STUDIES ON THE AMOUNT OF LIGHT EMITTED BY MIXTURES OF CYPRIDINA LUCIFERIN AND LUCIFERASE.

By KENNETH P. STEVENS.

(*From the Physiological Laboratory, Princeton University, Princeton.*)

(Accepted for publication, May 3, 1927.)

INTRODUCTION.

Interest has centered for a long time on the intensity and efficiency of animal luminescence, not only from a theoretical but also from a practical standpoint. With the exception of the beetles, most luminous animals emit light in such small amounts that a dark-adapted eye is required to register their intensities. It is a difficult matter to measure accurately low light intensities, especially when they are intermittent as in certain luminous organisms. The intensities of *Cypridina*, however, are sufficiently bright to measure.

Harvey, 1925, has successfully measured the amount of light and over-all luminous efficiency of dense emulsions of luminous bacteria, and finds that they are 0.156 per cent efficient. As is well known the light production in *Cypridina* is an oxidation process, due to the oxidation of a substrate, luciferin, in the presence of a catalyst, luciferase, and follows the laws of enzyme action. After mixing luciferin and luciferase solutions a gradual decrease of luminous intensity follows the initial marked brilliancy. Studies of these decay curves by Amberson by a photographic method show that the light intensities are continually and gradually falling off. In general the effect of the enzyme, luciferase, is proportional to its concentration. Amberson's curves show that the reaction velocities follow this law, *i.e.* the velocity constant is proportional to the concentration of the enzyme. Amberson assumed after Trautz that, ". . . . if light intensity at any instant is assumed to be proportional to reaction velocity at that instant, the decay curve of the light produced in the course of the luminescent reaction in *Cypridina* is, after the first second, in com-

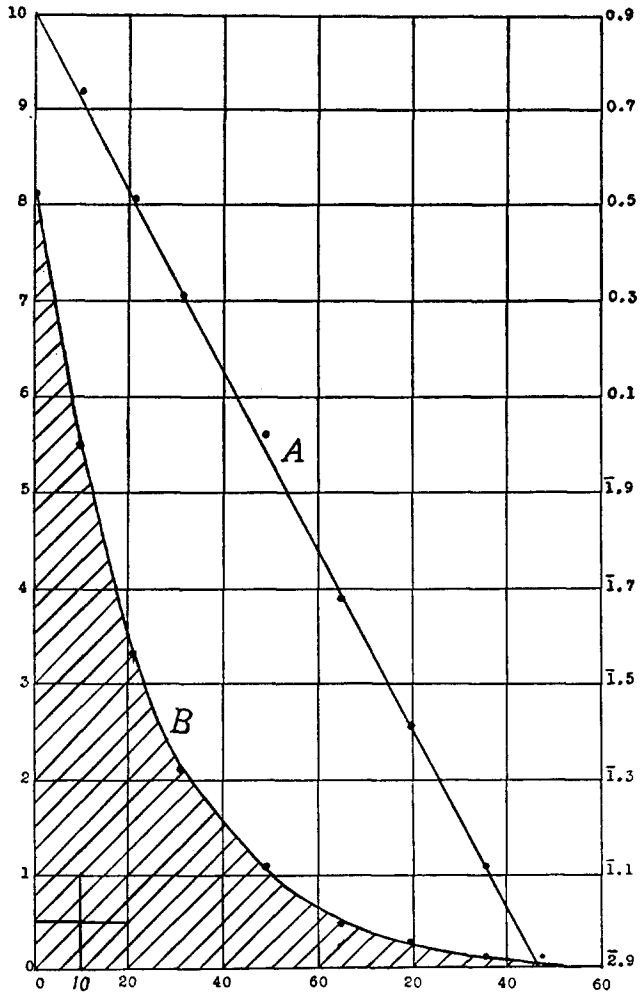

FIG. 1. Typical curve of *Cypridina* luminescence. *A*, logarithmic plotting; *B*, ordinary plotting of luminescence intensity against time. Ordinates, right, logarithms of intensity; ordinates, left, intensity in arbitrary units. Abscissæ represent time in seconds.

plete agreement with the theoretical expectation for a monomolecular reaction." In other words the rate of change of the concentration of *A* at any instant is proportional to its concentration at that instant, or

$$dx/dt \alpha (a-x)$$

where a is the number of molecules of A present originally, and x is the number of molecules of A changed in t minutes; $(a-x)$ is the concentration of A after t minutes or

$$dx/dt = k_1 (a-x)$$

where k_1 is the velocity constant. k_1 has a characteristic value for such reactions and is a measure of the rate of reaction. By integration one obtains

$$k = 1/t \; ln \frac{a}{a - x}.$$

When the intensities are plotted against time, a typical decay curve is obtained (Fig. 1), and when the logarithms of intensities are plotted against time, a straight line is obtained. Since the reaction velocities are proportional to the enzyme concentration, the velocity constants should vary with the luciferase concentrations, but not with the luciferin concentrations. Logarithmic plottings of different luciferin concentrations are parallel, provided the luciferase is present in the same concentrations, whereas the logarithmic plottings of different luciferase concentrations have different slopes.

The present paper records experiments carried out with *Cypridina* luciferin and luciferase in an attempt to measure the total amount of light emitted with various concentrations of the substrate and enzyme. The photographic method of Amberson was adequate for measuring the intensities in arbitrary units and the reaction velocities, but is unadaptable for determining the total amount of light emitted by a unit volume of luminous material in lumens.

A problem concerned with the dynamics of bioluminescence is beset with the difficulty of dealing with a very complex system. All attempts to purify either the enzyme or the substrate are unsuccessful. Furthermore, to obtain the luminous substances, the whole dried body of the organism must be extracted. Hence one is trying to measure one reaction, resulting in luminous intensity, in a system which may exhibit secondary oxidations, or additional modifying factors. In the following experiments anomalous and inexplicable factors control certain features of the reaction.

Materials and Methods.

Cypridina hilgendorfii Müller, the Japanese ostracod, was the organism which was used for the source of light in all the experiments. The desiccated animals were powdered and kept over $CaCl_2$. Great variations occur in the brilliancy of the luminescence from such powder depending upon the care with which the animals were originally gathered and dried, as well as the methods employed in preparing the extracts. It is necessary, therefore, to have the best available stock for studies upon luminescence.

The aqueous solutions of both the enzyme, luciferase, and the substrate, luciferin, have been freshly prepared for each series of experiments. In this way the minimum deterioration of solutions has been obtained. In no case has the enzyme been used after 6 hours from the time of preparation. Luciferase has been prepared in the standard manner by extracting the dried powder with cold sea water, or ordinary distilled water, and allowing the solution to stand until the contained luciferin has been completely oxidized to oxyluciferin. Such a solution is entirely non-luminous to a dark-adapted eye. The solution is then filtered and kept as the stock enzyme solution. Three series of experiments were completed with a distilled water extract; in all others sea water extracts were used. In all cases 0.25 gm. of powder to 50 cc. water have been used in preparing the luciferase.

The luciferin solutions were prepared in two different ways. Ordinarily 1 gm. of dried powder was extracted with 100 cc. N/10 HCl acid sea water. The *Cypridina* powder was added to the sea water at the moment of boiling and the boiling continued for a few moments in order to completely destroy all the enzyme. Cooling to room temperature immediately followed by allowing the flask to stand undisturbed in a vessel of cold water. During the cooling process the insoluble particles of powder settle to the bottom of the flask leaving a relatively clear yellowish solution of luciferin. The supernatant fluid is then decanted and is used as the stock luciferin solution. The oxidation of luciferin to oxyluciferin occurs spontaneously in the presence of oxygen in neutral and alkaline solutions, but in the absence of the enzyme such a spontaneous oxidation takes place without the production of light. This factor frequently plays a large part in the variations in the amount of light emitted from a given solution by reducing the available luciferin for the action of the luciferase. It has been customary, therefore, in the eleven series of experiments to use acid sea water in extracting the substrate, since acidity of the solution prevents the spontaneous oxidation. Later neutralization of the acid solution is effected in the actual experiments with N/10 NaOH, just previous to the addition of the enzyme.

In four series of experiments, another method of preventing spontaneous oxidation of luciferin was employed without the use of acid sea water. This method consisted of passing hydrogen through the luciferin solution for a considerable period of time immediately following the destruction of the enzyme. For this purpose a tall burette calibrated to 100 cc. was fitted with a rubber stopper at the open end. Through this stopper a U-shaped glass tube of small bore was fitted to allow the

escape of gas. By connecting the stop-cock of the burette to a hydrogen tank the contained oxygen could be replaced by hydrogen previous to the preparation of luciferin. The latter was then quickly poured into the burette filled with hydrogen, as soon after boiling as possible. The stopper was reinserted in the tube and hydrogen allowed to bubble through the solution until the whole mass had cooled to approximately room temperature. This procedure, however, lessens the amount of light emitted from the solution as will be seen from the data to be presented later.

In still other experimental series the hydrogen method was employed with sea water which had been neutralized to a pH 7.0. Very satisfactory results were obtained with this method of preparing the substrate.

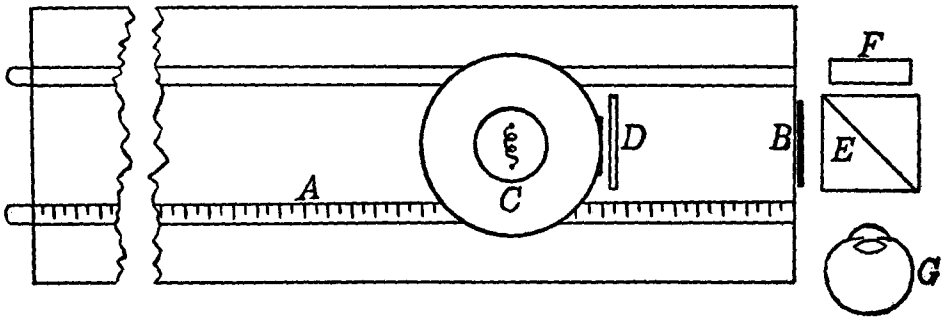

FIG. 2. Diagram of photometer bench. *A*, photometer bench; *B*, opal glass screen; *C*, chamber containing 21 C.P. lamp; *D*, No. 75 Wratten filter; *E*, comparison cube; *F*, mirrored vessel; *G*, position of observer.

The Photometric Method.

The measurement of luminescence of *Cypridina* solutions of varied concentrations is made possible by a comparison of the brightness of certain thicknesses of solution with the brightness emitted from a white surface illuminated by a light source of known intensity. The photometric method used in these experiments is an adaptation of that used by Harvey, 1925, and Morrison. The photometer bench (Fig. 2) consists of a track, one bar of which is marked off by divisions in mm. At one end of the bench (in this case at the 150 cm. mark) is placed a dull black screen with an opening in the center measuring 4.5 × 4.5 cm. square. Across this opening is fastened an opal glass screen. Care is taken that the screen shall be kept at the 150 cm. mark at all times. Behind this screen, on a movable standard, is a cylindrical dark chamber with an opening covered by a No. 75 Wratten blue green filter whose color value approximates most nearly that of the luminous solution of *Cypridina*. This chamber contains a small 6 volt 21 C.P. lamp of the type used in automobile headlights, so placed that illumination of the opal screen is complete. This chamber is attached to a sliding base so that the lamp can be

moved toward or away from the screen. Finally a plain black screen is fastened at the lower end on the calibrated scale of the photometer bench and the whole apparatus is entirely covered with a photographer's black cloth to prevent any reflections or radiations from extraneous sources from registering on the opal glass. In front of the opal glass screen is placed a comparison cube.[1] This cube is so constructed that alternately mirrored bands on the hypothenuse of one prism deflect a source of light issuing at right angles to the line of vision of an observer, while at the same time a beam of light in line with his vision is transmitted directly through the cube. Thus two different sources of light would appear to an experimenter as parallel adjacent bands whose intensities can easily be compared with only slight observational errors due to personal equations.

The source of reflected light is the 6 volt 21 C.P. lamp on the photometer bench. The source of the transmitted light is the solution of *Cypridina* coming from a specially constructed rectangular vessel placed behind the comparison cube. This vessel is made of glass, the front of clear glass, the back of opal glass and the sides and bottom mirrored so that the lateral dimensions of the vessel may be approximately infinite. The vessel used was 2 cm. in thickness by inside measurement.

The photometer lamp was always run at 2.3 amperes and 4.8 volts. The transmission coefficient of the No. 75 Wratten filter measured[2] 0.00573 with the lamp at 4.8 volts. The photometer lamp was calibrated without the Wratten filter by measuring with a Macbeth illuminometer the illumination of the opal glass, when the lamp was set at definite distances and the inverse square law applied for other distances. The test plate used with the Macbeth illuminometer had been calibrated by the Electric Testing Laboratories and gave a reflection factor of 0.79. By the formula, foot candles $\times$ constant, 1.076 (foot candles to millilamberts) $\times$ 0.79 (reflection factor) $\times$ 0.00573 (Wratten filter transmission factor) one obtains the brightness of the opal screen in millilamberts.

EXPERIMENTAL.

The experiments were always conducted in a dark room and with the aid of an assistant. The procedure of a typical experiment is here recorded in some detail as a matter of ease in understanding the variation which will be referred to later. It was customary to use 100 cc. N/10 HCl acid sea water and 1 gm. of powder for the luciferin extract. 10 cc. of this solution were carefully measured in a calibrated pipette and poured into the rectangular mirrored vessel.

[1] The comparison cube was kindly loaned by the Nela Research Laboratories of Cleveland.

[2] Kindly made by Dr. W. E. Forsythe of the Nela Research Laboratories for Dr. E. Newton Harvey.

To this was then added 5 cc. of sea water and 5 cc. of N/10 NaOH to neutralize the acid sea water. However, due to the buffers in the sea water and the alkaline nature of the *Cypridina* extract itself, the pH of such a solution ranged usually about 7.7. Such a solution contains about 0.1 gm. of *Cypridina* extract, although probably only a very small proportion of the powder used contains the luminous material, since the luminous organ comprises an extremly small percentage of the animal's body.

Immediately after preparing the vessel with the luciferin solution, 1 cc. of luciferase, containing 0.005 gm. of dry *Cypridina*, was quickly added to the luciferin solution and the time recorded. The vessel

TABLE I.

Series 3, Run 3.

Time	Distances (n)	n^2	$\frac{1}{n^2} \times 1000$	Logarithms
10	13.5	182.25	5.494	0.739
21	17.5	306.25	3.2679	0.514
31	22.0	484.00	2.0661	0.315
49	30.8	948.64	1.0548	0.023
65	45.8	2097.64	0.4784	$\bar{1}$.679
20	62.0	3844.00	0.2604	$\bar{1}$.415
36	87.8	7708.84	0.1298	$\bar{1}$.113
48	109.0	11881.00	0.0847	$\bar{2}$.927

Data calculated from observations in a typical experiment.

was rapidly agitated to facilitate mixing and placed immediately behind the comparison cube of the photometer apparatus. The observer then adjusted the photometer lamp until a match in intensities of the luminous bands on the comparison cube was obtained. At that moment the time in seconds and the distance in cm. on the bench scale were recorded by the assistant. The same procedure was repeated as the intensity diminished until the bands of light became too faint to read accurately. Very low intensities were discarded because of too great an error in comparison, and also frequently extremely high intensities were of little experimental value due to the fact that color differences between Wratten filter and the solution were accentuated.

Since intensity varies inversely as the square of the distance, the data obtained from such a series of records are tabulated by squaring the distance figures, and then obtaining the reciprocals of these squares and plotting the reciprocals against time. The reciprocals are taken as the arbitrary units used in the calculations. The graph in Fig. 1 is plotted from the data in Table I. These curves are comparable to Amberson's in showing that light dies out rapidly and follows the form of a pseudomonomolecular reaction. If the logarithms of the intensities are plotted against time, a straight line is obtained.

Immediately after a series of runs in any given set of experiments, the hydrogen ion concentration was determined by the colorimetric method for each solution used.

In any luminous solution of definite thickness prepared in as crude a manner as the *Cypridina* extract considerable absorption and some scattering of light must occur, due to particles held in suspension. Harvey, 1925, found this factor a very important one to consider in determining the lumens emitted by his emulsion of luminous bacteria. After recording the pH values, all the solutions of the same concentration were poured together and the light transmission determined directly as follows. The rectangular glass vessel with the mirrored sides and bottom and the opal glass back, which had been used for the experiments themselves, was filled with water and placed in front of the photometer lamp with the Wratten filter in place. The Macbeth illuminometer, also fitted with a No. 75 Wratten filter on the lamp side, was placed in front of the vessel of water and the inverse square scale on the illuminometer set at 10. The apparatus was then adjusted until a match in the fields of the illuminometer was obtained. This match with pure water represents 100 per cent transmission. The water in the vessel was then replaced by the *Cypridina* solution to be measured, and without changing the position of the apparatus the illuminometer lamp was moved back and forth until a match of the fields was obtained. The inverse square scale reads directly, and consequently gives the percentage transmission of the *Cypridina* solution. Readings can be duplicated to about 5 per cent for the low concentrations used. Since the vessel used for measuring the transmission of the solution is 2 cm. in thickness, and since the purpose of

the experiments is to determine the total light emitted from a 1 cm. cube of solution containing a definite amount of luminous powder, the thickness of the solution has to be taken into consideration and a correction made to obtain the transmission of a solution 1 cm. thick (T_1) by means of the formula

$$T_1 = \sqrt[n]{T_n}$$

in which T_n represents the thickness, n, of a solution used.

The determination of the amount of light emitted by 1 gm. of *Cypridina* powder is computed from the tabulated data as follows. It is necessary first to find the value in millilumens over 1 sq. cm. of the measuring vessel (2 cm. thick) for 1 arbitrary unit on the photometer scale, which will be constant for all experiments. If one obtains 2.1 foot candles of illumination of the opal glass for 2.5 arbitrary units, and since 1 millilambert in brightness equals 1 millilumen for every sq. cm., this value can be converted to millilumens per sq. cm. by the formula 2.1/2.5 foot candles $\times$ 0.00573, the transmission factor for the No. 75 Wratten filter, $\times$ 0.79, the reflection factor for the test plate, $\times$ 1.076, the conversion factor of foot candles to millilamberts. Solution of this formula gives 0.00408 millilumens per sq. cm. for 1 arbitrary unit. The total area of the decay curve (Fig. 1) is an index of the total amount of light emitted by the solution; this amount can be determined graphically by counting the squares and fractions of them. The straight line plotting of the logarithms is made to determine whether the experiment is progressing properly and also to determine the point of initial brightness of the solution at time 0. One square on the original plotting is equal to $\frac{1}{2}$ an arbitrary unit for every 10 seconds, determined from actual readings (Fig. 1, lower left square), hence $k = 0.00408/2$ or 0.00204 millilumens per sq. cm. for 10 seconds. But this factor is the brightness for a solution measuring 2 cm. thick which shows some absorption. Correcting for 1 cm. and taking into account the transmission of the solution by the formula $T_1 = \sqrt[n]{T_n}$, where the transmission (T_2) in a typical experiment is 64 per cent, one obtains

$$T_1 = \sqrt{0.64}, \text{ or } \log T_1 = \frac{\log T_2}{2}$$

$T_1 = 0.80$ or 80 per cent transmission.

To determine the brightness of a solution 1 cm. thick with no absorption, $(\bar{B}_1)$, the formula

$$B_n = \frac{\bar{B}_1 (T_n - 1)}{\log e\, T_1}$$

is used, in which B_n is the brightness of a solution n cm. thick, and T_n is the transmission of a solution n cm. in thickness. Applying this formula to the typical experiment

$$0.00204 = \frac{\bar{B}_1 (.64 - 1)}{\log e\, .80} \text{ or } 0.00204 = \frac{\bar{B}_1 (- .36)}{- .223} \text{ or } \bar{B}_1 = 0.001263 \text{ millilumens per sq. cm.}$$

This value can be easily converted from a surface brightness in cm. squares to a cube emitting light in all directions by multiplying by 4, which gives 5.052×10^{-6} lumens, the value from 1 cc. of solution of perfect transparency calculated for 10 seconds and corrected for 64 per cent transmission. Taking 37.5 area squares or arbitrary squares derived from experimental plottings in a typical run, and solving for total light emission per gm. of *Cypridina* in a solution containing 0.01 gm. of powder per cc. of solution, one obtains

$$0.005052 \times 10 \times 37.5 = \frac{1.895}{0.01} \times 10^{-3}$$

or 0.1895 lumens per gm. of powder per second.

Effect of Luciferase Concentration upon Amount of Light.

Table II contains data from three series of experiments totalling 32 runs with different concentrations of luciferase. It is understood, of course, that in an impure solution such as luciferase the actual concentration of enzyme is undetermined, but the relative concentrations are known. The velocity constants as determined from curves plotted for these series are tabulated in Table II. In one case the ratios are almost exactly the theoretical value. Amberson's conclusion that "the velocity of reaction is very nearly proportional to enzyme concentration" is supported by the present work.

Inspection of the data from the records of lumen-seconds per cc. of *Cypridina* solution (squares) shows considerable variation. These

variations in total light emission from solutions prepared in as nearly identical a manner as were the experimental solutions are not easily

TABLE II.

Amount of Light and Enzyme Concentration.

Enzyme concentration (C) in cc.	k	$\frac{k_2}{k_1}$	Squares	T_2	T_1	$\bar{B}_1 \times 4$	Lumens	Averages
				per cent	*per cent*		*gm. per sec.*	
Experiment III								
1	1.5		33.5	64	80	5.075×10^{-6}	.3396	
1	1.875		37.5	"	"	"	.3806	.356
1	1.86	1.58	34.25	"	"	"	.3476	
2	3.0		35.5	"	"	"	.3603	.3121
2	2.5		27.0	"	"	"	.2740	
Experiment IV								
1	1.80		46.6	74	86	4.75×10^{-6}	.4427	
1	1.55		40.6	"	"	"	.3857	.3844
1	1.83		34.2	"	"	"	.3249	
2	3.87	2.11	37.4	"	"	"	.3553	
2	2.70		38.6	"	"	"	.3667	.3591
2	4.40		37.4	"	"	"	.3553	
Experiment VI								
1	1.17		39.3	72	84.8	4.107×10^{-6}	.3776	
1	1.22		41.0	"	"	"	.3907	.3684
1	1.075		35.37	"	"	"	.3369	
2	1.69	1.64	33.4	"	"	"	.3182	
2	2.02		40.88	"	"	"	.3907	.3406
2	2.02		33.0	"	"	"	.3229	

The constant (k) of the reaction velocity is determined from the slope of the logarithmic plotting.

k_2/k_1 represents the ratio of the average velocity reaction slopes where k_2 is the velocity constant, when double amounts of enzyme are used.

Each cc. of enzyme solution contained 0.005 gm. of dry *Cypridina* powder and was added to 20 cc. of luciferin solution.

T_2 and T_1 are transmissions for 2 cm. and 1 cm. thick solutions; $\bar{B}_1 \times 4$ is explained in the text.

explained. Taking averages in all cases recorded, the total emission of light is about the same, but somewhat less, when the concentration

of luciferase is doubled. This is seen in the total light emission recorded as lumens per second per gm. of dry *Cypridina* which for 5 runs averages 0.3777 for concentrations designated as C and 0.3507 for $2C$ concentrations of luciferase. If the amount of light were independent of the luciferase concentration, these values should be the same.

Effect of Luciferin Concentration upon Amount of Light.

Seven series of experiments totalling 60 runs were made with variations in the concentration of luciferin from 2 gm. to 0.5 gm. of powder per 100 cc. water. Acid sea water, neutral sea water and distilled water saturated with hydrogen were used to prepare the luciferin. The data of three typical series are tabulated in Table III. Again considerable variation occurs in the lumen-seconds (squares) emitted per cc. of solution with the same concentration of luciferase. With higher concentrations of luciferin, the squares of light emitted are about twice as great. The total light emission recorded as lumens per second per gm. of dry *Cypridina* averages about the same in the different experiments with some tendency for the weaker luciferin concentrations to give relatively more light.

With a constant concentration of enzyme the velocity constant should be the same for a wide range of substrate concentrations, and the straight line plottings should be parallel, or nearly so. This expected relationship, however, did not appear in any of the graphs. Similar anomalous results occur also in Amberson's records. It is obvious, then, that the luciferin-luciferase system represents an exceptional case of reaction velocity.

While this work primarily is concerned with the amount of light emitted from 1 gm. of *Cypridina* powder, it is, nevertheless, important to interpret the curves which indicate the nature of the enzyme reaction under investigation. Why do velocity constants vary for the different concentrations of substrate? Without doubt the oxidation of luciferin is a monomolecular reaction in a heterogeneous system, since luciferase is a colloid and probably a protein (Harvey, 1920). It not infrequently happens that the change in a chemical reaction is less, the greater the initial concentration of the substrate (Hoeber), hence the reaction velocity cannot be defined by a definite constant to

signify the change in the two concentrations. Reactions of this sort are encountered in the inversion of cane sugar by invertase (Hudson),

TABLE III.

Amount of Light and Substrate Concentration.

Substrate concentration in gm. *Cypridina* per cc.	k	$\frac{k_2}{k_1}$	Squares	T_2	T_1	$\overline{B}_1 \times 4$	Lumens	Averages
				per cent	*per cent*		*gm. per sec.*	
Experiment XI								
.005	2.4		41.5	59	76.8	5.273×10^{-6}	.4376	
"	2.54		40.3	"	"	"	.4249	.390
"	2.43		34.29	"	"	"	.3616	
"	2.72		32.0	"	"	"	.3374	
.0025	2.8	1.16	19.83	89	94.3	4.369×10^{-6}	.3465	
"	2.98		18.00	"	"	"	.3145	.3156
"	3.2		16.70	"	"	"	.2920	
"	2.8		17.71	"	"	"	.3095	
Experiment XVIII								
.0025	1.47		7.62	75	86.6	4.713×10^{-6}	.1436	
"	1.25		7.007	"	"	"	.1332	
"	1.35		7.33	"	"	"	.1382	.1398
"	1.36		7.66	"	"	"	.1444	
.00125	1.6	1.26	4.09	92	95.9	4.286×10^{-6}	.1402	
"	1.6		5.02	"	"	"	.1721	
"	1.73		4.42	"	"	"	.1515	.1519
"	2.0		4.20	"	"	"	.144	
Experiment XVII								
.01	1.17		92.08	74	86	4.75×10^{-6}	.4373	
"	1.04		83.39	"	"	"	.3961	
"	1.00		91.17	"	"	"	.4330	.417
"	0.9		84.54	"	"	"	.4015	
.005	1.5	1.53	44.20	84	91.6	4.464×10^{-6}	.3946	
"	1.58		54.59	"	"	"	.4874	.439
"	1.57		47.81	"	"	"	.4268	
"	1.48		50.12	"	"	"	.4474	

Symbols the same as in Table II.

of grape sugar by zymase (Euler), and in the catalysis of H_2O_2 by platinum sol studied by Bredig and his students (Bredig and von Berneck). Hence the phenomenon is not peculiar to enzyme reactions

alone, and seems to be associated with the physical nature of the reactants.

Study of the data of this paper gives no evidence that the reaction is altered greatly by variation in pH between 6.6 and 8.4. In one series, at pH 6.8 an average of 0.508 lumens was obtained, while a series run at pH 8.2 emitted an average of 0.445 lumens. Consideration of all the data obtained does not justify the conclusion that the differences in velocity are due solely to pH differences. Salts can be excluded from consideration, since in a solution made up with sea water the additions of small amounts of *Cypridina* solution would not greatly affect the total salt content. The solutions were always brought to a room temperature of about 20°C. Measurements of viscosity between solutions containing 10 cc. and 5 cc. luciferin respectively gave a difference in magnitude of approximately 1 per cent. This variation is negligible. All these factors can, therefore, be excluded as the cause of the anomaly in velocity constants for different luciferin concentrations.

In the work with catalysis of metal sols, the so called microheterogeneous systems, catalytic activity is increased in weaker substrate solution, whereas in macroheterogeneous systems where the dispersion is less extensive the reaction follows the theortical proportionality. It is quite probable that in suspensions and colloids the substrate adsorbs to the surface of the enzyme in greater degree from the more dilute solutions. So many factors are involved in the enzyme reactions among colloidal solutions that an exact formulation of the kinetics has never been satisfactorily made, but as a working hypothesis it may be assumed that the greater velocity constant with weak luciferin concentration is associated with greater adsorption from this concentration.

SUMMARY.

1. A photometric method was devised for measuring the intensities of light emitted per cc. of luciferin solution and calculating the amount of light emitted per gm. of dried *Cypridina* powder. A total of 128 runs was made and the data are incorporated in this report.

2. The maximum amount of light emitted from 1 gm. of powder under the experimental conditions was 0.655 lumens. Different samples of powder vary greatly in amount of light production.

3. When the concentration of substrate is doubled, nearly twice as

much light is emitted, or an average ratio $2C/C$ of 1.86. Calculations of total light emissions *per gm. of powder* at different concentrations indicate that slightly more light is produced from the smaller concentrations. The maximum amount of light was produced by the solutions made with neutral sea water and averaged 0.445 lumens. The least light was obtained from solutions in distilled water saturated with hydrogen. The technique allows too rapid spontaneous oxidation prior to the saturation with hydrogen. The maximum amount of light from such experiments was only 0.077 lumens. Acid sea water solutions subsequently neutralized gave an average maximum of 0.386 lumens per gm. of powder per second.

4. When the concentration of enzyme is doubled, approximately the same amount of light is produced by both concentrations, although the stronger concentrations are slightly less effective than weaker ones. This undoubtedly is due to the colloidal nature of the enzyme and is a function of surface rather than of mass. In dilute solutions greater dispersion probably allows for greater adsorption to the surface of the enzyme. The average maximum amount of light produced in the series of enzyme experiments is of the magnitude 0.56 lumens per gm. of powder.

I wish to express my deep indebtedness to Dr. E. Newton Harvey under whose direction this study was undertaken, and to thank Mr. Stanton M. Hardy for the great help he rendered as my assistant.

I am also greatly indebted to Dr. Harvey for an abundant supply of *Cypridina hilgendorfii* Müller, the Japanese ostracod, the organism which has been used for the source of light in all the experiments.

BIBLIOGRAPHY.

Amberson, W. R., *J. Gen. Physiol.*, 1921–22, iv, 557.

Bredig, G., and Müller von Berneck, R., *Z. physik. Chem.*, 1899, xxxi, 258.

Euler, H., *Z. physiol. Chem.*, 1905, xliv, 53.

Harvey, E. N., *J. Gen. Physiol.*, 1925–27, viii, 89.

Harvey, E. N., The nature of animal light, Monographs on experimental biology, Philadelphia and London, 1920, 141.

Hoeber, R., Physikalische Chemie der Zelle und der Gewebe, Leipsic, 5th edition, 1922, 326–363.

Hudson, C. S., *J. Am. Chem. Soc.*, 1908, xxx, 1160.

Morrison, T. F., *J. Gen. Physiol.*, 1924–25, vii, 741.

Trautz, M., *Z. physik. Chem.*, 1905, liii, 1.