

east central Europe. The Dipylon and the Villanova style representing the earliest phase of the iron age in Greece and Italy, respectively, both abound in banded and panel patterns, especially the meander and the swastika. (The swastika is supposed to date as far back as the neolithic period.)

The art of the smith made rapid strides during the Hallstatt epoch. A process was developed of at least superficially hardening a blade of iron, although steel proper was as yet unknown. The engraved ornaments of the bronze age now give place largely to embossed patterns produced by hammering. With the epoch of La Tène the art of the third and last great stage (Kriegertum) spread over western and northern Europe.

The revision is everywhere both conservative and thorough; some thirty pages of addenda and references will contribute much toward its usefulness as a source book.

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THE MECHANISM OF LIGHT PRODUCTION IN ANIMALS

It has long been known that the dried powdered luminous organs of the fire-fly will glow if moistened with water containing oxygen. No light is given off if oxygen is absent. In a previous issue of SCIENCE I pointed out that if we allow this dried powder to stand for an hour in contact with water carefully freed of its dissolved oxygen and then admit oxygen, no phosphorescence is to be observed. It is quite obvious that the photogenic substance has been changed in some way even though no oxidation has taken place. The substance, therefore, which in presence of oxygen is oxidized with the production of light, in absence of oxygen is also decomposed but without light production. We have an analogous instance in the compound lophin (triphenylglyoxaline) investigated by Radziszewski. If hydrolyzed in presence of oxygen by alcoholic potassium hydrate, light is produced and benzoic acid and ammonia formed. In absence of oxygen, no light is produced and

benzaldehyde is formed instead of benzoic acid. The alkali acts as a catalyzer.

In the fire-fly it is natural to suppose that an organic catalyzer, an enzyme, is concerned in light production and it is the purpose of this paper to point out the fact that the existence of such an enzyme has been definitely proved and to add certain new facts to our knowledge of bioluminescence. The credit of this discovery belongs entirely to Professor Raphael Dubois, of the University of Lyons. As early as 1884 Dubois made the crucial experiments in which he showed that two substances are present in the luminous organs of *Pyrophorus noctilucus*, the West Indian cucullo, a thermostabile substance, luciferin, which oxidizes with light production and a thermolabile enzyme luciferase. In 1887 Dubois showed that the same was true for the luminous mollusc, *Pholas dactylus*. If the luminous slime from glands on the siphon and mantle of this mollusc are collected in sea water in two test tubes the solutions will phosphoresce for some time. Boil the solution in one tube and the light disappears instantly; allow the solution in the other tube to stand until the light disappears spontaneously. Then if both tubes, now dark, be mixed, the light reappears. The boiled tube contained luciferin but no luciferase while the other tube contained luciferase but all the luciferin had been oxidized by standing. On mixing, the two substances were again brought into contact and light resulted. In later papers Dubois has studied especially the properties of the *Pholas* luciferin and luciferase and the results are published in many papers in the *C. R. Acad. Sc. Paris* and the *C. R. Soc. Biol.* He says that luciferin is an albumin having acid properties and an active reducing power. It oxidizes readily with luciferase, potassium permanganate, barium peroxide and lead peroxide, giving off light and forming amino-acids and minute crystals giving the test for xanthin.

Luciferase, on the other hand, has all the properties of an enzyme, an oxidizing enzyme acting in the presence of iron salts, which will oxidize luciferin and also tannin, guaiac, a-

naphthol, etc. It resembles the oxydones of Batelli and Stern which are destroyed by ether, chloroform and acetone. It passes with difficulty through porcelain and is non-dialyzing. At 60° C. it is destroyed by heat, as also by digestion with trypsin.

It is astonishing that work such as that referred to above, published in well-known journals by a competent physiologist, should have received so little attention. No good account of Dubois's work is to be found in any of the physiologies in English or German, although he is mentioned as the author of the luciferin-luciferase "theory." I have recently been able to confirm a great many of Dubois's statements and to add some new facts. My material has been the West Indian cucullo, *Pyrophorus*,¹ the eastern American fire-flies, *Photinus* and *Photuris*, and luminous bacteria. There is absolutely no doubt of the existence of luciferase and luciferin and the possibility of separating these two substances.

I find that luminous bacteria also contain luciferin in very small amount and this can be precipitated by treating the bacteria with absolute alcohol and drying quickly. Such a dry powder gives no light with water, but a faint light with the luciferase of the fire-fly. I have been unable to obtain luciferase from the bacteria, due probably to the fact that, like so many of the bacterial enzymes, it is present as an endoenzyme and can only be extracted by high pressures. Curiously enough the bacterial luciferin can not be obtained by destroying the luciferase through heat. Lack of space does not permit of a discussion of this here, but the full details will be published later.

Luciferase of one form will act with luciferin of another, and *vice versa*. This is true for the two genera of eastern fire-flies (*Photinus* and *Photuris*) and for the West Indian *Pyrophorus* (*Elsteridæ*) and *Photuris* or *Photinus* (*Lampyridæ*). Fire-fly luciferin will give no light with extracts of non-luminous parts of the fire-fly or with non-luminous in-

sects or extracts of pill bugs, earthworms or slugs.

Whether the luciferin and luciferase of all forms are identical is still an open question. We know of many organic substances such as oils, alcohols, lophin, etc., which will phosphoresce at relatively low temperatures with alkalis, so that it would be by no means remarkable to find that the luciferin of different forms was different. I have this past winter discovered a luminous reaction which is remarkable in many ways and which closely parallels the method of light production in luminous forms. Pyrogallol will produce light with the vegetable oxidases (potato or turnip juice) if we add some hydrogen peroxide. As little as one part of pyrogallol in 254,000 parts water ($m/32,000$) will give perceptible light and $m/8,000$ a good light. Faint light is produced at 0° C. and a good light at 10° C. A characteristic of luminous animals is that they still produce light at 0° C. The pyrogallol + H_2O_2 corresponds to luciferin and the vegetable oxidase to luciferase. Like the luciferase of luminous forms the oxidase is destroyed by boiling. We might therefore separate a luminous mixture of pyrogallol + H_2O_2 and potato juice into a thermostable and thermolabile component which would again give light if brought together. Mammalian blood may take the place of the oxidase of plant juices.

In a general way, then, we may say that the problem of bioluminescence has been solved at least in its broad aspects. There still remain many details to be filled in, details which will take some time to complete. The exact chemical nature of luciferin is unknown, but the method of attack of the problem has been outlined and all that is necessary is a sufficient quantity of the luminescent material for the determination of its chemical nature. That it may be difficult to obtain enough for analysis is indicated by the luminescence of pyrogallol which takes place in the almost inconceivably small concentration of 1:254,000.

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¹ My studies of *Pyrophorus* were made under the auspices of the department of marine biology of the Carnegie Institution of Washington.

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