STUDIES ON BIOLUMINESCENCE.

VII. Reversibility of the Photogenic Reaction in Cypridina.

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(Received for publication, September 3, 1918.)

In a previous paper of this series,[1] I have described two photogenic substances in the ostracod crustacean, *Cypridina hilgendorfii*, which I called photogenin and photophelein. Photogenin is destroyed below the boiling point, is non-dialyzable, and prepared by making a cold water extract of the luminous animal and allowing it to stand in the air until no more light appears on shaking. This indicates that one of the photogenic substances, photophelein, has disappeared, leaving the photogenin. Photophelein is not destroyed by short boiling and will dialyze. It is prepared by making a hot water extract of the luminous animal. The hot water destroys the photogenin, leaving the photophelein. Whenever two such non-luminous solutions are mixed light appears.

On the grounds of method of preparation, relation to temperature, and dialysis, I regarded photogenin as comparable to luciferase and photophelein as comparable to luciferin, two photogenic substances described by Dubois[2] in the beetle, *Pyrophorus noctilucans*, and in the mollusc, *Pholas dactylus*. Dubois believes that luciferase is an oxidizing enzyme which oxidizes luciferin, an oxidizable substance, with light production. Neither luciferase nor luciferin alone in solution can produce light but light appears if solutions of the two are mixed and continues as long as any luciferin remains unoxidized. Dubois has also been able to produce light by oxidizing luciferin (alone) with a small crystal of $KMnO_4$, by H_2O_2 (with or without blood containing

[1] Harvey, E. N., *Am. J. Physiol.*, 1917, xlii, 318.

[2] Dubois, R., *Compt. rend. Soc. biol.*, 1885, xxxvii, 559.

hemoglobin), BaO_2, PbO_2, and other oxidizing agents. Through the kindness of Professor Dubois I have received some material of *Pholas dactylus* preserved in sugar and I can confirm his results on the effect of $KMnO_4$ and other oxidizing agents in producing light with luciferin of *Pholas*. I have likewise repeated my own experiments with the photophelein of *Cypridina* using a whole series of oxidizing agents applied in the same way, as with the luciferin of *Pholas* and, as previously, have failed to obtain any light with this substance.[3] The difference in our results is, therefore, not to be referred to a difference in method of experiment but to a difference in the substances themselves.

I found also that if one takes a concentrated solution of photogenin filtered through a porcelain or silicious filter candle to remove all granules and cell fragments and adds to it a little saponin powder, or amyl alcohol or NaCl or other inorganic salt crystals or tissue extracts of certain invertebrate non-luminous animals, that light would appear. Because NaCl could not possibly be oxidized by photogenin (= luciferase)—or any other substance—and because of my inability to make photophelein (= luciferin) luminesce with oxidizing agents, I regarded the photogenin itself as the source of the light and the oxidizable body. I have compared photogenin to zymase and photophelein to the coenzyme of zymase, believing that we are dealing with a system similar to that of the enzyme-coenzyme system of yeast. Hence the name photophelein or body assisting in the production of light.

[3] The following oxidizing agents (added ,where possible, in minute crystal or powder form) all gave light with *Pholas* luciferin, but no light with *Cypridina* luciferin: $KMnO_4$, $K_2Cr_2O_7$, PbO_2, Na_2O_2, BaO_2, MnO_2, $K_3Fe(CN)_6$, $K_2S_2O_8$, $Na_2B_4O_8$, and H_2O_2. The following oxidizing agents gave no light with either *Pholas* luciferin or *Cypridina* luciferin: K_2CrO_4, CrO_3, $KClO_3$, $KClO_4$, $FeCl_3$, KNO_3, Cl or Br water, I in KI, Na hypochlorite, hypobromite, or hypoiodite, colloidal Ag or Pt, benzoyl peroxide, potato or turnip juice, or blood containing hemoglobin or hemocyanin. If H_2O_2 in addition to the oxidizing agent is added to *Cypridina* luciferin, no light appears except a faint momentary flash with Na hypochlorite and hypobromite. As this faint flash also appears with thoroughly boiled extracts of *Cypridina*, lacking luciferin, it can have no significance. If H_2O_2 in addition to the oxidizing agent is added to *Pholas* luciferin the light is in some cases brighter than with H_2O_2 alone.

I now believe that under the term photophelein I have previously included two separate substances. One of these is the thermostabile dialyzing substance extracted from *Cypridina* by hot water. Although this substance cannot be oxidized with light production by oxidizing agents, it does oxidize spontaneously (also without light production) in the air and loses its power of producing light with photogenin. In the absence of air its solutions are stabile for months. Once oxidized it can again be reduced and will again give light if photogenin is added. It is therefore an oxidizable material and, I believe, similar to the luciferin of *Pholas*. I propose therefore to use Dubois' word luciferin for the thermostabile dialyzing substance of *Cypridina* in place of photophelein and luciferase for the thermolabile non-dialyzing substance in place of photogenin. The source of the photogenic substances can be designated by prefixing the name of the animal as *Cypridina* luciferin, *Pholas* luciferin, etc. I suggest also that luciferin when oxidized be designated oxyluciferin.

Luciferin is found only in luminous animals. In non-luminous animals and probably also in luminous animals there is a second substance which I have formerly included in the term photophelein, and which may be properly so called, that acts in a manner similar to saponin, NaCl crystals, etc., upon the extract of *Cypridina* which has stood until the light disappears. When we allow a *Cypridina* extract containing luciferin and luciferase to stand, the luciferin is not completely oxidized, even though the extract is thoroughly aerated, but some of it is bound (adsorbed or combined?) by other substances in the extract. The saponin, NaCl crystals, and extracts of non-luminous animals act by setting free the bound luciferin which is then oxidized and light appears. I suggest that the term photophelein be applied to these substances in tissue extracts. They are not destroyed by boiling. On standing some are stable while others are unstable.

The best way to rid a luciferase solution of the bound luciferin is to shake it thoroughly with chloroform. Such a solution will give no light with extracts of non-luminous animals or saponin, NaCl crystals, etc., but a brilliant light with *Cypridina* luciferin.

An insight into the *modus operandi* of saponin, NaCl crystals, or photophelein may be gained from the following experiments. Both luciferin and luciferase are adsorbed by many finely divided precipi-

tates and colloidal particles, such as bone-black, $Fe(OH)_3$, kaolin, and others. If we take a colloidal $Fe(OH)_3$ solution of the proper concentration (which can only be determined by experiment), add some dilute luciferase to it, and then after a minute luciferin, no light will appear. This is because the luciferase has been completely adsorbed by the colloidal $Fe(OH)_3$, for if we now add some dilute luciferase to the above mixture light will appear but it will very quickly disappear because the new luciferase added is again very rapidly adsorbed, but not so rapidly adsorbed that we fail to get light at first. On adding more luciferase we may again get a momentary light but the additions cannot be made indefinitely because we finally reach a point where the colloidal $Fe(OH)_3$ has become saturated with luciferase and then the mixture glows for a considerable time. It is obvious that for this experiment to succeed there must be more luciferin present than can be completely adsorbed by $Fe(OH)_3$ and so little luciferase present that it is completely adsorbed by the $Fe(OH)_3$. Suppose we have a mixture of $Fe(OH)_3$, luciferase, and luciferin complying with the above conditions. Can we in any way remove the luciferase from its adsorbed condition on the colloidal $Fe(OH)_3$? This might theoretically be done in two ways and we actually find in practice that both methods are possible. Anything which precipitates the colloidal $Fe(OH)_3$ will decrease the surface available for adsorption of luciferase and if the surface area is sufficiently decreased some luciferase may be forced into solution again where it is able to oxidize the luciferin. If we add NaCl crystals to the colloidal $Fe(OH)_3$ + dilute luciferase + more concentrated luciferin mixture, the $Fe(OH)_3$ is precipitated and light appears. If in place of NaCl crystals we add a trace of saponin, the colloidal $Fe(OH)_3$ is not precipitated but light also appears. This is an example of the second method of removing luciferase from an adsorbed condition; namely, by using a material (saponin) which is more strongly adsorbed than the luciferase and which is able to replace it as adsorbed body. I believe these to be the explanations of the effect of NaCl crystals, saponin, etc., in giving light with luciferase solutions, except that the luciferase is in excess and a small amount of adsorbed (or combined) luciferin is present which is liberated by NaCl or saponin and gives light with luciferase. Photophelein probably acts in a manner analogous to the saponin.

I have considered the thermostabile, dialyzing substance as similar to the luciferin of *Pholas* despite the fact that Dubois reports *Pholas* luciferin destroyed at 70°C., whereas *Cypridina* luciferin is only destroyed by several minutes boiling in an open beaker. I find that this destruction of *Cypridina* luciferin on short boiling is due to the increased rate of oxidation at the boiling point and that no destruction of *Cypridina* luciferin will occur if boiled in an atmosphere of hydrogen.[4] *Cypridina* luciferin is truly thermostabile but is oxidized to oxyluciferin on boiling in the air. We may say that *Pholas* luciferin is *similar* but certainly not *identical* with *Cypridina* luciferin. If so, we should expect to obtain light on mixing *Pholas* luciferin and *Cypridina* luciferase, yet no light appears. Neither is there light on mixing *Cypridina* luciferin and *Pholas* luciferase, although the *Pholas* luciferase prepared from the material which Dubois sent me gave a rather faint light with *Pholas* luciferin.[5]

We have, therefore, at least three substances concerned in light production: luciferin, luciferase, and photophelein. Luciferin is a body oxidizing with light production, dialyzable, and relatively resistant to heat. Luciferase is destroyed by boiling, non-dialyzable, and accelerates the oxidation of luciferin. While it may be used up in the reaction if mixed with a sufficient quantity of luciferin, luciferase has

[4] I have endeavored to repeat this experiment with the luciferin of *Pholas* sent me by Professor Dubois, but without success. *Pholas* luciferin boiled in a current of hydrogen for 15 minutes would give no light when a crystal of $KMnO_4$ was added. The hydrogen was produced in a Kipp generator and may have contained a little air. In my experience short (20 to 40 seconds) boiling of *Pholas* luciferin does not completely destroy its power of producing light when a crystal of $KMnO_4$ is added.

[5] I believe the faint light obtained on mixing *Cypridina* luciferin and fire-fly or *Noctiluca* luciferase and *vice versa*, recorded in my former paper (*Am. J. Physiol.*, 1917, xlii, 328) where luciferin is called photophelein and luciferase is called photogenin, is not due to the oxidation of luciferin by luciferase of the second species but is due to the presence of photophelein. I am led to this conclusion because the light is so faint, but cannot be sure until the cases are reinvestigated. The mixing of luciferin and luciferase of different species or genera of luminous ostracods, especially if the colors of their luminescence differed, would shed considerable light on this interesting question of specificity. A non-luminous Japanese species of *Cypridina* does not contain either luciferin or luciferase but it does contain photophelein.

many of the characteristics of an enzyme and certainly as much right to be called an enzyme as the peroxidases of plants which are also used up in the oxidation process. The *Cypridina* luciferase reaction appears to be specific to an extraordinary degree. Of many tried I have found no substances or plant or animal extracts which can take the place of luciferase[6] nor any substances[7] or plant or animal extracts[8] which can be oxidized with light production by luciferase. The light recorded with various extracts of luminous and non-luminous animals in my former paper is to be referred to the presence of photophelein, the third substance concerned in light production which probably acts by assisting the luciferin-luciferase reaction in the manner already suggested. Let us now turn to the oxidation product or products of luciferin.

When luciferin is oxidized it must be converted into some substance or substances and I believe this change involves no fundamental destruction of the luciferin molecule, as it is a reversible process. I

[6] I have tried the blood or extracts of many species of animals or plants including those containing strong oxidizing enzymes both with and without H_2O_2 and have always failed to obtain light with *Cypridina* luciferin. Among others the juice of Indian pipe (*Monotropa*), potatoes, and turnips (containing strong oxidases and peroxidases), the blood of the ox and a worm (*Arenicola*) (containing hemoglobin), the blood of the squid (*Loligo*), *Limulus* and *Sycotopus* (containing hemocyanin), and extracts of *Chætopterus* (a luminous annelid), and the mollusc, *Unio*, (rich in manganese) were tried. Dubois reports that he has obtained light on mixing *Pholas* luciferin with the blood of divers molluscs and marine crustaceans (*Ann. Soc. Linn. Lyons*, 1913, xl). I can confirm this statement for an extract of *Unio*, but obtained no light with *Limulus* blood, *Sycotopus* blood, squid (*Loligo*) blood, or turnip or potato juice and *Pholas* luciferin. Evidently *Pholas* luciferin is much more readily oxidized with light production than *Cypridina* luciferin.

[7] The following oxidizable substances have been tested: æsculin, lophin, bergamot oil, pyrogallol, gallic acid, aniline, adrenalin, phenol, α-napthol, *p*-phenylenediamine, ortol, orcinol, hydrochinone, resorcinol, pyrocatechol, tannin, benzidine, gum guaiac, amidol, α-napthylamine, and the chromogen of the false indigo plant (*Baptisia*). Luciferase, with or without H_2O_2, will not accelerate the oxidative color change in any of the above compounds.

[8] I have regularly obtained a fair light on mixing luciferase well shaken with chloroform to set free any bound luciferin and boiled potato or turnip juice or boiled *Limulus* blood. The light is especially marked about the coagulum in the boiled *Limulus* blood. The significance of these results is not apparent.

shall speak of the principal if not the only product formed as oxyluciferin. Most observers have considered a rather fundamental change to occur when the photogenic substance is oxidized. Thus the crystals of xanthine or some related substance in the reflecting layer of the fire-fly have been regarded as the oxidation products of the luminous material thought to be nucleoprotein. Dubois[9] regards luciferin as a protein and states that it forms the same oxidation products as other proteins, amino-acids being mentioned as possible substances formed. It should be pointed out in this connection that the formation of amino-acids from proteins involves no oxidation but an hydrolysis.

If we assume that the oxidation of luciferin changes the molecule but slightly, we at once think of comparing the change luciferin $\rightleftarrows$ oxyluciferin with the change reduced hemoglobin $\leftrightarrows$ oxyhemoglobin. The condition is, however, not so simple as this, for oxyhemoglobin will again give up its oxygen providing the partial pressure of oxygen is sufficiently low, whereas oxyluciferin will not do this. We cannot reduce oxyluciferin solution by exhausting the oxygen with an air pump.

There is another oxidation reduction system which can also be easily reversed, but not by merely removing the oxygen—that is the reduction of a dye such as methylene blue to its leuco base. I believe the change which occurs when luciferin is oxidized is similar to that which occurs when the leuco base of methylene blue or sodium indigosulfonate is oxidized to the blue dye.

My attempts to reduce the oxidation product of luciferin started from the observation that if one places a clear solution of luciferase in a tall test-tube, although it may give off no light at first when shaken, after standing a day or so a very bright light would appear on shaking. This was especially true when the luciferase had become turbid and ill smelling from the growth of bacteria. Thinking that the bacteria produced a substance which could be oxidized by the luciferase, I tried growing bacteria and also yeast on appropriate culture media and after some days of growth mixing the culture media containing the products of bacterial or yeast growth with luciferase,

[9] Dubois, *Ann. Soc. Linn. Lyons*, 1914, lxi, 169.

expecting to obtain light. But no light appeared. However, if a little crude luciferase solution was added to the bacterial or yeast cultures and then allowed to stand for some hours, light appeared whenever they were shaken. Indeed such cultures behaved much as a suspension of luminous bacteria which has used up all the oxygen in the culture fluid and will only luminesce when, by shaking, more oxygen dissolves in the culture medium. Realizing that in bacterial cultures in test-tubes anaerobic conditions soon appear, and also the strong reducing action of bacteria upon many substances (for instance nitrates or methylene blue) under anaerobic conditions, it occurred to me that the bacteria might be utilizing the oxygen of the oxidation product of luciferin, reducing it to luciferin again. We must remember that since crude luciferase solution is a cold water extract of a luminous animal allowed to stand until all the luciferin has been oxidized, it must contain oxyluciferin as well as luciferase and will give light if the oxyluciferin is again reduced and oxygen admitted. This appears to be the correct explanation of the above experiments.

Not only bacteria but also tissue extracts have a strong reducing action in absence of oxygen. Thus, muscle tissue stained in methylene blue will very quickly decolorize (reduce) the methylene blue if oxygen (air) is kept away, but the blue color immediately returns if air is admitted. Oxyluciferin, *i.e.* a solution of luciferin which has been completely oxidized by boiling or standing in air until it no longer gives light with luciferase, if mixed with a suspension of ground frog muscle and kept in a well filled and stoppered test-tube for some hours, is reduced to luciferin and gives a bright light if now poured into luciferase solution. Frog muscle suspension alone or oxyluciferin alone gives no light with luciferase, nor will a mixture of frog muscle suspension and oxyluciferin, if shaken with air for several hours. Only if this last mixture is kept under anaerobic conditions is the oxyluciferin reduced.

The reducing action of tissues is said to be due to a reducing enzyme (reducase or reductase), itself composed of a perhydridase and some easily oxidized body such as an aldehyde.[10] In the presence of the

[10] Bach, A., *Biochem. Z.*, 1911, xxxi, 443; xxxiii, 282; 1912, xxxviii, 154; 1913, lii, 412.

perhydridase the oxygen of water oxidizes the aldehyde and the hydrogen set free reduces any easily reducible substance which may be present. There is a perhydridase in fresh milk, spoken of as Schardinger's enzyme,[11] which is destroyed by boiling. If some aldehyde is added, fresh milk will reduce methylene blue to its leuco base or nitrates to nitrites, upon standing a short time. If shaken with air, the blue color returns. There is no reduction unless an aldehyde is added or unless some boiled extract of a tissue such as liver is added. The boiled liver extract has no reducing action of its own but supplies a substance similar to the aldehyde which has been spoken of as a coenzyme. Milk will reduce methylene blue without aldehyde if bacteria are present in large numbers. There is also no reduction if the milk, methylene blue, and aldehyde are agitated with air. The temperature optimum is rather high, 60–70°C.

I find that milk is a favorable and convenient medium for the reduction of oxyluciferin and that it acts without the addition of an aldehyde or the presence of bacteria. There is probably a substance acting as the aldehyde in the luciferase-oxyluciferin solution. No light appears if milk is added to a luciferase-oxyluciferin solution, but if the mixture is allowed to stand in absence of oxygen, light will appear when air is admitted. The air can be conveniently kept out by filling small test-tubes completely with the solution and closing them with rubber stoppers.

Oxyluciferin may also be readily reduced by the use of the blood of the horseshoe crab (*Limulus*) allowed to stand until bacteria develop.[12] This experiment is of special interest because the blood contains hemocyanin which is colorless in the reduced condition and blue in the oxycondition. The color change thus serves as an indicator of the oxygen concentration in the blood. A sample of foul smelling *Limulus* blood full of bacteria will become colorless on standing in a test-tube for 10 or 15 minutes but the blue color quickly returns if shaken with air. Such a blood has the power of reducing oxyluciferin through the activity of the bacteria which it contains. Fresh blood has very little if any reducing action.

[11] Schardinger, F., *Chem. Ztg.*, 1904, xxviii, 704.
[12] Alsberg, C. L., *J. Biol. Chem.*, 1915, xxiii, 495.

As almost all animal tissues contain reductases, it is not surprising to find that a freshly prepared and filtered extract of *Cypridina* containing oxyluciferin and luciferase which gives no light on shaking, will, on standing in a stoppered tube for 24 hours at room temperature, give light when air is admitted.[13] While this may be due to the development of bacteria with a reducing action it does not seem likely, as under the same conditions methylene blue is not reduced in 24 hours and there is no turbidity or smell of decomposition in the tube. In 48 hours bacteria do appear and methylene blue is also reduced. If we add chloroform, toluene, or thymol to the tubes of *Cypridina* extract to prevent the growth of bacteria, and allow them to stand 48 hours, upon admitting air the tube with chloroform gives no light but the tubes with toluene and thymol do give light although it is not so bright as if they were absent. I believe that these substances have a destructive action on the reductases, most complete in the case of chloroform.

I have not been able to demonstrate that a *Cypridina* extract will reduce methylene blue or nitrates to nitrites either with or without the addition of acetaldehyde. This may be due to the fact that oxyluciferin, which is also present, may be reduced more readily than either nitrates or methylene blue and so is reduced first.

Dubois[14] has described in *Pholas* a precursor of luciferin, which he calls proluciferin, which is converted into luciferin by another enzyme, coluciferase. The proluciferin is not destroyed by boiling and the coluciferase will withstand a higher temperature than luciferase and may be freed of luciferase in this manner. He cites an experiment[15] to prove the existence of proluciferin and coluciferase in *Pholas* but I have been unable to repeat this with *Cypridina*. One might suppose that on allowing an extract of *Cypridina* (luciferase) to stand in absence of oxygen, some proluciferin, assuming this to be present, would be converted into luciferin which would give light if air was admitted. But we can allow a boiled extract of *Cypridina* (containing no coluciferase) to stand with milk or muscle tissue suspensions

[13] This experiment may also be performed with *Pholas* luciferase with a similar result.

[14] Dubois, *Compt. rend. Soc. biol.*, 1907, lxii, 850; 1917, lxxx, 964.

[15] Dubois, *Compt. rend. Soc. biol.*, 1917, lxxx, 964.

in absence of oxygen and, upon admitting air and adding luciferase, obtain light. As luciferase is only found in luminous animals it does not seem likely that a coluciferase would be widespread but we do know that a reducing enzyme occurs in milk and tissue extracts—is, in fact, widespread. It seems more logical to interpret the above experiments as due to the reduction of an oxyluciferin to luciferin rather than the conversion of a proluciferin to luciferin.

Indeed, we can reduce oxyluciferin by means which do not involve the use of animal extracts and consequently are free from the objection that "coluciferase" may be responsible for the result, but which, nevertheless, are perfectly well known reducing methods. Perhaps the best of these is reduction by palladium black and sodium hypophosphite. The latter is oxidized in presence of palladium and nascent hydrogen is set free.[16] The nascent hydrogen reduces any easily reducible substance which may be present, such as methylene blue or oxyluciferin. Oxyluciferin is not reduced by palladium alone or hypophosphite alone but methylene blue is reduced by palladium black alone.

If hydrogen sulfide is passed through a solution of methylene blue the dye is very quickly reduced and becomes colorless. If the H_2S is driven off by boiling the colorless methylene blue solution, the blue color again returns on cooling. Oxyluciferin can also be reduced to a certain extent by H_2S. Sulfur dioxide or oxides of nitrogen (prepared by the action of HNO_3 on Cu) had no reducing action on either methylene blue or oxyluciferin.

Dilute acid favors the reduction of oxyluciferin. If one saturates an oxyluciferin solution with CO_2 or adds a little dilute acetic acid and allows the solution to stand for 24 hours, a certain amount of reduction will occur. No reduction occurs if the solution is saturated with pure hydrogen and allowed to stand 24 hours. If one adds some Mg powder to oxyluciferin and then dilute acetic acid in successive additions as the acetic acid is used up in formation of Mg acetate, the oxyluciferin will be reduced relatively quickly. Nascent hydrogen is produced in the reaction and is no doubt the active reducing agent, while the acid accelerates the reduction. Soured milk also has

[16] Bach, *Ber. chem. Ges.*, 1909, xlii, 4463.

quite a marked reducing action. Acid thus favors reduction and hinders oxidation, while alkali favors oxidation and hinders reduction of the oxyluciferin.

While I have not studied the properties of oxyluciferin so fully as those of luciferin, as far as I can judge both substances give the same general reactions and possess identical properties. If we make a concentrated hot water extract of *Cypridina*, it will contain all the substances of the animal soluble in hot water and not coagulated by heat and may be spoken of as crude luciferin solution. If air is bubbled through this solution for some time, all the luciferin is oxidized and it may then be spoken of as crude oxyluciferin solution. Both crude luciferin and crude oxyluciferin solution are yellow in color, but I do not believe that either luciferin or oxyluciferin is yellow in color because an ether or benzene extract of *Cypridina* is also yellow although luciferase, luciferin, and oxyluciferin are all insoluble in ether and benzene. The yellow pigment which can be observed to make up part of the luminous gland of *Cypridina* is not luciferin or luciferase. It may be a pigment related to urochrome.

When tests are applied and precipitating reagents are added to crude luciferin and crude oxyluciferin solution they give identical results in each case. Thus if crude luciferin is saturated with $(NH_4)_2SO_4$ or $MgSO_4$, a flocculent precipitate forms which may be demonstrated to contain most of the luciferin. Oxyluciferin solution also gives flocculent precipitates on saturation with $(NH_4)_2SO_4$ and $MgSO_4$ and these contain most of the oxyluciferin. To demonstrate this the precipitates, after washing, are dissolved in a small amount of water mixed with fresh milk (or frog muscle suspension) and allowed to stand in a stoppered tube for 24 hours. If any oxyluciferin is present it will be reduced to luciferin and give light when luciferase is added. One-half saturation with $(NH_4)_2SO_4$ or $MgSO_4$ or saturation with NaCl salts out no material from either crude luciferin or oxyluciferin solution. Picric acid gives no precipitate but only an opalescence in both cases. In a similar manner it may be shown that most of the oxyluciferin is precipitated by phosphotungstic acid but not by acetic acid or CO_2, in this respect also agreeing with the behavior of luciferin. Like luciferin the oxyluciferin will pass porcelain filters, dialyze through parchment or collodion membranes, is soluble

in absolute alcohol but not in ether or benzene, and is undigested by salivary diastase, pepsin HCl, Merck's pancreatin in neutral solution, and erepsin. The salivary diastase and the pancreatin (containing amylopsin, trypsin, and lipase) were allowed to digest for 4 days at 38°C. without showing any evidence of digestive action. Oxyluciferin is partially but not completely precipitated by basic lead acetate and tannic acid.

As luciferin is so easily oxidizable a substance, we should expect to find that it will reduce just as glucose will reduce. However, a concentrated solution of luciferin has no reducing action on Fehling's (alkaline Cu), Barfoed's (acid Cu), Nylander's (alkaline Bi), or Knapp's (alkaline Hg) reagent. Glucose will reduce methylene blue in alkaline (not in neutral) solution but luciferin will not reduce methylene blue in alkaline or neutral solution. It would seem, then, that luciferin must contain no aldehyde group. If so, we should expect to obtain reduction of some of the above reagents. Just what group is concerned in the oxidation is unknown at the present time and speculation regarding it in the absence of more experimental data can be of little value.