THE EXCITATION OF FLUORESCENCE IN FLUORESCEIN

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ABSTRACT

Fluorescence in violation of Stokes' law, excited in an alcoholic solution of specially purified fluorescein, was compared by a photoelectric method with the reciprocal emission when fluorescent and exciting wave-lengths are interchanged. The following theoretical equation, recently proposed by the author, was found to be largely verified: $f_{yx}/f_{xy}=\lambda_x J_y/\lambda_y J_x$. Here f is the exciting power, i.e. $f_{yx}d\lambda_y$ is the emission of fluorescent energy from unit volume in the wave-length range $d\lambda_y$ when the fluorescing substance is illuminated with unit density of exciting radiation of wave-length λ_x, f_{xy} is the same thing with the wave-lengths interchanged, and J_x, J_y are the densities of black-body radiation for these two wave-lengths at the temperature of the fluorescing substance. A peculiar interest attaches to this equation because it contains no unknown constants.

Fluorescence, excitation and absorption curves for the same fluorescein were also determined with moderate accuracy. The fluorescence curves show a single maximum around 5240A but change shape as the exciting wave-length is varied. The excitation curve for fixed incident energy shows a maximum around 5000 for fluorescence at 5270; for other points in the fluorescence spectrum the maximum excitation occurs at shorter wave-lengths, e.g. at 4730 ± 80A for fluorescence at 5009 or 5582. The curves suggest two superposed bands of different width but with almost coincident peaks. The maximum absorption occurs around 4750.

IN a recent paper[1] in the PHYSICAL REVIEW the writer obtained the following theoretical equation concerning the excitation of fluorescence:

$$\frac{f_{yx}}{f_{xy}}=\frac{\lambda_x J_y}{\lambda_y J_x}, \tag{1}$$

where f represents the spectral exciting power,[2] i.e. $f_{yx}d\lambda_y$ is the emission of fluorescent light per unit volume of wave-length between λ_y and $\lambda_y+d\lambda_y$ when the fluorescing substance is illuminated with a density unity of exciting radiation of wave-length λ_x, f_{xy} is the same thing with the wave-lengths interchanged, and J_x, J_y are the densities of black-body radiation at these two wave-lengths. This equation is remarkable

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[1] E. H. Kennard, Phys. Rev. **28**, 672 (1926).

[2] *Note.* The term "exciting power" is applied by Nichols and Merritt (Phys. Rev. **31**, 381 (1910)) to the quantity f/a where a is the absorption. The usage adopted here seems to be a little more in harmony with the common use of the term "power", which usually refers to a determining factor that is directly and readily controlled and measured, such as the exciting intensity in the present case. Perhaps the ratio f/a might be called the "spectral efficiency" of excitation.

in that it contains no unknown constants whatever. If such an equation could be shown to hold for the fluorescence of liquid and solid substances, it should constitute a distinct step forward toward a theoretical understanding of these phenomena; in particular, it would give quantitative support to the familiar suggestion that the great breadth of the bands is due in some way to the energy of thermal agitation. The equation, in an older but substantially identical form, was found to agree with existing data on eosin and resorufin.[3] The purpose of the present investigation was to test its validity on fluorescein, in which the shape of the fluorescence curve could not be assumed to be independent of the exciting wave-length.

The conclusion reached is that Eq. (1) is correct within the experimental errors as regards the implied variation of the anti-Stokes light with temperature, that it holds numerically wherever the anti-Stokes light is relatively bright, and at least approximates to the truth in all other cases tried.

I. Apparatus

In order to measure the spectral exciting power for wave-lengths close together it is necessary to adopt the relatively unusual procedure of resolving both the exciting light and the fluorescence. This necessitates the measurement of very weak light. A visual spectrophotometer was first tried but was found inadequate, the light being about ten times too faint even when the slits were made regrettably wide. The next simplest method appeared to be photography; so a "spectrograph" was improvised on an old mirror-spectrometer base, with a large flint prism, and with good projection lenses mounted, along with the slits, in wooden boxes fastened tightly to the spectrometer arms. It was found, however, that sufficient blackening could be obtained only by many hours of exposure; so with reluctance the troublesome method of photo-electric photometry was substituted.

For this purpose an old Dolezalek electrometer was converted into a "Compton," the original description[4] being followed except that the quadrants were made deeper. The instrument never worked really well; the worst of its irregularities finally ceased only when it was surrounded with a thick layer of cotton batting. The sensitivity was usually 10–15000 mm per volt at 250 cm. The photo-electric cell was one of the simplified Kunz type supplied by the Central Scientific Company; a special cell obtained from Professor Kunz himself was also tried but was not found much superior under the conditions of the experiment and was not used after being damaged by blue-glow discharges. Much time was lost in trying to compensate for a persistent

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[3] E. H. Kennard, Phys. Rev. **11**, p. 37 (1918).

[4] A. H. and K. T. Compton, Phys. Rev. **14**, 85 (1919).

positive leak; for a high resistance, ionized air, alcohol, and India-ink lines on paper (K. T. Compton) were all tried but steadiness could not be obtained. Finally this drift was simply endured and in case of need partially compensated by allowing a little light to leak onto the cell from a lamp fed by a storage battery. The cell was mounted in an air-tight chamber, dried with P_2O_5, immediately under the electrometer; the spectrograph slit was placed about 20 cm from it, to allow a little freedom for manipulation, but the light path was carefully screened. By means of B-batteries and dry cells a voltage varying from 225 to 229.5 volts was applied to the cell. As the voltage rose above 226.5 volts the sensitivity rapidly increased but large irregularities began to appear in the response to light, hence the higher voltages were avoided as much as possible. The rate-of-drift method was employed, but proportionality of deflection rate, corrected for dark drift, was relied upon only in subsidiary observations.

The exciting light was furnished by a new Hilger constant-deviation spectrometer illuminated by a 400-watt tungsten lamp, whose plane-spiral filament was turned almost edge-on and then focussed on the slit through large lenses. The current through the lamp was carefully held constant, and the combined system was calibrated as to transmitted energy with a Coblentz thermopile and galvanometer. Tests made later showed no detectable change in spectral distribution over the range here used even when the blackening was carried to the point where the lamp usually failed.

Spectral calibration of both instruments was made with lines from a hydrogen-helium tube and from mercury and iron arcs, a reference point being inserted in the exit slits. The slits were then adjusted to the desired width (from 40 to 78A), entrance and exit slits of each instrument being set about equally wide in terms of transmitted wave-length, and readings were taken of the first appearance and of the final disappearance of the green mercury line as the setting was altered; the mean of these two readings was taken as the setting at which this line was transmitted centrally through the slits, and from it a correction to the calibration curve was computed for use in making settings upon other wave-lengths. Half of the difference of the two readings was taken as the slit width.

Since the sensitiveness of the electrometer could not be depended upon to remain constant, all principal measurements were made by a substitution method. The light from a tungsten lamp behind a ground-glass screen was reflected by a right-angle prism upon a magnesium-oxide block which could be slipped at will in front of the spectrograph slit. The lamp ran on a track whose "zero" was found by measurement, the distance through the prism being divided by the refractive index. The lamp with its ground-glass was calibrated with a Lummer-Brodhun contrast spectrophotometer against a tungsten lamp whose color temperature at 97.3 volts had been determined at the Bureau of Standards. The magnesium block was also verified to be non-selective over the same range of wave-lengths. The extreme range of intensities covered in the observations was 200 : 1. To reduce the brighter intensities a filter was interposed in the exciting beam; the filters used consisted of photographic films between glass plates, in ratio steps of 2. Three of the most used transmissions were determined directly with the spectrophotometer in comparison with carefully measured sector discs run at 40 R.P.S.; the transmission of the other filters at the same wave-lengths was then compared with these by an obvious substitution method, and relative transmissions of all filters at other wave-lengths were observed in reliance upon proportionality of intensity and slit width (carefully corrected for zero error). The filters were found to be uniform over their surfaces within the accuracy of observation but they varied somewhat with wave-length.

The entire optical system was amply screened against stray light, both outside and by means of diaphragms inside the instruments.

The material employed was a sample of fluorescein kindly furnished by Professor E. Merritt; it had been specially purified in the Cornell laboratory of chemistry and was said to test 100% pure by analysis. A concentrated mother solution in absolute alcohol was prepared and from this working solutions were made by heavy dilution with the same solvent, the concentration being adjusted so that brilliant fluorescence was obtained without excessive absorption in the spectral region under observation.

II. "ANTI-STOKES" OBSERVATIONS

Method of observation. For the principal observations designed to test Eq. (1), the intensity of the fluorescent light was increased by reflecting the exciting beam from a right-angle prism into the specimen and then taking the fluorescent light out backward, close to the prism, at an angle of 20–25° with the exciting beam. The paths of the two beams through the solution were thus made equal and the direct effect of absorption upon the result was eliminated; but absorption still had the disadvantageous effect of causing a much more rapid variation of intensity with wave-length than would occur with the beams at right angles. The specimen was contained in a glass tube about 18 mm in diameter and this was two-thirds enclosed on the sides by the upper end of a much thicker copper rod wound below with a heating coil, the exciting beam passing out through a slit in the copper in order to minimize scattered light, and a cylindrical lens of 7 mm focal length was inserted with the spectrograph slit and the useful part of the solution at conjugate foci. For the readings at room temperature a thermometer was lashed to the rod near the tube (or at first merely placed over it on the tin screening). When the temperature was raised by means of the heating coil, a slender thermometer was passed down through a rubber cork into the top layer of the solution and a thick layer of wool was laid around the tube to check convection currents. In a subsequent test this thermometer was pushed down into the middle of the solution and then read 0.8° higher, so a correction of this amount was added to all "hot" readings. The temperature was easily held constant within half a degree.

To compare the reciprocal exciting powers for a chosen pair of wave-lengths, say λ_1 and $\lambda_2 > \lambda_1$, two similar half-sets of observations were taken, excitation being made at λ_2 in the first half-set and fluorescence observed at λ_1 these wave-lengths being then interchanged for the second half-set. The usual observational formula was $F\ D\ F\ F\ D\ C\ C\ D\ C\ C\ D\ C\ C\ D\ F\ F\ D\ F$, F denoting a drift due to fluorescence, D a dark drift, and C a drift due to the comparison lamp, which was set to give about the same effect as the fluorescence. From each complete set of observations the observed ratio of the exciting powers, $R = f_{21}/f_{12}$, was calculated. Let F_1, F_2 denote respectively the mean net rates of drift due to fluorescence in the two half-sets, L_1, L_2 the means of the net drifts due to the comparison lamp each multiplied by the square of the distance from the magnesium block, B_1, B_2 the relative spectral intensities of the comparison lamp at λ_1 and λ_2, S_1, and S_2 the relative intensities of the exciting beam at λ_1 and λ_2, and t_1, t_2 the transmissions of the filters used (usually none in the first half-set). Then

$$R \equiv \frac{f_{21}}{f_{12}} = \frac{F_2 L_1 B_2 S_2 t_1}{F_1 L_2 B_1 S_1 t_2} \tag{2}$$

Theoretical values of R were then calculated from (1) with the assumption of Wien's law for J, so that

$$\frac{1}{R} = \frac{f_{12}}{f_{21}} = \frac{\lambda^6{}_2}{\lambda^6{}_1} e^{-\alpha(\lambda_2 - \lambda_1)} \tag{3}$$

where $\alpha = 1.435/T\lambda_1\lambda_2$, T being the temperature of the fluorescing substance. A large correction for slit-width is, however, necessitated by the rapid diminution of the anti-Stokes light with decreasing wave-length. Entrance and entrance slits being equal, the spectral transmission of either instrument is represented as a function of wave-length by an isosceles triangle whose base is twice the spectral slit-width, w, and can be written $k(1 - |\lambda - \lambda_0|/w)$, where λ_0 is the central wave-length and k is a constant. The amount of light issuing from the spectrograph when the central wave-length of excitation is λ_2 and the spectrograph is set to transmit centrally a wave-length λ_1 will therefore be

$$F_{12}=k'\int_{\lambda_1-\epsilon}^{\lambda_1+\epsilon}\int_{\lambda_2-\eta}^{\lambda_2+\eta}f_{1'2'}S_2'\left(1-\frac{|\lambda_1'-\lambda_1|}{\epsilon}\right)\left(1-\frac{|\lambda_2'-\lambda_2|}{\eta}\right)d\lambda_2'd\lambda_1'$$

where $f_{1'2'}$, S_2' denote respectively the exciting power and the spectral intensity of the exciting beam for wave-lengths λ_1' and λ_2', and ϵ, η are the respective slit widths of the two instruments. Putting $f_{1'2'}=f_{2'1'}\exp\left[-\alpha(\lambda_2'-\lambda_1')\right]$, in accordance with (3) but omitting the λ factors as inconsequential in the present connection, and then expanding f and S, we have

$$F_{12}=k'\int_{\lambda_1-\epsilon}^{\lambda_1+\epsilon}\int_{\lambda_2-\eta}^{\lambda_2+\eta}\left[f_{21}+(\lambda_1'-\lambda_1)\frac{\partial f_{21}}{\partial\lambda_1}+(\lambda_2'-\lambda_2)\frac{\partial f_{21}}{\partial\lambda_2}\right]\cdot$$
$$\left[S_2+(\lambda_2'-\lambda_2)\frac{\partial S_2}{\partial\lambda_2}\right]e^{-\alpha(\lambda_2'-\lambda_1')}\left(1-\frac{|\lambda_1'-\lambda_1|}{\epsilon}\right)\left(1-\frac{|\lambda_2'-\lambda_2|}{\eta}\right)d\lambda_2'd\lambda_1'. \tag{4}$$

If the wave-lengths are now interchanged on the two instruments, the issuing light becomes, with sufficient approximation,

$$\begin{aligned}F_{21}&=k'\int_{\lambda_1-\epsilon}^{\lambda_1+\epsilon}\int_{\lambda_2-\eta}^{\lambda_2+\eta}f_{21}S_2\left(1-\frac{|\lambda_1'-\lambda_1|}{\epsilon}\right)\left(1-\frac{|\lambda_2'-\lambda_2|}{\eta}\right)d\lambda_2'd\lambda_1'\\&=k'\epsilon\eta f_{21}S_2.\end{aligned} \tag{5}$$

Carrying out the integration in (4) and then dividing (4) by (5), we find

$$\frac{F_{12}}{F_{21}}=\frac{1}{R}=e^{-\alpha(\lambda_2-\lambda_1)}\frac{4}{\alpha^4\epsilon^2\eta^2}(\cosh\alpha\epsilon-1)(\cosh\alpha\eta-1)$$
$$\left[1+\frac{1}{\alpha f_{21}}\frac{\partial f_{21}}{\partial\lambda_1}\left(\alpha\epsilon\coth\frac{\alpha\epsilon}{2}-2\right)-\left(\frac{1}{\alpha f_{21}}\frac{\partial f_{21}}{\partial\lambda_2}+\frac{1}{\alpha S_2}\frac{\partial S_2}{\partial\lambda_2}\right)\left(\alpha\eta\coth\frac{\alpha\eta}{2}-2\right)\right]. \tag{6}$$

The reciprocal of the right-hand member of (6), with the exponential factor omitted, was applied as a correction factor to the theoretical values of R. Approximate values of the derivatives of f_{21} as modified by absorption, which is obviously what is required in the formula, were obtained from subsidiary observations in which λ_1 and λ_2 were varied; in most of these, no allowance was made for the (moderate) spectral variation of sensitiveness of the observing system. The bracket in (6) containing these derivatives usually differed from unity by 1−3% (maximum, 10%) and the entire correction factor ranged from 0.86 to 0.95. Rough estimates indicated that the effect of higher derivatives of f was very small.

An attempt was made also to estimate another source of error, that due to light scattered inside the instruments. Such light will tend always to make the anti-Stokes light appear too strong and so to diminish the observed value of R, and it is particularly serious in these observations because of the relative faintness of the light under observation. Before taking any of the final data, the green light from a mercury lamp, isolated by absorbing glasses, was passed into the Hilger spectrometer and the amount of light issuing through the slit for various settings of the drum was determined photometrically in comparison with a direct beam from the lamp. Light from the spectrometer was then reflected into the spectrograph for a similar test upon that instrument, correction being now made for the scattering in the spectrometer. As an example, the light scattered through the slit when set 120A away from the entering wave-length came out about 0.00044 of the light transmitted without scattering, for both instruments; at 200A it was half as much. An approximate integration was then made, from the source calibration curve and from fluorescence and exciting power curves obtained in a separate rapid survey, to find the resulting error in R, this being done for only a few pairs of wave-lengths and interpolation being then employed for the others. The results ought to be correct at least within fifty percent.

A point clearly brought out by these calculations and not always properly appreciated is that the scattering in the observing instrument may for some settings be a more serious source of error than that in the exciting instrument because of the large amount of fluorescent light which is passing through and which is much stronger than the wave-length actually being observed.

The final results are given in Table I. λ_1 and λ_2 are the two wave-lengths for each set, I is a rough estimate of the intensity of the "anti-Stokes" light, R is the observed value of that quantity (anti-Stokes exciting power divided into the inverse power), ρ is the ratio of the theoretical value of R, corrected for slit width but not for scattering, to the observed value, and Sc is the estimated percent increase in ρ caused by scattered light. The same specimen was used throughout the first long series of observations at room temperature; for the series at a higher temperature, which followed, a fresh dilution was made from the mother solution with one drop of NaOH added, and fresh portions of this were taken at intervals; and the latter material was also used for the final five sets except that for the very last set six drops of NaOH were added to the specimen under observation. Other sets in considerable number were rejected because of some known or suspected error in the adjustments, but consistency in the policy as to rejections, independently of whether the theoretical equation was confirmed or not, has been preserved.

TABLE I

Results on the violation of Stokes' law

λ_1 λ_2	I	R	ρ	Sc	λ_1 λ_2	I	R	ρ	Sc
(Temperature, 20.0–24.4°)					(Temperature, 60.2–64.4°)				
5320–5469	8	8.23	1.02	12	5316–5469	11	4.67	1.53	9
5265–5469	8	24.3	.89	13	5268– "	9	11.6	1.20	9
5219–5469	9	38.9	1.19	12	5176–5365	24	12.2	1.05	3
5180–5469	6	83.6	1.12	13	5132–5316	37	11.0	1.11	2
5176–5469	6	68.3	1.38	12	5132–5268	83	6.66	1.00	2
5114–5469	3	290.	.90	14	5089– "	51	13.3	.96	2
5134–5269	54	7.37	1.08	2	5048– "	25	23.7	1.02	3
5091–5269	34	16.4	1.01	3	5009– "	20	43.3	.96	4
" "	33	16.2	1.08	3	4951– "	7	92.	1.17	11
5050–5269	17	29.3	1.13	4	4916– "	4	115.	1.65	15
" "	14	31.5	1.06	4	5048–5178	89	6.68	1.02	2
5010–5269	9	57.0	1.16	6	5009– "	47	10.8	1.13	3
" "	8	55.8	1.25	6	4971– "	24	19.8	1.15	5
4976–5269	6	77.3	1.61	9	4936– "	16	31.2	1.26	8
5090–5200	60	6.45	.95	2	4898– "	7	55.	1.33	10
5050–5200	22	16.4	.77	4					
5011–5200	24	14.8	1.67	13	(Temperature, 24–25°)				
4976–5200	10	32.3	1.42	8	5089–5268	19	15.5	1.14	3
4938–5200	4	113.	.82	12	5048–5200	27	11.6	1.07	3
5011–5133	43	7.47	1.09	3	5176–5364	11	17.8	1.03	3
4976– "	26	13.2	1.18	5	5177–5330	14	9.58	1.12	2
4940– "	11	25.7	1.14	7	" "	54	9.97	1.08	2

A glance at the results shows at once that the theoretical law expressed by Eq. (1) must at least come near to the truth. The discrepancies seem to be correlated chiefly with the intensity of the anti-Stokes light and not with either spectral position or temperature; a few of the largest were no doubt due to an undetected blunder in adjustment. In Table II the results are divided into three groups according to the intensity of the anti-Stokes fluorescence and mean values of ρ and of Sc are given for each group; the number of sets in the group is given in parentheses.

TABLE II

The data of Table I averaged by groups.

	Cold			Hot		
I		ρ	Sc		ρ	Sc
25–60	(8)	1.067	2.6%	(6)	1.039	2.5%
12–24	(6)	1.15	3.3	(4)	1.104	5.0
3–11	(13)	1.15	10.	(5)	1.38	11.

Sources of error. The most serious source of error next to scattering undoubtedly lies in the spectral settings. An error of only one angstrom in the assumed difference in wave-length between the slits of the two instruments makes a difference of about 1.7% in the value of ρ. An accuracy of this order was striven for with more or less success in the calibrations, but it is doubtful whether subsequent settings are reliable to this extent. The average deviation from their mean of the six electrometer readings ranged in different sets from 2 to 8%, so that the Accidental error in ρ would be at least several percent.

No correction is required for absorption, since the paths of exciting and of fluorescent light through the solution were equal and the total effect of absorption would therefore remain the same when the wave-lengths were interchanged. As a check, a very concentrated solution gave $R=13.81$ for the 5268–5089 ratio, whereas the same solution diluted to half and then to quarter strength gave $R=15.28$, 15.20. The strength usually employed lay between the last two. On the other hand, there does exist the possibility of a systematic error arising from the fact that the different wave-lengths coming from the source were not distributed spatially in quite the same manner in the solution; and an analogous inequality existed in regard to the fluorescent light. The resulting error is hard to estimate. In any case, it can hardly explain the systematic trend of the observed discrepancies, for it should be equivalent to a fixed shift in wave-length and so should produce the same percent of error in all ratios. Furthermore, a single set of readings taken later on the 5245–5090 ratio, under unfavorable conditions, with the exciting light taken out from a square cell at right angles to the exciting beam, gave $\rho=1.06$, in good agreement with the previous results.

Conclusion. In view of all these sources of error the conclusion seems justified that the first group of observations in Table II definitely support the theoretical equation given in (1) and the second group, $I=12$ to 24, are not inconsistent with it. A doubling of the allowance for scattered light would on the average pretty well cover all of the discrepancies; and it is a fact that a few imperfect tests with absorbing screens suggested considerably larger effects due to this cause. The third group of results are therefore not decisively inconsistent with (1) and show that this equation at least comes pretty near to the truth even when the anti-Stokes light is very weak.

The factor λ_x/λ_y in (1) is a characteristic contribution from quantum theory and its experimental verification would therefore be of particular interest. Here we can only say that the omission of this factor would make the observed agreement *worse* by 2–4%.

III. FLUORESCENCE, EXCITATION AND ABSORPTION CURVES

In August, with the photo-electric system working poorly, a rapid survey was made of the relative exciting powers for the solution of fluorescein that had been used in the "hot" observations. The material was put in a square cell and viewed near one corner at right angles to the exciting beam, a correction (0–20%) being made for absorption. The coefficient of absorption was determined with the spectrophotometer by the usual method of observing slit-widths first with the solution and then with pure alcohol in the cell and taking the logarithm of the ratio of these readings; the slit zero was found by measuring the known transmission of the 0.5 filter with extremely narrow slits. The smallest transmission through 2 cm of the solution came out about 10%. The spectrometer slit was not over 100A wide.

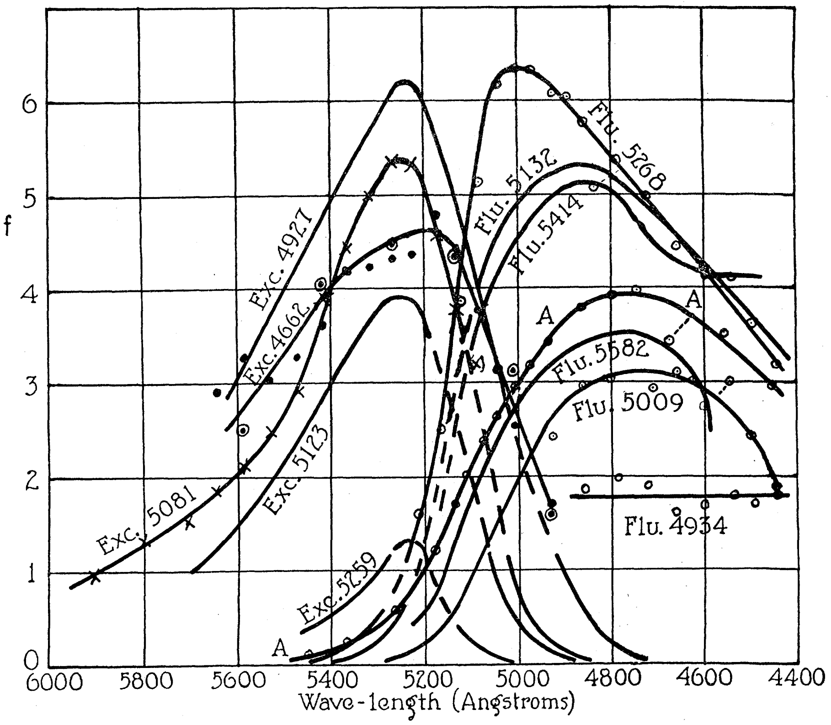

FIG. 1.

The results are shown in Fig. 1, curve *AA* giving the absorption on an arbitrary scale. The fluorescence curve was determined, using the comparison lamp, for excitation at 5081A (× in the figure representing in part the means of several repetitions), and at 4662 (dots in the figure, the dots in circles denote values obtained from the excitation curves), also a few points at 4787. Excitation curves were then found, by simply varying the exciting wave-length and assuming proportionality of deflection rate and fluorescent energy, for fluorescence at 4934, 5009, 5132 (points not shown in figure), 5268 (more or less repeated), and 5132 and 5414 (points not shown). The scale factors of the various curves were assumed to be unknown and were adjusted so as to secure the best harmony between all of the results. In this way there was obtained a set of numbers, some in duplicate, proportional to the true exciting power, f, for various pairs of wave-lengths; these are plotted twice over in the figure, first, as fluorescence curves (left) each giving the spectral intensity of fluorescence of various wave-lengths excited in unit volume by unit density of energy of a given wave-length, and, second, as excitation curves (right) each giving the spectral intensity of fluorescent emission from unit volume of a certain wave-length excited by unit energy density of various wave-lengths, all on the same arbitrary scale. Additional values calculated from Eq. (1) were used to extend each set of curves on one side beyond the region of direct observation.

Circumstances made it impossible to secure more than two electrometer readings on each point, with some checking back to guard against systematic change of conditions, consequently the observed parts of the curves may be in error by as much as 6% of the largest value of f shown (the upper parts of the curves marked "Exc. 5081" and "Fluo. 5268" being perhaps twice as accurate). Nevertheless the data seemed to possess at least temporary interest because they suffice to establish the following conclusions:

(1) The fluorescence curve is not constant in shape. Not only does it draw in its toes toward the violet as the exciting wave-length encroaches upon it, thus preserving the validity of Eq. (1), but the center also rises, until the exciting wave-length reaches 5000A, after which the whole curve drops rapidly. This result is not necessarily in conflict with earlier data of Nichols and Merritt,[5] who found no change of shape for a solution in *water*; their material was also doubtless less pure. Those authors found,[6] however, for an alcoholic solution two maxima at 5340 and 5500, resp., with shoulders at 5240 and 5680, whereas here there is a single maximum at 5240 ± 30A. This difference may be due either to greater purity of the material used in the present investigation or to the fact that those authors appear to have excited with much shorter wave-lengths.

(2) The wave-length of maximum excitation shifts from 4730 ± 80A for fluorescence at 5000 or at 5600 to 5000 ± 40A for fluorescence at 5268.

(3) For a particular wave-length of fluorescence, excitation and absorption are obviously not (always) proportional. A rough integration yields the further result that the total fluorescence increases in relative strength, by a total amount of 28%, as the exciting wave-length rises from 4800 to 5100, after which it drops sharply; but too great reliance cannot be placed upon this result because the long-wave

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[5] Nichols and Merritt, Phys. Rev. **18,** 403 (1904).

[6] Nichols and Merritt, Phys. Rev. **32,** 38 (1911).

part of the fluorescent spectrum is incompletely represented. Vavilov found[7] total fluorescence and absorption to be proportional for an aqueous solution of fluorescein; but results similar to those described here were found by Valentiner and Rössiger.[8]

These results would all find qualitative explanation if there were in reality two fluorescence bands of invariable form with maxima close together, one being a broad band with a broad excitation curve having its maximum around 4730 and the other a narrower but stronger band with a narrow excitation curve at about 5000A.

A more thorough study of the exciting power in fluorescein would seem to be well worth while. Probably for this purpose the far greater rapidity of visual photometry as compared with the photo-electric method would amply compensate for the trouble of finding enough line sources or good screens so that spectral resolution of the source light could be avoided.

This investigation was made in the physical laboratory of Cornell University and was supported by a grant from the Heckscher Research Council. The assembling of apparatus and most of the observational work was done by Mr. L. S. Taylor, who acted as research assistant under the grant, and the author is deeply indebted to him for his skilful handling of the instruments and for his cheerful persistence in the face of difficulties. The author is also indebted to many of his colleagues in Cornell for innumerable courtesies during the progress of the work.

GÖTTINGEN,
November 15, 1926.

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[7] S. I. Vavilov, Phil. Mag. **43**, 307 (1922).

[8] Valentiner and Rössiger, Zeits. f. Physik, **36**, 81 (1926).