

results here reported, as well as those obtained by Taylor, show undoubtedly that the enzyme of ricinus has synthetic power.

Since this article was written there has appeared in the *Z. angew. Chem.*, **24**, 385 (1911) an article by Adolf Welter. He discusses some experimental work on the reversibility of enzyme action and gives some results of the synthesis of fats from fat acids and glycerol through the action of the castor oil bean ferment. Welter uses blanks identical with mixtures used, only heated to 60–80° to destroy the activity of the lipase. He recognizes the change brought about by the decrease in acid values.

The experimental work on this subject was performed at the University of Michigan.

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THE STABILITY OF THE PHOTOGENIC MATERIAL OF THE LAMPYRIDAE AND ITS PROBABLE CHEMICAL NATURE.¹

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One of the oldest and at the same time one of the most important observations relative to the chemical processes involved in the production of light by living creatures, is that the photogenic tissues may be dried, preferably in the absence of air (oxygen), and preserved, again out of contact with air (oxygen), for considerable periods, without losing the power to evolve light when moistened in the presence of air (oxygen). This fact, which seems so remarkable at first sight, appears to have been discovered by Reaumur in his work on the luminous bivalve *Pholas dactylus*, in 1733. The observation was repeated by Spallanzani in 1794 and 1796, by Carradori in 1798, by Carus in 1864, and by Dubois in 1886. Spallanzani extended it to the luminous *Medusae*, Carus to the fireflies (*Lampyridae*), and Dubois to the *Myriapoda* and the *Elateridae*. Pflüger, in 1875, seems to have regarded the luminous tissue of the *Lampyridae* as of too little vitality to exhibit this phenomenon, but the recent work of Kastle and McDermott on this same subject indicates that his conclusion was probably based on material dried in the air.

Kastle and McDermott showed that if the luminous organs of the common firefly of this region, *Photinus pyralis*, were dried *in vacuo* with a residual atmosphere of hydrogen, and subsequently sealed *in vacuo* or in hydrogen, the tissue will retain its photogenic power, and exhibit

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it when moistened, for thirteen months after preparation. Since the latter paper was written, material prepared at the same time as that referred to above has been found to be active almost eighteen months after preparation, and so far as could be judged, without appreciable depreciation. The material preserved *in vacuo* seemed to be slightly more active than that preserved in hydrogen. Kastle and McDermott also showed that the photogenic material was not always entirely exhausted when the dried tissues were first moistened, but that if carefully dried again, the production of light might be repeated two or even three times with the same specimen. The present author has also shown that if the dried photogenic tissue be moistened with a three per cent. solution of hydrogen peroxide, a brighter light is produced than if water alone is used, and the hydrogen peroxide is decomposed.

It has been our experience that if the tubes in which the dry luminous tissue was sealed were broken and the tissue exposed to the air, it rapidly lost its photogenic power, and it had been observed that dried organs of lampyrids thus exposed to the air on damp days glowed feebly for a long time, apparently exhausting their luminous activity. Consequently considerable disappointment was experienced when it was found that a tube in which some 500 of these photogenic organs had been sealed in hydrogen had been accidentally broken; a few of the organs were tested and found to have apparently entirely lost their photogenic activity. An ordinary rubber stopper was placed in the tube, and the latter laid away for possible future use. On three occasions since the breaking of the tube, this stopper has been removed to secure a few of the dried organs for various experiments, without any attention being paid to possible residual photogenicity; but when the tube was opened a fourth time, and a few organs removed on April 29, 1911, about 6 months after the tube was broken, it was thought worth while to test them in the matter of light emission. Accordingly about a dozen of the dried organs were placed in a mortar, ground to a powder, and a few drops of water run in upon them. A faint but distinct yellowish glow resulted, showing that in spite of six months' exposure to air, without especial precautions and at room temperature, a certain amount of the photogenic substance still remained unoxidized. At a still more recent date, May 16, 1911, another lot of these dried organs was removed from this same tube and ground with 3 per cent. hydrogen peroxide; a much brighter light resulted than that produced by water alone and the hydrogen peroxide was actively decomposed. The light produced appeared somewhat reddish in tone.

Through the kindness of Dr. W. W. Coblentz, of the Bureau of Standards, the author was enabled to test the effect of the temperature of liquid air upon the fresh luminous tissue, and upon the same lot of dry material

as referred to above. So far as the power to produce light is concerned, this temperature has no effect upon the luminous tissue, fresh or dry. A living lampyrid (*Photuris pennsylvanica*) dropped into a test-tube immersed in liquid air flashed rapidly for a few seconds, but was soon overcome by the cold, and fell back in the tube, frozen stiff; meanwhile, the luminous organ began to shine brilliantly, but the brilliancy rapidly diminished, the diminution in brilliancy being accompanied by a change in the color of the light, which become very reddish. The light finally disappeared entirely, or very nearly so. On warming to the room temperature, the light first appeared reddish, and with increase in intensity become more and more like the normal light of the insect. The insect was dead, but the luminous tissue continued to glow for some time. The effect on a freshly detached luminous organ was the same, except that the vapors from the liquid air acted as a powerful stimulus to the tissue when first placed upon it. Grinding the tissue in liquid air did not destroy, or apparently in the least weaken its power to emit light on restoration to normal temperature. With the dried tissue from the 1910 lot, previous immersion in liquid air, or grinding while immersed in the liquid, had absolutely no effect, since when moistened with hydrogen peroxide solution after such treatment, the tissue glowed as strongly as before. Experiments have also been performed with liquid sulfur dioxide and liquid ammonia, on the dried tissue. Both of these liquefied gases permanently destroy the photogenic power of the dried tissue, the latter changing the color of the organs to a deep salmon. Sulfur dioxide has been known for some time to destroy the photogenicity of the fresh tissue, and of the dried tissue after moistening; ammonia in solution in water, however, has apparently acted as a stimulus to the activity of the fresh tissues.

Another previous observation of the author is of interest here: A number of luminous organs from *Photinus pyralis* had been ground to an emulsion with glycerol immediately after removal from the insects. A faint glow persisted for some moments after the emulsion had been poured into a bottle, but the next morning no luminosity was visible, nor was any excited by agitation, and it was concluded that the photogenic material had spent its powers. The bottle was subsequently forgotten, and not noticed again for about six months, when it was washed up for other use. As the glycerol emulsion was poured out into running water, several bright points were noticed, and upon darkening the room and washing the bottle out with cold water, quite a display of these points of light was observed, some of the larger fragments glowing for two or three minutes before extinction. Glycerol has usually been regarded as an unsuitable medium for the preservation of photogenic organs, and has even been regarded as poisonous to the light-producing power, but

it would seem from the foregoing notes that such organs may retain a portion of their photogenic power for at least six months in this medium. Histologic fixing fluids without exception appear to permanently destroy the photogenic power of the tissue; many of these fluids are powerfully oxidizing in nature.

With regard to the stability of the material in the numerous other photogenic forms, but little can be said. *Pholas* has been said to preserve its photogenic power, when preserved in honey, for a year, needing only the application of warmth to call forth an exhibition of the light. A few experiments have been made with the photogenic bacteria, from which it seems probable that if a culture of these organisms could be taken to complete dryness when at their maximum activity, the dried culture might show luminescence when moistened; the experiments, however, were not sufficiently satisfactory to warrant definite conclusions on this point. Macfadyen has found that if photogenic bacteria are cooled to the temperature of liquid air, and then allowed to return to their normal temperature for growth, they grow and luminesce again with practically unabated vigor. But if while at the temperature of liquid air, the luminous microorganisms are ground in a mortar, they fail to exhibit luminosity or growth when the temperature returns to normal.

All of these facts show parallelism to other well-known facts in biological chemistry, *e. g.*, the drying of ferments to retain their activity. Still another point of similar interest brought out by Kastle and McDermott was that the dry photogenic tissue could be heated to 100° without perceptibly injuring its activity upon moistening.

It must be, therefore, that atmospheric oxygen has less effect upon the photogenic material of these forms, especially when dry, than has ordinarily been supposed, and therefore the isolation of this material in its non-oxidized condition while difficult may not be a problem hopeless of solution.

Much work has been done, and much of it is conflicting, upon the question of whether or not the photogenic process is an oxidation. The balance of the evidence now is strongly in favor of the oxidation theory in the majority of cases—so strongly, in fact, as to leave but little room for doubt. The photogenic bacteria glow more brightly in oxygen, and are extinguished, though not killed, in neutral gases. The photogenic organs of the *Lampyridae* and *Elateridae* involve a network of anastomosing tracheal capillaries connecting directly with the breathing spiracles. The luminous organs of fish and cephalopods have a similar network of capillaries connected with the circulatory system. The dried photogenic material of the *Lampyridae* glows most strongly when moistened in oxygen and certain oxidizing gases, and but faintly or not at all in

neutral gases; it glows more brightly when moistened with a solution of a powerful oxidizing agent (hydrogen peroxide) than when water alone is used. Certain *per se* non-luminous myriapods and worms emit secretions which become actively luminous when ejected into the air or water. These are a few of the points tending to show the oxidative nature of the photogenic process.

Dubois' view that the light production in *Pholas* and in the *Elateridae* is due to a reaction between two substances, extractable from the photogenic tissues by different processes, is of considerable interest. Especially significant is his observation that one of his extracts may be replaced by chemical substances, such as potassium permanganate, lead peroxide and barium peroxide, and from this it seems not improbable that his oxidizing liquid extract may be a solution of an organic peroxide, possibly of a specific nature, especially since he failed to secure the production of light when he substituted laccase for the oxidizing extract.

The isolation of the photogenic compound is a baffling problem, as we know little or nothing as to its nature, but a few speculations as to its probable nature may not be amiss. The view that the "phosphorescence" was due to the element phosphorus was probably the first view advanced which would seem in any degree reasonable by modern chemical standards, and this view has had its supporters up to quite recent times. It needs very little consideration to see that it is impossible, however, and the chemical fact that there is no free phosphorus in the tissues practically settles it. A more rational view was that of Lavoisier, that the light was produced through the spontaneous combustion of phosphine, produced by the decomposition of protoplasm; this view has also had its supporters up to modern times. Again this theory fails for the want of isolation of any phosphorus compounds resembling phosphine from the luminous tissues. Watase's view that the luminosity was connected with the contractility of protoplasm does not satisfy the chemical requirements of the case.

From the standpoint of biological chemistry, the first rational view was probably that the photogenic compound was a fat. Certain fats were known to exhibit faint luminosity when undergoing slow oxidation. Many fats blacken with osmic acid, and it was found that the photogenic tissues of the *Lampyridae* stained readily with this compound. Thus there seemed to be some evidence of the fatty nature of the compound. Macaire, however, advanced the theory that the compound was albuminous, as it was coagulated by heat, and in this he was followed by Kölliker. Heinemann found phosphoric acid in the ash of the luminous organs of the cucuyo though earlier investigators obtained conflicting results on this point. Phipson regarded the photogenic material as nitrogenous, and stated that on decomposition it gave off an odor of propylamine.

Another point of view stands us in good stead here. In the majority of cases, the luminous tissues of animals appear to be developed embryologically from the fatty layers of the body (see Dahlgren and Kepner). In some cases, of course, this does not hold, as in the shark *Spinax niger* whose luminous organ is developed from squamous epithelium; but in many, if not in most cases of the higher luminous forms, the derivation from the fat tissue is the usual embryologic source of the photogenic organs. This fact, taken with what has been brought out previously, points to the supposition, for it can be little else, that in the *Lampyridae* and many other photogenic forms the photogenicity is the result of the oxidation of a lipoid (phosphatide) containing an unsaturated aliphatic radical, and probably an albuminous grouping. It is interesting to note that Radziszewski includes protagon (lecithin) among the organic compounds possessing phosphorescence.

Some attempts to isolate a photogenic material by extraction of the fresh organs with ether, alcohol or chloroform, in an atmosphere of hydrogen, all failed; however, it is hoped to extend this method of investigation to other solvents, especially to water containing no dissolved oxygen.

With the bacteria it seems unlikely that this view will hold good, and the same may also be true of the *Noctilucae*, *Pyrocystae*, etc. The photogenic bacteria can produce light in a medium as simple as water containing one per cent. of asparagine and three per cent. of sodium chloride, so that it would seem that here the product of metabolism oxidizable with photogenicity might not be so very complex.

Another suggestion is worthy of consideration so far as the possible differences between the photogenic material in different luminous forms: It might possibly be that there would be found to be organic "Photophore" groupings, just as we now have fluorophore and chromophore groupings. That this is indeed probable is indicated by the work of Delépine and of Trautz. The latter showed that the spectrum of the light emitted when pyrogallol is oxidized by perhydrol in the presence of formaldehyde covers very nearly the same spectral area as that of the emitted light of the firefly, and that light produced in a good many cases of chemiluminescence is greenish,¹ *i. e.*, like the firefly's light, has its point of maximum intensity near the yellow-green, the part of the spectrum having the maximum radiant efficiency. It may be then that the "Noctulucin" of Phipson, the "Luciferin" of Dubois and the "Photogen" of Molisch are different chemical compounds having similar or related photogenic groupings in the organic radical.

¹ Polimani has recently asserted that the luminosity of *Pyrosoma* cannot be due to oxidation, and cites its greenish color as a basis for this view—a statement which does not seem to be well founded, in view of the large number of known chemiluminescent oxidations which produce a greenish light.

In summary: The photogenic compound present in the *Lampyridae* is much more stable towards atmospheric oxygen than has usually been thought, especially when dried out of contact with the air; it presents points of similarity to other known biological products; from embryological and chemical considerations it appears probable that it is an albuminous lipid (phosphatide).

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NEW BOOKS.

Messungen elektromotorischer Kräfte galvanischer Ketten with wässrigen Elektrolyten. By R. ABEGB, FR. AUERBACH AND R. LUTHER. Wilhelm Knapp, Halle, 1911.

The purpose of this exceedingly valuable number of the series (No. 5, "Abhandlungen deutschen Bunsen-Gesellschaft") is to summarize all the observed electromotive forces of reversible systems in order that they may be available for the consideration of the general problem of affinity. The work is divided into three parts: the first containing a chronological list of all the systems which have been studied; the second a systematic selection of the most reliable results, grouped together under the elements considered; while the third is devoted to the consideration of single or normal potentials.