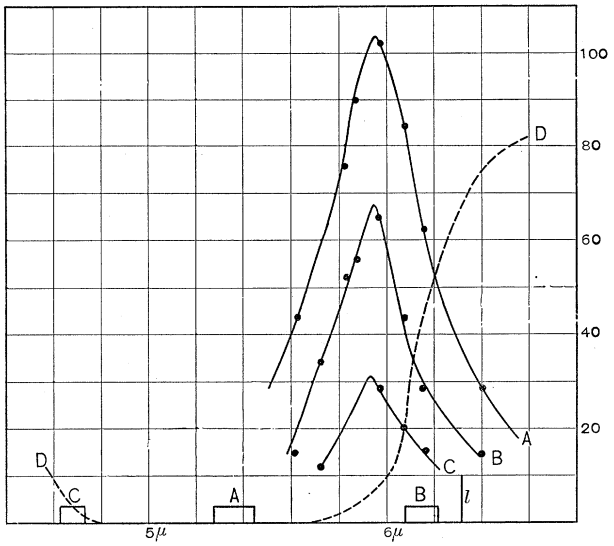

Fig. 10.

Naphthalin-roth. Fluorescence spectra obtained when the exciting light lies in different regions of the spectrum. Curve *A* was obtained when the exciting light was confined to the region marked *A*; etc. Vertical scales arbitrary.

Lommel's contention that it is possible to excite fluorescence in eosin by means of the light of the sodium flame is fully confirmed by the data plotted in Fig. 9, from which it will be seen that the exciting light by means of which curve *A* was obtained had a mean wave-length almost precisely equal to that of the sodium lines. Since it was found possible by means of light, all of which

was of greater wave-length than the maximum of the fluorescence spectrum, to produce fluorescence of sufficient strength for measurement with the spectrophotometer, it follows that *observable* fluorescence can be produced by light of even greater wave-length than that recorded in our diagram.

We deem the evidence already given in the foregoing paragraphs to be conclusive, so far as these substances are concerned; but in view of the differences of opinion among physicists as regards the validity of Stokes's law we venture to add the following description of a determination of the wave-length of the least refrangible monochromatic light which we found capable of exciting fluorescence in the three solutions with which this paper deals.

Since some writers have laid great stress upon the errors due to stray light, two different methods were employed of avoiding it. In the first the exciting light was passed through a solution of the substance to be examined before dispersion, thus filtering out those rays particularly active in producing fluorescence. The filtered light was then dispersed by means of the large spectroscope already described, and a second solution was subjected to an isolated, nearly monochromatic, region of the spectrum. The wave-length of this region was increased until the last trace of fluorescence, observed directly with the eye, was about to disappear. To distinguish between fluorescence and the presence of light diffused from small particles, the light was viewed at an angle of 90° through a Nicol prism, by which means diffuse light was completely excluded. The limit of excitation thus determined lay, as had been anticipated, further to the red than in the cases where a *spectrophotometrically measurable* fluorescence had been obtained, excepting in the case of eosin, where it was found to coincide almost exactly with the ultra-edge of the band used in exciting the spectrum shown in curve *A* (Fig. 9).

The second method consisted in sending the isolated region of the dispersed light to be used for excitation through a second spectroscope and determining as before the limit of excitability. This method of removing stray light has been extensively used and is well known to be effectual. The source of light in both cases was an arc-light.

The results obtained by these two methods were identical. They were as follows :

TABLE.

Substance.	Maximum Wave-length Exciting Observable Fluorescence.
Fluoresceïn,	.542
Eosin,	.589
Naphthalin-roth,	.632

These wave-lengths, all of which lie far to the red from the maximum of the fluorescence spectrum, are indicated in Figs. 3, 8, and 9 by means of the vertical lines marked *l*.

Measurements upon other fluorescent substances, and especially upon those which Lommel regarded as not belonging to his first class, are in progress and the results will be given in a subsequent communication.

PHYSICAL LABORATORY, CORNELL UNIVERSITY,
December, 1903.