XXVI.—ON THE CAUSE OF THE GREENING OF OYSTERS.

By M. Puységur.

WITH A SUPPLEMENTARY NOTE ON THE COLORATION OF THE BLOOD CORPUSCLES OF THE OYSTER.

By John A. Ryder.

The acquisition of a green tint by the soft parts of oysters has been observed in a great many places; at Marennes, l'île d'Oleron, Courseulles, etc., and practical as well as scientific men have long been engaged in trying to discover the cause of this phenomenon. Their observations are scattered through diverse periodical publications, to which I have been obliged to refer, for it was important to determine if their researches were of any real value, inasmuch as none of those yet undertaken seem to lead to correct and indisputable conclusions.

The first paper upon this subject which I have been able to find is by Gaillon, which was issued in the *Journal de Physique, de Chimie et d'histoire naturelle* for September, 1820, tome xci, and was republished with notes by Bory de Saint-Vincent in the *Annales générales des sciences physiques*, tome vii, Brussels, 1820, pp. 89–94.

While desiring to be concise, I cannot resist the temptation to cite some passages from the notice by Gaillon.

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"This green color," says this savant, "is attributed by some to a disease which affects these mollusks." "No," say others, "it is due to the particles (fragments) of green marine plants upon which they feed during a part of the spring and autumn." Others simply pretend that the plants cause the water to become green at certain times, and that the oyster absorbs the color from the water and retains it.

After having combated by arguments and objections, which do not seem very convincing, the views which he would reject, Gaillon at once enters upon a discussion of the subject.

"This malady," he continues, "is it peculiar to the oyster? No; for of other mollusks, some actinians which I placed in this greenish water were not slow to absorb some of the color. This last observation led

* *Notice sur la Cause du Verdissement des Huitres. Par M. Puységur, Sous-commissaire de la Marine, Chevalier de la Legion d'Honneur. Extr. de la Rev. Maritime et Coloniale, pp.* 11, 1 *pl. Paris, Berger-Levrault et Cie.* 1880.

me to suppose that the cause of the greenness was due to the water, which I supposed saturated and impregnated the substance of the oysters, rather than to a derangement of their organic functions. Reflecting upon this idea, I fixed my attention upon the upper valve of an oyster then 'greening' at the bottom of a park; I observed upon its surface very small masses of a very deep, brilliant, green color. I brought my microscope and placed upon a slide, moistened with a drop of water, one of these little, deep, emerald-green masses, which I had found on the the shell of the oyster; what was my satisfaction to find that it contained hundreds of minute attenuated animalcules, pointed at both ends. They were diaphanous at their ends and slightly tinged with green in the middle, where there were present many contractile points."

He says further: "These little beings behaved variously. Sometimes they moved with the axis of the body inclined to the direction of movement. Sometimes they would turn round like a magnetic needle upon its pivot, or they would exhibit a sudden movement of impulsion forward or in a retrograde direction; sometimes again they would erect themselves upon one end; they seemed to like to group themselves together and become entangled amongst one another without order. I have seen them dart at and attack with their pointed ends, as with a lance, other infusorial animalcules the size of which was greater than their own."

I would here close my citations from Gaillon. After having sought to discover the relationship of the animalcule, which he so laboriously describes, he decided for himself, upon the ground of the analogies which he thought he saw, to class it amongst the vibrios, and he proposed for it the name of *Vibrio ostrearius.*

In justice to Gaillon we must admit that he saw "the animalcule" which he described, and that we would not describe their movements in the field of the microscope much better than he has done, but this need not deter us from blaming him for not pushing his studies beyond the determination of the mere fact of the coexistence of these organisms and the green color of the oyster, and to have quietly accepted as a sufficient explanation of the facts the dubious *post hoc, ergo propter hoc.*

This superficial mode of investigation was wide of the mark, as regards bringing about conviction and leading to any real discovery. In another place Gaillon refers to a polemic, which may be said to have been more playful than serious, and of which a trace may be found in the *Annales maritimes (Sciences et Arts)* of 1821, pages 874 to 880, and in the same publication for 1822, pages 86 to 89. M. Goubeau de Billenerie, president of the civil tribunal of Marennes, denies absolutely the existence of the "Vibrio," and asks for its "certificate of origin."

But we find that in other respects he does not oppose Gaillon.

"My opinion," says he, "is that it is necessary to take into account the action of a combination of causes; in the first place, the situation of our *claires* on the banks along the Seudre, the fresh water of which

is mingled with that of the sea, and which is poured into our reservoirs during the spring-tides, to again be mixed with the waters of the river; in the second place, the action of a moderate temperature; then the sun and the northeast wind which brings about the above-mentioned thermal conditions; in fine, to the mode in which the parks are managed, according to methods which have been adopted after prolonged experience."

In the *Médecin Malgré lui*, when Sganarelle explains to Géronte why his daughter is mute, the reasons which he gave are, to say the least, quite as plausible as the causes invoked by M. Goubeau de la Billenerie to account for the *greening* of oysters. He invokes all the elements; the sun, the northeast wind, and then the moderate temperature along the banks of the Seudre, from September to April, that is to say, that prevailing during the coldest part of the year. This is taking into account a great many causes in order to produce such a simple effect, and reason is confounded when it is inquired what share each of these many causes has had in producing the observed phenomena. The criticism of M. Goubeau de la Billenerie is far from as valuable as the incomplete investigation by Gaillon.

In his *Voyage d'exploration sur le littoral de la France et de l'Italie*, M. Coste does not himself directly enter upon the consideration of the question which now occupies our attention. He contents himself with reporting the various opinions which others have expressed, and merely enunciates the following conclusion, p. 118:

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"Of these three opinions, that which attributes to the nature of the soil the power of greening [oysters] appears to be the most in accord with the actual facts of the case."

We shall see in the sequel that the nature of the soil is not in any sense the immediate cause of the viridity of these mollusks. This opinion, like the others, was not expressed by M. Coste as his own, but only as the most plausible amongst those which he had enumerated. A man of the character of the learned professor of the College of France would not be content to discuss this subject with the intention of merely playing upon words.

In this case, as in many others where vital processes come into play, the science of chemistry has been fruitless in the investigation of the cause of the viridity of oysters. M. Berthelot, at the request of M. Coste, sought to discover what was the true nature of the matter to which the coloration of the branchiæ of the oysters of Marennes was due, and the only results which he obtained from analyses were of an absolutely negative character.

"Summarizing our results," says the celebrated chemist, "the coloring matter found in the oysters of Marennes does not resemble that of blood, that of the bile, nor vegetable or animal coloring matters generally. The coloring matter of the blood contains, it is true, some iron, but the

properties of this matter as well as its color are very different from the latter.

M. Bonchon-Brandely, charged in 1877 with the duty of preparing a *Rapport sur l'ostreiculture sur le littoral de la Manche et de l'Ocean*, reviews briefly the method of culture by which oysters are made to assume this color, but does not touch upon its causes. He thinks it probable that the coloration is due to the absorption of the chorophyll diffused through the sea-water, an opinion which is without foundation, because chlorophyll, which is soluble in alcohol, ether, benzine, &c., is altogether insoluble in water.

Finally, M. Paul Petit published an article in the *Revue Pharmaceutique* for 1878, No. 7, page 112, from which we extract the following:

"The learned French diatomologist, M. A. de Brébisson, has observed in the oyster parks of Courseulles a peculiar diatom to which he has given the name of *Amphipleura ostrearia*.

"This species assumes in the portion not occupied by the endochrome a bluish-green tint, but this disappears after dessication. We have supposed, says M. de Brébisson in the note which accompanies his drawing, that this species communicated its color to green oysters, and that it seemed to him that it assumed this color when it grew in a park, which had a tendency to cause the oysters contained therein to become green."

In the beginning of his article, M. Paul Petit mentions the analyses made by M. Balland, pharmacist-major, and M. Gaillard, chief pharmacist of division, at Alger, and who declared that they had found copper in green oysters. M. Gaillard himself concludes from his observations that this was because some process was fraudulently employed to color the mollusks, and that it consisted merely in immersing them in a solution of a salt of copper, and leaving them in it till they were saturated by it.

We will not deny that these chemists may have found copper in these oysters, since they make the assertion, but our own direct experiments have shown—

1. That an oyster which is placed in a bath of sulphate of copper is not colored at all.

2. That death quickly follows when they are plunged into such a mixture.—

I think I have now completed the historical review of the researches which have been made up to the present time to discover the causes of the greening of oysters, and I hope I have shown that none are very exhaustive, the truth still remaining to be discovered in regard to this subject; but, before passing to the observations and experiments which we have made jointly, I would give expression to my sense of obligation for the friendly and able assistance which was very willingly rendered to me during his stay at Croisic in the spring of 1877, by Dr. Bornet, the distinguished algologist, well known to the learned world, and as the continuator of the labors of the lamented M. Thuret.

I received a commission in 1875 from the department of the marine

to establish some experimental parks on the Croisic. After some months of study to determine the elevation of the grounds and their nature, I arranged an experimental park on the shore, and I sought to imitate the method practiced along the shores of the Seudre. The claires which I had established at Sissable were placed in such a condition as to fit them to receive the water, each spring tide, during 10 to 12 hours or less. Situated on the eastern part of the Croisic, where the sea is never disturbed by heavy waves, was the ground which I had at my disposal, and under such conditions that the methods in use at Marennes and on the Tremblade could be put into operation.

I will not speak here of the results of these attempts. They have exceeded my expectations. The products grown at Sissable would seem to rival those of Marennes, and if they are not yet well known it is because the establishment where they are grown has not yet passed far beyond the experimental stage, having as yet yielded for consumption only from 100,000 to 150,000 oysters. The product will, besides, always be as limited as the grounds (parks) where they are reared.

Besides their other qualities, in which, as I have said, they approach those of Marennes, the oysters of Sissable acquire a higher degree of viridity than those grown in the claires on the Seudre. I therefore found myself in the presence of the problem of which I will treat in this notice, and which I determined to solve.

Like Gaillon, of whose researches I was ignorant at this time, as well as of the publication of M. P. Petit, which appeared after my observations, I remarked the relation which existed between the viridity of the claires and that of the oysters.

The material which coats the bottom of the claires forms a slimy, blackish green layer, and superficially has all the appearance of being composed of *Nostocaceæ*, an inferior group of Algæ, representatives of which are so frequently found at the bases of humid walls, in ditches, and marshy places. Upon microscopic examination, M. Bornet and myself recognized that this substance was composed of an alga belonging to an entirely different group, in fact, of diatoms, the innumerable fusiform frustules of which traversed the field of the microscope in all directions in a very lively manner. We also thought that we recognized this diatom as nearly allied to *Amphipleura* and *Navicula*. But finally, in order to have the determination made as exactly as possible, we submitted it to M. Grunow, the able Austrian algologist, who very willingly examined it, stating that it was a variety of his *Navicula fusiformis*, to which he had given the name of *ostrearia*. (Grunow; New Diatoms from Honduras, in Month. Mic. Journ., 1877, p. 178.)

EXPLANATION OF FIGURE 1.

Navicula fusiformis, var. *ostrearia*, Grunow.

Enlarged 330 times. (The densely stippled extremities of the frustules indicate the portions of the organism in which the blue vegetable pigment is contained.)

This diatom presents a singular peculiarity, first observed by M. de Brébisson, as we have already seen from the note cited from M. Petit,

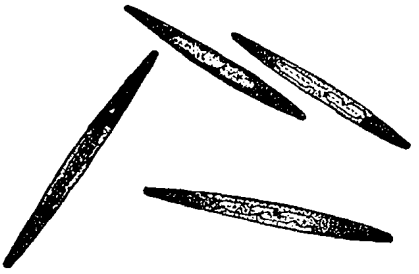

FIG. 1.

but which has not yet apparently been published by that savant. We know that the frustule of a diatom is a cell, the silicious wall of which incloses a yellowish pigment (diatomine), disposed, according to the germs, in the form of bands or granules, and a colorless liquid which fills up the rest of the cavity of the cell. For this reason when diatoms of the usual type are examined in mass they have a more or less intense yellowish brown color, but this is not the case with *Navicula ostrearia*. The two bands of chlorophylloid substance (diatomine) exist in this just as in all other *Naviculæ*, and they are of the usual yellowish color, but, aside from them, the intra cellular liquid, instead of being colorless, has a beautiful azure blue tint.

This tint is more pronounced at the extremities of the frustules than in the middle portion, where it is sometimes entirely absent. This substance belongs to the group of pigments, which are soluble in water, and is different from chlorophyll and diatomine, which do not dissolve in that liquid. Treated with water acidulated with acetic or hydrochloric acid, *Navicula ostrearia* immediately loses its blue color and the liquid absorbs the latter, becoming bluish-green. When dried this diatom also loses its color.

These facts being determined, the next experiment to be tried was plainly indicated. What result would we get if we took some white-fleshed oysters and fed them exclusively upon *Navicula ostrearia*? This we sought to determine in the following manner: Along the edges of a claire, which had become green, we very carefully collected with a spoon some of the green material with which the sides and bottom of the claire was coated. The material so collected in bottles was shaken for an instant so as to separate the heavy particles, and the dirt (which is almost impossible to get rid of entirely), is deposited on the bottom of the vessel; afterwards we poured off and saved the supernatant-colored liquid, which then contained nothing but the diatoms with the admixture of scarcely any foreign matters. We thus also obtained water, which was sufficiently charged with the necessary *naviculæ*. This operation requires some attention and some dexterity. When the diatoms have been

gathered with too great an amount of impurities or with too much water, which is nearly always the case when the duty of gathering them was intrusted to a guard, it is not always possible to have the mixture sufficiently concentrated or as clean as is to be desired.

Upon returning to our lodgings we poured the water charged with the diatoms into deep plates, which we placed on a table near a window. The diatoms soon collected on the sides and bottom of the vessels, in a greenish mucilaginous layer, the thickness and tint of which varied according to the richness of the gatherings. We then placed in each plate, according to the size of the latter, from three to six perfectly white-fleshed oysters, which had never been in a claire, the shells of which had also been previously washed and brushed clean. In similar plates, filled with ordinary sea water, we placed some oysters of the same kind as the others. In conducting this experiment we were of the opinion that the acquisition of the green color was altogether due to a peculiar regimen.

Twenty-six hours after the commencement of the experiment all of the oysters which had remained in the water charged with diatoms were deeply tinged with green; the others had not suffered any change of color. Repeated many times the experiment always gave the same result; the coloration was also more intense, or just in proportion as the water was more heavily charged with the diatoms. In one of our experiments an opening was made in the shell of one of the oysters so that the mantle of the mollusk could be seen from without. After having caused this oyster to become green we again placed it in pure sea water, and after some days the coloration had entirely disappeared. It reappeared when the oyster was replaced in some water containing *Navicula ostrearia*. This experiment was finally repeated at a distance from the scene of our work. White-fleshed oysters, which were sent to Paris, together with a flask containing water charged with the diatoms, were then fed and colored with them at the *Jardin des Plantes* in the laboratory of M. Decaisne.

In the course of the experiment we noticed that the oysters opened and closed their valves and caused currents to be established in the surrounding water, which were carried into the animal, together with the diatoms which they held in suspension. The existence and direction of these currents was apparent wherever the coating of diatoms was soon removed, thus leaving the bottom of the disk uncovered, but the covering of diatoms remained on those parts of the dishes where the action of the currents was not perceptible.

Carried to the buccal apparatus by the cilia with which the branchiæ are provided, the *Naviculæ* passed into the stomachs of the mollusks, where they give up their contained nutriment. The yellow chlorophyll is disintegrated and digested; the soluble pigment passes directly into the blood, to which it communicates its color. It is likewise the most vascular portions of the animal, such as the branchiæ, which are most deeply colored.

An examination of the digestive canal of the oysters used in the experiments proved that the soft substance of the diatoms was really absorbed. The stomach, intestine, and excrements were packed full of the tests of the *Naviculæ*. These tests, composed of silex, were not attacked by the juices of the stomach, but it would have astonished us to find that their contents, protected as they are by such a refractory envelope, had suffered dissolution by the action of the digestive fluids of the oyster, if we did not know that this envelope was not completely closed, but that there is an unsilicified line of suture which separates the two valves which compose the frustules of the diatoms.

There therefore remains no longer any doubt as to the fact that the viridity of oysters is entirely due to the absorption or digestion of the soft parts of the *Naviculæ* held in suspension by the surrounding water; this definite experience also completely overturns the hypotheses which attribute it to the influence of the soil, to the mixture of fresh and salt water, to northeast winds; in a word, all the other conjectural causes to which this simple phenomenon has been childishly attributed are shown to be inadequate.

It is evident, moreover, that the coloring matter is directly absorbed by the mollusks, and that the process takes place inside of the animals. If, in fact, dissolution of the coloring matter took place in sea water, the water would be tinged as soon as the diatoms were blanched. Now, this is not the case. In fresh water, on the contrary, the coloring matter is immediately dissolved and as a result the diatoms are blanched. A single drop of water placed on a slide containing the diatoms causes them to lose their color instantly. Finally, if a piece of filtering paper is saturated in the fresh water which has been placed on the diatoms, and it is afterwards dried, it will present absolutely the same color as the green oysters.

These laboratory observations are, moreover, perfectly in accord with the phenomena observed by the oyster culturists. Heavy rains cause the greenness of the claires to disappear, and the dry and salt-laden northeast winds, which augment the saturation of the waters, are, on the contrary, favorable to the production of the green coating in the claires.

If the subject is still far from exhausted, that part of it with which I have busied myself has been decided. Others who continue the research may, perhaps, do better than I, but not by the use of other methods. I submit these researches to my successors, and, without further comment, I would like to be permitted to call attention to the two following questions, which, it appears to me, it would be interesting to study:

1. Is *Navicula fusiformis* var. *ortrearia* present at all seasons of the year in the claires, or is it found in winter?

2. The coloration, which reveals its presence during the time when the claires are green, is it accidental and temporary? In other words,

does this alga disappear completely from the claires when the waters change their color, or does it only lose its color at this time?

These difficult questions which involve the consideration of the marvelous world of protophytes, require for their resolution much patient observation. But the difficulty of the problem only augmented the enjoyment of those engaged in its solution, and to conclude with an expression familiar to my sympathetic collaborator, I would say: we discovered all that we sought; that sufficed for the pleasure of the seeking.

SUPPLEMENTARY NOTE ON THE COLORATION OF THE BLOOD CORPUSCLES OF THE OYSTER.

By John A. Ryder.

The foregoing essay by M. Puységur has just recently fallen into my hands; earlier references which I made to his important investigations have been only at second hand and from notices which have not done his work justice. His methods have been positive, and there seems to have been little chance for him to have erred in his conclusions.

My own investigations of this subject have also convinced me of the correctness of M. Puységur's conclusions; they, in fact, supplement them. I subjoin a brief statement of the facts observed by me, together with some account of collateral observations by other investigators.

I have ascertained that *Ostrea virginica* is affected by an acquired viridity at certain times and in certain places in precisely the same way as the common *O. edulis* of Europe and the *O. angulata* of the Tagus, as I have been able to learn from fresh material from Liverpool, obtained for me through the efforts of Professor Baird. The cause of this peculiar staining of the soft parts of these animals is, therefore, very probably the same throughout both the European and the American oyster-growing regions. My own studies have also shown beyond a shadow of doubt that the acquisition of this color comes about as follows: That the coloring is either derived from without, or else may be a hepatic coloring principle, which, on account of some derangement of the normal metabolic processes of the animal, has been dissolved and absorbed by the lympho-hæmal fluids, and then imbibed by the blood cells or hæmatoblasts, and thus imparted to them their peculiar color. The blood cell of the oyster measures about $\frac{1}{3000}$th of an inch in diameter, but varies somewhat in size. It is amœbal in its behavior to a surprising degree, and throws out pseudopodia when at rest, which may even be branched. In a temperature abnormal to them in winter, that is, in a very warm room, I have had them live under a compressorium, bathed in the serum from the vessels of the animal, for four hours, dur-

ing which time they exhibited the most surprising activity of movement, at times even becoming confluent with one another.

The corpuscles which have been most deeply tinged appear to have lost their amœbal dispositions, and in this condition they tend to lodge in the numerous interstices between the prominent muscular trabeculæ found in the ventricle of the oyster. In a few instances I have found large cysts lying just below and covered by the epithelium of the mantle, which were packed full of these green-colored blood cells, which had apparently been accumulated in and been the cause of the formation of these cysts. When the cysts were cut open the corpuscles would very quickly escape, often in very feebly resistant masses, but which, upon shaking in a watch glass, would at once separate into distinct corpuscular bodies, each of which was provided with a nucleus. These corpuscles differed in no respect morphologically from a normal blood cell of the oyster, except in color.

The heart in oysters which are deeply tinged with green is often affected in its ventricular portion, where the deposit of corpuscles in the chinks between the trabeculæ of the ventricle and over the inner walls of the latter may be as much as a sixteenth of an inch in thickness. This thick deposit of green corpuscles gives to the normally somewhat translucent ventricle a delicate pea-green color. This condition of affairs may sometimes be well seen in sections of the heart of a green oyster, where the stratum of abnormal cells is thus shown to be present as a thick adherent layer covering the whole of the internal parietes of the ventricle, which even extends down behind the upwardly directed lips of the auriculo-ventricular valves, so as possibly to some extent impede their free action. Occasionally an impoverished green oyster may be found, the vessels of which exhibit this coloring faintly in their courses through the mantle.

The nature of this coloring matter seems to have been very satisfactorily determined by M. Puységur, who concludes, as we have seen, that it is neither chlorophyll nor diatomine, though he does not seem to have resorted to spectroscopic analysis and has relied entirely upon other physical tests, mainly such as would determine its solubility in various menstrua. He shows that it is some specific coloring matter which, unlike chlorophyll, is soluble in water. I would here suggest that it is probably a peculiar form of chlorophyll, allied to what is known as *phycocyanin*, which is found in certain simple algæ, known to botanists as the *Cyanophyceæ*, which embrace five subdivisions, viz, *Chroococceæ*, *Nostocaceæ*, *Oscillatorieæ*, *Rivularieæ*, and *Scytonemeæ*, according to Sachs, who says: "These organisms are of a bluish, emerald, or brownish green, or some similar color due to a mixture of true chlorophyll and phycocyanin; this pigment becomes diffused out of dead or ruptured cells, and thus produces the blue stain on the paper on which *Oscillatorieæ* are dried. From crushed specimens treated with cold water phycocyanin is extracted as a beautiful blue solution, blood red in reflected light (Cien-

kowski and Rostafiuski). When the crushed plants are treated with strong alcohol, after the extraction of the blue pigment, a green solution is obtained, which contains true chlorophyll, and probably a special yellow pigment, phycoxanthin (Millardet and Kraus)." The group of phycocyan and phycoerythrine vegetable pigments, according to H. C. Sorby, give remarkable spectra with one main absorption band.

I have failed to prove by spectroscopic research that the substance which tinges the oyster is chlorophyll. In fact I have been unable to obtain alcoholic solutions of this substance from green oysters which were apparently dense enough to give a spectrum, and light, which had been transmitted through a mass of the green blood-cells, also failed to show any absorption bands. Dessication destroys the bluish-green color in *Navicula ostrearia*, according to Puységur, and it is significant in this connection that Sorby found that the Phycocyanin group of pigments were also associated with albuminous substances somewhat in the same way as hæmoglobin in the blood, being, like the latter, decomposed at exactly the same temperature as that at which albumen coagulates. My own view may therefore be expressed as follows: That the coloring material in green oysters, on account of its solubility in water, its instability and color, is probably allied to Phycocyanin, since we know that it is not chlorophyll, because the latter is insoluble in water, meanwhile remembering that the spectroscope also gave us entirely negative evidence upon this point.

The diffuse bluish coloring matters of *Stentor coeruleus* and *Freia* are also interesting in this connection. The detection of vegetable algous parasites in the fresh-water mussel by Leidy is a case of an entirely different nature, from the condition of things found in green-fleshed oysters, yet it is interesting as an illustration of animal and vegetable symbiosis.

The most searching tests which I made for the detection of the presence of green vegetable parasites in the oyster, as I at one time supposed, have given negative results, and I think that in the presence of all the foregoing evidence the phenomenon can be in no sense due to *symbiosis*, but rather to a tinging of the blood cells by an unstable coloring matter, which has been dissolved out of the food, and which in this case is derived from a diatom, in the more fluid plasma of which it exists in much the same relation to the latter as the hæmoglobin found in the blood corpuscles of vertebrates, or the coloring matters which tinge the blood of Cephalopods, or those of some of the *Arcidæ*, but not forming, as in them, a normal portion of their substance.

The coloring matter, however, in the case of the oyster when absorbed from its vegetable source produces certain abnormal changes in those blood-cells which imbibe it, as is conclusively shown by the facts which we have related regarding the accumulation of the tinged corpuscles in cysts of an abnormal character, as well as in the heart. Yet this effect is clearly unlike that produced by inert staining fluids, such as are used

in making histological preparations; for, as a rule, none of these will be absorbed until the tissue has been killed by some other re-agents, or not until the cells have been pricked open, so as to break the continuity of their walls, which are not immediately pervious to coloring agents. The fact also that the green color may be again gradually withdrawn by removing the food which is the cause of the viridity, is equally remarkable, and is likewise not a property of other staining fluids used to tinge dead cells, with the exception of some of the anilines, such as Safranin and Dahlia. Yet in this last case the parallel which has been instituted is again unfair, because the anilines are extracted from dead plasma, whereas the green color is withdrawn from the living plasma of the blood cells.

It is also evident that whatever the nature of the change may be which is induced by the green-coloring principle in the blood-cells of the oyster, that it does not interfere with the nutrition of the animal, which, according to the universal, concurrent testimony of oystermen, is almost invariably plump and in good condition when its soft parts have become greenish. This is also proof that the coloring material must be more inert than carmine, and must necessarily not be poisonous, or else the nutritive processes would be greatly interfered with, especially if the color were of mineral origin. For it is known that certain mineral substances have an extraordinarily high chemical equivalency when combined with protoplasm; some mineral salts, such as those of arsenic, lead, copper, and mercury, will enter into combination with many hundreds of times their own weight of living protoplasm; this circumstance seems to explain why these substances are so poisonous; why they produce violent symptoms in the process of elimination, or produce a fixed condition of the plasma and death.

Sometimes the flesh of oysters has a decidedly yellowish cast, verging to brownish-yellow in certain parts, especially the palps, gills, and edges of the mantle. This I have at times supposed to be due to the consumption of large quantities of the tests of diatoms, which were filled mostly with yellowish-brown endochrome or diatomine. If the peptones or ferments secreted by the gastric glands (the liver) of the oyster merely dissolve this material, I see no reason why, under certain circumstances, a pale brownish-yellow tint might not be assumed by the blood-cells of the animal after having absorbed that substance in the same way that they absorb the bluish-green tint. That the brownish endochrome might, in short, be carried in solution into the vessels and there imbibed by the blood-cells is very probable. That the siliceous tests of diatoms are quickly emptied of their plasma and endochrome after they are swallowed by mollusks is shown by the fact that I have never found a diatom in any part of the alimentary canal of the oyster, except the stomach, from which all the contents had not been removed.

Taking a survey of the lower groups of the vegetable world, which contain bluish-green pigments, and which are at the same time free-swimming in their habits, so as to place them within reach of the stationary oyster as food, there is none which actually seems more likely to be the source of the green tinge here discussed than the *Diatomaceæ*. And as there is no other class of forms so commonly and constantly met with in the alimentary canals of marine mollusks generally, I think we might take it for granted, for this reason alone, that they are the source of the coloration. In fact, it is rarely that I have met with any other vegetable organisms in the stomachs of oysters except diatoms, after having examined hundreds, by the excellent method of first removing the recently-swallowed contents of the gastric cavity with a pipette thrust into the mouth and through the short gullet. The "bill of fare" of the animal can then be very deliberately studied under the microscope after the contents of the pipette have been pressed out upon and prepared for observation under a compressorium. This method was also independently adopted by M. Certes in the course of his investigations upon the commensal fauna of the oyster, which led to the discovery of the remarkable organism which he has called *Trypanosoma Balbianii*, and which is almost as invariably present in the alimentary canal of the oyster as the frustules of diatomaceous plants. While the fact that diatoms impart a green tinge to oysters, which have been erroneously supposed to be hurtful in that condition when consumed as food, it is also very probably true that in the case of the common *Mya arenaria*, the flesh of which is said to occasionally acquire a greenish tint, the coloration is in like manner derived from the same source, viz, the diatomaceous plants.