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ON THE BIOLOGICAL RELATION OF LEAF DYE TO BLOOD DYE[1]

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ABSTRACT

The author formulates conclusions for biological sciences from the discovery resulting from his cooperation with Leon Marchlewski. It is about the similarity of hematoporphyrin (a derivative of haemoglobin) isolated by Marceli Nencki and phylloporphyrin (a derivative of chlorophyll) obtained by Marchlewski, which suggests their genetic relationship, and thus a common origin of the world of animals and plants.

Keywords: Chlorophyll, haemoglobin, origins of the living world.

In addition to Białobrzeski's[2] work on hemin, I would like to devote a few remarks to the biological significance of the very close genetic relationship between phylloporphyrin, a derivative of chlorophyll, and the hematoporphyrin obtained by me and Sieber, the existence of which was recently demonstrated by Schunk and Marchlewski.[3] According to these authors, phylloporphyrin $C_{16}H_{18}N_2O$ is probably related to hematoporphyrin, $C_{16}H_{18}N_2O_3$, as e.g. antrapurine is related to oxyanthraquinone, i.e. both of these bodies are oxidation products of one and the same substance, only to a different extent. The spectra of both dyes, dissolved in ether, acid or alkaline liquid, as well as the spectra of the corresponding zinc salts are almost identical; the only difference is that the hematoporphyrin streaks lie somewhat closer to the red part of the spectrum. This analogy of the spectra ap-

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[1] The Philosophy and Science editors has preserved in this text its original editing. The original title in Polish is: *O stosunku biologicznym barwnika liści do barwnika krwi*, Gazeta Lekarska, 1897, (23), pp. 608–612.

[2] Białobrzeski, *Berichte d. d. chem.,* Deutschen chemischen Gesellschaft [...] Archives des Sciences Biologiques, XXIX, 17, p. 2842; Archives d. Sciences biol., St. Petersburg, V, p. 283.

Editor's note: In the text we use the original bibliographical notation.

[3] Schunk, Marchlewski, Justus Liebigs Annalen der Chemie, v. 290, p. 306.

plies also to the extra-violet part, as evidenced by the photographs taken by Tschirch with the aid of a quartz spectrograph. Dissolved in neutral liquids, both bodies have the same color and both fluoresce; dissolved in ether and left in diffused light in embedded tubes, after a few months they lose their color completely.

Also interesting is the relationship between the chemical properties of hemin and phyllatataonin, from which the dyes that occupy us come from. By treating hemoglobin with hydrogen chloride, hydrogen bromide or acetic acid,[4] we obtain the appropriate hemins: $C_{32}H_{31}O_3N_4FeCl$, $C_{32}H_{31}O_3N_4FeBr$, $C_{32}H_{31}O_3N_4FeOCOCH_3$, i.e. hematin esters, which after saponification give hematin $C_{32}H_{31}O_3N_4FeOH$. A similar ease of creating esters is also characteristic of phyllatonin. Alakachlorophyll, under the influence of hydrogen chloride dissolved in methyl or ethyl alcohol, gives the appropriate phyllatonin ester, from which phyllatonin $C_{40}H_{39}N_6O_5OH$ can be obtained by saponification. By treating phyllatonin with acetic acid anhydride, the mentioned authors obtained an acetic acid ester: $C_{40}H_{39}N_6O_5OCOCH_3$.

Hematin, or rather hemochromogen, in combination with various proteinaceous bodies forms haemoglobins of various types of blood. Bertin-Sans and Moitessier[5] recently reported that they had obtained methemoglobin from protein and hematin in an alkaline solution. By the action of ammonium sulphur, haemoglobin was obtained from methemoglobin, and oxyhemoglobin from the latter. Unfortunately, there is nothing in the above message that could support the authors' opinion, namely there is no mention of whether crystals of the appropriate types of hemoglobin were obtained. On the other hand, Küstler[6] took the breakdown of hematin into less complex bodies quite far. Working with chromic acid dissolved in acetic acid, he obtained two nitrogen-free acids with a relatively simple composition: $C_8H_{10}O_5$ and $C_8H_{10}O_6$. It is expected that the structure of these acids will soon be investigated.

How and with what bodies chlorophyll is connected in plant cells, we do not know yet. The chemical ratio of chlorophyll to phylloporphyrin is also by no means as straightforward as the ratio of hematin to hematoporphyrin.

The results of Schunk's and Marchlewski's work are of great importance for biological chemistry, as they shed light on the most ancient periods of the history of the systemic world and at the same time indicate the common origin of the animal and vegetable states. DARWIN's theory of the origin of species is based on changes in form under the influence of various conditions of life in the struggle for existence. However, the differences of the systems consist not only in the different form and structure of the organs,

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[4] Kürstler, *Beiträge zur Kenntnis des Hämatins*, Tübingen 1896.

[5] Bertin-Sans, Moitessier, Bulletin de la Société Chimique de France, Mai, 1893.

[6] Kürstler, op. cit.

but also in the difference in the chemical composition of the compounds that make up their living cells. The nature of metabolism depends on the properties of these compounds, and the latter affects the form of cells and the formation of individual organs from them. In other words, the form of the groups of cells that make up an organ depends on the metabolism that the given organisms have developed, struggling for existence under various conditions. Along with the change of living conditions, not only the form changes, but also the chemical composition of cells, as well as the metabolism. Therefore, for a more accurate understanding of the history of the development of the systemic world, it is necessary to compare not only the morphological properties of cells, but also their chemical composition and the metabolism taking place in them. For this reason, the works of Schunck and Marchlewski, aimed at demonstrating the affinity between the dye of blood and leaves, bodies of such different physiological significance, have an undeniable scientific value.

Thanks to the bacteriological researches carried out during the last twenty years, our knowledge of single-celled organisms and their metabolism has become much more complete, and consequently we now view the life phenomena of the more complex creatures of the animal and vegetable world differently. Winogradski's research has shown that nitrifying bacteria, which do not contain chlorophyll, contribute to the formation of complex organic compounds from carbon dioxide, ammonia and inorganic salts, grow and multiply in a similar environment. And here, as in the green parts of plants, the deoxidation of carbonic acid and the production of organic matter take place, with the only difference that oxygen is not released, as in green plants, in a free state, but is used to oxidize ammonia to nitrous acid. Other species of bacteria develop and multiply using coal hydrates or ammonium salts of organic acids with a fairly simple structure, e.g. malic, tartaric, citric acids. Finally, many bacteria are nourished by complex proteinaceous bodies, the same substances on which animal organisms feed. In such cases, the necessary oxygen is obtained by bacteria from the air or from the ground. Thus, in beings lacking chlorophyll and haemoglobin, we see a great variety of metabolisms, which are either as in plants or as in animals; here we encounter all kinds of transitive forms, among which anaerobiosis deserves special attention, which is a characteristic feature of all typical fermentations. It should be noted that the chemical composition of the microbial body varies not only in different forms of these beings, but even in one and the same form it is subject to changes depending on the external conditions of existence. The variability of the morphological features of microbes is also as great as in any other class of organic beings. Let me recall, for example, the development of carbuncle bacilli in the atmosphere of various gases (Szpilman), their development in the form of threads containing spores (Koch) or also development without spores (Roux).

Hundreds of similar instances could be cited, and all of them show that the generation of different kinds of bacteria proceeds with far greater ease than among the higher beings which have come into existence later. We have the right to assume that the simplest systems, building their bodies from such relatively simple compounds as carbonates, water and ammonia, belong to the oldest inhabitants of our planet. Plant organisms convert carbonic acid into starch in the light with the help of a separate substance, chlorophyll. In the later periods of the earth's existence, from the same primordial substance that gave rise to chlorophyll, the pigment of the blood was formed, a compound with a more definite function of binding the oxygen of the air and giving it to the cells of the organs. Besides, chlorophyll is a property not only of plants, we find it in many protozoa and in some lower animal beings. Brandt, as is well known, found that the chlorophyll-containing bodies found in many protozoans, in some cocci, and in many *planaria*, must be regarded as single-celled algae, neither morphologically nor physiologically independent of the organism in which they are found. This seaweed, named by Brandt: "*zoochlorella*," can live on its own after the death of the host animal. If the animal does not have them at all, or has little, then it must nourish itself, like other animal systems, with ready-made organic compounds. Given enough zoochlorella, the host animal can feed, like a typical plant, by assimilating inorganic substances. From this, Brandt draws the conclusion that the green bodies of animals, due to their physiological significance, correspond to the chlorophyll grains of plants, but differ greatly from the latter in physiological terms. Brandt's research was confirmed by Geza-Entz, Kessler, Hamann, Dangeard, Remy-Saint-Loup. Engelmann, on the contrary, claims that many years earlier he had discovered the green virionettes (*vorticella*), the cuticle and subcuticula of which are colored not by chlorophyll grains, but by a soluble green substance chemically identical to chlorophyll. Engelmann proved that by means of this green substance, the virionettes could give off oxygen in the light. So there are animals that, with the help of a dye, indistinguishable from chlorophyll, and combined with living protoplasm, assimilate carbon dioxide in the light, just like green plants. According to Engelmann's later research, there are also bacteria [Engelmann's gave them the name of purple (*Purpurbacterien*)] with protoplasm, stained with a red dye, bacteriopurpurin, releasing oxygen in the light like green plants. The secretion of oxygen here is entirely dependent on the presence of bacterio-purpurin in the protoplasm, and as a result, the development and reproduction of the bacteria in question is possible for a long time only if light has access to them.

As on the one hand there are plants without chlorophyll, on the other hand there are whole classes of animals that do not have red blood. For insects whose tissues receive oxygen from the air by means of tracheae, hemoglobin is of course superfluous as an intermediary for supplying tissues with oxygen. The blood of the dorsal duct of these creatures is colorless and contains a large number of colorless bodies. In coccidia, ascidia, and headless molluscs, instead of red blood, we find a colorless fluid containing more or less proteinaceous bodies and cellular elements. In many cephalopods, gastropods, and crustaceans the vascular system contains a soluble proteinaceous body, hemocyanin, which turns blue in air and is thought to contribute to some extent in respiration. Of the composition of this substance, as well as of the composition of chlorocruorin, discovered by Ray-Lankesteer in some annelids, we know almost nothing, in spite of the formulas and analyzes given by Griffits. According to Mac-Munn and others, the spectrum of haemocyanin does not contain any absorption streaks, while in the spectrum of chlorocruorin we have streaks very close to those of hematin. Mac-Munn discovered in the perivisceral fluid of the sea urchin another pigment of primary importance for respiration, namely quinochrome. Only worms and all vertebrates have red blood containing hemoglobin. As far as we know, the physiological task of red blood cells is by no means extensive. Their purpose is to carry oxygen to the tissues, while the white bodies carry to certain parts of the organism food insoluble in animal juices and various other substances, such as fat, certain dyes, foreign bodies, bacteria, etc. The higher a given organism ranks in the order of animals, the more conspicuous is the division of labor between its cellular elements.

Thus, we have seen how many examples we have in the organic world of the synthesis of organic bodies from carbon dioxide without the participation of hemoglobin. We have seen further that in the most perfect representatives of the animal and vegetable world—leaved plants and red-blooded animals—the respective pigments, i.e., chlorophyll and hemoglobin, have a common origin. We have inherited the idea that the worlds of plants and animals are so interconnected that one can hardly exist without the other. I would express a different opinion. I believe there was a time when there was no animal world except the protozoa, and the role of microbes in the farm of nature, as it now belongs to animals, was played by microbes, causing putrefaction and slow burning. It would be premature to wish to draw further conclusions from this; but I found it useful to express my thoughts, and at the same time to draw the attention of chemists to a very interesting and worthy field of study. The analysis of hemoglobin and chlorophyll has already gone quite far. Therefore, further work in this field should be aimed at studying the structure of these bodies by means of synthesis. It is easy to understand that this way would lead to new views.

Concerning the formation of hematoporphyrin in the animal body, I recently published a note[7] showing that pancreatic ferment, acting on a protein, produces a body, still known to Gmelin, which yields a red-colored

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[7] Nencki, Ber. d. d. chem. Gesellsch, v. 28, p. 566.

substitution product with bromine (Stadelmann's "proteinochromogen"). I have shown that the percentage composition of hematoporphyrin, and especially animal melanin, is quite similar to the percentage composition of proteinochromogens, so it is probable that it is from the latter that blood and bile pigments, as well as melanin pigments, are formed in the animal system. If this supposition were to be confirmed, it would be highly probable that also in a plant cell, a chromogenic group is formed from a protein molecule by hydrolysis, and a chlorophyll from the latter.

ABOUT THE AUTHOR — (1847–1901). Born in Boczki near Sieradz, in the central part of Poland (then under Russian rule); died in St. Petersburg. Participant of the January Uprising (1863–1864) directed against the tsarist rule. After the fall of the uprising, forced to emigrate. Initially studied philosophy—in Krakow, Jena and Berlin—then medicine. After two years of scientific work in Berlin (under the supervision of Adolf von Baeyer), moved to the university in Bern, Switzerland; soon appointed professor in 1871. A department of medicinal chemistry was created there especially for him. He spent the last ten years of his life in St. Petersburg as head of the Department of Chemistry at the Institute of Experimental Medicine. Nencki Institute of Experimental Biology of the Polish Academy of Sciences in Warsaw is named just after him. (See also—Editorial)