STUDIES IN LUMINESCENCE.

BY EDW. L. NICHOLS AND ERNEST MERRITT.

XIV. FURTHER EXPERIMENTS ON FLUORESCENCE ABSORPTION.

IN the fourth paper of this series we have used the term fluorescence absorption in referring to the increase of the absorbing power of a fluorescent substance which results from fluorescence. Such an effect was first observed by Burke,[1] who found a considerable increase in the absorption of uranium glass when the glass was excited to fluorescence. The writers observed the same effect in solutions of fluorescein and eosin.[2] The increase in the absorption appeared to be greater for those wave-lengths which corresponded to the brightest regions of the fluorescence spectrum. Camichael,[3] upon repeating these experiments, was unable to detect any change of absorbing power during fluorescence either in the uranium glass used by Burke or in the fluorescent solutions tested by us. The question was again attacked in 1904 by Miss Wick,[4] who made a detailed study of the phenomenon in the case of an alcoholic solution of resorufin. Her results consistently showed an increase in the absorbing power of the solution during fluorescence, and were in complete agreement with the results obtained by us with fluorescein and eosin. More recently a method of detecting the effect, if it exists, has been suggested by Wood,[5] and a few trials of the method by him led to negative results. The most recent experimenter in this field is Houstoun,[6] whose very careful experiments also fail to give any indication of a change in absorption due to fluorescence.

The results obtained by ourselves, and especially those obtained by Miss Wick, were so definite and positive that until recently we have been of the opinion that the failure of others to observe the effect was due to the fact that they had not chosen suitable

[1] Burke, Philosophical Transactions, Vol. 191 A, p. 87, 1898.
[2] Nichols and Merritt, PHYSICAL REVIEW, Vol. 19, p. 397, 1904.
[3] Camichael, Comptes Rendus, Vol. 140, p. 139.
[4] Frances G. Wick, PHYSICAL REVIEW, Vol. 24, p. 407, 1907.
[5] R. W. Wood, Phil. Mag., Vol. 16, p. 940, 1908.
[6] R. A. Houstoun, Proc. Royal Society of Edinburgh, Vol. 29, p. 401, 1909.

conditions for the experiment. We were led to suspect the existence of some systematic error, however, by the results of a careful study of the collimator slit of the spectrophotometer used in our recent experiments upon the distribution of energy in fluorescence spectra. It was found that the screw was a very accurate one and that the opening of the slit was very closely proportional to the reading of the micrometer screw. To test this point the slit was mounted in a lantern and the enlarged image was measured for a large number of different readings of the micrometer. The results are shown in Fig. 94.

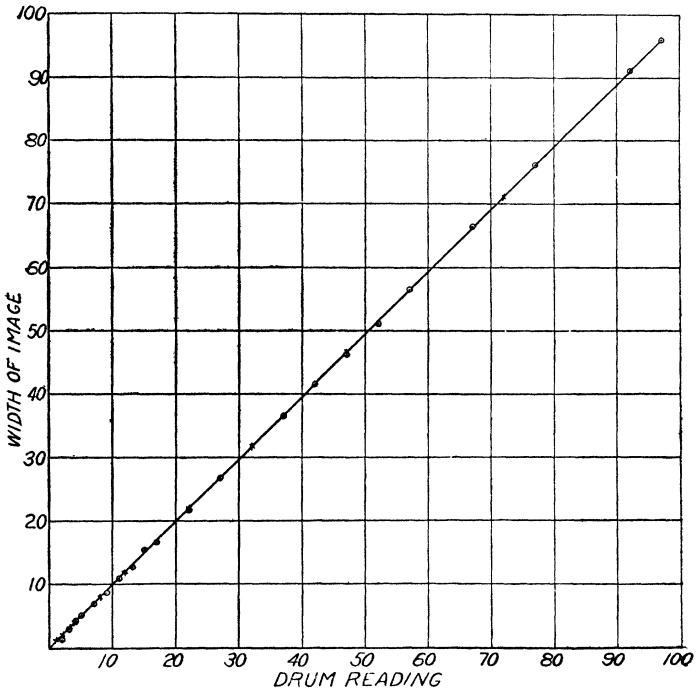

Fig. 94.

We then tested the amount of light passing through the slit at different widths by balancing two acetylene flames against each other, the adjustment being made by varying the slit width in one case and by varying the distance of the flame in the other. The experiments were performed in a dark room with an elaborate sys-

tem of screens to prevent reflections, and several independent tests convinced us that the inverse square law of distances was very exactly satisfied. If the intensity is computed by the law of inverse squares, and if a curve is plotted showing the relation between intensity and slit width, the results obtained are of the type illustrated in Fig. 95.

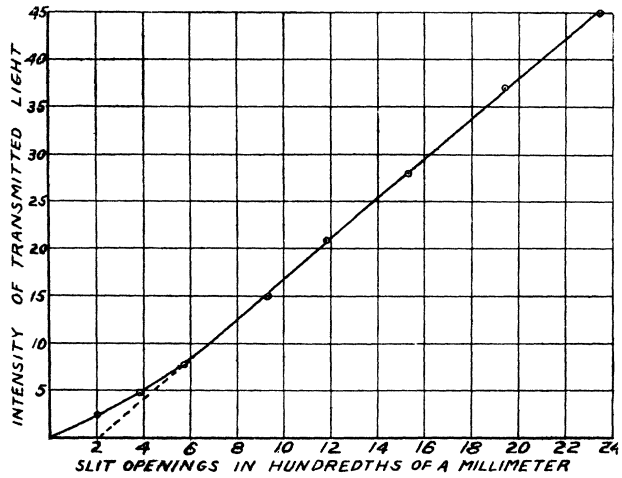

Fig. 95.

It will be seen that for narrow slits the intensity of the transmitted light is not proportional to the slit width. When the width exceeds a few hundredths of a millimeter the line becomes practically straight, so that equal increments in slit width correspond to equal increments in intensity. The conditions, whatever they are, which lead to the curve in the neighborhood of the zero of Fig. 95 are equivalent in their effect to a shift in the zero point of the screw by about two divisions. The intensity transmitted by a slit 50 divisions wide is not twice as great as that transmitted by one 25 divisions wide, but the ratio is in reality 48 to 23.

These experiments were not made with the same instrument that had been used by ourselves and later by Miss Wick, but refer to an exactly similar Lummer-Brodhun spectrophotometer. It seems probable that this effect, due possibly to diffraction or to reflection from the jaws of the slit, is common to all instruments of this type.

It is clear that if the effect is disregarded, indications of fluorescence absorption may be obtained even if no such effect exists. The method used by Miss Wick and by ourselves involved three readings; namely, the intensity of the fluorescent light alone, the intensity of the light transmitted by the non-fluorescent solution, and the combined intensity when light was transmitted through the solution at the same time that the latter was excited to fluorescence. The sum of the first two readings being found greater than the third, it was assumed that the transmission in the latter case was less than in the first. If, however, each of these readings of intensity had been too great by two divisions, as indicated by Fig. 95, the result of such procedure would be to give an apparent fluorescent absorption measured by two divisions.

While it is difficult to see how this source of error alone could account for results of the character obtained by Miss Wick and ourselves, the detection of one source of systematic error made it appear possible that other similar errors might be present, and has led us to take up the study of this question anew. The result of the numerous experiments which will be briefly described in this paper has been to convince us that the phenomenon of fluorescence absorption either does not exist, or that the effect is so small that the methods thus far used for its detection are inadequate.

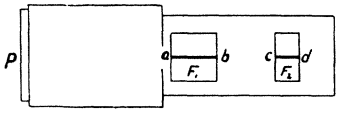

Fig. 96.

In the first method used an attempt was made to obtain a photographic record of the effect. The arrangement is shown in diagram in Fig. 96.

The large square cell F_1, containing a solution of fluorescein, was excited from above by a narrow beam of light, so that a central layer *ab* was excited while the rest of the solution was not. Directly back of this was another cell F_2, also illuminated from above so that the narrow vertical strip *cd* was excited. The photographic plate *P*, suitably screened from all sources of light except the fluorescence in F_1 and F_2, would be fogged nearly uniformly over its surface by the light from *ab* if this alone was excited, and the fogging would also be practically uniform if *cd* was excited. If, how-

ever, that part of the liquid in F_1 which is excited to fluorescence acquires the power of absorbing the fluorescent light more strongly than before, then we should expect *ab* to cast a shadow upon the photographic plate. While this shadow could not be expected to be very sharp or very dense, the results obtained by ourselves and Miss Wick indicated that under suitable conditions it ought to be clearly visible in the negative.

Extended efforts were made to obtain such conditions of concentration, thickness of layer, intensity of excitation, etc., as would bring out the expected shadow on the plate *P*. Over 100 negatives were made and many of these were under conditions which

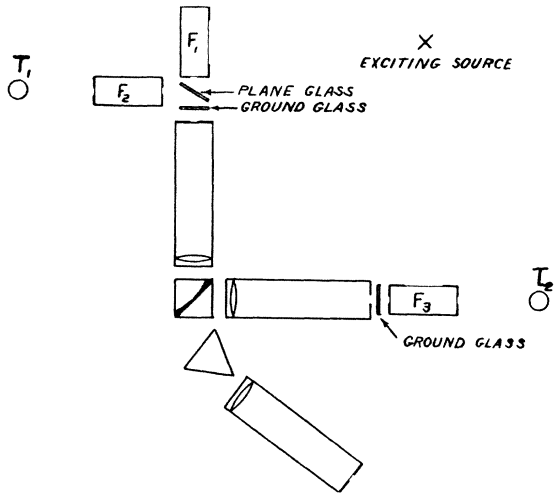

Fig. 97.

appeared to us to correspond to those under which the spectrophotometer had indicated a large fluorescence absorption; but in no case was a definite shadow observable.

The next method of testing the matter is shown in diagram in Fig. 97. Three cells containing a solution of fluorescein, or in some cases resorufin, were used as shown in Fig. 97. F_1 and F_3 were excited by the same source so as to eliminate errors due to variations in excitation; the source used was sometimes a quartz mercury lamp and in other cases the tungsten lamp. Since the solution was

exactly the same in all three cells, and since F_1 and F_3 were at nearly the same distance from the exciting source, the two collimator slits were illuminated with almost equal brightness. Any slight inequality was balanced by opening or closing one of the slits. This adjustment being made, the exciting source was extinguished and light was sent through the cell F_3 from a small tungsten lamp, T_2. To balance this illumination, light from another tungsten lamp, after passing through the cell F_2, was reflected by a piece of plane glass into the second collimator slit. The balance was obtained by adjusting the distance of T_2, which slid upon a graduated photometer track. When this balance was obtained the exciting source was again started and the field of the spectrophotometer observed. If the effect of fluorescence is to increase the absorbing power of F_3 we should expect that while fluorescence alone and transmission alone give a perfect balance, there would be a lack of balance when excitation and transmission occur simultaneously. When satisfactory conditions of steadiness were obtained no such disturbance of balance could be observed. A sample set of readings is given in Table I. The small positive result obtained in this case is smaller than the errors of observation. In other cases the results indicated a small decrease in absorbing

TABLE I.

Resorufin. Excited by Mercury Arc.

Slit in front of F_3 set for equality of fluorescence alone. Readings 51.6, 49.7, 50.7, 50.7. Slit set at the average 50.7.

Lamp T_2 set for $F+T$, *i. e.*, fluorescence and transmission together. Distances =233, 245. Average 239.

Lamp T_2 set for transmission alone (T) 233, 233. Average 233.

$F+T$, 226, 228. Average 227.

Slit set again for equality of fluorescence alone. Readings 40.7, 50.4, 50.3, 50.7. Slit set at the average 50.3.

$F+T$: 235, 239. Average 237.

T: 243, 245. Average 244.

$F+T$: 226, 237. Average 232.

Slit set for F alone: 50.8, 50.8, 50.7, 50.6. Average 50.8.

$F+T$: 238, 243. Average 240.5.

T: 240, 236. Average 238.

$F+T$: 243, 237. Average 240.

Average of all: Distance of lamp for T alone =238.3; for $T+F$ =236.0.

If this difference is real it indicates that the absorption of the solution during fluorescence exceeds its absorption when unexcited by 1.7 per cent.

power during fluorescence. The experiment was tried with solutions of different concentration and different intensities of excitation. But when satisfactory conditions as regards steadiness were obtained no disturbance of balance could be detected which was greater than the errors of observation.

It was suspected that the phenomenon of fluorescence absorption apparently demonstrated by Miss Wick and ourselves might indicate not an increase in the absorbing power of the solution but rather a decrease in its power of fluorescing. The results obtained by us might be interpreted equally well in either of these two ways. The fact that fluorescence absorption seemed to be in proportion to intensity of fluorescence in different parts of the spectrum lent strength to this view. If the effect is a diminution of fluorescence and not an increase in absorption the failure of our photographic tests could also be explained in an obvious manner.

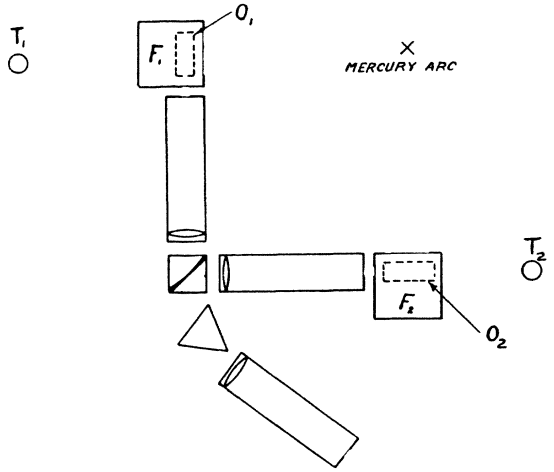

Fig. 98.

If the fluorescence is diminished we should expect the diminution to be observable not only along the line of the transmitted light but in other directions. To test this matter we set up two large fluorescent cells F_1 and F_2 (Fig. 98) covered with black paper except on the sides towards the exciting mercury arc, and having two openings O_1 and O_2 in the bottom through which the light of the

tungsten lamps T_1 and T_2, after reflection from mirrors, might pass up into the cell. The balance having been obtained with fluorescence alone, the lamp T_1 was then turned on and we tried to determine whether there was any change in the balance resulting from extinguishing T_1 and at the same time lighting T_2. The only effect was a slight one, opposite in sign to that which would be indicated in fluorescence absorption, and due undoubtedly to a small amount of stray light entering the slit after reflection from the walls of the cell.

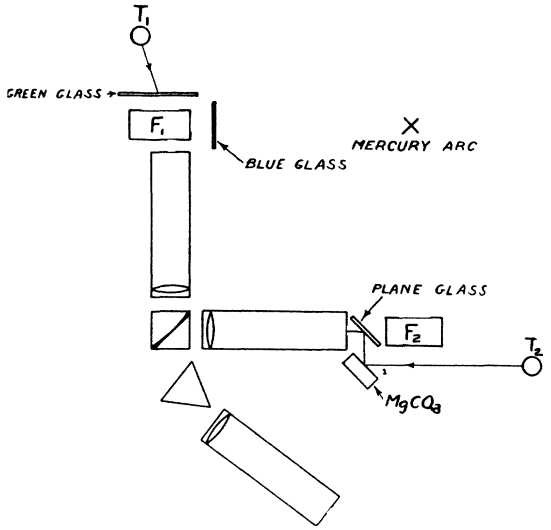

Fig. 99.

It seemed to us possible that the effect might be analogous to the effect of infra-red rays in suppressing fluorescence, and that possibly it might be produced not by the visible rays which caused this annoyance through stray reflection, but by the red or infra-red rays. We therefore interposed ruby glass in the path of T_1 and T_2, thus eliminating disturbances due to stray light. Under these circumstances no disturbance of balance resulted from extinguishing one lamp and lighting the other.

It seemed possible that while fluorescence absorption might really be the result of a diminution in the intensity of fluorescence this decrease might not be the same in all directions but might be great-

est in the direction in which transmitted light proceeded. The arrangement of apparatus shown in Fig. 99 was intended to test for the effect in directions nearly but not quite the same as the direction of transmitted light. The light from the tungsten lamp T_1 passes through the cell F_1 so as not to fall in the slit of the instrument both being nearly parallel to the axis of the collimator. The small amount of stray light from T_1 which unavoidably did reach the slit was balanced by light from the second tungsten lamp T_2 as shown in the figure. The results were entirely negative. Thinking that the intense green line of the mercury arc might pro-

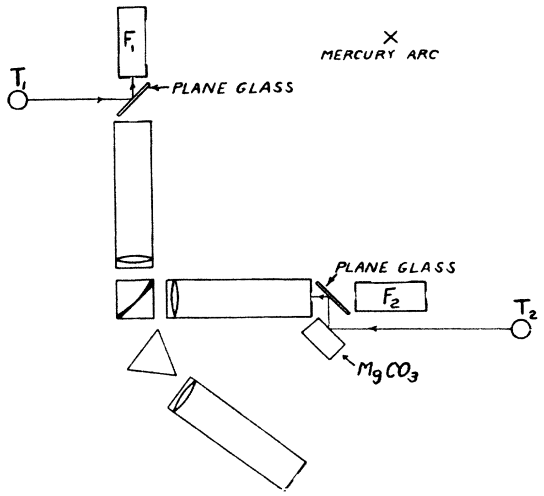

Fig. 100.

duce so much effect in weakening the fluorescence in F_1 that additional light from T_1 would produce only a slight effect, we interposed in some cases blue glass as shown, and in order to diminish the excitation produced by the light from T_1 we sometimes used green glass as indicated. The results, however, were in all cases negative.

In Fig. 100 is shown an arrangement for testing the effect of transmitted light upon fluorescence in case the direction of transmission is opposite to the direction in which fluorescence is observed. No effect could be detected.

In Fig. 101 are shown the essential parts of the apparatus used in applying the method suggested by Wood. A disk, shown in detail at the right of the figure, was arranged so that the four sectors might either occupy the position shown in the upper diagram, or might be shifted with reference to each other so that the inner openings and the outer openings would be alternate. This disk was mounted between the exciting source and the fluorescent cell *F*, as shown. Light passed through the outer sectors to excite fluorescence, and light passing through the inner sector, after reflection from a block of magnesium carbonate, passed through the cell

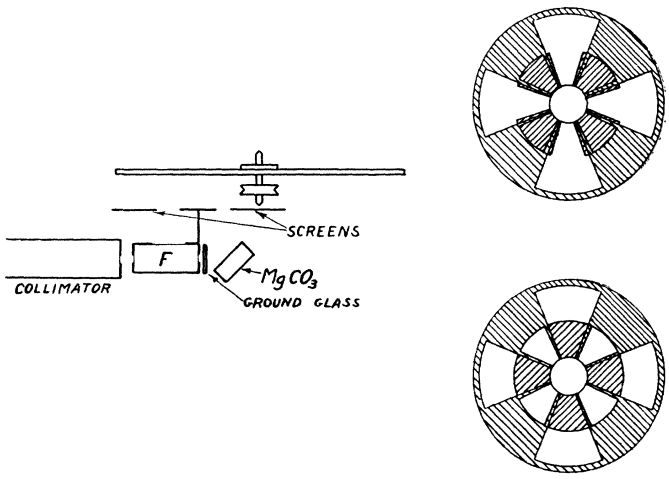

Fig. 101.

to the collimator slit. Having balanced the illumination against a standard placed before the second collimator for the case where the inner and outer sectors were open alternately, the disk was then changed so as to make the excitation and transmission simultaneous. The results of several sets of observations with this apparatus are given in Tables II.–V. The observations indicate a slight *negative* effect, that is, the transmission of the solution appears to be greater during fluorescence. But the effect indicated is so small that we are inclined to look upon it as resulting from accidental errors.

A few measurements were made by a method essentially the

TABLE II.

Fluorescein. Moderate Concentration. Woods' Method.

The numbers in the table give the width of the slit near F (Fig. 101) when set to a balance with the standard in front of the other slit.

$\lambda = 0.523\,\mu$.

Sectors Open Alternately.		Sectors Open at Same Time.	
100.9		101.2	
97.7		100.2	
104.2	Average 101.58	100.5	
103.5		101.2	Average 100.78
100.5		102.0	
101.7		98.8	
100.0		100.7	
100.4	Average 100.65	98.7	Average 100.05
Final average = 101.11		Final average = 100.41	

TABLE III.

Resorufin. Dilute. Woods' Method. $T = 0.18F$. $\lambda = 0.599\,\mu$.

Sectors Open Alternately.		Sectors Open at Same Time.	
113.4		111.2	
112.2		112.4	
114.7		112.3	
	Average 113.4	111.9	Average 111.95
112.4		112.3	
113.8		113.7	
113.3		113.4	
114.7	Average 113.7	112.8	Average 113.05
Final average = 113.55		Final average = 113.0	

Fluorescence absorption $= -.033T$.

TABLE IV.

Resorufin. Dilute. Woods' Method. $T = 1.02F$. $\lambda = 0.599\,\mu$.

Sectors Open Alternately.		Sectors Open at Same Time.	
64.6		66.2	
65.2		64.4	
67.3		65.4	
66.8	Average 65.98	65.7	Average 65.42
66.6		66.8	
66.4		65.9	
66.5		66.2	
66.9	Average 66.68	66.3	Average 66.30
Final average = 66.33		Final average = 65.86	

Flourescence absorption $= -.014T$.

TABLE V.

Resorufin. Dilute. Woods' Method. $T = 7.27F$. $\lambda = 0.599\,\mu$.

Sectors Open Alternately.		Sectors Open at Same Time.	
20.6		19.0	
20.1		19.4	
20.6		19.8	
20.1	Average 20.35	20.0	Average 19.78
20.3		20.0	
19.9		20.3	
20.4		20.5	
20.4	Average 20.25	20.7	Average 20.38
Final average = 20.30		Final average = 20.08	

Fluorescence absorption = $-.012T$.

same as that used by us in 1904, except that the source of the light transmitted was the fluorescence of a portion of the solution. A dilute solution of fluorescence was contained in a cell 20 cm. long, so arranged that either the half nearest the slit or that at a distance could be screened from the exciting light, which in this case was the mercury arc. The spectrophotometer was set for a wave-length near the crest of the fluorescence spectrum and not overlapping either of the mercury lines, so that errors due to stray light were excluded. Upon screening that part of the cell lying nearest the slit the field was illuminated by the fluorescence light of the distant part of the cell after transmission through the rest of the cell T. Upon screening the distant part of the cell the light received in the instrument was due to the fluorescence of the nearer part F. Upon removing both screens fluorescence and transmission occurred at the same time C. If absorption is increased by fluorescence we should have

$$F + T > C.$$

The readings in one case are given in Table VI.

The observations contained in Table VI. indicate a positive fluorescence absorption of 15 per cent. But if we apply the additional zero correction called for by the calibration curve of Fig. 2 the difference between $F+T$ and C is reduced to 0.9 div. or 4.3 per cent. In other cases an equally large negative result was obtained.

The results of all of these experiments, which have been repeated

TABLE VI.

F	T	C
23.7	23.2	
23.7	23.4	
22.9	23.6	41.2
23.4	22.9	41.2
Average 23.42	Average 23.28	Average 41.2
Zero 2.4	2.4	2.4
$F=21.02$	$T=20.88$	38.8

$$F+T-C=41.9-38.8=3.1=0.15T.$$

many times, and performed with more precautions to avoid false results than can be indicated in this brief account, has been to convince us that the previous results of both ourselves and of Miss Wick are due to some systematic error, and that the supposed increase in absorption due to fluorescence either does not in reality exist or is too small to be detected by these methods. We have not been able to determine the exact nature of the error which led to our preceding results. The peculiar relation between slit opening and intensity brought out in Fig. 95 will explain some of the results but not all. Another source of error which might have been an important one is that resulting from the neglect of the slit width correction, to the importance of which we have directed attention in an earlier article of this series. Unfortunately the photometer with which the original observations were made is in use in another piece of work under such conditions that we have not been able to examine it with particular reference to these points.