STUDIES ON BIOLUMINESCENCE.

XIV. The Specificity of Luciferin and Luciferase.

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INTRODUCTION.

In the living world are found more than thirty groups of organisms producing light, scattered, in the animal kingdom at least, through orders widely different in morphological characteristics. Physiologically, one order may contain individuals producing light in quite different ways, as the squids, some of which have light organs producing an external secretion of luminous material, while others possess light organs of internal combustion. Among the fish, also, are found various types of luminescence. Some forms emit light only as a result of stimulation while others luminesce continually day and night, and the intensity is quite independent of stimulation of any kind. In this respect these fish resemble the fungi and luminous bacteria which also emit a steady continuous light which is not varied on stimulation.

While it is impossible at the present time, because of lack of data, to classify accurately and logically the various types of luminescence found in living things, the accompanying list is an attempt in this direction, which will serve as a guide to the groups, whose particular characteristics of luminescence are discussed in this paper. Accordingly, relationship as well as physiological peculiarities of luminescence have been considered in making the group (Table I).

Two questions at once arise in connection with these data. The first has to do with the presence of the luciferin-luciferase reaction. As is evident from the above list, luciferin and luciferase, first dis-

TABLE I.

Groups of Luminous Organisms.

Group.	Genus studied.	Type of luminescence.	Luciferin and luciferase.
Bacteria.	*Microspira.* *Photobacterium.*	Intracellular and contintinuous.	Negative.
Fungi.	*Armillaria.* *Clytocybe.*	Intracellular and continuous.	?
Radiolaria.	*Thallassicola.*	Intracellular; on stimulation.	?
Dinoflagellates.	*Gonyaulax.* *Ceratium.*	Intracellular; on stimulation.	?
Cystoflagellates.	*Noctiluca.*	Intracellular; on stimulation.	Negative.
Sponges.	*Grantia.**	(?); on stimulation.	Negative.
Medusæ and hydroids.	*Æquorea.* *Mitrocoma.*	Extracellular (?); on stimulation.	Negative.
Pennatulids.	*Cavernularia.* *Pennatula.* *Ptylosarcus.*	Extracellular (?); on stimulation.	Negative.
Ctenophores.	*Bolina.*	Intracellular (?); on stimulation.	Negative.
Tomopterid worms.	*Tomopteris.*	Extracellular (?); on stimulation.	?
Syllid worms.	Odontosyllis.	Extracellular (?); on stimulation.	Positive.
Polynöed worms.	*Polynöe.*	Intracellular; on stimulation.	Negative ?
Chætopteroid worms.	*Chætopterus.*	Extracellular; on stimulation.	Negative
Earthworms.	*Microscolex.*	(?); on stimulation.	?
Ostracod crustacea.	*Cypridina.* *Pyrocypris,*	Extracellular; on stimulation.	Positive.
Copepod crustacea.	*Metridia.* *Pleuromma.*	Extracellular; on stimulation.	?
Schizopod crustacea.	*Meganyctiphanes.*	Intracellular; on stimulation.	Negative.
	Gnathophausia.	Extracellular; on stimulation.	?

* Luminescence doubtful.

TABLE I—*Continued.*

Group.	Genus studied.	Type of luminescence.	Luciferin and luciferase.
Decapod crustacea.	*Sergestes.*	Intracellular; on stimulation.	?
	Heterocarpus.	Extracellular; on stimulation.	?
Myriapods.	*Geophilus.*	Extracellular; on stimulation.	Negative ?
Springtails.	*Podura.*	Intracellular (?); on stimulation.	?
Beetles.	*Luciola.* *Pyrophorus.*	Intracellular; on stimulation.	Positive.
Flies.	*Boletophila.*	Intracellular; on stimulation.	?
Brittle stars.	*Ophiacantha.*	Intracellular (?); on stimulation.	?
Lamellibranch mollusks.	*Pholas.*	Extracellular; on stimulation.	Positive.
Nudibranch mollusks.	*Phyllirrhöe.*	Intracellular (?); on stimulation.	?
Cephalopods.	*Watasenia.*	Intracellular; on stimulation.	Negative.
	Heteroteuthis.	Extracellular; on stimulation.	?
Balanoglossids.	*Glossobalanus.*	Extracellular; on stimulation.	Positive.
Salpids.	*Pyrosoma.*	Intracellular; on stimulation.	Negative.
Fish	*Photoblepharon.* *Anomalops.*	Intracellular; continuous.	Negative.
	Maurolicus. *Porichthys.*	Intracellular; on stimulation.	?
	Gonostoma.	Extracellular (?); on stimulation (?).	?

covered by Dubois[1] in *Pyrophorus*, an elaterid beetle, and *Pholas*, a mollusk can be demonstrated in several other groups of the animal kingdom but not in all. Why cannot these substances be demonstrated in all orders? It would seem that so fundamental a reaction should be universal; that is one question awaiting solution.

A second question concerns the specificity of luciferin and luciferase. Will the luciferase of one species produce light with the luciferin of another species, or genus, or group, and *vice versa?* We have here material for an interesting study of enzyme specificity and this is necessary, as we shall see, for a proper analysis of the first question, why luciferin and luciferase cannot be demonstrated in all groups of luminous animals.

The Luciferin-Luciferase Reaction.

The general methods for preparing luciferin and luciferase are very simple. Luciferin is made by adding hot water to the luminous organ of the animal or by quickly heating the luminescent extract of the luminous animal to temperatures which permanently quench the light, or to boiling. By this means the luciferase is destroyed on heating before the luciferin (which is not destroyed by heating) has been completely oxidized. Care must be taken to destroy the luciferase as quickly as possible, before it has had time to oxidize the luciferin. Hence the advantage of adding hot water suddenly to the luminous gland. Care must also be taken not to heat the luciferin to too high a temperature, or too long, as it may be destroyed under these conditions. Hence the advantage of heating a luminous extract to just the point where the light is permanently extinguished, and cooling quickly. Before deciding that luciferin cannot be demonstrated in an animal, these precautions have always been taken.

Luciferase is prepared by allowing a cold water extract of the luminous gland to stand until the luciferin has been completely oxidized. This oxidation can be accelerated by shaking the solution to aerate it well, or by gentle heating (not sufficient to destroy the luciferase), or by adding such substances as chloroform, saponin, or sodium glycocholate. These substances apparently act by liberating

[1] Dubois, R., *Compt. Rend. Soc. Biol.*, 1885, ii, 559; 1887, iv, 564.

luciferin bound (combined or absorbed) in some way in the solution, perhaps sometimes by causing cytolysis of still intact photogenic cells or by causing solution of photogenic granules or granulysis. Extracts of non-luminous animals sometimes contain substances acting like the above cytolytic agents. For these I have suggested the general term of photopheleins. Care has been taken to exclude such sources of error and misinterpretations in the studies described below.

It is obvious, from the method of preparation of luciferase, that, should there be just enough luciferase or less than enough luciferase to oxidize all the luciferin of a luminous gland, we could not obtain a solution of luciferase by the above method. Only if an excess of luciferase over luciferin exists can a solution of luciferase be obtained. It is possible, therefore, that this is the explanation of negative results for the presence of these bodies in certain groups of luminous organisms, a possibility that can be tested in part and that will be discussed below.

It is not to be supposed that inability to demonstrate luciferin and luciferase in a luminous form is always due to the same cause. Assuming that luciferin and luciferase really do occur in all luminous forms, it may be that they are present in such small amounts, compared with the bulk of non-luminous tissue necessarily included in extracting them, that no luminescence is visible. This might be the case in *Chætopterus*, an annelid worm, where luminous gland cells occur over the surface of the body. These cells cannot be removed individually and the most luminous regions of the worm must be extracted as a whole, involving a large mass of non-luminous material.

Again, either luciferin or luciferase or both may be very unstable in some forms, undergoing change before their presence in an extract may be tested.

Or, it is not impossible that the luciferase may occur in an endoenzyme condition, similar to the zymase of yeast or enzymes of bacteria, which render it impossible to extract except under special conditions and high pressures. I have concluded that such is the case in luminous bacteria and that this explains the absence of a luciferin-luciferase reaction in these forms.[2] It seems possible also, however,

[2] Harvey, E. N., *Am. J. Physiol.*, 1916, xli, 449.

that very little luminous material is present in these forms at any one time, but that it is manufactured continuously by the living bacterial cell.

But in addition to the forms which may contain only small amounts of luciferin and luciferase, or unstable luciferin and lucerferase, or luciferase in endoenzyme condition, there are at least two groups of animals which contain abundant luminous materials, whose light is long lasting, whose cells may be easily broken up, and in which the photogenic substances may be readily dried and give a bright light on again moistening. These are the medusæ, *Æquorea forskalea* and *Mitrocoma cellularia*, and the pennatulids, *Cavernularia haberi* and *Ptylosarcus* Sp.(?) especially the medusæ. *Æquorea and Mitrocoma*, found at Friday Harbor, Washington, contain many clumps of luminous cells about the rim of the umbrella at the base of the tentacles. Under the microscope masses of yellow material can be seen in the position from which the light comes, which probably are the photogenic cells. Gentle rubbing of the region liberates abundant luminous secretion which sticks to the fingers and which causes the sea water to luminesce quite brightly. The rim of the umbrella is easily cut away and this material, when squeezed through cheese cloth, gives a permanent bright luminous extract whose light lasts several hours. The animal itself luminesces only on stimulation.

There is, then, in these medusæ no lack of photogenic material. The material is readily extracted and stable, since the light lasts for several hours. Nevertheless, the luciferin and luciferase reaction cannot be obtained with these forms despite many attempts and care to guard against all sources of error.

What is the reason for this negative behavior? Is luminesence of jellyfish quite a different process from that in *Cypridina*, *Pholas*, or fireflies, which do give the luciferin-luciferase reaction? Or is the amount of luciferase in these forms just sufficient to oxidize the luciferin which is present and leave no excess in the extract? In *Cypridina* there is enough luciferase in one animal to oxidize the luciferin of 100 animals, but not an indefinite amount. *Cypridina* luciferase behaves as an enzyme but is not a perfect example of a catalyst which should transform indefinite amounts of substrate. There are, however, enzyme-like bodies known, the peroxidases of plants, in which there is a definite

mass relation between peroxidase and body oxidized, and it is not impossible that some luciferases behave in this way.

It should be pointed out in this connection that the light of these jellyfish comes unquestionably from granules of relatively large size. They can be seen at night under the microscope as dots of light with definite boundaries, not merely points of light. These granules shine brightly for some time, but if saponin or sodium glycocholate or fresh water is added, the granules dissolve with a sudden flare of light and then become dark. It is possible that the granule represents a combination of luciferin and luciferase in just the proper proportions for utilization. In *Cypridina* no such luminous granules exist in the extract (although granules occur in the luminous gland) or if they do exist in the extract they are ultra-microscopic in size.

It should also be borne in mind that Harden and Young[3] found an excess of zymase proper in some yeasts and an excess of co-zymase in other kinds. There is a certain resemblance between the luciferin-luciferase complex and the co-zymase-zymase complex and we may have an excess of luciferin in some animals and an excess of luciferase in others. Only if the latter condition existed could we demonstrate the presence of these two bodies.

I had hoped to solve this question by determining if the luciferin of medusæ will give light with the luciferase of some other form; *i.e.*, with a solution which we know to contain luciferase, as that of *Cypridina*. Such a test has given absolutely negative results. The luciferin of *Æquorea*, *Mitrocoma*, *Cavernularia* or *Ptylosarcus*, prepared in various ways, will give no light with *Cypridina* luciferase. Neither will the reverse "cross" (*Medusa* luciferase and *Cypridina* luciferin) give luminescence. These results are given in Table II.

This would seem to indicate that there was no *Mitrocoma* nor *Æquorea* nor pennatulid luciferin in the extract. There is a possibility, however, that *Cypridina* luciferase is absolutely specific and will not act with the luciferins of other forms. If that is the case, and my work shows that luciferin and luciferase are specific, except for very closely related forms, we cannot expect to throw light on the problem by this method.

[3] Harden, A., and Young, W. J., *Proc. Roy. Soc. Biol.*, 1906, lxxvii, 405; lxxviii, 369

There is a second method of attacking the problem. Suppose we prepare a solution which should contain medusa luciferin. On adding this to a glowing extract of medusæ, which must contain luciferin and also luciferase to oxidize the luciferin, a brighter light should result because within certain limits, with a given amount of luciferase, the more luciferin is present, the brighter will be the light. Trials have shown that no brighter light results from adding additional medusæ luciferin to a glowing medusa extract. Apparently, therefore, the medusa luciferin solution contains no luciferin or the glowing extract of medusæ contains no luciferase; in other words, these substances do

TABLE II.

"Cross."					Reaction.
Mitrocoma luciferase	+	*Mitrocoma* luciferin	.....		Negative.
" "	+	*Cypridina*	"	.....	"
Æquorea "	+	*Æquorea*	"	.....	"
" "	+	*Cypridina*	"	.....	"
Cypridina "	+	*Mitrocoma*	"	.....	"
" "	+	*Æquorea*	"	.....	"
Cavernularia "	+	*Cavernularia*	"	.....	"
" "	+	*Cypridina*	"	.....	"
Cypridina "	+	*Cavernularia*	"	.....	"
Ptylosarcus "	+	*Ptylosarcus*	"	.....	"
" "	+	*Cypridina*	"	.....	"
Cypridina "	+	*Ptylosarcus*	"	.....	"
" "	+	*Cypridina*	"	.....	Brilliant light.

not exist in the medusæ. While my work thus far points to this conclusion, we should certainly expect so fundamental a reaction as that of luciferin with luciferase to be universal. The statement that luciferin and luciferase do not occur in medusæ must therefore be considered as tentative and dependent on the present state of our knowledge.

Specificity of Luciferin and Luciferase.

The specificity of luciferin and luciferase is of considerable interest apart from the question discussed above. Accordingly, I have made a study of the luminescence resulting when *Cypridina* luciferin and luciferase is mixed with these bodies prepared from other animals of

TABLE III.

Organism.	"Cross."					Reaction.
Bacteria.	*Cypridina*	luciferase	+	*Bacteria*	luciferin.	Not tried.
	"	luciferin	+	*Bacteria*	luciferase.	" "
	Bacteria	luciferin	+	"	luciferase.	Negative.
Cystoflagellates	*Cypridina*	luciferase	+	*Noctiluca*	luciferin.	Negative.
	"	luciferin	+	"	luciferase.	"
	Noctiluca	luciferin	+	"	luciferase.	"
Medusæ.	*Cypridina*	luciferase	+	*Æquorea*	luciferin.	Negative.
	"	luciferin	+	"	luciferase.	"
	Æquorea	luciferin	+	"	luciferase.	"
	Cypridina	luciferase	+	*Mitrocoma*	luciferin.	"
	"	luciferin	+	"	luciferase.	"
	Mitrocoma	luciferin	+	"	luciferase.	"
Pennatulids.	*Cypridina*	luciferase	+	*Pennatula*	luciferin.	Negative.
	"	luciferin	+	"	luciferase.	"
	Pennatula	luciferin	+	"	luciferase.	"
	Cypridina	luciferase	+	*Cavernularia*	luciferin.	"
	"	luciferin	+	"	luciferase,	"
	Cavernularia	luciferin	+	"	luciferase.	"
	Cypridina	luciferase	+	*Ptylosarcus*	luciferin.	"
	"	luciferin	+	"	luciferase.	"
	Ptylosarcus	luciferin	+	"	luciferase.	"
Ctenophores.	*Cypridina*	luciferase	+	*Bolina*	luciferin.	Negative.
	"	luciferin	+	"	luciferase.	"
	Bolina	luciferin	+	"	luciferase.	"
Annelids.	*Cypridina*	luciferase	+	*Tomopteris*	luciferin.	Not tried.
	"	luciferin	+	"	luciferase.	Positive ?
	Tomopteris	luciferin	+	"	luciferase.	Not tried.
	Cypridina	luciferase	+	*Odontosyllis*	luciferin.	Negative.*
	"	luciferin	+	"	luciferase.	" *
	Odontosyllis	luciferin	+	"	luciferase.	Positive.
	Cypridina	luciferase	+	*Polynöe*	luciferin.	Negative.
	"	luciferin	+	"	luciferase.	"
	Polynöe	luciferin	+	"	luciferase.	"
	Cypridina	luciferase	+	*Chætopterus*	luciferin.	"
	"	luciferin	+	"	luciferase.	"
	Chætopterus	luciferin	+	"	luciferase.	"
Crustacea.	*Cypridina*	luciferase	+	*Pyrocypris*	luciferin.	Positive.
	"	luciferin	+	"	luciferase.	"
	Pyrocypris	luciferin	+	"	luciferase.	"

* Perhaps a slight reaction.

TABLE III—*Continued.*

Organism.	"Cross."					Reaction.
Crustacea.	*Cypridina*	luciferase	+	*Meganyctiphanes*	luciferin.	Negative.
	"	luciferin	+	"	luciferase.	"
	Meganyctiphanes	luciferin	+	"	luciferase.	" *
Myriapods.	*Cypridina*	luciferase	+	*Geophilus*	luciferin.	Not tried.
	"	luciferin	+	"	luciferase.	" "
	Geophilus	luciferin	+	"	luciferase.	Negative (rather dilute solutions).
Insects.	*Cypridina*	luciferase	+	*Luciola*	luciferin.	Negative.
	"	luciferin	+	"	luciferase.	"
	Luciola	luciferin	+	"	luciferase.	Positive.
Molluscs.	*Cypridina*	luciferase	+	*Pholas*	luciferin.	Negative.
	"	luciferin	+	"	luciferase.	" (?).
	Pholas	luciferin	+	"	luciferase.	Positive.
Ascidians.	*Cypridina*	luciferase	+	*Pyrosoma*	luciferin.	Negative.
	"	luciferin	+	"	luciferase.	"
	Pyrosoma	luciferin	+	"	luciferase.	"
Fish.	*Cypridina*	luciferase	+	*Photoblepharon*	luciferin.	Negative.
	"	luciferin	+	"	luciferase.	"
	Photoblepharon	luciferin	+	"	luciferase.	"
	Cypridina	luciferase	+	*Anomalops*	luciferin.	"
	"	luciferin	+	"	luciferase.	"
	Anomalops	luciferin	+	"	luciferase.	"

all degrees of relationship as regards *Cypridina*. The results are best expressed in the form of a tabulation. In this table the words "luciferin" and "luciferase" are used to indicate extracts so prepared that they should contain luciferin and luciferase, provided these substances are formed by the organism in question. Table III represents the results of experiments performed at various times during the past seven years in all parts of the world. Its incompleteness is due to the difficulty of obtaining luminous animals in sufficient quantity for the test in question.

From the evidence in Table III it would seem that *Cypridina* luciferase will only give light with the luciferin of a very closely related form, *Pyrocypris*, a genus that differs from *Cypridina* only in the character of appendages on the upper lip. Both genera belong to the family, *Cypridinidæ*. Crosses of *Cypridina* luciferase with the luciferin of *Odontosyllis*, *Pholas*, or fireflies, all of which possess a luciferin-luciferase reaction, gives only negative or very faint positive results as does the reverse mixture, *Cypridina* luciferin crossed with the luciferase of these other forms. All my attempts to oxidize *Cypridina* luciferin with oxidizing enzymes or oxidizing agents of various kinds have failed, and also all attempts to oxidize with light production, various easily oxidizable substances with *Cypridina* luciferase.[4] We must therefore conclude that these two substances responsible for the production of light by *Cypridina*, are specific to the highest degree.

SUMMARY.

Among sixteen groups of luminous forms investigated by the author, in only four (fireflies, *Pholas*, ostracods, and *Odontosyllis*) is it possible to demonstrate the luciferin-luciferase reaction. In many groups this is probably due to the small amount of these substances present in the luminescent organism or to their instability. In the medusæ and pennatulids, despite a large amount of luminescent material, luciferin and luciferase cannot be demonstrated. This does not appear to be due to the presence of luciferin and luciferase in equivalent proportion, or to their instability. In fact, one is led to the conclusion that luciferin and luciferase do not exist in these forms, but such a conclusion must be regarded as merely tentative, in view of the fundamental character of the luciferin-luciferase reaction. Luciferin of one form will not luminesce with the luciferase of another form or *vice versa*, unless very closely related (*Cypridina* and *Pyrocypris*). All experiments emphasize the specificity of the light producing substances of *Cypridina*.

[4] Harvey, E. N., *J. Gen. Physiol.*, 1918–19, i, 269.