The Spectral Distribution of Firefly Light

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ABSTRACT The *in vivo* emission spectra of sixteen species of Jamaican fireflies and four species of American fireflies have been measured with a photoelectric recording spectrometer. A simple technique of stimulation with ethyl acetate vapor was found to elicit bright continuous emission over a period of several minutes. Although the luciferin-luciferase cross-reactions were positive in all cases tested, peak intensity wavelengths show distinct species differences, ranging from 5520 to 5820 A. Widely separated emission peaks arise from the thoracic and abdominal organs respectively in the same animal, the click-beetle, *Pyrophorus.*

INTRODUCTION

It is a well established observation that the light emitted by different species of fireflies ranges in color from intense green to bright yellow. The spectral distribution of the bioluminescence emission has been the subject of numerous investigations, summarized in Table I. Included in Table I and italicized for easier identification are the measurements on twenty species reported on in the present paper. The use of photographic spectrometry by Coblentz (1) eliminated the physiological Purkinje effect as being solely responsible for observed visual color differences. It is still conceivable that the differences can be due to self-absorption in the photogenic cells or to absorption in the "transparent" layer covering the light organ. However, Seliger and McElroy (2, 3) demonstrated a color shift in the bioluminescence of purified extracts of *Photinus pyralis* luciferase and luciferin, varying with pH or, more recently, with Zn^{++} ionic concentration. Further they demonstrated that *Photuris pennsylvanica* enzyme extract plus *Photinus pyralis* luciferin produces the green emission of *pennsylvanica* while *Photinus pyralis* enzyme extract plus *Photuris pennsylvanica* luciferin produces the yellow-green emission of *pyralis.* Both the color shift and the *enzyme-specific* bioluminescence emission imply that enzyme differences among species, either in specific amino acid components or in tertiary

TABLE I

SUMMARY OF MEASUREMENTS OF EMISSION SPECTRA OF FIREFLY BIOLUMINESCENCE

The species *Photinus marginellus*, *Photinus pyralis*, *Photinus scintillans*, and *Photuris pennsylvanica* are native to the eastern United States and were collected and measured during the summer of 1963. All the other species newly reported in the present work were collected and measured in Portland parish, Jamaica, in February, 1963. Unless otherwise indicated only a single specimen of the species was studied. The sex of specimens is specified where known. It is likely that where sex was not specified in literature reports on species of *Photinus*, male specimens were involved because in this genus the males are usually much more common than the female and have much the larger light organ.

Species	Observer and technique		Wavelength at peak intensity	Description of spectrum
			*Angstroms**	
"Glow-worm"	Secchi, 1872	VS	—	Continuous band in visible
Glow-worm (*Lampyris*)	Phipson, 1876	VS	—	4860–6560 A band limits
Glow-worm	Conroy, 1882	VS	—	5180–6560 A band limits
Glow-worm	Ramdas and Venkiteshwaran, 1931	PGS	—	5290–5860 A band limits
Glow-worm	Brooks, 1940	PGS	—	4690–5879 A band limits
"Indian Firefly"	Severn, 1881	VS	—	Continuous band in visible
Diphotus montanus	Buck, 1941	PGS	—	5150–6200 A band limits
Diphotus semifuscus	Buck, 1941	PGS	—	5125–6550 A band limits
Diphotus unicus	Buck, 1941	PGS	—	5050–6450 A band limits
Diphotus sp. (2 specimens)	*Present work, 1963*	*PES*	*5550*	*FWHM‡ 730 A; 10% points: 5100–6350 A*
Lecontea sp.	*Present work, 1963*	*PES*	*5700*	*FWHM 795 A; 10% points: 5290–6800 A*
Phengodes laticollis	McDermott, 1911	VS	—	5110–6450 A band limits
Phengodes sp.	Buck, 1941	VS	—	5100–5900 A band limits
Photinus ceratus ♂	Buck, 1941	VS	—	5350–6200 A band limits
Photinus ceratus-morbosus§ (6 specimens)	*Present work, 1963*	*PES*	5710	FWHM 670 A; *10% points: 5360–6300 A*
Photinus commissus ♀	*Present work, 1963*	*PES*	*5640*	*FWHM 730 A; 10% points: 5200–6900 A*
Photinus consanguineus	McDermott, 1910	VS	—	5500–6150 A band limits
Photinus consanguineus	Coblentz, 1912	PGS	5780	FWHM 467 A; 10% points: 5300–6200 A
Photinus evanescens ♂	Buck, 1941	VS	—	5350–6550 A band limits
Photinus evanescens (4 specimens)	*Present work, 1963*	*PES*	*5700*	*FWHM 670 A; 10% points: 5350–6300 A*
Photinus flavolimbatus ♂	Buck, 1941	VS	—	5275–6200 A band limits
Photinus fuscus	Grinfeld, 1944	PGS	5690	5120–7150 A band limits
Photinus gracilobus	Buck, 1941	VS	—	5075–6550 A band limits
Photinus gracilobus	*Present work, 1963*	*PES*	*5720*	*FWMH 730 A; 10% points 5330–6500 A*
Photinus leucopyge (6 specimens)	*Present work, 1963*	*PES*	*5690*	*FWHM 700 A; 10% points 5300–6450 A*
Photinus lobatus (3 specimens)	*Present work, 1963*	*PES*	*5700*	*FWHM 670 A; 10% points 5280–6800 A*
Photinus marginellus	*Present work, 1963*	*PES*	*5650*	*FWHM 640 A; 10% points 5220–6520 A*
Photinus melanurus (3 specimens)	*Present work, 1963*	*PES*	*5700*	*FWHM 670 A; 10% points 5280–6500 A*
Photinus nothus (3 specimens)	*Present work, 1963*	*PES*	*5700*	*FWHM 670 A; 10% points 5270–6500 A*
Photinus pallens ♂	Buck, 1941	PGS	5775	5150–6425 A band limits
Photinus pallens ♀	Buck, 1941	PGS	5775	5200–6450 A band limits
Photinus pallens	*Present work, 1963*	*PES*	*5650*	*FWHM 690 A; 10% points 5230–6750 A*
Photinus pardalis	*Present work, 1963*	*PES*	*5600*	*FWHM 600 A; 10% points 5180–6350 A*

VS, visual spectrometry; PGS, photographic spectrometry; PES, photoelectric spectrometry.

* The accuracy in the determination of peak position is ± 30 A. The precision with any one specimen is ± 10 A.

‡ FWHM is the full width at half maximum intensity.

§ We were unable to distinguish between these two species in the February, 1963, samples. A discussion of speciation in regard to fl type will be found in a separate paper.

TABLE I—*Concluded*

Species	Observer and technique		Wavelength at peak intensity	Description of spectrum
			Angstroms•	
Photinus pyralis	Ives and Coblentz, 1910	PGS	—	5250–6400 A band limits
Photinus pyralis	McDermott, 1910	VS	—	5350–6200 A band limits
Photinus pyralis	Coblentz, 1912	PGS	5670	FWHM 333 A
Photinus pyralis (*in vitro*)	McElroy and Rainwater, 1948	PES	5660	FWHM 860 A
Photinus pyralis (*in vitro* pH 7.5)	Seliger and McElroy, 1960	PES	5620	FWHM 600 A; 10% points: 5100–6250 A
Photinus pyralis (*in vitro*—acid pH)	Seliger and McElroy, 1960	PES	6140	FWHM 600 A
Photinus pyralis ♂	*Present work, 1963*	*PES*	*5620*	*FWHM 680 A; 10% points: 5160–6580 A*
Photinus pyralis ♀	*Present work, 1963*	*PES*	*5620*	*FWHM 680 A; 10% points: 5160–6580 A*
Photinus scintillans ♂	*Present work, 1963*	*PES*	*5750*	*FWHM 650 A; 10% points: 5290–6700 A*
Photinus scintillans ♀	*Present work, 1963*	*PES*	*5750*	*FWHM 650 A; 10% points: 5290–6700 A*
Photinus scintillans	Coblentz, 1912	PGS	*5780–5800*	
Photinus sp.	Young, 1870	VS	—	4870–6560 A band limits
Photinus sp. (new species)	*Present work, 1963*	*PES*	*5690*	*FWHM 730 A; 10% points: 5100–6340 A*
Photinus synchronans	Buck, 1941	PGS	5850	5150–6450 A band limits
Photinus variabilis ♂	Buck, 1941	PGS	5800	5200–6600 A band limits
Photinus xanthophotis catherinae ♂	Buck, 1941	PGS	5850	5350–6400 A band limits
Photinus xanthophotis catherinae ♀	Buck, 1941	PGS	5800	5350–6400 A band limits
Photinus xanthophotus	*Present work, 1963*	*PES*	*5690*	*FWHM 680 A; 10% points: 5250–6410 A*
Photinus xanthophotus ♀	*Present work, 1963*	*PES*	*5670*	*FWHM 680 A; 10% points: 5250–6410 A*
Photuris jamaicensis ♀	Buck, 1941	VS	—	5225–6550 A band limits
Photuris jamaicensis ♂	*Present work, 1963*	*PES*	*5550*	*FWHM 700 A; 10% points: 5140–6610 A*
Photuris jamaicensis ♀ *(2 specimens)*	*Present work, 1963*	*PES*	*5550*	*FWHM 740 A; 10% points: 5130–6640 A*
Photuris pennsylvanica (larva)	Coblentz, 1912	PGS	5520	FWHM 400 A
Photuris pennsylvanica	McDermott, 1910	VS	—	5400–6150 A band limits
Photuris pennsylvanica	Coblentz, 1912	PGS	5520	FWHM 400 A; 10% points: 5100–6000 A
Photuris pennsylvanica	*Present work, 1963*	*PES*	*5520*	*FWHM 640 A; 10% points: 5090–6400 A*
Pyrophorus sp.	Pasteur, 1864	VS	—	Continuous band in visible
Pyrophorus sp.	Aubert and Dubois, 1884	VS	—	4860–7240 A band limits
Pyrophorus noctilucus (thorax)	Dubois, 1886	VS	—	4870–6870 A band limits
Pyrophorus noctilucus (thorax)	Langley and Very, 1890	VS	—	4680–6400 A band limits
Pyrophorus noctilucus (abdomen)	Langley and Very, 1890	VS	—	4630–6630 A band limits
Pyrophorus noctilucus (thorax)	Coblentz, 1912	PGS	5380–5400	FWHM > 600 A
Pyrophorus plagiophthalamus (thorax)	Buck, 1941	PGS	5850	5050–6500 A band limits
Pyrophorus plagiophthalamus (thorax)	*Present work, 1963*	*PES*	*5530*	*FWHM 760 A; 10% points: 5040–6330 A*
Pyrophorus plagiophthalamus (abdomen)	Buck, 1941	PGS	5950	5400–6450 A band limits
Pyrophorus plagiophthalamus (abdomen)	*Present work, 1963*	*PES*	*5820*	*FWHM 730 A; 10% points: 5300–6700 A*
Pyrophorus punctatissimus	Grinfeld, 1944	PGS	5580	4900–7085 A band limits

The references in the table are as follows:—

Aubert, and Dubois, R., *Compt. rend. Acad. sc.*, 1884, **99**, 477; Brooks, G., *Compt. rend. Acad. sc.*, 1940, **210**, 228; Buck, J. B., *Proc. Rochester Acad. Sc.*, 1941, **8**, 14; Coblentz, W. W., *Carnegie Institution of Washington, Pub. No. 164*, 1912; Conroy, J., *Nature*, 1882, **26**, 319; Dubois, R., *Bull. Soc. Zool. France*, 1886, **11**, 1; Grinfeld, R., *Pub. Fac. Cienc. Fisicomat. Univ. La Plata*, 1944, **3**, 447; Ives, H. E., and Coblentz, W. W., *Bureau Standards Bull.*, 1910, **6**, 321; Langley, S. P., and Very, F. W., *Am. J. Sc.*, 1890, **40**, series 3, 97; McDermott, F. A., *Canad. Entomol.*, 1910, **42**, 357; McDermott, F. A. *Ann. Rep. Smithson. Inst.*, 1911, 345; McDermott, F. A., *Scient. Am.*, 1911, suppl. 71, 250; McElroy, W. D., and Rainwater, C. S., *J. Cell. and Comp. Physiol.*, 1948, **32**, 421; Pasteur, L., *Compt. rend. Acad. sc.*, 1864, **59**, 509; Phipson, T. L., *J. Franklin Inst.*, 1876, **71**, series 3, 68; Ramdas, L. A., and Venkiteshwaran, L. P., *Nature*, 1931, **128**, 726; Secchi, P., *Compt. rend. Acad. sc.*, 1872, **75**, 314; Seliger, H. H., and McElroy, W. D., *Arch. Biochem. and Biophysics*, 1960, **88**, 136; Severn, H. A., *Nature*, 1881, **24**, 165; Young, C. A., *Am. Naturalist*, 1870, **3**, 615.

structure, are responsible for the observed color variations. It is therefore important from the viewpoint of mechanism to obtain *in vivo* emission spectra of additional species of fireflies and to examine their respective purified luciferin-luciferase systems. The present paper is an attempt to systematize the *in vivo* emission spectra, as a first step toward solution of the general problem.

EXPERIMENTAL TECHNIQUE

The spectrometer used was a portable Fastie-Ebert type grating spectrometer (4) with a focal length of 500 mm and a linear dispersion in first order of 27 A/mm. The grating was a 4 inch square replica, 1200 lines/mm, blazed at 5000 A. The phototube was an E.M.I. 9558B. This is an 11 stage Venetian blind dynode type with a tri-alkali photocathode, S-20 response. The combined spectral efficiency of the spectrometer-transmission phototube sensitivity was determined with a National Bureau of Standards tungsten color temperature standard lamp, operated at a color temperature of 2854°K. Over the visible region (4000 to 7000 A) the *relative* energy distribution of the radiation from the color temperature lamp is the same as that for an ideal blackbody. The data of Barbrow (5) for a color temperature of 2854°K were therefore converted from microwatts per steradian-10 mμ-candela to photons per second-steradian-10 mμ-candela. At a fixed arbitrary phototube gain the current per photon per second was measured as a function of wavelength. The normalized averaged results for five of the filaments of the NBS standard lamp are plotted in Fig. 1.

The spectrometer grating was driven by a rotating "sine θ" cam, such that $\frac{d\lambda}{dt} =$ constant. The angular speed of the grating, as driven by a small synchronous motor and a stepwise adjustable ratio gear box, could be varied from 10 RPM to $\frac{1}{5}$ RPM. The DC amplifier used to measure phototube current was the same as that described previously (6). The recorder was a transistorized two-channel Sanborn model 320. Rotating on the same axis as the sine θ cam was a circular cam with evenly spaced indentations around the periphery. This, in conjunction with a lever-action microswitch, furnished "event-pulses" to the Sanborn recorder. These event, or marker pips were spaced at equal time intervals which, owing to the sine θ displacement of the grating, corresponded to equal wavelength intervals. Thus the wavelength calibration of the spectrometer was linear with marker number and therefore with chart distance. The wavelength calibration curve of the instrument was obtained with a low pressure Hg discharge lamp. All observed current measurements were corrected for spectral efficiency using the data of Fig. 1. The time for a complete spectrum was approximately 10 seconds.

The Jamaican fireflies were mainly collected on the Long Bay-Ecclesdown back road in the hills at the base of the John Crow Mountain range in the northeast section of Jamaica at an altitude of approximately 750 feet. It is possible in Jamaica, in a relatively small area at the same altitude, to collect numbers of more than a dozen different species of firefly, which is a distinct advantage for the types of comparisons in which we are interested. In the Baltimore area over the entire summer of 1963, only four species were found, and some of these only rarely. Definite identification of the Jamaican species was made on the basis of the shape and coloration of the dis-

sected genitalia as described by McDermott and Buck (7). The American species were identified on the basis of the data of Green (8) and confirmed for us by F. A. McDermott.

For studies of the thoracic organ of the large luminous elaterid beetle *Pyrophorus*, which glows steadily for long periods, the animal was mounted directly at the 1 mm wide entrance slit of the spectrometer. To observe the ventral light organ of *Pyrophorus*, which is situated in a cleft between the thorax and abdomen, it was necessary to remove the hard wing cases and fold the body backwards. With the smaller lampyrid fireflies, which ordinarily luminesce only *via* brief flashes separated by long intervals of darkness, the following procedure was used: a glass tube 5 cm long and approximately 3 mm inside diameter was constricted at one end to hold a small

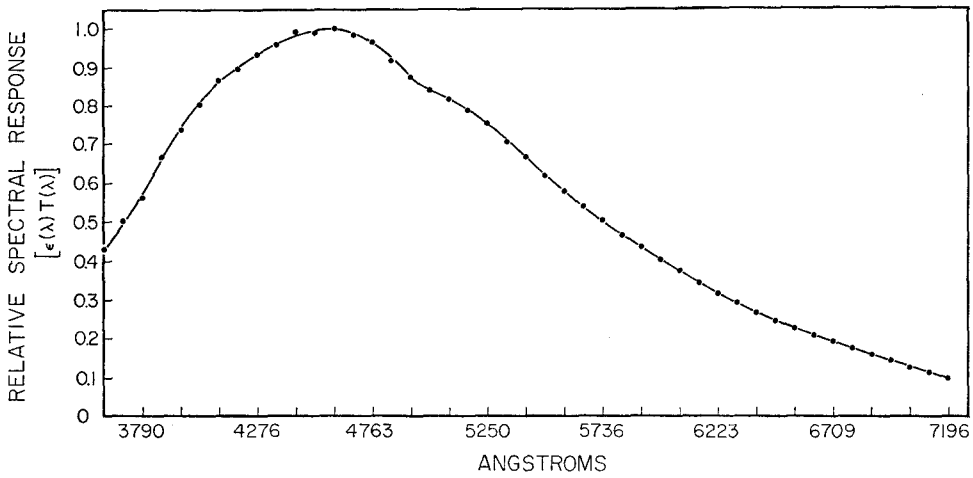

FIGURE 1. Relative spectral photon response of phototube-spectrometer combination as measured with a National Bureau of Standards color temperature standard lamp. The curve represents the product of the phototube photon spectral sensitivity and the transmission of the grating, blazed at 5000 A.

cotton plug and fitted with a small rubber tube. The tube was fixed vertically at the center of curvature of the spherical entrance mirror, constricted end down. In aligning, a miniature lamp with a 2 mm filament was inserted into the glass tube to a fiducial mark and the image of the filament was focussed directly on the entrance slit of the spectrometer. The live firefly was then gently put in place of the miniature lamp, tail down, so that the light organ was at the fiducial mark and then wedged from above with cotton. A drop of ethyl acetate was placed on the cotton wedge and the vapor was gently drawn through the glass tube by inhaling through the rubber tube attached to the constricted end. In a time ranging from 15 seconds to about 60 seconds the firefly would start to flash weakly, then more strongly and would finally emit a bright continuous light lasting between 2 to 5 minutes. The continuous glow usually appeared first at one small spot on the light organ and then spread over the entire organ. After this the light would flicker and then finally go out. The firefly

never completely recovered from this treatment although the next day it was usually still alive and able to glow weakly.

We found the effect of ethyl acetate to be consistent in all lampyrid fireflies with the exception of *Diphotus* for which ethyl acetate seemed completely inhibitory. It is interesting to note that *Diphotus* does not "flash" like the others but in nature emits a

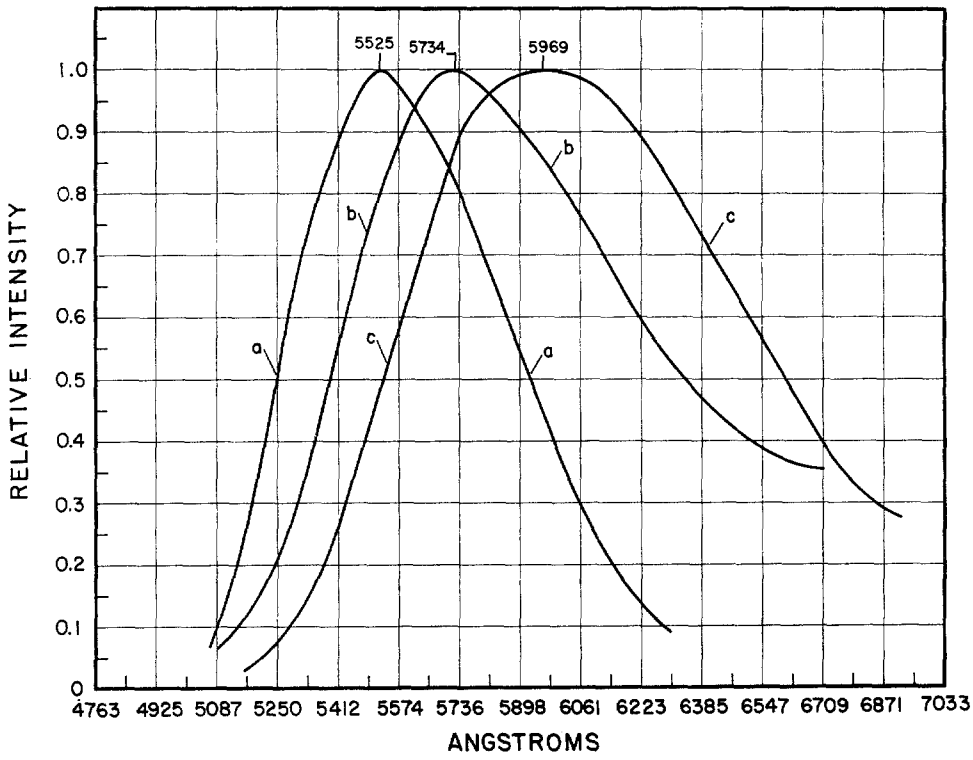

FIGURE 2. Normalized bioluminescence emission observed from the green-emitting thoracic light organ of *Pyrophorus* showing effect of absorption by cuticle. (*a*) Direct emission of light normal to the light organ so that there is no cuticle absorption; (*b*) partial absorption by cuticle; (*c*) emission from under side of light organ showing the large effect of the blue-green absorption by the cuticle.

continuous steady pulse lasting many seconds. It is possibly this difference in nervous control of rapid flashing that makes for the apparent difference in the ethyl acetate effect.

RESULTS AND DISCUSSION

From close visual observation of light emission from various angles there does not appear to be any absorption by the transparent layer of cuticle immediately overlaying the light organs of *Pyrophorus*. It has been variously reported that the abdominal organ emits orange or red light rather than yellow. This

effect can be approximated if the organ is viewed at an angle through the general body cuticle with the abdomen in a partly closed position. Then preferential absorption of the blue and green components by the brownish body cuticle makes the light appear orange-red. The same is true for the

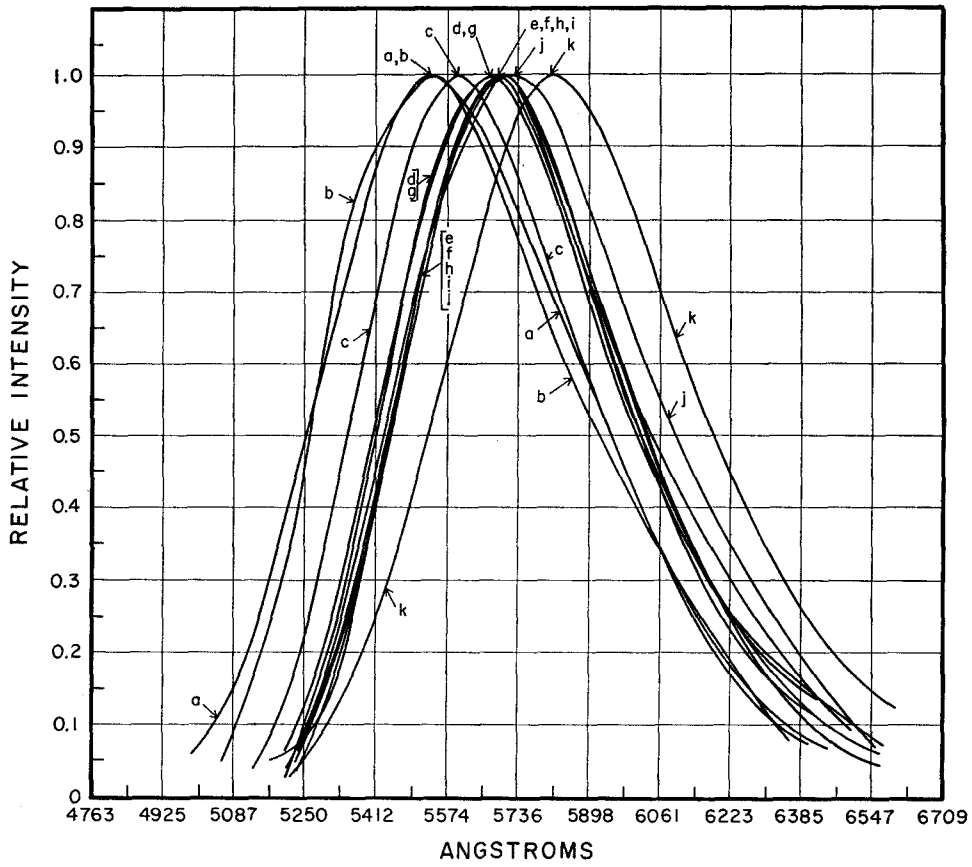

FIGURE 3. Bioluminescence emission spectra of various Jamaican fireflies. (*a*) *Pyrophorus plagiophthalamus* (thoracic organ); (*b*) *Diphotus sp.*; (*c*) *Photinus pardalis*; (*d*) *Photinus xanthophotis*; (*e*) *Photinus leucopyge*; (*f*) *Photinus melanurus*; (*g*) *Photinus nothus*; (*h*) *Photinus evanescens*; (*i*) *Photinus ceratus* or *morbosus*; (*j*) *Photinus gracilobus*; (*k*) *Pyrophorus plagiophthalamus* (ventral organ).

thoracic organ. For example, Fig. 2 *a* shows the normal green emission spectrum of the thoracic organ and the same emission, *b* and *c*, modified by absorption in the cuticle surrounding the organ. The effects were produced by mounting the beetle at various angles so that the body cuticle acted as an absorber.

However, during a more recent collecting trip to the same region, two of

us (H. H. Seliger and W. D. McElroy) were able to find a small number of *Pyrophorus* which emit from their ventral organ a definite orange light, as contrasted with the more predominant yellow light-emitting forms. We were not able at this time to measure the *in vivo* emission spectra.

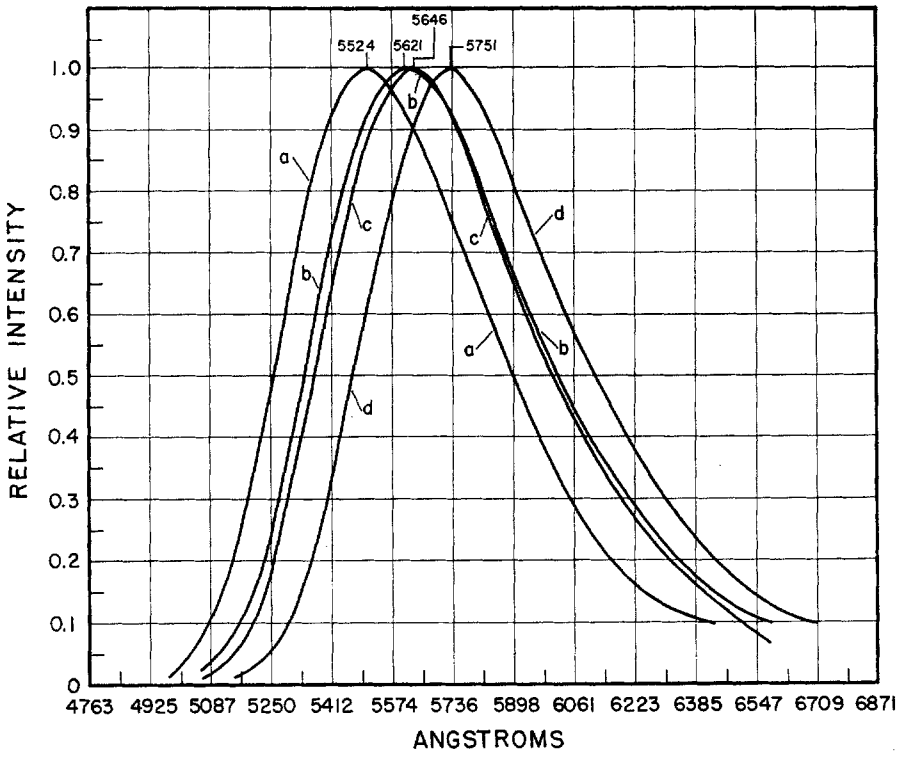

FIGURE 4. Bioluminescence emission spectra of some American fireflies. (*a*) *Photuris pennsylvanica*; (*b*) *Photinus pyralis* ♂ and ♀; (*c*) *Photinus marginellus*; (*d*) *Photinus scintillans* ♂ and ♀.

The emission spectra of the bioluminescence of various Jamaican and American fireflies are given in Figs. 3 and 4 respectively. Fig. 5 is a tabulation of the emission peak positions for all the species measured. The wide range of emission peaks, from 5520 A in *Photuris pennsylvanica* to 5820 A in the ventral organ of *Pyrophorus plagiophthalamus* is remarkable in that all the firefly luciferins that we have isolated and tested by paper chromatography, fluorescence emission, and fluorescence excitation are the same as synthetic luciferin. There is thus no reason to suspect that the mechanism of oxidation of luciferin is different for the different species. Rather it appears that different enzyme-substrate combinations can have slightly different energy levels and consequent differences in emission spectra. From the symmetric shapes of the spectra

shown in Figs. 3 and 4 for Jamaican and American fireflies respectively, it would seem unlikely that the resultant color is a mixture of the emissions of molecular species, one with its maximum in the green region and the other in the yellow or red regions. However, we have in the case of the *pyralis* luciferase-luciferin reactions been able to demonstrate the existence of two such

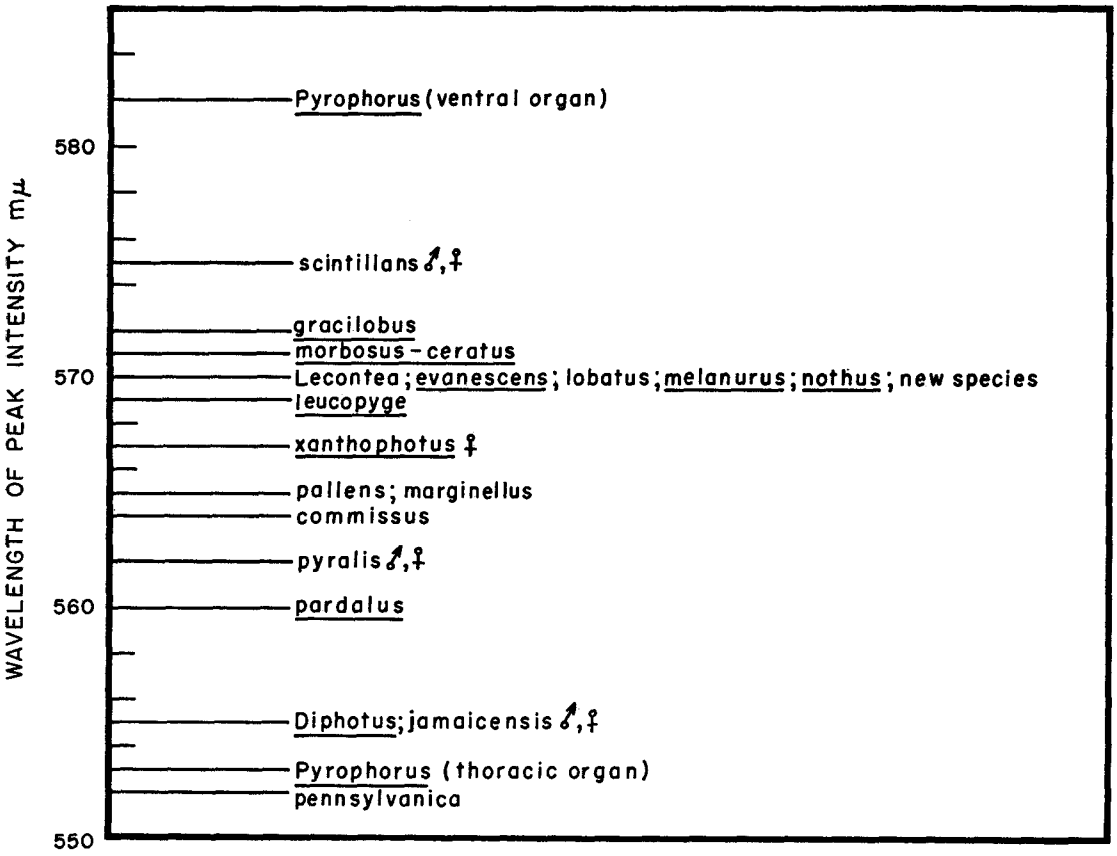

FIGURE 5. Summary of peak emission wavelengths for twenty firefly species reported in this paper. The spread in emission peaks is over 300 A. Data: all lower case names are of the genus *Photinus* except *jamaicensis* and *pennsylvanica*. These latter are of the genus *Photuris*. Except where underlined, only a single specimen was studied.

emitting molecular species *in vitro* (2). There is the normal yellow-green emission at neutral or alkaline pH together with a small fraction of a red emission at around 6140 A. At acid pH values, the yellow-green emission is inhibited and only the low intensity red emission is observed. The intermediate spectra observed at intermediate pH values are actually composites of these two emissions. It is possible, in the case of fluorescent dyes, to alter the fluorescence emission by attachment of various electron-donating substituents. It is there-

fore likely that the differences in emission of the various species are due to either the complexing of the luciferyl adenylate molecule with some such group on the protein molecule, or the slight admixture of a red-emitting molecular species to the predominant yellow-green emission. Different firefly species, due to slightly different enzyme structure, might then emit in slightly different spectral regions.

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