On page 175 of Director Thorne's excellent book on "Farm Manures," he also shows that, when the increase is computed by the method which he states "has been deemed best," the net profit is greater per acre, and very much greater per dollar invested, from raw rock phosphate than from acid phosphate.

ACRE-YIELDS IN OHIO MANURE-PHOSPHATE EXPERIMENTS: AVERAGE OF 9 YEARS, 1906–1914

Phosphate	None	None	Rock	Rock	Acid	Acid
Manure	Yard	Stall	Yard	Stall	Yard	Stall
Corn, bushels	54.4	62.7	68.3	72.1	65.8	68.9
Wheat, bushels	24.3	25.5	27.2	28.2	29.8	29.4
Hay, tons	2.00	2.34	2.43	2.60	2.43	2.68
Value 3 crops	$57.20	$65.20	$68.52	$72.20	$69.60	$72.52
Phosphate gains	.....	.....	11.32	7.00	12.40	7.32
Phosphate cost	.....	.....	1.20	1.20	2.40	2.40
Phosphate profit	.....	.....	10.12	5.80	10.00	4.92
Profit per $1	.....	.....	8.43	4.83	4.17	2.05

The accompanying table gives the average of the actual yields secured in these Ohio experiments during the last half of the eighteen-year period.

If we value the corn at 40 cents a bushel, the wheat at 80 cents, and the hay at $8 a ton, and count the cost of rock phosphate at $7.50 per ton and acid phosphate at $15, we find, by this direct method of computation, that the rock phosphate was slightly more profitable per acre, and more than twice as profitable per dollar invested, as the acid phosphate.

In his letter to the agricultural editors, Dr. Wheeler quotes a personal letter from Professor Mooers expressing his opinion as to the conclusions which should be drawn from experimental data, in part unpublished, secured by the Tennessee Experiment Station. If this opinion is based upon a continuation of the experiments in which two crops (wheat followed by cowpeas) were grown every year on the same land, as reported in Tennessee Experiment Station Bulletin No. 90, in which on page 89 it is shown that for each dollar invested rock phosphate paid back $2.29, and steamed bone meal only $1.90, and in which the use of steamed bone meal is commended and the use of rock phosphate discouraged, we must await further publication by the Tennessee Station before accepting Wheeler's confidential report of Mooers' personal opinion.

For more complete data from the phosphate experiments conducted by many state experiment stations, the interested reader is referred to Illinois Experiment Station Circular 186, "Phosphates and Honesty."

CYRIL G. HOPKINS,
ALBERT L. WHITING
UNIVERSITY OF ILLINOIS

SPECIAL ARTICLES

THE LIGHT-PRODUCING SUBSTANCES, PHOTOGENIN AND PHOTOPHELEIN, OF LUMINOUS ANIMALS

IN a previous issue of SCIENCE (N. S., XLIV., 208, 1916), I called attention to Dubois's discovery of substances called luciferin and luciferase in the West Indian "cucullo" *Pyrophorus noctilucans,* and the mollusc, *Pholas dactylus.* I also recorded the existence of similar bodies in the fire-flies, *Photinus* and *Photuris,* and of luciferin in luminous bacteria. Luciferase, according to Dubois, a thermolabile enzyme capable of accelerating the oxidation of luciferin, is prepared by allowing an extract of luminous cells to stand until the light disappears. The luciferin is thus completely oxidized and used up. The luciferin, according to Dubois, a thermostabile substance capable of oxidation with light production, is prepared by extracting the luminous cells with hot water which destroys the luciferase but not the luciferin. Light will appear if we mix the solutions of luciferin and luciferase in presence of oxygen. Each

substance alone in solution is non-luminous and fairly stable. On this theory, therefore, the luciferin is the source of the light and, according to Dubois, *Pholas* luciferin will give light on oxidation with $KMnO_4$, blood, H_2O_2 and similar oxidizing agents. He found also substances (luciferase) in the blood of various marine molluscs and crustaceans which would give light with *Pholas* luciferin, but the latter he found only in the luminous organs of *Pholas dactylus*.[1]

Since the publication of my previous paper, I have investigated very thoroughly the chemistry of light production in five different forms:[2] the Japanese fire-flies, *Luciola parva* and *L. vitticollis;* an ostracod crustacean, *Cypridina hilgendorfii;* a squid, *Watasenia scintillans;* a pennatulid, *Cavernularia haberi;* and the protozoan, *Noctiluca miliaris*. *Watasenia, Cavernularia* and *Noctiluca* will not give the luciferin-luciferase reaction despite the most favorable conditions and many attempts to demonstrate it. These organisms need not be considered at present, as there are many reasons why the luciferin-luciferase reaction might fail.

Cypridina and *Luciola* both contain bodies similar to luciferin and luciferase, but in these forms the production of light differs in very essential points from that described by Dubois in *Pholas,* and I have come to quite different conclusions regarding the nature of the substances concerned. We may conveniently use Dubois's terminology for the present. First, in *Cypridina* and *Luciola,* it is the luciferase which is found only in the luminous cells, and luciferin is widely distributed in non-luminous forms. Second, I have been unable to oxidize luciferin with light production by $KMnO_4$ or other oxidizing agents. Third and most important, *Cypridina luciferase* will give light with substances (NaCl crystals, thymol, butyl alcohol, saponin), *some of which could not possibly be oxidized.* The *luciferase* and not the luciferin is therefore the *source* of the light. Instead of luciferin oxidizing with light production through the catalytic action of luciferase, luciferin is a body assisting in the evolution of light from luciferase. I therefore propose the new names of *photogenin* (light producer from *phos,* light, and *gennao,* to produce) for luciferase, and *photophelein* (light assister from *phos,* light, and *opheleo,* to assist) for luciferin, to indicate more clearly the nature of the light-producing process. *Cypridina* photophelein (= luciferin) in addition to its thermostabile property is easily dialyzable, while photogenin (= luciferase) is not. In these points and some others, the system resembles and may be compared to the zymase system (enzyme and coenzyme) of yeast cells.[3] As in so many other biological reactions an easily diffusible thermostabile substance (coenzyme) and a difficultly diffusible thermolabile substance (zymase) are concerned.

The light-producing power of photogenin and photophelein is very extraordinary. *Cypridina* photogenin will give visible light with photophelein in one part to 1,600,000,000 parts water. Even this is an underestimate, as we do not know the concentration of photogenin in the luminous cells apart from proteins, water, etc. In the small amounts necessary to produce light and in destruction by boiling, photogenin resembles an enzyme but differs in the fact that it is used up in the reaction. Experiment has shown that it takes photophelein from one hundred *Cypridinas* to use up the photogenin from one *Cypridina.* Perhaps the fact that photogenin is used up is not sufficient evidence to condemn it as an enzyme since many enzymes are poisoned or destroyed by reaction products; nevertheless I have deemed it best for the present to avoid the termination *ase.*

Cypridina and the firefly differ from *Pholas* in the points enumerated above and agree in most properties with each other, but with some exceptions. For instance, firefly photogenin is readily destroyed by chloroform or

[1] Dubois, R., *Annales de l. Soc. Linn. de Lyon,* 1913 and 1914.

[2] Studies made in Japan under the auspices of the department of marine biology, Carnegie Institution of Washington.

[3] Harden, A., and Young, W. J., *Proc. Roy. Soc., B,* 77, 405, 1906, and 78, 369, 1906.

ether and is a very unstable substance. Firefly photophelein is not harmed by chloroform and can be preserved for many days. On the other hand, it is the *Cypridina* photophelein which is the unstable substance. A water solution of *Cypridina* photogenin preserved with chloroform for 56 days will still give light on mixing with fresh photophelein. It should be borne in mind that photogenin, the source of the light, is not only a very powerful substance, but also a stable substance. If we can see the light from a stable body in a concentration of 1:1,600,000,000, what might not be accomplished with the pure substance? We have, perhaps, in the power of photogenin the first indication of a really possible utility of "cold light." My work is not sufficiently advanced to state the chemical nature of photogenin except to say that it is probably protein. Many of the properties of photogenin and photophelein will be found in forthcoming papers on *Cypridina, Cavernularia* and the firefly.

The photogenin and photophelein of *Cypridina* are secreted together into the sea water as a perfectly clear granule-free secretion from gland cells on the upper lip, but as already mentioned, in the body, photophelein is found throughout the animal, probably in the blood, photogenin only in the luminous cells. Just as in presence of photogenin, photophelein is rapidly used up with light production, so in presence of extract of the non-luminous cells of *Cypridina,* photophelein quickly disappears, but without light production. If we boil the non-luminous cell extract or exclude oxygen, the photophelein is not so rapidly used up. In the case of the firefly, the photophelein disappears so rapidly from an extract of non-luminous cells that it is necessary to extract them with boiling water to prepare a stable solution giving light with photogenin. Because of failure to boil the extract, I previously had overlooked the existence of photophelein in the non-luminous parts of fireflies. The evidence seems to indicate that boiling destroys a substance existing in non-luminous parts which oxidizes the photophelein.

Probably photogenin from different forms is different, at least there is a certain amount of specificity in the photogenin-photophelein reaction. Photogenin from *Cypridina* will give a faint light with photophelein from the firefly, but photogenin and photophelein of the same species or allied species give much the brightest light. For instance, firefly photogenin will give a brighter light with photophelein from other species of fireflies or even from *non-luminous* insects (*i. e.,* the boiled cell extracts of non-luminous beetles) than with *Cypridina* photophelein. Indeed, it may be found that the photogenins from different forms exhibit differences in light-giving power, depending on relationship, similar to the differences in the hemoglobins, or similar to the specificity of the precipitin reactions of different animals.

If Dubois's statement that *Pholas* luciferin will give light with oxidizing agents, that it is not destroyed by heat and is found only in luminous cells, be confirmed, we may perhaps look to two general methods of light production in the animal kingdom—one as in *Pholas,* the oxidation with light production of *luciferin* by luciferase so closely paralleled by pyrogallol and peroxidases;[4] the other, as in *Cypridina* and the firefly, through the interaction of photogenin and photophelein, the *photogenin* giving light by some mechanism which can not at present be definitely stated. The closest parallel is the zymase system. Just as zymase is inactive without its co-enzyme, so photogenin is inactive (will not emit light) without photophelein, and just as there are certain quantitative relations between zymase and co-zymase, so there are similar quantitative relations between photogenin and photophelein. As oxygen is necessary for light production, we may, perhaps, provisionally regard photogenin as a substance auto-oxidizable with light production only in the presence of photophelein.

E. Newton Harvey
Princeton University,
October 16, 1916

[4] Harvey, E. N., *Amer. Jour. Physiol.,* 1916, XLI., 454.