

**Further observations on enterochlorophyll, and allied pigments / by C.A. Mac Munn.**

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FURTHER OBSERVATIONS

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ON

ENTEROCHLOROPHYLL,

AND

ALLIED PIGMENTS.

BY

C. A. MAC MUNN, M.A., M.D.

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FURTHER OBSERVATIONS

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ALLIED PLANTS

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V. *Further Observations on Enterochlorophyll, and Allied Pigments.*

By C. A. MAC MUNN, M.A., M.D.

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[PLATES 9, 10.]

IN a paper, read before the Royal Society in 1883,\* I described the results of an examination of the so-called "bile" of invertebrates, and proved that the alcohol extracts of the "liver," or other appendage of the intestine answering to it, showed a spectrum so like that of vegetable chlorophyll as to have led me to conclude that no essential difference exists between the spectrum of enterochlorophyll and plant chlorophyll.

At that time I could not decide the points which are now considered.

The object of the present investigation was to determine:—(1) Whether enterochlorophyll is due to the presence of symbiotic algæ or not; (2) whether it is an *immediate* food-product and merely an instance of the intracellular digestion of food chlorophyll; (3) if it is not derived from either of these sources, is it built up by and in the liver of the animal yielding it? (4) in what points does it differ from plant chlorophyll and the chlorophyll of *Spongilla*?

I believe I can prove the absence of symbiotic algæ, the absence of food-products, the animal origin of the pigment, and that it yields, at least in some cases, similar decomposition-products to plant chlorophyll. This evidence is not based on spectroscopic examination only, but also on the study of the morphology of enterochlorophyll and on the absence of starch and cellulose.

At the same time I do not wish to appear too confident as to the purely animal origin of the pigment in *all* cases.

*Spectrum of Chlorophyll in a Living Leaf and in an Animal containing Chlorophyll built up by itself.*

What is the spectrum of chlorophyll in a living leaf? To enable one to reply to this question a suitable method of examination must be adopted, and I may say at

\* Proc. Roy. Soc., vol. 35 (1883), p. 370.

once that the chemical spectroscope is not suited for the examination, (1) because of its greater dispersion, (2) owing to the difficulty of sufficiently illuminating the object. The leaf should be examined by means of the microspectroscope and illuminated by the help of a substage condenser; if necessary a section should be made, or, if the leaf is too thin to show the full spectrum, it may be necessary to place one leaf over another. *In a leaf so examined at least five bands are visible, and it is these five bands, with another nearer the violet, which are seen in an alcohol solution of chlorophyll.* I examined the leaves of thirteen plants in this way successively, and in every one I could see the five bands. The plants taken were:—*Ilex*, *Hedera*, *Agapanthus*, *Lilium*, *Aspidium* (*filiæ mas*) (frond), *Scolopendrium* (frond), *Begonia*, *Laurus*, *Ficus* (*elastica*), *Scarforthia* (*elegans*), *Geranium*, *Primula*, and common grass.

Hence KRAUS'S\* map of the spectrum of a living leaf is correct, and HANSEN'S statement to this effect can be verified. Plate 9, Chart I., spectrum 1, shows this spectrum. If a *Spongilla*, which, as Professor LANKESTER† has shown, is a true chlorophyll-building animal, be examined in the same manner, the bands are found to agree with those of the spectrum of a leaf; such is also the case in an infusorian coloured by its own chlorophyll, *e.g.*, *Ophrydium*, spectra 2 and 3, Chart I.‡

Further, it will be shown that an alcohol solution of *Spongilla* chlorophyll shows the same bands as a similar solution of plant chlorophyll, and closely resembling a similar solution of enterochlorophyll. I have not thought it advisable to mention all recent researches on plant chlorophyll, because they have not yet emerged from a state of confusion and nothing could be gained by doing so. My object is to show that whether what has been named chlorophyll has or has not been isolated, there exists in plants a green colouring matter which, before it has been touched by a reagent, gives the same spectrum as a green colouring matter in an animal, and that the same solutions of both colouring matters give the same spectra, which differ slightly, but in no essential respect, from those of similar solutions of enterochlorophyll.

Before proceeding to describe the results of saponifying vegetable and animal chlorophyll, I will now give the result of an examination of the "liver" pigments of some invertebrate animals.

#### *The "Liver" Pigments of some Invertebrates.*

KRUKENBERG and I found, independently of each other, that the alcohol extracts of the "livers" of some animals showed a band in red; he called the pigments giving this band *hepatochromates*, but neither from his description or drawings of the

\* 'Zur Kenntniss der Chlorophyllfarbstoffe,' &c., 1872. Tafel I., 5.

† In Professor LANKESTER'S early description of *Spongilla* chlorophyll he called it chondrichlor, but subsequently he agreed with SORBY as to the identity of this colouring matter with plant chlorophyll. See also his paper in Quart. Journ. Micro. Soc., vol. 22 (1882), pp. 229-254.

‡ I was not certain about the fourth band, and so have left it out of the drawing of this spectrum.

spectra can it be inferred that he knew he was dealing with a true chlorophyll. In my paper, already referred to,\* I had shown enterochlorophyll to be present in *Ostræa*, *Mytilus*, *Cardium*, *Anodonta*, *Unio*, *Octopus*, *Buccinum*, *Fusus*, *Purpura*, *Littorina*, *Helix aspersa*, *Helix pomatia*, *Helix citrina*, *Arion*, and *Limax* among *Mollusca*. In *Homarus*, *Cancer*, *Carcinus*, *Pagurus*, and *Astacus* among *Arthropoda*. In *Uraster*, *Asterias*, and *Echinus* among *Echinodermata*. A chlorophyll band is seen in KRUKENBERG'S drawings† of the alcohol extracts of the "livers" of *Grapsus marmoratus*, *Carcinus mænas*, *Pilumnus villosus*, *Eriphia spinifrons*, *Homarus vulgaris*, *Buthus occitanus*, *Tethys fimbria*, *Pleurobranchus*, *Eledone moschata*, *Aplysia depilans*, and *Mytilus edulis*. It is satisfactory to know that KRUKENBERG has come to the conclusion that his "hepatochromates" are *animal pigments*, and his evidence is of the more value in this case as he has made a great number of observations on animal colouring matters.

As I have gained more experience in the examination of solutions since my last paper was communicated, I may have to describe in this the spectra of some already referred to, especially as differences are sometimes seen when a great number of the same species is examined; this remark applies more especially to starfishes, in which the chlorophyll obtained from the radial cœca shows certain differences with regard to the dominant band, which, in my opinion, establish beyond all doubt the animal origin of the pigment. Besides, with the large spectroscopie obtained with the grant allowed me by the Royal Society, I am able to measure more accurately the wavelengths of the bands than I could before.‡ The consideration of the spectra of solutions of vegetable chlorophyll should naturally precede that of those of animal chlorophyll, but it will be deferred for convenience until I come to describe the results of saponification.

#### MOLLUSCA.

*Paludina vivipera*.—In a deep layer of the rectified spirit extract§ of the "liver" of this species the three bands shown in spectrum 4, Chart I., are seen, and in a thinner layer another band between green and blue. The solution was a deep-yellow colour and fluoresced red. Its first three bands read approximately:—1st,  $\lambda$  678 to  $\lambda$  656; 2nd,  $\lambda$  620 to  $\lambda$  600; 3rd,  $\lambda$  552 to  $\lambda$  539. With nitric acid the change already described, and to be referred to below, took place. Hence the enterochlorophyll present differs in no respect from that of other mollusca.

\* *Loc. cit.*

† "Vergleichend-physiologische Studien," 3<sup>te</sup> Abth. 1880. Taf. 1. KRUKENBERG, too, arrived at the same conclusion as myself, namely, that various pigments are prepared in the liver for use elsewhere (S. 189), especially for the integument.

‡ The bands in blue and violet are most difficult to measure, and if with even better instruments they are found to differ from my measurements the fault, I hope, will not be attributed to inaccuracy.

§ Of course this and all the following extracts were filtered before examination.

*Limnæus stagnalis*.—The method pursued in this case, and in all the following, was the same, and differs from that described in my former paper, the “liver” or other enteric appendage answering to it being extracted for some hours with the solvent, the extraction being generally repeated if necessary; by the adoption of this method a greater quantity of colouring matter is got into solution.

Rectified spirit extracts of the “liver” are greenish-yellow and show a red fluorescence; the spectrum consists of five bands, which differ slightly in position from those of an absolute alcohol extract, the spectrum of which is shown in spectrum 5, Chart I. The bands read as follows:—1st, from  $\lambda$  678 to  $\lambda$  651·5; 2nd,  $\lambda$  618 to  $\lambda$  600; 3rd,  $\lambda$  547 to  $\lambda$  537; 4th, (about)  $\lambda$  519 to  $\lambda$  503; and 5th, (about)  $\lambda$  492·5 to  $\lambda$  473·5. On adding nitric acid the colour became greener, and the bands assumed the position shown in Chart I., spectrum 6, their centres reading:—1st,  $\lambda$  654; 2nd,  $\lambda$  602·5; 3rd,  $\lambda$  570·5; 4th,  $\lambda$  536; and 5th, uncertain. If allowed to stand longer in contact with the acid the fluid assumed a bluish tint.

Caustic soda seems to affect the position of the bands,\* the colour of the solution remaining green, an alcohol solution so treated gave a spectrum whose bands read as follows:—1st band,  $\lambda$  683·5 to  $\lambda$  657; 2nd,  $\lambda$  625 to  $\lambda$  602·5; 3rd,  $\lambda$  551 to  $\lambda$  538·5; 4th,  $\lambda$  521·5 to  $\lambda$  498·5 (?). No noticeable shifting of the bands took place with acetic acid. An aqueous extract of the “liver” also gives a chlorophyll-like spectrum. In some parts of the body of this mollusc there is evidence of the presence of hæmoglobin, and in its black parts and elsewhere a lutein or lipochrome. As will be shown further on, the 4th and 5th bands of the enterochlorophyll spectrum belong to a similar pigment. I could find no enterohæmatin in the “liver” of this species.

*Trochus ziziphinus*.—The absolute alcohol extract of the “liver” is green, fluoresces red, and shows spectrum 7, Chart I., the band reading as follows:—1st band,  $\lambda$  672 to  $\lambda$  651·5; 2nd, (about)  $\lambda$  618 to  $\lambda$  593; 3rd,  $\lambda$  548·5 to  $\lambda$  535; 4th, uncertain. With nitric acid the colour became greener, and the usual series of bands appeared.

*Trochus cinerarius*.—An absolute alcohol extract of the “liver” is yellow, fluoresces red, and shows spectrum 8, Chart I. The bands reading as follows:—1st,  $\lambda$  678 to  $\lambda$  657 (light shading up to 647); 2nd,  $\lambda$  620·5 to  $\lambda$  595; 3rd,  $\lambda$  545·5 to  $\lambda$  535; 4th and 5th, from about  $\lambda$  519 to  $\lambda$  484·5 (?). It became greener with nitric acid, and showed the same band as in other cases. Caustic soda intensified the double band nearer violet.

*Littorina littorea*.—Although I have described the “liver” pigments of this mollusc in my former paper, I have made some further experiments on them which are worth recording. An absolute alcohol extract of the “liver” is greenish-yellow, and gives a well-marked red fluorescence; the fully developed spectrum is shown in spectrum 9, Chart I., the three principal bands of which read as follows:—1st,  $\lambda$  678 to  $\lambda$  654; 2nd,  $\lambda$  623 to  $\lambda$  600; 3rd,  $\lambda$  548·5 to  $\lambda$  537. On treating with caustic soda a turbidity

\* It also weakened the fluorescence. In the case of *Mytilus* and *Ostræa* I did not notice any change with NaHO.

was produced; on filtering, evaporating, and extracting the residue with petroleum ether\* the solution was green and showed a red fluorescence; and now a dark band was seen to replace the 4th and 5th bands of spectrum 9, which extended from  $\lambda$  496.5 to  $\lambda$  477, another was perhaps also visible in the violet. On evaporating this solution down, and extracting with bisulphide of carbon, a deep yellow solution was obtained showing similar bands, the two nearer violet being plainly marked, the first much darker than the second, and reading  $\lambda$  524.5 to  $\lambda$  503, and  $\lambda$  492.5 to  $\lambda$  473.5.

The residue was also soluble in ether and in chloroform. Hence the enterochlorophyll of *Littorina* thus treated is soluble in alcohol, ether, petroleum ether, chloroform, and bisulphide of carbon. It is soluble in the solvents of the "lipochromes," and of fat, and this agrees with the microscopic characters of enterochlorophyll, for it is often dissolved in oil globules, as will be shown further on. According to my own observations† a lutein pigment is present in *Littorina*, and KRUKENBERG‡ finds the same which he calls a lipochrome, and which I have no doubt is prepared in the "liver." But there is another interesting point about *Littorina*: the pharyngeal muscle yields hæmoglobin, and if the "liver" be examined in the compressorium with a substage condenser and SWAN lamp a spectrum is seen which is shown in spectrum 10, Chart I.; now, these bands recall to mind hæmochromogen, or what I have called enterohæmatin. An aqueous solution of the "liver" treated with ammonium sulphide shows these bands intensified, so that they are at least closely related to hæmatin; moreover, in other parts of the body of this species, histohæmatins are found, hence it is highly probable that in the "liver" the radical of these pigments is prepared just as the lipochrome radical is also prepared in it. That this hæmatin pigment is related to enterochlorophyll is very probable.

*Patella vulgata*.—Absolute alcohol extracts of the "liver" are yellowish-green and fluoresce red. The spectrum of an alcohol solution is shown in spectrum 11, Chart I., the bands reading:—1st,  $\lambda$  678 to  $\lambda$  654; 2nd,  $\lambda$  620.5 to  $\lambda$  598; 3rd,  $\lambda$  547 to  $\lambda$  536. The double band extended from  $\lambda$  521.5 to  $\lambda$  475 (?). On adding nitric acid the usual series of bands appeared, reading:—1st,  $\lambda$  669 to  $\lambda$  649; 2nd,  $\lambda$  613 to  $\lambda$  595; 3rd,  $\lambda$  580.5 to  $\lambda$  569, and 4th,  $\lambda$  545.5 to  $\lambda$  537 (?). Acetic acid did not appear to affect the colour or spectrum much. Caustic potash acted as in most other cases, *i.e.*, intensifying the bands nearer violet. Sulphuric acid produced the same effect as nitric; so also did hydrochloric. But, besides enterochlorophyll, *Patella* often shows the presence of enterohæmatin in its "bile." On removing the animal from its shell, the blackish membrane covering the visceral mass is seen to be more prominent posteriorly to the right tentacle; if this part be punctured, a brownish viscid fluid exudes,

\* This shows that this enterochlorophyll is changed by caustic soda, and also that its yellow constituent is changed by it.

† Proc. Birm. Phil. Soc., vol. 3 (1883).

‡ "Grundzüge einer vergleichenden Physiologie der Farbstoffe und der Farben," 1884.



which examined quickly shows two hæmochromogen-like bands, which disappear on exposure to air, and reappear with ammonium sulphide (Chart I., spectrum 12).

In *Patella*, the pharyngeal muscles contain hæmoglobin (RAY LANKESTER), and histohæmatins occur in various parts of the body; therefore, the same remarks made about the "liver" pigments of *Littorina*, will apply here. Lutein (=a lipochrome) can also be detected in various parts, as well as in the "liver."

*Helix pomatia*.<sup>\*</sup>—From the "liver" of this mollusc, pigments are obtainable which differ in no essential respect from those of other pulmonate mollusca, they have been described by me in a former paper. I have made a few further observations since, which are as follows:—A solution of the "liver" pigments in weak alcohol is greenish-yellow, fluoresces red, and gives spectrum 13, Chart I., of which the bands read:—1st,  $\lambda$  672 to  $\lambda$  660, feeble shading to  $\lambda$  647; 2nd,  $\lambda$  623 to  $\lambda$  593; 3rd,  $\lambda$  561.5 to  $\lambda$  557; 4th, about  $\lambda$  545.5 to  $\lambda$  529.5. There was also a fifth band, perhaps  $\lambda$  494.5 to  $\lambda$  475. A second extraction with absolute alcohol showed a spectrum belonging to enterochlorophyll only (spectrum 14, Chart I.). The red fluorescence of this green extract was well marked, and its bands read:—1st,  $\lambda$  672 to  $\lambda$  657, shading to  $\lambda$  649; 2nd,  $\lambda$  620.5 to  $\lambda$  596.5 (?); 3rd,  $\lambda$  545.5 to  $\lambda$  532; and 5th,  $\lambda$  490.5 to  $\lambda$  472 (?). In the absolute alcohol extract of another "liver," spectrum 15, Chart I., was observed, which differs from both of the above extracts; these bands read as follows:—1st,  $\lambda$  672 to  $\lambda$  657, shading to  $\lambda$  647; 2nd,  $\lambda$  623 to  $\lambda$  593; 3rd,  $\lambda$  569 to  $\lambda$  557 (?); 4th,  $\lambda$  563 to  $\lambda$  551, and a feeble 5th,  $\lambda$  494.5 to  $\lambda$  475. All these solutions were united and saponified, the results will be described further on. Probably spectrum 15 belongs to a colouring matter *intermediate* between enterohæmatin and enterochlorophyll.

KRUKENBERG<sup>†</sup> found evidence, as he thought, of the presence of several pigments, but the appearances can probably be explained on the above supposition. I have repeated my former experiments on other Mollusca whose "liver" pigments I have already described, but have nothing further to add to former descriptions.

### *The Enterohæmatin of Invertebrates.*

According to KRUKENBERG<sup>†</sup> the change produced by sulphide of ammonium (on enterohæmatin) is not due to a reduction but to the action of an alkali on this pigment, which he names "helico-rubin," but sulphuretted hydrogen and neutral reducing agents produce the same effect; and *if the "bile" of an animal containing*

\* The red bile shows *without any treatment* two enterohæmatin bands:—1st,  $\lambda$  569 to  $\lambda$  555; and 2nd, perhaps  $\lambda$  540 to  $\lambda$  524.5. Hence the idea of KRUKENBERG, that they only appear when alkalies are added, is not correct.

† *Loc. cit.* See also SORBY's paper on "The Evolution of Hæmoglobin," Quart. Journ. Micro. Soc., vol. 16 (1876), p. 77. KRUKENBERG apparently did not know of this paper, or he could not have fallen into the error noted above. See also my paper, *loc. cit.*

*enterohæmatin* be examined quickly, the two bands of the reduced pigment can be seen; on exposure to air they fade away, to be again brought back by reducing agents. Caustic alkalies, no doubt, also cause the bands to appear, but this is no argument against reduction and oxidation. The impropriety of calling this pigment "helicorubin" is patent when we consider that it is not limited to *Helix*, but occurs in *Limax*, *Arion*, *Astacus*, and *Patella*, &c., as has been shown by me. I have tried to convert it into hæmatoporphyrin, but hitherto with only partial success; but I had not enough material to work upon. It is a very unstable body, resembling in this respect Professor LANKESTER'S chlorocruorin. As I have already said, it is probably the mother-substance of those histohæmatins, which are found in animals in whose "livers" it is built up. Another view might be held, namely, that it is an excretion and represents the form in which the above colouring matters are got rid of, but its very instability is against that view.

#### ECHINODERMATA.

It will be seen that the dominant band of chlorophyll in solutions of the radial cœca of starfishes is sometimes replaced by two narrow ones\* in the same part of the spectrum; I believe this has been noticed among plants in the case of very young green leaves, but I am unable to find the reference to this statement. It would seem to show that, as an examination of a considerable number of the same species teaches, one meets with enterochlorophyll in all stages of manufacture; in some cases immature and having just been formed, or in stages of preparation; in other cases fully formed, when it more nearly approaches the condition of plant chlorophyll.

*Solaster papposa* and other species.—The first specimen examined was a brilliant red colour, which colour was due to a pigment having a close resemblance to zoonerythrin (=tetronerythrin), but in thin layers of its solutions showing two or three bands like those of lutein. Hence the pigment cannot be *rhodophan*† or *xanthophan*, and its colour shows that it is not *chlorophan*, so that all one can say is that it is a lipochrome.‡ A rectified spirit extract of the radial cœca (which were brownish) was yellow, had a faint red fluorescence, and gave a well-marked chlorophyll spectrum (spectrum 16, Chart I.). The three most prominent bands read as follows:—1st,  $\lambda$  669 to  $\lambda$  651·5; 2nd,  $\lambda$  618 to  $\lambda$  593 (?); and 4th,  $\lambda$  509 to  $\lambda$  490·5 (?). These measurements are doubtful owing to the small amount of pigment in solution.

The dominant band in red was found in a fourteen-rayed red *Solaster papposa*, slightly different from the above, from  $\lambda$  672 to  $\lambda$  651·5 (*i.e.*, in an absolute alcohol solution of the cœca). Its other bands agreed closely with those of the first specimen. Both solutions on treatment with nitric acid gave the usual series of bands. In a twelve-

\* This double band in red was noticed also in other cases, *e.g.*, *Octopus* and *Anodonta*, &c.

† See below.

‡ See below. Note the one-banded lipochrome in spectrum 16.

rayed *Solaster* the dominant band of an absolute alcohol solution of the radial cœca—which was yellow and fluoresced red—extended from  $\lambda$  669 to  $\lambda$  657. Other specimens were examined with the same result, their radial cœca furnishing only a small amount of enterochlorophyll. No enterohæmatin could be detected; and in none of their integuments was hæmatoporphyrin found. A full account of the integumental colouring matters is reserved for another communication.

*Uraster rubens*.—A great number of this species have been examined, and the results obtained are so important in deciding the question as to the animal origin of enterochlorophyll, that I think it necessary to give the results in greater detail than in other cases.

(1) *Uraster* with a nodulated integument.—A filtered alcohol extract of the cœca was a dull sap green, and showed a red fluorescence and two well-marked bands, and a feeble third in a deep layer; also in a shallow layer an ill-defined band at the blue end of green. The bands read:—1st,  $\lambda$  669 to  $\lambda$  640; 2nd,  $\lambda$  620.5 to  $\lambda$  591. With nitric acid the spectrum changed, and the bands read:—1st,  $\lambda$  665 to  $\lambda$  640; 2nd,  $\lambda$  618 to  $\lambda$  591; 3rd,  $\lambda$  579 to  $\lambda$  557 (?). The great breadth of the band in red is here noticeable.

(2) *Uraster* with a red integument.—Here the alcohol extract of the radial cœca was greenish, fluoresced red, and gave the same kind of band in red as (1), but after this solution had stood twelve hours *the band in red could be no longer seen*, and the colour of the solution had changed to orange-yellow. The residue left after evaporation of the alcohol was a pale, yellowish-brown colour, and dissolved in chloroform showed one faint band in the blue part of green. *Hence it would appear that the enterochlorophyll in this case spontaneously changed into another colouring matter*; the amount of material was, however, too small to enable me to determine what this colouring matter was.

(3) A violet *Uraster*.—The radial cœca yielded when extracted with alcohol a deep gamboge-yellow coloured solution, showing a badly-marked band in red, corresponding in position with the above-mentioned band, but showing a strong absorption of the violet end of the spectrum up to E. In a thin layer a broad ill-defined shading from  $\lambda$  516 to beyond  $\lambda$  475 became detached. On adding nitric acid a distinct blue colour was produced, the band in red moved nearer the violet, and the broad shading could be no longer seen. Caustic soda did *not* cause this shading to disappear. There is no doubt that these cœca contained a colouring matter which belongs to the lipochromes, and I believe it is more like KÜHNE'S rhodophan or xanthophan than anything else. Now, as we find in the integument a similar pigment, there can no longer be a doubt that the integumental pigment (as I formerly showed) is prepared in the radial cœca.

(4) A reddish-brown *Uraster*.—The radial cœca when extracted with alcohol furnished a dark-yellow solution with a red fluorescence. This showed spectrum 17, Chart I., the result of combining the spectrum of a deep and shallow layer. The bands read:—1st,  $\lambda$  667 to  $\lambda$  649; 2nd,  $\lambda$  611 to  $\lambda$  593; and 3rd,  $\lambda$  541.5 to  $\lambda$  529.5.

There was a 4th band, which, after adding caustic soda, became more distinct, and read about  $\lambda$  505 to  $\lambda$  484.5 (?).

(5) The cœca of a brown *Uraster* were also extracted with absolute alcohol, the extract added to that obtained from the cœca of (4), and saponified by HANSEN'S method. The extract from the cœca of (5) agreed in its spectrum with that of (4). The result of this experiment will be described under "*Saponification*."

(6) An orange and brown specimen of *Uraster*.—The dirty green radial cœca were extracted with absolute alcohol, and furnished an orange-coloured solution with a red fluorescence, which showed a spectrum similar to 17, Chart I. The bands read approximately: 1st,  $\lambda$  667 to  $\lambda$  651.5; 2nd,  $\lambda$  615.5 to  $\lambda$  593; 3rd,  $\lambda$  543 to  $\lambda$  532 (?), and a 4th, about  $\lambda$  511.5 to  $\lambda$  486.5.

(7) A brown *Uraster*, with greenish cœca.—These formed in absolute alcohol a yellow solution with a greenish-brown tinge and a red fluorescence. The bands read: 1st,  $\lambda$  669 to  $\lambda$  647; 2nd,  $\lambda$  613 to  $\lambda$  593; 3rd,  $\lambda$  540 to  $\lambda$  530.5. The 4th band could not be read distinctly, but appeared to be  $\lambda$  511.5 to  $\lambda$  475 (?), probably formed by the coalescence of two bands.

If any of these last three solutions were treated with nitric acid it became greenish, and the bands read: 1st,  $\lambda$  660 to  $\lambda$  640; 2nd,  $\lambda$  609 to  $\lambda$  589; 3rd,  $\lambda$  576 to  $\lambda$  557; 4th (perhaps),  $\lambda$  540 to  $\lambda$  524.5, and possibly another,  $\lambda$  505 to  $\lambda$  484.5.\*

The action of zinc and sulphuric acid was tried on these last three solutions, but the result obtained showed that no reduction-products similar to those obtained from hæmatin could be found; nor, indeed, in the case of vegetable chlorophyll could I succeed in getting anything different. Hence the statement that chlorophyll yields similar decomposition-products to hæmatin is erroneous.

(8) An orange *Uraster* with brownish-green cœca.—These extracted with absolute alcohol formed a yellow solution, with a red fluorescence, the bands of which read as follows: 1st,  $\lambda$  667 to  $\lambda$  649; 2nd,  $\lambda$  615.5 to  $\lambda$  593; 3rd,  $\lambda$  544 to  $\lambda$  532, and 4th, about  $\lambda$  516 to  $\lambda$  488.

A second extraction of the cœca of the last three specimens furnished the same results on examination, the solutions in absolute alcohol being greenish, with a red fluorescence, the bands reading: 1st,  $\lambda$  667 to  $\lambda$  649; 2nd,  $\lambda$  611 to  $\lambda$  593; 3rd,  $\lambda$  543 to  $\lambda$  529.5, and 4th,  $\lambda$  516 to  $\lambda$  488 (?).

A third extract gave the same results; hence it is evident that the only pigment which could be extracted by absolute alcohol was enterochlorophyll.

(9) A brown *Uraster* yielded similar results, the bands of the solutions of the radial cœca corresponding with the above.

While these nine *Urasters* all contained a colouring matter whose bands in alcoholic solutions agree closely enough when allowance is made for the difficulty of measuring

\* A second and third extraction of these cœca yielded the same spectra as the above, and these extracts were used for saponifying, as described further on.

the edges of the feeble bands, the following two showed certain peculiarities with regard to the dominant band in red.

(10) A *Uraster* of a brown and orange colour had its cœca extracted with absolute alcohol. The solution obtained was a deep yellow, with a red fluorescence, and gave spectrum 18, Chart I., which is the result of combining the spectrum of a deep and shallow layer. The bands read as follows: 1st,  $\lambda$  683.5 to  $\lambda$  675; 2nd,  $\lambda$  664 to  $\lambda$  651.5; 3rd,  $\lambda$  613 to  $\lambda$  593 (?); 4th,  $\lambda$  545.5 to  $\lambda$  532 (or  $\lambda$  543 to  $\lambda$  529.5), and the 5th, about  $\lambda$  505 to  $\lambda$  473.5. I could not see any other band nearer violet. That this spectrum did not belong to chlorofucin was seen by the action of caustic soda on it, as the bands were hardly affected, whereas in a similar solution of chlorofucin they become completely changed under the influence of this reagent.

On treatment with nitric acid the similarity in constitution of this pigment to other enterochlorophylls was proved, although the bands are not coincident; they read: 1st, about  $\lambda$  681 to  $\lambda$  669; 2nd,  $\lambda$  660 to  $\lambda$  645; 3rd,  $\lambda$  609 to  $\lambda$  589; the others were uncertain. On evaporating on the water-bath, a yellowish residue with particles of a brown colour in it, was obtained, and on extracting with petroleum-ether and filtering, a yellow solution was obtained giving *two bands* in the red, with these readings: 1st,  $\lambda$  683.5 to  $\lambda$  672; 2nd,  $\lambda$  665 to  $\lambda$  654; also a broad shading between green and blue. The two former being of equal intensity, and nearly equal breadth. This is shown in spectrum 19, Chart I. On diluting with more petroleum-ether a band became detached from about  $\lambda$  501 to  $\lambda$  481. Now according to HANSEN "chlorophyll green" is insoluble in petroleum-ether, but this chlorophyll was soluble in it. In a still more dilute solution (of petroleum-ether) another band, nearer violet, from about  $\lambda$  467 to  $\lambda$  451, could be seen. On touching the dried residue with a solution of iodine in iodide of potassium it showed a reddish tinge; with nitric acid it became green, and with sulphuric acid green. On dissolving some residue left after evaporation of a petroleum-ether solution in bisulphide of carbon a reddish-yellow solution was obtained showing only *one* broad band in green and blue.

(11) The cœca of another *Uraster* on being extracted with absolute alcohol yielded a deep-yellow solution with a red fluorescence, and a similar spectrum to that just described, the bands of which read: 1st,  $\lambda$  683.5 to  $\lambda$  675; 2nd,  $\lambda$  665 to  $\lambda$  651.5; 3rd,  $\lambda$  613 to  $\lambda$  593; 4th,  $\lambda$  543 to  $\lambda$  529.5; and 5th,  $\lambda$  505 to  $\lambda$  475 (?). An attempt was made to get reduction-products from this solution with zinc and sulphuric acid, but with a negative result.

(12) A brown *Uraster*.—The cœca, extracted with absolute alcohol, yielded a deep-yellow solution having a red fluorescence. The bands read: 1st,  $\lambda$  667 to 651.5; 2nd,  $\lambda$  615.5 to  $\lambda$  593; 3rd,  $\lambda$  545.5 to  $\lambda$  535; and another from about  $\lambda$  509 to  $\lambda$  484.5. I failed to get a reduction-product from this by treating with magnesium and acetic acid.

(13) The deep-yellow solution of the cœca of another *Uraster*, fluoresced red, and

gave the following bands: 1st,  $\lambda$  667 to  $\lambda$  649; 2nd,  $\lambda$  615.5 to  $\lambda$  593; 3rd,  $\lambda$  543 to  $\lambda$  532; and 4th,  $\lambda$  509 to  $\lambda$  484.5.

(14) Another solution of the same kind, of the same colour, and with the same bands in its spectrum, was united with that of (13) and saponified by HANSEN'S method, as will be described further on.

Other specimens were examined with the same result, and the presence of one lipochrome or lutein band was found to be the rule, which fact alone goes far to show that the enterochlorophyll of *Uraster rubens* is not a *vegetable* chlorophyll, and the saponification method tends to support this view.

I formerly stated\* that among worms I failed to find enterochlorophyll, and I have recently examined the "bile" of *Aphrodite aculeata* with a negative result.

"Bile" of *Aphrodite aculeata*,†—I find that the stomach of *Aphrodite* contains in its wall hæmoglobin; its presence in the nerve ganglia (of abdomen) had been previously detected (LANKESTER). Although the "bile," which was of a dark-brown colour, did give a faint band in red, yet on evaporating it to dryness and extracting with alcohol a colourless and bandless solution was obtained. Chloroform hardly took up anything from the same residue, being only faintly yellow. Ether, and acetic ether, also failed to extract any colouring matter. It did not contain enterohæmatin. It was but little changed by nitric or sulphuric acid. It contained only a brown pigment, insoluble in alcohol, ether, chloroform, and acetic ether, soluble in water, and unaffected by acids or alkalies.

#### *The Saponification of Vegetable Chlorophyll.*

KÜHNE ‡ applied the saponification method to the isolation of the *chromophans* of the retina and succeeded in separating out three pigments from the soap,—chlorophan, rhodophan, and xanthophan,—which he designated the "lichtbeständige" colouring matters of the retina. He showed their points of difference from the lutein of the *corpora lutea* and from yolk pigments and other bodies which hitherto had been known as luteins. It is unnecessary here to describe his results in detail except to mention that these pigments agree in showing SCHWALBE'S iodine reaction when in the *solid* state, *i.e.*, a green-blue to blue with iodine in iodide of potassium, they also become a dark green-blue to blue with concentrated sulphuric or nitric acid,§ and they are soluble in the lutein solvents. KÜHNE has, however, more recently found that when the pigments are pure they may fail to show these colour reactions. Hence all we have to rely on is the presence of certain faint bands in the violet and blue parts of the spectrum and peculiarities of solubility when we wish to determine the presence of such pigments. I have myself repeatedly found that colouring matters giving similar

\* *Loc. cit.*

† *Cf.* KRUKENBERG, *loc. cit.*, *infra*.

‡ "Untersuchungen aus dem Physiologischen Institute der Universität zu Heidelberg," Band 1, Heft 4, 1878, and Band 4, Heft 3, 1882. ("Ueber lichtbeständige Farben der Netzhaut," &c.)

§ CAPRANICA, "Archiv für Anatomie und Physiologie," 1877, Heft 3, S. 285, and SCHWALBE, "Handbuch der gesammten Augenheilkunde," Leipzig, 1874.

spectra to the chromophans individually, and which KRUKENBERG has assumed to be identical with the chromophans, failed to give any blue coloration with iodine in iodide of potassium, and their reactions with sulphuric and with nitric acid were equally unsatisfactory.

KRUKENBERG,\* taking KÜHNE'S researches as a basis, has proposed that all those animal pigments which are soluble in certain solvents, which give bands in the blue and violet parts of the spectrum, and the above reactions when in the solid state, should be included under one name, that of the lipochromes. He has found these lipochromes widely distributed through the animal kingdom. In his early publications he restricted the term to the luteins, but he now includes zoonerythrin (= tetronerythrin). But it seems to me that this generalisation is too great, and that the abandonment of the old term lutein is likely to lead to confusion. However KRUKENBERG deserves great credit for having evolved order out of chaos, and whether we accept the name lipochromes or not, will matter little if it be kept in mind that they are mostly all lutein-like pigments.

In most cases KRUKENBERG applied KÜHNE'S saponification method to the isolation of the lipochromes, and found that they "withstand" heating with caustic soda; but I cannot altogether agree with this, because in some cases bands previously invisible come into view, and in many cases bands are much intensified, showing a decided change in the composition of the pigment.

Dr. ADOLPH HANSEN† applied the same method to the chlorophyll of young wheat plants, and was led to some important conclusions as to the composition of chlorophyll; he says he has restored to KRAUS‡ the right of having been the first to show how to separate chlorophyll into its constituents.

I need not describe the preliminary treatment to which he subjected his plant-material, but limit myself to the description of the saponification.

The alcohol extract of the leaves is concentrated by evaporation, and the concentrated extract treated when boiling drop by drop with caustic soda solution; when the alcohol has evaporated water is added, and the solution heated again. After the evaporation of the greater part of the water alcohol is again added and the saponification ended. When the alcohol has gone chloride of sodium is added in excess to ensure the separation of the soap. The green soap is now extracted in the separating funnel with petroleum-ether, the extraction being repeated as long as the petroleum-ether appears yellow. The soap is then shaken with pure ether, after which it is extracted with ether containing "some cubic centimetres" of alcohol which extracts the green constituent. In this way the two colouring matters composing

\* 'Vergleichend-physiologische Studien,' 1880-1882, and 'Grundzüge einer vergleichenden Physiologie der Farbstoffe und der Farben,' 1884; also 'Grundriss der med.-chem. Analyse,' 1884.

† 'Arbeiten des botanischen Instituts zu Würzburg,' Bd. 3, Heft i., and 'Verhandlungen der physikalisch-medizinischen Gesellschaft zu Würzburg,' N. F., Band 18, (1884), p. (109). See "Nature," vol. 30, p. 224.

‡ 'Zur Kenntniss der Chlorophyllfarbstoffe und ihrer Verwandten,' Stuttgart, 1872.

chlorophyll are separated from each other, and these HANSEN calls "chlorophyll yellow" and "chlorophyll green." The former goes into the petroleum-ether, from which it can be obtained in the form of *yellow needles* on evaporation, or on dissolving the residue in alcohol and evaporating. The latter is obtained purer by extracting the residue obtained by evaporation of the alcohol-ether solution, with alcohol holding ether, whereby salt and other impurities remain behind. According to HANSEN "chlorophyll yellow" is a lipochrome,\* since it gives the reactions of these pigments with nitric and sulphuric acid and iodine, and the spectrum consists of three bands in the blue half of the spectrum.

"Chlorophyll green" in alcohol and ether possesses four bands in the red half of the spectrum, one between B and C, covering C in one edge, one between C and D nearer D, one just after D, and the last between D and E nearer E, while the blue half of the spectrum is absorbed from before F onwards to the violet end. It crystallises in sphere-crystals out of the ethereal solution, showing a black cross with polarised light.

HANSEN maintains that by this treatment the originally existing chlorophyll green is not decomposed.† But I cannot quite agree with this statement. I find the spectrum of "chlorophyll green" after saponification and when dissolved in ether-alcohol solution not quite the same as before saponification; *it is quite different*.

I now proceed to describe the results of my own experiments. I shall first describe the saponification of vegetable chlorophyll.

#### *Experiments on the Saponification of Vegetable Chlorophyll.*

I have not attempted in all these cases to follow up the purification of the pigments so as to obtain them crystallised, my object being to determine the *spectra* of the various solutions and compare them with solutions of animal chlorophyll. In some however I saw both sphere-crystals and needles, and can verify the accuracy of HANSEN's descriptions. I will select two sets of experiments to illustrate what I have got to prove, as I find the appearances in most cases coincide with those to be described.

On extracting the green leaves of *Primula* with absolute alcohol after crushing them in this solvent, a green solution with a blood-red fluorescence is obtained in

\* Prof. SACHS told Dr. HANSEN that MILLARDET had observed similar crystals. HANSEN, *loc. cit.* HARTSEN, Chem. Central-Blatt, 1872, S. 525, 1875, S. 613, also obtained a yellow crystalline body from Chlorophyll. He also "saponified" chlorophyll. 'Neue Chemische Untersuchungen,' Nordhausen, 1875.

† This statement is in contradiction to the statements of such experienced investigators as Professors STOKES, SORBY, and others. It has been long known that even the small amount of acid in some leaves will decompose chlorophyll, and such violent treatment as boiling with caustic soda must certainly *change* it, as is the case. (Cf. also RUSSELL and LAPRAIK, Journ. Chem. Soc., vol. 41, p. 338, and SACHSSE 'Die Chemie und Physiologie der Farbstoffe, Kohlehydrate und Proteinsubstanzen,' 1877.)



which the bands in the red half\* of the spectrum read as follows: 1st band,  $\lambda$  678 to  $\lambda$  640; 2nd,  $\lambda$  627 to  $\lambda$  600; 3rd,  $\lambda$  593 to  $\lambda$  569; then *another band in the green, faint, whose presence can be detected in a living leaf.* To take its wave-length a deeper solution is examined, when we get for the whole series of bands: 1st,  $\lambda$  681 to  $\lambda$  638; 2nd,  $\lambda$  629 to  $\lambda$  600; 3rd,  $\lambda$  593 to  $\lambda$  566; 4th,  $\lambda$  551 to  $\lambda$  532; while in a more dilute solution: 5th,  $\lambda$  486.5 to  $\lambda$  467; and 6th (about),  $\lambda$  451 to  $\lambda$  438. This solution was saponified with caustic soda and the subsequent treatment carried out as in HANSEN'S method. (*Supra.*)

The petroleum-ether solution of the soap was yellow, had no fluorescence, and showed two bands: the first from  $\lambda$  488 to  $\lambda$  468, and second (about)  $\lambda$  458.5 to  $\lambda$  445. If we now examine the mother liquid from which the petroleum-ether, the ether, and the ether with alcohol have removed their pigments, we find that it no longer shows the original spectrum. While the original solution showed in a deep layer spectrum 1, Chart II., this solution showed spectrum 4, Chart II.; hence saponification has altered the chlorophyll to a certain extent. The first ether-alcohol extract of the green soap was only pale-green, but the second was a fine blue-green solution, *with a splendid blood-red fluorescence*, and now gave spectrum 5, Chart II., the bands of which read: 1st,  $\lambda$  651.5 to  $\lambda$  627; second,  $\lambda$  607 to  $\lambda$  583.5; third,  $\lambda$  537 to  $\lambda$  524.5. There is also a feeble band between D and E, such as HANSEN describes from  $\lambda$  572 to  $\lambda$  557 (?). On now comparing these bands with those of the original solution they are seen to be *quite different*. In a deeper layer of solution they occupied the following positions: 1st,  $\lambda$  656 to  $\lambda$  620.5; 2nd,  $\lambda$  609 to  $\lambda$  580.5; 3rd (a shading only),  $\lambda$  572 to  $\lambda$  557; and 4th,  $\lambda$  537 to  $\lambda$  519.

The action of nitric acid on this isolated "chlorophyll green" did not cause the same bands to appear as in an ordinary alcohol solution of chlorophyll, when treated with this reagent; the spectrum obtained is shown in spectrum 6, Chart II., the colour of the solution being bluish. After some time the band at D, and that after it, became much fainter, the first and fourth bands being very distinct. On comparing this result with that obtained in the case of enterochlorophyll a difference is apparent. I found that the solid "chlorophyll green" went into bisulphide of carbon; but in this the red fluorescence was not so apparent, and as none of this residue went into petroleum-ether there could not have been a lipochrome present. On evaporating a solution of chlorophyll green, and examining with a  $\frac{1}{6}$ th objective, thousands of round green granules were seen; some of these were red in certain lights, and were therefore dichroic, which is a property of the solid pigment, according to HANSEN.†

If grass-chlorophyll is saponified, some *trifling* differences between the spectra of its

\* Compare spectrum 1, Chart I., with spectra 1 and 7, Chart II., and it is seen that the bands of the solutions giving the latter spectra are nothing more than those found in a living leaf. I am quite certain about the presence of a band between D and E in a living leaf.

† The yellow residue also gave the reactions described by HANSEN with iodine, nitric, and sulphuric acids.

solutions and those just described are noticed. An absolute alcohol solution prepared in the usual way gives spectrum 7, Chart II., in a deep layer, and spectrum 8, Chart II., in a shallow one. The solution was a brilliant green, with a blood-red fluorescence, and its bands read as follows: 1st,  $\lambda$  679.5 to  $\lambda$  636; 2nd,  $\lambda$  627 to  $\lambda$  600; 3rd,  $\lambda$  593 to  $\lambda$  566; 4th,  $\lambda$  551 to  $\lambda$  532; and in a thinner layer: 5th,  $\lambda$  486.5 to  $\lambda$  467; 6th,  $\lambda$  451 to  $\lambda$  438 (?). I found that caustic soda alone certainly affected these bands; that before D could no longer be seen, and the whole spectrum appeared as shown in spectrum 9, Chart II.\* The band in red had completely changed its position, as it now read from  $\lambda$  662.5 to  $\lambda$  627, and blended into the second band; 3rd,  $\lambda$  582 to  $\lambda$  560; 4th,  $\lambda$  537 to  $\lambda$  519; moreover the red fluorescence was no longer as noticeable as before. The change brought about, with regard to the position of the bands, is somewhat similar to that caused by nitric acid; thus the alcohol solution of the above chlorophyll on treatment with nitric acid gave: 1st band,  $\lambda$  665 to  $\lambda$  640; 2nd,  $\lambda$  615.5 to  $\lambda$  593; 3rd,  $\lambda$  579 to  $\lambda$  560; 4th,  $\lambda$  543 to  $\lambda$  519, the fluorescence being also much diminished. I also found that acetic acid moved the edge of the principal chlorophyll band towards red, not, as is usually stated, towards violet—a change which is often seen in solutions of enterochlorophyll. Thus the darkest part of the band is  $\lambda$  703 to  $\lambda$  665, and then a shading  $\lambda$  665 to  $\lambda$  645.† Ammonia diminishes‡ the red fluorescence. The above results show that there is yet much to be learned about the chlorophyll of plants, and that the saponification process is not quite as harmless towards chlorophyll as recent writers would have us believe. On saponifying and agitating the soap with petroleum-ether a yellow solution was obtained, which gave three bands in the violet half of the spectrum, spectrum 10, Chart II., the third band being only visible by daylight. This third band is not constant; in other similar solutions from grass I failed to see it. The two first bands read:  $\lambda$  490.5 to  $\lambda$  470; 2nd,  $\lambda$  458.5 to  $\lambda$  445 (?). On treating the solid residue from this solution with iodine in iodide of potassium it became green, with nitric acid dirty green, and with sulphuric acid green and slaty-blue. A chloroformic solution of the yellow residue showed three distinct bands, two of which read: 1st,  $\lambda$  501 to  $\lambda$  481; 2nd,  $\lambda$  467 to  $\lambda$  451 (?). A bisulphide of carbon solution showed only two distinct bands: 1st,  $\lambda$  521.5 to  $\lambda$  498.5; 2nd,  $\lambda$  488 to  $\lambda$  470.

An attempt to obtain the needle-shaped crystals of chlorophyll yellow only partially succeeded.

\* It is a remarkable fact that the decomposability of vegetable chlorophyll differs very much in different cases. I often failed to bring about this change with caustic soda in similar solutions, which also causes precipitation in such solutions. Cf. DRAGENDORFF'S "Plant Analysis," 2nd Eng. ed., 1884, p. 114, note; here it is shown that possibly the chlorophyll may exist in different states of combination in plants.

† Possibly two bands may have been formed; note the above readings; this appearance also varies very much in different cases. I frequently noticed a narrow dark band placed over a lighter one.

‡ The diminution of the fluorescence by alkalies appears to be due to precipitation.

On then agitating the soap with ether alone, this took up some colouring matter, becoming green and showing a fine red fluorescence. The first alcohol-ether extract did not take up much "chlorophyll green," but the second was a fine green colour with a red fluorescence; this would teach that the apparent loss of fluorescence on treating an alcohol solution of chlorophyll with caustic soda is merely due to precipitation, otherwise the persistence of fluorescence after saponifying cannot be accounted for. The bands of this solution now, however, were totally different in position from those of the original solution, as was always found to be the case; thus they read: 1st,  $\lambda$  649 to  $\lambda$  627; 2nd,  $\lambda$  600 to  $\lambda$  582 (?); 3rd,  $\lambda$  540 to  $\lambda$  524.5, they are shown in spectrum 11, Chart II. I would also call attention to spectrum 12, Chart II., which is that of a shallow depth of this solution. The band far over in violet is not a lipochrome band, for if it were the other bands should be present; it read from  $\lambda$  468 to  $\lambda$  451. (Dr. SORBY\* shows a similar band in the spectrum of blue chlorophyll.) Its presence in similar solutions is, however, not constant.

The solution of saponified chlorophyll, from which the petroleum-ether and the ether had removed the above pigments, showed also a changed spectrum, its bands now reading: 1st,  $\lambda$  649 to  $\lambda$  620.5; 2nd,  $\lambda$  602.5 to  $\lambda$  582.

To see the exact change which had taken place by saponification an ether-alcohol solution was evaporated down and the residue dissolved in absolute alcohol; a green solution was thus obtained showing spectrum 13, Chart II., of which the bands read: 1st,  $\lambda$  669 to  $\lambda$  649; 2nd,  $\lambda$  640 to  $\lambda$  627; 3rd,  $\lambda$  613 to  $\lambda$  593; 4th, uncertain; and on comparing these measurements with those of the bands of the first absolute alcohol solution a great discrepancy is apparent. Moreover, the results of saponifying grass chlorophyll do not exactly coincide with those obtained by saponifying that of *Primula*, but even in the case of grass the results are not by any means constant. Whether this is due to the fact that chlorophyll varies in composition according to season remains to be proved, but the following observations show that such discrepancies may occur.

A solution obtained by digesting grass in ether, to which rectified spirit had been added, gave a series of bands agreeing closely with those seen in the above absolute alcohol solution. They read: 1st,  $\lambda$  679.5 to  $\lambda$  640; 2nd,  $\lambda$  629 to  $\lambda$  598; 3rd,  $\lambda$  591 to  $\lambda$  566; and 4th (about),  $\lambda$  548.5 to  $\lambda$  532. In a thin layer: 5th,  $\lambda$  486.5 to  $\lambda$  467; and 6th,  $\lambda$  451 to  $\lambda$  438. On saponifying and extracting as described above with petroleum-ether, the resulting yellow solution showed only two, not three, bands: the first,  $\lambda$  490.5 to  $\lambda$  472; and 2nd,  $\lambda$  458.5 to  $\lambda$  445 (examined by daylight). The ether extract showed only one feeble band in red, but the ether and alcohol extract gave spectrum 14, Chart II., the bands reading: 1st,  $\lambda$  669 to  $\lambda$  654; 2nd,  $\lambda$  640 to  $\lambda$  627; 4th,  $\lambda$  543 to  $\lambda$  532; and 5th,  $\lambda$  511.5 to  $\lambda$  492.5. The mother liquid gave practically the same bands. This discrepancy is not of great importance, as spectra 13 and 14 really belong to the same pigment, but spectra

\* Proc. Roy. Soc., vol. 21 (1873), pp. 442 *et seq.* Compare SACHSSE, "Die Chemie und Physiologie der Farbstoffe," &c., S. 24, &c.

14 and 11 do not agree (spectrum 14 represents probably a pigment more changed than spectrum 11).

The above experiments are sufficient to show that the chlorophyll of plants is altered by saponification, but I have now to show that it is not altered in the same manner nor to the same extent as *Spongilla* chlorophyll and enterochlorophyll.\* At times I found the same *splitting up* of the dominant band by caustic soda, but the bands did not give the same readings as in a similar solution of *Spongilla* chlorophyll.

Thus taking an alcohol solution of grass chlorophyll, whose bands read as follows: 1st,  $\lambda$  683.5 to  $\lambda$  640; 2nd,  $\lambda$  627 to  $\lambda$  600; 3rd,  $\lambda$  593 to  $\lambda$  566; 4th,  $\lambda$  551 to  $\lambda$  535; and saponifying as before, I found that the ether-alcohol extract gave a double band in red, the first one very narrow and faint, the second darker, and a band in violet *not* due to the yellow chlorophyll constituent. These read (approximately): 1st,  $\lambda$  669 to  $\lambda$  660; 2nd,  $\lambda$  649 to  $\lambda$  631; and the one in violet  $\lambda$  460 to  $\lambda$  443.5 (?); in a deeper layer, however, other bands were seen, and one broad band in red covered the space occupied by the first two seen in a thin layer, the whole series reading: 1st,  $\lambda$  672 to  $\lambda$  623; 2nd,  $\lambda$  609 to  $\lambda$  580.5; 3rd,  $\lambda$  540 to  $\lambda$  527; 4th, (about)  $\lambda$  511.5 to 494.5.

The ether solution also gave the double band in red, while only one could be seen in the mother solution (soap-lees), namely, the second of the two bands in red. †

Now in this case I used only enough caustic soda to ensure the saponification of the fat, so that the splitting up of the band in red was not due to an excess of alkali.

The grass was obtained in spring, and the leaves were young, which probably accounts for the results obtained.

*Saponification of the Chlorophyll of Spongilla lacustris.*—*Spongilla* may be taken as a typical example of an animal which builds up chlorophyll. The observations of Prof. RAY LANKESTER ‡ and SORBY § have shown this beyond doubt; hence it is most suitable for deciding the question which I had to answer. Does animal chlorophyll contain the same green and yellow constituents as vegetable chlorophyll, and is it similarly affected by saponification?

I have been able to examine the chlorophyll of *Spongilla* in great abundance, as I had a good deal of material upon which to work. The spectrum yielded by the living sponge is shown in spectrum 2, Chart I. If portions of this sponge are digested for some time with ether containing alcohol, and the extraction repeated as long as the solution becomes green, a fine green solution is obtained, showing a blood-red fluores-

\* Saponification does not always affect the dominant band in red to the same extent. Professor FOSTER, in looking over some of my preparations, noticed that in one case the centre of the bands, before and after saponifying, was the same; hence the decomposability of chlorophyll differs in different cases. See above, which was written before this foot-note.

† The petroleum-ether extract of the soap from this chlorophyll showed only *two* bands when examined by sunlight, their positions corresponding to those already given.

‡ Quart. Journ. Micro. Soc., *loc. cit.*

§ *Ibid.*, vol. 15, N.S., p. 47.

cence, and in a deep layer giving spectrum 15, Chart II., of which the bands read: 1st,  $\lambda$  678 to  $\lambda$  640; 2nd,  $\lambda$  627 to  $\lambda$  600; 3rd,  $\lambda$  589 to  $\lambda$  566; 4th,  $\lambda$  548.5 to  $\lambda$  529.5; which are practically the same as the bands of a similar solution of plant chlorophyll. On evaporating such a solution, and dissolving the residue in absolute alcohol, a similar spectrum is obtained, of which the bands read: 1st,  $\lambda$  678 to  $\lambda$  647, and a shading to  $\lambda$  640; 2nd,  $\lambda$  625 to  $\lambda$  598; 3rd,  $\lambda$  591 to  $\lambda$  569; 4th,  $\lambda$  548.5 to  $\lambda$  532; and also a lipochrome band beginning at  $\lambda$  494.5 and extending to  $\lambda$  468, also probably a second which could not be measured.

That this pigment should be changed by saponification appeared likely, *à priori*, from the action of caustic soda on an alcoholic solution, which was not the same as in the case of plant chlorophyll. On adding this reagent two bands appeared in the red at first of equal intensity, the first band being the original chlorophyll band, the other when it first appears being much less shaded; the latter then gets darker, and all the bands finally appear as shown in spectrum 16, Chart II. The action of nitric acid is similar to its action on enterochlorophyll and plant chlorophyll, the solution becoming bluish eventually, and giving these bands: 1st,  $\lambda$  669\* to  $\lambda$  638; 2nd,  $\lambda$  613 to  $\lambda$  591; 3rd,  $\lambda$  577.5 to  $\lambda$  558.5; 4th,  $\lambda$  543 to  $\lambda$  521.5, and 5th, about  $\lambda$  505 to  $\lambda$  484.5. The most noticeable change with acetic acid was the marked intensification of the 4th band from the red, but it is not correct to say that a *new* band appeared with acetic acid, *it had been already there and became intensified*.

An absolute alcohol solution of this chlorophyll was saponified by HANSEN'S method. The petroleum-ether solution (after saponification) was yellow and gave two bands in the violet half of the spectrum, but also two bands in the red in a deep layer. Spectrum 17, Chart II., is the result of combining a deep and shallow layer. The presence of the bands in red may be due to the saponifying not having been thoroughly carried out.† The other bands read: 1st,  $\lambda$  490.5 to  $\lambda$  468; 2nd,  $\lambda$  458.5 to  $\lambda$  445. *In the second petroleum-ether extract the green constituent was also present.* The yellow constituent could not be made to crystallize distinctly. It became in the solid state greenish with iodine in iodide of potassium, a well-marked blue and blue-green with sulphuric acid (also a violet and brown tint appeared for a short time), and green with nitric acid. Hence it gave the same reactions as the yellow constituent of plant chlorophyll.

The ether extraction removed nearly all the green colouring matter from the soap; it was green and did not show a very distinct fluorescence. Its spectrum is shown in spectrum 18, Chart II., and its bands read: 1st,  $\lambda$  669 to  $\lambda$  658.5; 2nd,  $\lambda$  649 to  $\lambda$  636; 3rd,  $\lambda$  613 to  $\lambda$  589; 4th,  $\lambda$  576 to  $\lambda$  560; 5th,  $\lambda$  545.5 to  $\lambda$  529.5; and 6th, about  $\lambda$  513 to  $\lambda$  496.5 (?). After this extraction the soap-lees was, as usual, agitated with ether containing alcohol, but, contrary to what I found in the case of

\* The shifting of the band in red not being as well marked as in other cases.

† Although in this and other cases I followed HANSEN'S directions exactly.

plant chlorophyll, the latter solution was almost colourless, the previous ether extraction having taken up the green colouring matter. The "chlorophyll green" was obtained from the ether extract on evaporation in the form of spherical crystals giving a black cross with crossed NICOLS. The mother liquid from which the above extracts had been removed also showed a double band in red and a feeble shading before D, so that it contained the same pigment as that present in the ether extract. Hence it is quite evident that *Spongilla* contains a colouring matter which in alcoholic solution gives the same spectrum as vegetable chlorophyll; it further resembles the latter in being composed of a green and a yellow constituent, but its behaviour with caustic soda in the cold, and on saponification with it, shows that it is decomposed into a body whose spectrum is not the same as that of a solution of vegetable chlorophyll similarly treated. The green constituent, and probably the yellow, are however crystallizable in the same forms as the constituents of vegetable chlorophyll.

*Saponification of Enterochlorophyll.\**—To obtain a sufficient supply of enterochlorophyll for saponification from the *oyster*, twenty "livers" were removed and repeatedly extracted with absolute alcohol as long as they gave up anything to it. The united extracts furnished an olive-yellow solution with a blood-red fluorescence, the solution giving almost the same bands as a similar solution of leaf green as regards the red half of the spectrum, but wanting that after D. Thus such a solution gives spectrum 1, Chart III. and 2, III. in a thinner layer. The bands read: 1st,  $\lambda$  672 to  $\lambda$  654, shading up to  $\lambda$  640; 2nd,  $\lambda$  620.5 to  $\lambda$  595; 3rd,  $\lambda$  545.5 to  $\lambda$  532; the 4th band about  $\lambda$  501 to  $\lambda$  475. On saponifying and shaking with petroleum-ether the green constituent—or, at least, what answers to it in this case—was taken up with the yellow one, and the solution gave two bands in red, as shown in spectrum 3, Chart III.; and in a thin layer one band appeared, as shown in spectrum 4, Chart III., in the blue and green from about  $\lambda$  505 to  $\lambda$  475. In the hope of separating the green from the yellow constituent this solution was evaporated down and again extracted with petroleum ether, but again the band in red was seen. On filtering a petroleum-ether solution an orange stain was left on the paper. Hence the lipochrome present in the enterochlorophyll (in this case) of *Ostræa* differs from that of *Spongilla* and plant chlorophyll in giving only one band. *It resembles KÜHNE'S rhodophan or xanthophan, while in Spongilla and in plants the lipochrome is more like chlorophan.†*

On extracting the soap with ether this became a yellow-green colour, and showed a faint band in red and abrupt absorption of the violent end of the spectrum.

\* It seems highly probable that the individual differences found by me on saponifying enterochlorophyll are due simply to the fact that it is present in various stages of formation, presenting a parallel in this respect to plant chlorophyll.

† According to KÜHNE, *loc. cit.*, chlorophan gives two bands, rhodophan, a very broad dark shading, absorbing the spectrum from D onwards towards violet, and recalling to mind tetronerythrin, *which I am inclined to think it is*, and xanthophan one band.

The alcohol-ether extract of the soap was yellowish-green and did not show a marked fluorescence; it showed a band in red and another evidently due to the presence of a lipochrome. On evaporating it down and extracting the residue with absolute alcohol a solution was obtained, giving spectrum 5, Chart III., the band in red reading  $\lambda$  675 to  $\lambda$  657.

The mother liquid also gave two bands in red, spectrum 6, Chart III., and the other faint bands shown in that spectrum. The first read  $\lambda$  669 to  $\lambda$  654, and second  $\lambda$  638 to  $\lambda$  623.

The residue from a petroleum-ether extract did not give a distinct colour change with iodine in iodide of potassium, it became green with nitric acid, and a less distinct green with sulphuric acid. In other cases the greenish colour with iodine was better marked.

The occurrence of the one-banded lipochrome in the enterochlorophyll of *Ostræa* is the rule, as other experiments taught, and it is very difficult to separate it from the green constituent; still, although a complete separation of the yellow from the green constituent cannot be easily accomplished by saponification, it is quite evident that the enterochlorophyll of *Ostræa* is composed of these constituents, and bears a most remarkable resemblance to plant chlorophyll and to that of *Spongilla*, differing slightly in the fact that it is more decomposed by saponifying than either and in a different manner.\*

I saponified an alcohol extract of the liver of *Helix pomatia* by the same method. Just as in the case of *Ostræa* and *Spongilla* the green constituent went into the petroleum-ether, giving a band in red from  $\lambda$  672 to  $\lambda$  657, also a lipochrome band from  $\lambda$  503 to  $\lambda$  482.5, and the other solutions only contained decomposition-products of the green constituent mixed with the yellow. This enterochlorophyll was much changed by the saponification, and, like that of *Ostræa*, contained a one-banded lipochrome.

The remaining experiments on the saponification of enterochlorophyll were carried out on that of starfishes and *Mytilus*, and owing to greater abundance of material gave more satisfactory results. Taking an absolute alcohol extract, giving the spectrum described under specimens (4) and (5), *supra*, and saponifying it, and then extracting the soap with various solvents, I found that the petroleum-ether only gave *one* lipochrome band between green and blue, and no bands in red. The ether solution, however, showed perhaps one band  $\lambda$  490.5 to  $\lambda$  470,† and no band in red. In chloroform the yellow residue—left on evaporating the ether—formed a deep yellow solution showing only *one* band,  $\lambda$  505 to  $\lambda$  481, and in deeper layer general absorption of the violet and blue, thus resembling KÜHNE'S rhodophan or xanthophan. On

\* Although there was only one lipochrome band in the above extracts, yet in some cases the fatty matter coloured by the chlorophyll, which separates out on evaporating an alcohol solution, gave two bands belonging to the lipochrome.

† Perhaps another from about  $\lambda$  456.5 to  $\lambda$  443.5.

evaporating the chloroform an orange-coloured residue was left, and on touching this with a solution of iodine in iodide of potassium it became perhaps redder, with nitric acid bluish and then green, with sulphuric acid a blue, blue-green, and in parts brownish colour.

The ether-alcohol solution was yellowish, showing a band in red in a deep layer, and absorption of the blue and violet parts of the spectrum, and now all the other bands of the chlorophyll spectrum had disappeared, as shown in spectrum 7, Chart III. Hence the enterochlorophyll had become changed by saponifying; it contained a yellow and a green constituent, the former a one-banded lipochrome as in the case of *Ostræa*.

A solution (alcohol), giving the spectrum described under (7), from the radial cœca of several specimens of *Uraster* was saponified as before. The petroleum-ether extract of the soap showed only *one* band, spectrum 8, Chart III., from  $\lambda$  488 to  $\lambda$  475, and in a chloroform extract of the residue from  $\lambda$  516 to  $\lambda$  484.5. The ether solution also showed the same band from  $\lambda$  494.5 to  $\lambda$  475, and no other.

The ether-alcohol solution was yellowish, and showed one band in red and strong absorption of the blue and violet (and one lipochrome band\*).

The mother liquid (after removal of these extracts) gave a double band in red, the first  $\lambda$  669 to  $\lambda$  651.5, and second  $\lambda$  636 to  $\lambda$  623, and as the original solution showed only *one* band in red, it is quite evident that the enterochlorophyll had become changed by saponifying.

A solution (alcoholic) from the cœca of (13) and (14) was saponified as before. The yellow petroleum-ether extract gave *two* lipochrome bands, the first from  $\lambda$  498.5 to  $\lambda$  473.5, and the second  $\lambda$  458.5 to  $\lambda$  445, and no band in red.†

The ether solution of the soap was greenish-yellow, and left the soap green; it also contained the lipochrome constituent. The alcohol-ether extract was greenish, and on concentration showed a band in red, spectrum 9, Chart III., and as before only one band in red is seen, showing how the green constituent had been altered.

The mother liquid (soap-lees) gave two bands in red as before, the first  $\lambda$  665 to  $\lambda$  649, and second  $\lambda$  636 to  $\lambda$  623.

On evaporating a petroleum-ether solution distinct yellow needles, such as HANSEN describes, were obtained (see Plate 10, fig. 7), and the residue also gave a slate-blue and green with sulphuric acid, a green with nitric acid, and it became slightly reddish with iodine in iodide of potassium. The alcohol-ether solution also on evaporation left crystals, some of which occurred as minute round green granules, and larger spherical crystals showing a fine black cross with crossed NICOLS. I have also sketched these in the drawing (Plate 10, fig. 8), they are evidently the same as those obtained by HANSEN from vegetable chlorophyll. I also saponified the enterochlorophyll of

\* Even the band in red is only seen in a deep layer. The lipochrome band is not shown in the map.

† It is worthy of notice that complete separation is accomplished in this case, when there is a double lipochrome band.



*Mytilus edulis*, using for the purpose the alcohol extracts of twenty-eight "livers." The bands of the solutions so obtained were not affected by treatment with caustic soda\* in the cold, nor on boiling with it. Their readings were as follows: 1st,  $\lambda$  683.5 to  $\lambda$  649; 2nd,  $\lambda$  623 to  $\lambda$  596.5; 3rd,  $\lambda$  547 to  $\lambda$  535; the bands nearer the violet, of which there were two, could not be measured.

On saponifying as before, I found that the separation of the green from the yellow constituent could not be completely brought about, the petroleum-ether giving a band in red from  $\lambda$  675 to  $\lambda$  657. Two lipochrome bands were also barely visible, which could not be measured; moreover, the first ether extract removed all the colouring matter from the soap, as in the case of *Spongilla*. This solution was a dull yellow-green colour; and its bands were but little different from those of the original chlorophyll solution; they read: 1st,  $\lambda$  683.5 to  $\lambda$  649; 2nd,  $\lambda$  640 to  $\lambda$  629; 3rd,  $\lambda$  620.5 to  $\lambda$  596.5; 4th,  $\lambda$  543 to  $\lambda$  532, and two others belonging to the yellow constituent too faint to be measured. The mother liquid (soap-lees) gave a band in red,  $\lambda$  675 to  $\lambda$  651.5, and another,  $\lambda$  640 to  $\lambda$  625. It will be noticed that the most important change was the appearance of the  $\lambda$  640 to  $\lambda$  629 band—which was very faint—in the ether solution.

On attempting to extract the yellow constituent from the green by evaporating the ether solution, and extracting with petroleum-ether, I failed, as both constituents were dissolved by it. This result was so constant that I think it may be accepted as a peculiarity of enterochlorophyll.

The "chlorophyll-yellow" in this case gave, in the solid state, a yellow colour with iodine in iodide of potassium, a green and blue with sulphuric acid, and a transient blue with nitric acid. Hence it is a lipochrome.

The enterochlorophyll of *Mytilus*, obtained by evaporating an alcohol solution, is insoluble in water, soluble in alcohol, ether, chloroform, bisulphide of carbon, benzol, petroleum-ether, and in *olive oil*. Its solubility in the last medium explains how it is held in solution in oil drops in many cases.

It is coloured, in the solid state, a fine blue-green and green with strong sulphuric acid, which does not destroy the bands, as they are seen in the resulting green solution after filtering through asbestos, but not in the same position as before. They are the same bands as those seen in a solution of enterochlorophyll after treatment with nitric acid. Nitric acid colours the solid pigment blue-green, which eventually disappears, and is replaced by a red colour. Hence by saponifying it is clear that enterochlorophyll differs from plant chlorophyll with regard (1) to the difficulty of separating its green from its yellow constituent; (2) to the position of the bands in a petroleum-ether extract of the yellow constituent; (3) in the fact that while this yellow constituent gives a similar colour reaction with nitric and sulphuric acids, it gives a different colour with iodine in iodide of potassium in most cases.

\* A similar effect was noticed in the case of *Ostræa*, the solution becoming more orange after boiling with NaHO.

*Absence of Starch and Cellulose, and Morphology of Enterochlorophyll.*

In none of the above cases was chlorofucin found; and I have shown in my paper "On the Chromatology of Actiniæ" it ought to be present if symbiotic algæ are present, and in animals living on marine algæ it ought to be present if the "liver" chlorophyll be due to intracellular digestion of food chlorophyll. It was also necessary to see whether starch and cellulose were present, and to attempt to study the morphology of the pigment. The results strikingly confirm the spectroscopic evidence and support the idea that enterochlorophyll is built up by the animal.

I found the best results were obtained by freezing the "livers" and examining the frozen sections. For this purpose the small ether-freezing microtome of CATHCART answers admirably. The freezing method has to be adopted since if the tissues are hardened in alcohol the chlorophyll is removed; and alcohol would be required because other hardening agents alter the pigment. The relationship of the enterochlorophyll to the gland cells is, however, difficult to make out in frozen sections, but I think it is found in all cases within the epithelium cells lining the "liver" tubes.

I made various sections of invertebrate "livers" obtained from animals feeding and fasting, but never obtained a trace of starch or cellulose with iodine in iodide of potassium, SCHULZE'S fluid, or with iodine and sulphuric acid. These experiments were made on the "livers" of *Helix aspersa*, *Anodonta cygnea*, *Patella vulgata*, *Ostræa edulis*, *Mytilus edulis*, *Astacus fluviatilis*, the cœca of starfishes, &c. The precautions recommended by GEDDES:\* of previously digesting the tissues in alcohol, and in caustic potash, and neutralising with acetic acid, having been adopted in each case. Now, if food-products were present we ought to get starch or at least cellulose; and if symbiotic algæ were present the product of their activity, starch, and the cellulose wall of the alga itself, should be present; but they are not; hence these tests alone furnish a strong argument in favour of the purely animal origin of enterochlorophyll.

A section of the "liver" of *Ostræa edulis* under a low power such as a 1-inch or 2-inch shows tubes cut in different planes, oblique, transverse and longitudinal, and the pigment appears to be distributed mainly along the periphery† of the tubes, the centre of each tube in cross-section contrasting strongly with the periphery, the former being colourless, the latter deeply coloured. The pigment appeared in the form of fine granules, some yellow, some brown, and others of intermediate tints. The granules are not all round, some being angular. In teased-out specimens the pigment seemed disposed in bands along the long diameter of each tube, and in cross-sections of the tubes a section of four such bands forming a kind of cross can be seen. Besides

\* "Nature and Functions of the 'Yellow Cells' of Radiolarians and Cœlenterates." Proc. Roy. Soc., Edinburgh, vol. 11 (1882), p. 377.

† Not the extreme periphery is meant, but that portion between the centre and the wall of the tube, i.e. the secreting part. This is only seen in cross-sections. In teased-out specimens almost the whole tube appears coloured.

occurring in granules, singly and in aggregates, some of which are round, the pigment is also dissolved in oil globules and may be present in the diffused condition. No unicellular algæ can be seen.\* (See Plate 10, fig. 2.)

In *Uraster* the smaller cœcal tubes are seen packed with oil globules of a yellow colour, some of which are very large, and this appearance is so constant that I think one would be correct in assuming that here the enterochlorophyll is mainly dissolved in oil.

In *Patella*, sections show the tubes of the "liver" cut in various planes, the pigment occurring mostly in very fine granules of a yellow colour, and as in *Ostræa* it is distributed along the periphery† of the tubes, sometimes in the diffused state. Some yellow oil globules are also seen. The granular pigment appears to be confined to the epithelium cells lining the tubes.

In *Littorina*, the "liver" itself has the same kind of structure, but so little connective tissue holding the tubes together, that the sections readily fall to pieces. The enterochlorophyll occurs in very small granules, of an orange colour, in small round cells, and in oil globules, some of which have a greenish tint. On the whole, it appears confined to the periphery of the tubes. (See Plate 10, fig. 1.)

In *Purpura lapillus*, the amount of pigment is very small, and of a pale-yellow colour; the epithelium lining the periphery of the tubes seems to contain the pigment mostly in a diffused condition.

In *Limnæus stagnalis* the enterochlorophyll occurs in round and angular minute granules, also in oil globules, some of which are yellow, others a more pronounced green. Coloured cells of an epithelial type, some of which enclose granules, and others diffusely stained with the colouring matter, are also found. These latter are doubtless the epithelial lining cells of the "liver" tubes. (Plate 10, fig. 4.)

In *Helix pomatia* the enterochlorophyll is found in granules and oil globules; also in peculiar cells probably of an epithelial type, some of which contain granular pigment, and others are diffusely stained of a green and yellow-green colour. The latter probably belonging to the lining of the cell spaces of the "liver."‡

In *Helix aspersa*§ the appearances are much the same, but the large round brown bodies are very peculiar (see Plate 10, fig. 5). It would seem that the latter are coloured by the hæmatin constituent of the "bile"; the enterochlorophyll occurs in the periphery of the cell spaces and in their epithelial lining. The large brown cells measured from  $13\ \mu$  in diameter to  $15\ \mu$ , other yellowish-brown cells from about  $8\ \mu$  to  $4\ \mu$ , down to  $1\frac{1}{2}\ \mu$ . There are green oil globules  $8\frac{1}{2}\ \mu$  in diameter, some  $5\frac{1}{2}\ \mu$  of a yellow colour, others are coloured by a mixture of yellow, green, and brown, measuring  $4\ \mu$  and some  $2\ \mu$ . The brown bodies do not lose their colour when extracted with alcohol, while the

\* In *Mytilus edulis* the pigment occurs very richly in round brownish-yellow granules, also in small oil globules.

† *I.e.*, in the secreting cells.

‡ In the "bile" certain spherical crystalline bodies are seen which I hope to examine shortly.

§ I am rather inclined to the belief that in *Helix* and *Limax* after feeding some food chlorophyll is taken up into the "liver."

yellow and green constituents do. In none could I find a trace of starch or cellulose. Here also the enterochlorophyll occurs in granules. In hibernating snails there are an immense number of coloured oil globules in the "liver," as well as granular pigment.

In *Limax* there are large green cells stained uniformly (of a pale-green colour), these cells measure from  $17\ \mu$  to  $13\ \mu$ , down to  $10\ \mu$ , some being larger and some smaller, which are probably the "liver" cells. There are not so many coloured oil globules, but abundance of pigment in minute granules. The latter can be seen enclosed in the cells (secreting epithelium) lining the cell spaces.

In none of the cells could starch or cellulose be detected, nor were any unicellular algæ present.

On the whole, then, it would appear that enterochlorophyll occurs dissolved in oil globules, also in the granular form, and sometimes dissolved in the protoplasm of the secreting cells of the "liver." *In no case can unicellular algæ be found.*

#### *Remarks.*

This investigation, although not as complete as I had hoped to make it, yet, I believe, decides the question as to the animal origin of enterochlorophyll, and also shows that in *Spongilla* a true animal chlorophyll is built up.

What I call chlorophyll in this paper is the mixture of colouring matters which can be extracted from the green leaves of land plants by means of alcohol, or alcohol and ether, and the spectrum consists of the six bands described, whose wave-lengths are given above. These bands, except one, are the bands seen in a living leaf, concerning which SACHS\* remarks, after adducing the evidence of GERLAND and RAUWENHOFF,† "It is not easy to understand how certain physicists can maintain the contrary." VOGEL is however cited by DRAGENDORFF‡ in support of the statement§ that "in examining a fresh leaf only the most marked line between B and C is seen." But his statement is sufficiently refuted by the drawings of the spectra which accompany this paper, compare, *e.g.*, spectrum 1, Chart I., with spectra 1, 2, 7, 8, of Chart II. The colouring matters present in an alcohol solution are (in those cases where I have examined such a solution), at least two, a green and a yellow. The former giving four bands, the latter generally two. No doubt can exist as to the truth of this statement in the mind of any one who carefully studies the literature|| of chlorophyll and then makes the experiments recorded in this paper.

\* 'Botany,' 2nd Eng. ed., p. 758.

† 'Archives néerlandaises,' vol. 6 (1871), p. 604; also 'POGGENDORFF's Annalen,' band 143 (1871), p. 585.

‡ 'Plant Analysis,' Eng. ed., 1884, p. 19.

§ 'Berichte der deutschen Chemischen Gesellschaft,' band 11 (1878), pp. 623 and 1367.

|| See also ASKENASY, *Bot. Zeit.*, 1867, p. 225; KROMAYER und LUDWIG, *Archiv d. Pharm.*, band 156 (1861), p. 164; AÉ, *do.*, band 192 (1870), p. 163; WIESNER, *Chem. Centralblatt*, 1874, p. 353; FILHOL, 'Comptes Rendus,' tome 61 (1865), p. 371; HARTSEN, *Annalen der Phys.*, band 146 (1872), p. 158; SACHSSE, *Chem. Centralblatt*, 1878, p. 121, also 'Die Chemie und Physiologie der Farbstoffe,' &c., 1877.

Professor STOKES,\* FREMY,† SORBY,‡ KRAUS,§ and latterly HANSEN|| and others may all be quoted as authorities in support of the view that plant chlorophyll is composed of more than one constituent. PRINGSHEIM maintains that FREMY's view : namely that the constituents exist side by side in chlorophyll, is erroneous, and holds that they are only products of decomposition.¶ Which of these views is right it is impossible to say with our present knowledge. Still we know that chlorophyll consists of two or more constituents united together in some way.

*Spongilla* contains a similar chlorophyll, and this is built up by the animal itself, a comparison of the measurements in this paper, and of spectra 1, 7, and 15, Chart II., proves this beyond all doubt. In *Cantharides*\*\* an animal chlorophyll also exists, but I have not saponified it; and in all the chlorophyll-containing animals enumerated in SACHS's 'Botany,' by Professor LANKESTER, with one or two exceptions, e.g., *Idotea* and *Bonellia*, I have no doubt a similar chlorophyll exists. In *Anthea cereus* it is due to symbiotic algæ.

A comparison of the bands of enterochlorophyll as they are shown in Chart I., with those of vegetable chlorophyll in Chart II., shows a difference with regard to the bands in the violet half of the spectrum, and on running the eye down along the F line one sees that the bands belonging to the yellow constituent are joined in some cases, in others placed close together, and in some cases only one band is present, which replaces the other two. After saponifying the same result is arrived at. The yellow constituent of the enterochlorophyll, while generally giving in the solid state the colour reactions of SCHWALBE and CAPRANICA, shows different bands to those of vegetable "chlorophyll yellow" (=xanthophyll).

I do not think that HANSEN's "chlorophyll green" is a body which exists *as such* in chlorophyll, because the examination of its solutions after saponification reveals a wide difference in the position of the bands of the "chlorophyll green," compared with those belonging to the red half of the spectrum in the original chlorophyll solution. What its relationship may be to the greenish-white body crystallizing in four-sided plates, and appearing red by transmitted light, which HOPPE-SEYLER†† isolated from grass, is doubtful, or what to the other substance isolated by him crystallizing in needles, dark green by reflected and brown by transmitted light, and named *chloro-*

\* Proc. Roy. Soc., vol. 13 (1863-4), p. 144. BURNETT, "Lectures on Light," 2nd course, pp. 8 and 9.

† 'Comptes Rendus,' tome 50 (1860), p. 405; tome 61 (1865), p. 180. Journ. für prakt. Chem., band 87 (1862), p. 319.

‡ *Loc. cit.*

§ *Loc. cit.*

|| *Loc. cit.*

¶ Chem. Centralblatt, 1880, pp. 299, 316, 331; also KONEAD, 'Flora,' 1872, p. 396.

\*\* See Brit. Assoc. Reports, 1883. I have recently examined again a solution obtained by digesting the *elytrae only* in ether containing alcohol and find that chlorophyll is present.

†† 'Berichte der deutschen Chemischen Gesellschaft,' Jahrg. 12 (1879), p. 1555; Jahrg. 13 (1880), p. 1244.

*phyllan*, is equally doubtful. The former substance is, according to DRAGENDORFF,\* probably the same as BOUGAREL'S† *erythrophyll*. GAUTIER'S‡ crystalline body is, according to HOPPE-SEYLER, a mixture of erythrophyll, chlorophyllan, and wax.

Still HANSEN'S method is useful because it enables one to see if the chlorophyll of animals can be made to yield the same constituents as that of plants, and if the solutions yield the same spectra after saponifying.

It is unnecessary to repeat the account of the results I obtained, but I may say that it appears that vegetable chlorophyll is considerably changed by saponification, so is *Spongilla* chlorophyll, and so is enterochlorophyll. A likeness in all the spectra of the solutions of these chlorophylls is, however, apparent after saponifying, and the *ultimate* constituents crystallize in the same form, so far as I can judge from HANSEN'S description. The mere fact that it is most difficult to separate the saponified constituents of animal chlorophyll from each other, while it is easy enough in the case of vegetable chlorophyll, goes to show that the constitution of the respective chlorophylls differs, and this fact, taken in connexion with the microscopic, spectroscopic, and chemical evidence, seems to me to establish almost beyond doubt that in the case of enterochlorophyll we are dealing with an animal product.

I have not quoted nearly all the papers written on chlorophyll, as this would be a great and unnecessary task. § The following table giving the wave-lengths of the bands in solutions of enterochlorophyll, *Spongilla* chlorophyll, and plant chlorophyll, shows at a glance the agreements and differences between them :--

\* *Loc. cit.*, p. 115.

† Bulletin de la Soc. Chim., tome 27 (1879), p. 442.

‡ *Ibid.*, tome 28 (1879), p. 147.

§ RUSSELL and LAPRAIK (*Journal of Chem. Soc.*, vol. xli., p. 338) describe a splitting up of the dominant band of chlorophyll into two by caustic alkalies, and cite CHAUTARD ('Comptes Rendus,' tome 76., p. 570), in support of this statement. They also state that on heating chlorophyll with solid potash it is completely decomposed, the dominant band disappearing. HANSEN insists on the avoidance of too much caustic alkali, and in all cases I tried to follow his directions as closely as possible. See also paper in *Journal of Chem. Soc.*, by TSCHIRCH, 1884, p. 57; and PRINGSHEIM'S "Researches on Chlorophyll," by Professor B. BALFOUR, *Quart. Journ. Micr. Soc.*, vol. 22, p. 75, &c. In SACHSSE'S treatise, *loc. cit.*, a great number of authorities are quoted.

## COMPARISON of Enterochlorophyll, Chlorophyll of Grass and Spongilla.

Band.	Paludina. Rectified Spirit.	Limnæus. Rectified Spirit.	Trochus Z. Absolute Alcohol.	Trochus C. Rectified Spirit.	Littor'na. Absolute Alcohol.	Patella. Absolute Alcohol.	Grass. Absolute Alcohol.	Spongilla. Absolute Alcohol.
I.	λλ 678 to 656	λλ 678 to 651·5	λλ 672 to 651·5	λλ 678 to 657	λλ 678 to 654	λλ 678 to 654	λλ 679·5 to 636	λλ 678 to 640
II.	620 to 600	618 to 600	618 to 593 (?)	620·5 to 595	623 to 600	620·5 to 598	627 to 600	625 to 598
III.	0 Uncertain	0 "	0 "	0 "	0 "	0 "	593 to 566	591 to 569
IV.	552 539	547 to 537	548·5 to 535	545·5 to 535	548·5 to 537	547 to 536	551 to 532	548·5 to 532
V.	?	519 to 503	?	519 —	496·5 —	521·5 —	486·5 to 467	494·5 468
VI.	?	492·5 473·5	?	— 484·5	— 477 (with NaHO)	— 475	451 438	?

## REMARKS.

On allowing for the difficulty of measuring the edges of feeble bands, the errors of refraction caused by varying temperatures of room and difference of solvent, a tolerably close agreement is apparent with regard to the bands (I.-IV.) in the red half of the spectrum. Those in the violet half differ much, not only between enterochlorophyll and grass chlorophyll, but between the latter and *Spongilla* chlorophyll, which is in agreement with the result of saponifying. The third chlorophyll band is generally missing from the spectrum of enterochlorophyll, but the position of the other bands teaches that it is not a decomposition-product of chlorophyll. Especial stress should be laid on the λλ of band in red, especially on the wave-length of its redward edge; this being the most deeply shaded *is the last to disappear* on dilution.\*

\* Prof. STOKES suggested that by adopting the "fractional" method of separation I might have more easily arrived at the same results. Accordingly this was done, when it was found that the constituents of enterochlorophyll could be *partially* separated from each other. But the behaviour of enterochlorophyll is not the same as that of plant chlorophyll under these conditions, as the green constituent goes into the spirit, and the yellow into the bisulphide, which proves that the pigments are dissolved in a medium differing from that which holds them in solution in the plant.—(July 24, 1886.)

## EXPLANATION OF PLATE 9.

## CHART I.

- Sp. 1. Spectrum of a living leaf of common grass and of the other leaves mentioned in this paper. The fourth band is exaggerated.
- Sp. 2. A bit of *Spongilla* examined in the same way.
- Sp. 3. Spectrum of an *Ophrydium*.
- Sp. 4. Enterochlorophyll of *Paludina vivipera* in rectified spirit.
- Sp. 5. Enterochlorophyll of *Limnæus stagnalis* in absolute alcohol.
- Sp. 6. The same solution treated with nitric acid.
- Sp. 7. Enterochlorophyll of *Trochus ziziphinus* in absolute alcohol.
- Sp. 8. Enterochlorophyll of *Trochus cinerarius* in alcohol.
- Sp. 9. Enterochlorophyll of *Littorina littorea* in absolute alcohol.
- Sp. 10. Spectrum of the "liver" of *Littorina littorea* examined with an achromatic substage condenser and SWAN lamp.
- Sp. 11. Enterochlorophyll of *Patella vulgata* in absolute alcohol.
- Sp. 12. "Bile" of *Patella vulgata* showing enterohæmatin.
- Sp. 13. Diluted alcohol solution of the "liver" of *Helix pomatia*.
- Sp. 14. A second absolute alcohol extract of the same "liver."
- Sp. 15. Absolute alcohol extract of the "liver" of another *Helix pomatia*.
- Sp. 16. Enterochlorophyll of *Solaster papposa* in rectified spirit.
- Sp. 17. Enterochlorophyll of *Uraster rubens* in alcohol.
- Sp. 18. Enterochlorophyll of another specimen of *Uraster rubens* in absolute alcohol, showing the double band in red.
- Sp. 19. The solution giving spectrum 18 was evaporated to dryness, and the residue extracted with petroleum-ether gave this spectrum. (Cf. *Spongilla*-chlorophyll.)\* The second band in red is a shade too broad.

## CHART II.

- Sp. 1. Chlorophyll of *Primula* in absolute alcohol, deep layer.
- Sp. 2. The same, shallow depth, showing the bands of "chlorophyll yellow" at F and before G.
- Sp. 3. Petroleum-ether solution of the "chlorophyll yellow" of *Primula* after saponifying.
- Sp. 4. The mother liquid, after separation of the ether, alcohol ether, and petroleum-ether extracts, showing how the chlorophyll bands have been displaced by the saponification.

\* Some of these spectra represent the result of examining a deep and shallow layer of liquid.



- Sp. 5. The second ether-alcohol extract of the soap from *Primula*; compare with spectrum 1. All the bands are displaced.
- Sp. 6. Alcohol solution of the same "chlorophyll green" (after saponifying) with nitric acid; early stage of reaction.
- Sp. 7. Absolute alcohol solution of chlorophyll of common grass.
- Sp. 8. The same, thin layer of liquid. These bands really agree with those of spectrum 2; (see measurements).
- Sp. 9. Action of caustic soda in the cold on an alcohol solution.
- Sp. 10. Petroleum-ether extract of the soap from this chlorophyll, showing a three-banded "chlorophyll yellow" spectrum (=xanthophyll).
- Sp. 11. Second alcohol-ether extract of the same soap, showing changed position of the bands of "chlorophyll green." (*Cf.* spectrum 7.)
- Sp. 12. Shallow depth of same solution. Note the band between F and G.
- Sp. 13. Alcohol solution of the saponified "chlorophyll green," obtained as described in the paper.
- Sp. 14. Ether-alcohol extract of saponified chlorophyll from common grass in another experiment.
- Sp. 15. Alcohol-ether extract of chlorophyll of *Spongilla* before saponifying.
- Sp. 16. The action of caustic soda in the cold on an alcohol solution of the same.
- Sp. 17. Petroleum-ether extract of the same after saponifying.
- Sp. 18. Ether extract of the same soap, showing complete change in the spectrum caused by saponifying.

## CHART III.

- Sp. 1. An absolute alcohol solution obtained from the "livers" of 20 specimens of *Ostræa edulis*.
- Sp. 2. The same thinner layer showing only one band in the violet half of the spectrum.
- Sp. 3. The above solution after concentration by evaporating was saponified, and the petroleum-ether extract of the soap gave this spectrum, showing that the constituents, both that corresponding to the "chlorophyll green" in other cases and that corresponding to the "chlorophyll yellow," are taken up by this solvent.
- Sp. 4. The spectrum of a thinner layer of the same.
- Sp. 5. Ether-alcohol extract of the same soap evaporated, and the residue dissolved in alcohol.
- Sp. 6. The mother liquid (soap-lees) after extraction with these solvents, showing how the original spectrum has become completely changed.
- Sp. 7. Alcohol-ether extract obtained from the saponified enterochlorophyll of *Uraster rubens*. All the bands, except that in red, have disappeared, and even this is only visible in deep layers.

Sp. 8. Petroleum-ether extract of this saponified enterochlorophyll showing only one band.

Sp. 9. Alcohol-ether extract obtained as described under spectrum 7 in another experiment.

The measurement of bands in the microspectroscope from which all the above spectra were mapped is attended with greater difficulty than in the chemical spectroscopy owing to the shortness of the spectrum; hence some trifling errors of position may creep into the maps, which for that reason are not as reliable as the measurements given in this paper, each of which has been worked out by an interpolation curve from the readings of the photographed scale of the large chemical spectroscope. The remarkable agreement in these measurements—all of which were worked out independently of each other—shows that the wave-length method is the most reliable one, combined with the use of a spectroscope of moderate dispersion.

#### EXPLANATION OF PLATE 10.

In Plate 10, I have endeavoured to show the appearances presented by enterochlorophyll when fresh frozen sections of the "livers" of Invertebrates are examined under a power of about 320 diameters. The drawings do not show the *relative proportions* of the constituent cells, granules, &c., but merely the most typical appearances in each case. To save printing in too many colours, the latter have been made more uniform than they actually appear in the specimens.

Fig. 1. Enterochlorophyll of *Littorina littorea*, principally in granules and dissolved in oil globules.

Fig. 2. Ditto, from "liver" of *Ostræa edulis* in the same form, the liver cells containing the pigment.

Fig. 3. Ditto, from "liver" of *Helix pomatia*, the larger cells containing granules are probably the secreting cells, whose protoplasm is stained with the pigment, and some of which contain larger granules than others.

Fig. 4. Enterochlorophyll of *Limnæus stagnalis*, in granules, oil globules, and secreting cells of "liver," some of which have their protoplasm diffusely stained with the pigment, and contain the latter also often in granules.

Fig. 5. Ditto, from "liver" of *Helix aspersa*, the transition from green to brown and the presence of the large brown spherical bodies is very remarkable. Very few "liver" cells are here shown.

Fig. 6. Ditto, from "liver" of *Limax*. The large epithelial liver cells are uniformly stained with the pigment, besides containing it in the granular form; in other cases oil globules containing the pigment are seen.

Fig. 7. Crystals (which, according to HANSEN, are those of the lipochrome\*) from a petroleum-ether extract of saponified enterochlorophyll of *Uraster rubens* (the outline of lower figure is that of a droplet of liquid).

Fig. 8. Crystals obtained from an alcohol-ether extract of the same soap (belonging, according to HANSEN, to "chlorophyll green").

Fig. 9. The same with crossed NICOL'S, but without selenite, showing the peculiar black cross.

\* It has yet to be proved whether these crystals may be merely coloured by the lipochrome; possibly (?) they belong to one of the fatty acids, *cf. e.g.*, crystals of the so-called margarin.

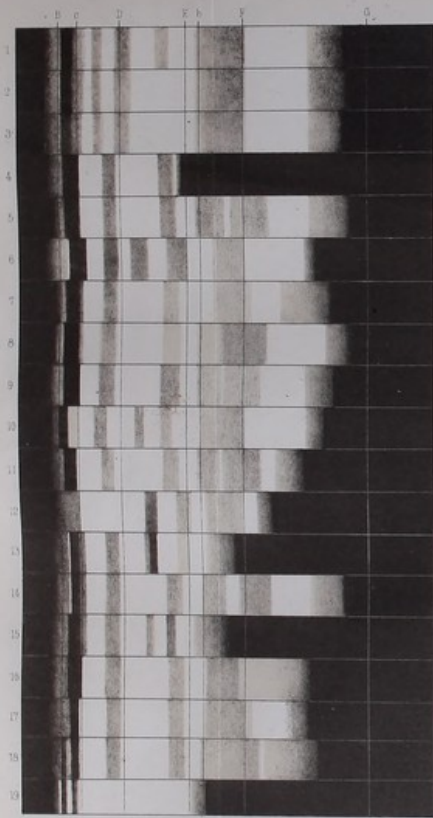


Chart I.

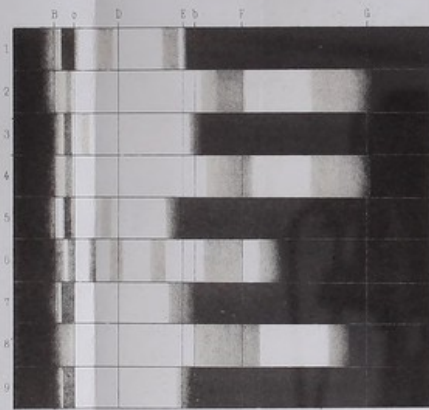


Chart III.

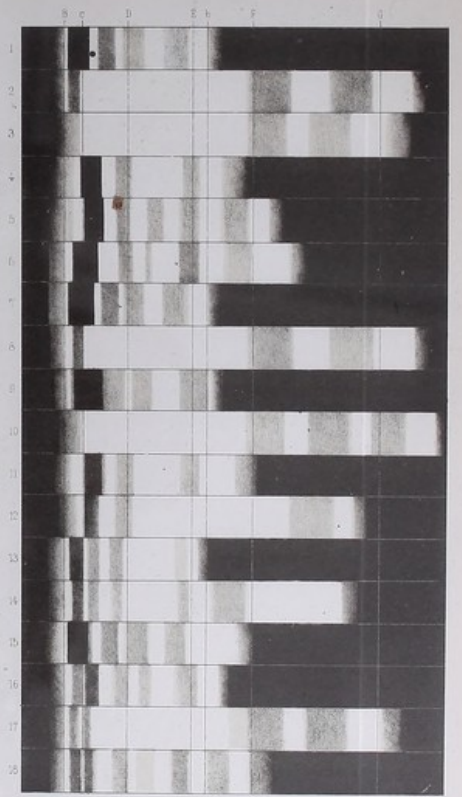


Chart II.



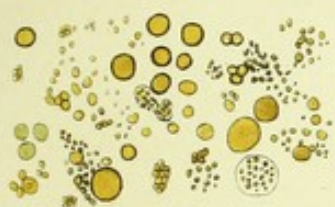


Fig. 1. x320

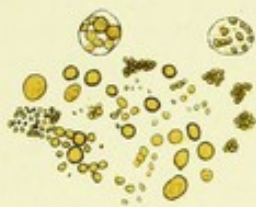


Fig. 2. x320



Fig. 3. x320



Fig. 4. x320

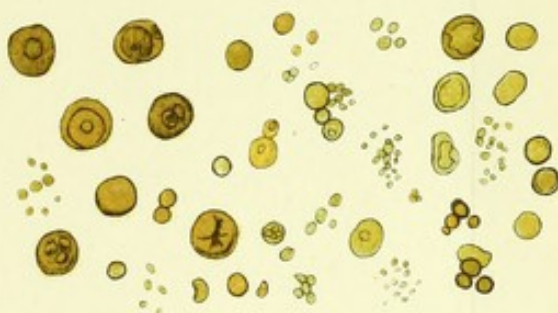


Fig. 5. x320

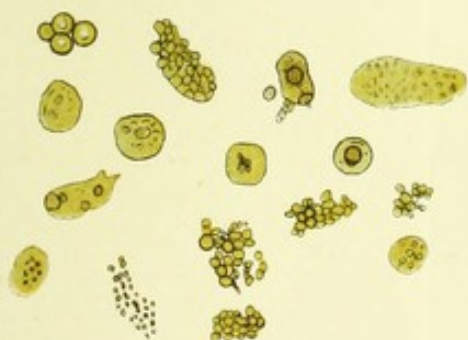


Fig. 6. x320



Fig. 7. x320

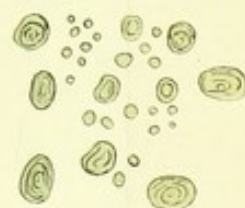
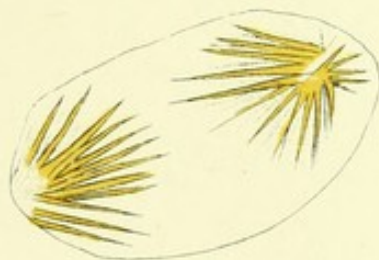


Fig. 8. x320

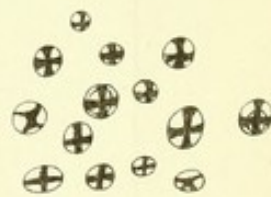


Fig. 9. x320

