Notes on some Animal Colouring Matters examined at the Plymouth Marine Biological Laboratory.

(*Brief Abstract.*)

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The following is a list of the Invertebrates which I had an opportunity of examining. Their pigments were not in all cases thoroughly studied, partly because the Laboratory had been opened but a short time before my arrival, and was not yet fully provided with the necessary chemical apparatus, partly through want of time.

Cœlenterates.

Chrysaora hysocella. *Corynactis viridis.*
Tubularia indivisa.

Echinoderms.

Antedon rosaceus. *Asterina gibbosa.*
Asterias glacialis. *Holothuria nigra.*
Goniaster equestris. *Ocnus brunneus.*
Solaster papposa.

Vermes.

Arenicola piscatorum. *Polynoe.*
Terebella. *Chætopterus insignis.*
Cirratulus. *Nemertes neesii.*
Nereis. *Phyllodoce viridis.*
Pontobdella.

Tunicates.

Styela grossularia. *Ascidia virginea.*
Botryllus violaceus. *Clavellina lepadiformis.*
Botrylloides.

Bryozoans.

Lepralia foliacea

And one Mollusc—*Doris.*

The results arrived at will be published *in extenso* in the Quart. Journ. Micros. Sc., but I may here briefly refer to the most important facts which I came upon.

Does the spectroscope support the supposition that symbiotic Algæ are present in Antedon rosaceus? The answer is decidedly no, as neither chlorophyll nor chlorofucin is present in any of the extracts of this Echinoderm. I found that if the stomach with its contents was removed before the extraction with alcohol, ether, &c., the above result was obtained, but if allowed to remain then chlorophyll (from the food) was present. This decides the question. Krukenberg has figured a chlorophyll band in his map of the alcohol extract of *Antedon* which led me to suppose that I should also find it, but the result was as described. Dr. P. Herbert Carpenter* has come to the same conclusion, by studying the morphology of the supposed algæ and the pyriform oil-cells of Wyville-Thomson, which Vogt and Yung† took to be the amœboid spores of these algæ. Apart from spectroscopic proof the latter were found neither to possess a cellulose wall nor to contain starch, and as they are easily seen to grow out from the surface of the integument, being attached to it by their narrower parts, it is not easy to see how they can have been mistaken for anything else but what they are. The red pigment of *Antedon* I found not to be identical with Moseley's antedonine, and in the complete paper the points of difference are referred to at length.

The ether extract was found to contain a lipochrome‡ probably allied to Kühne's xanthophan.

The red pigment itself easily goes over into glycerin, alcohol, and partially into water. It gives no well-marked bands; its colour is destroyed by acids, and more or less changed by alkalies.

Krukenberg's paper, *Ueber die Farbstoffe von Comatula mediterranea*, will be found in his Vergleichend-physiologische Studien, zweite Reihe, dritte Abth., 1882.

Other Echinoderms.—The fine violet colour of *Asterias glacialis* can be extracted by fresh water, but the solution gives no bands; the colour is diminished by ammonia and by caustic potash, and is not much affected by hydrochloric acid. Alcohol extracts from the integument a lipochrome allied to Kuhne's rhodophan,§ but ether

* *Notes on Echinoderm Morphology*, Quart. Journ. Mic. Sci., Jan., 1887.

† Traité d'Anatomie Comparée Pratique, Livr. 7, 8, pp. 519—572.

‡ The lipochromes (Krukenberg), or fat-pigments, include those formerly known as luteins, also tetronerythrin and the chromophanes of the retina, &c. They give bands in the violet half of the spectrum, and become in the solid state blue or green by H_2SO_4 and HNO_3, and in some cases blue or green with I dissolved by means of KI. The last test often fails in the case of animal lipochromes. All lipochromes are soluble in such solutions as ether, chloroform, bisulphide of carbon, &c.

§ Kühne, Unters. a. d. physiol. Inst. d. Univ. Heidelberg, Bd. i, 1878; Bd. iv, 1882.

fails to extract it. The violet colouring matter could not be extracted by glycerin, and no hæmatoporphyrin could be obtained by extracting the integument with alcohol and sulphuric acid, whereas in *Uraster rubens,* especially in brownish specimens, that pigment is present as I have shown.* Nor was any found in *Goniaster, Solaster, Asterina,* nor in *Holothuria nigra* and *Ocnus brunneus.* The integuments of the first three yield lipochromes, which are described in the full paper. *Holothuria nigra* contains within it, and colouring its ovaries, its blood, its digestive gland, &c., one or more lipochromes. The polian vesicle contains what may be described as a lipochromogen, and in the blue ovaries of *Ocnus brunneus* a similar substance is found which, under the influence of alcohol, ether, &c., as in the case of the beautiful blue pigment of the larval lobster and the "cyano-crystals" of other Crustaceans, becomes changed into a reddish lipochrome. Enterochlorophyll† is present in *Goniaster, Solaster,* and *Asterina,* and in these, as well as in many other Echinoderms, notably in *Holothuria nigra,* there is reason for supposing that its yellow or red lipochrome-constituent is built up in the digestive gland, from whence it is carried to the integument. In the last-mentioned species it is present in the blood. It may be remembered that Dr. Halliburton‡ has detected a lipochrome in the blood of various Crustaceans, in which also it is prepared in the "liver." So that we may consider the digestive gland of these animals not only an organ in which digestive ferments are prepared, but also as discharging a chromatogenic function. In *Holothuria nigra* a yellow pigment can be extracted from the integument by alcohol, which possesses a magnificent emerald-green fluorescence. This has been described by Krukenberg§ as a "Uranidine," and it has also been described by Prof. Jeffrey Bell.‖ To the latter I am indebted for a solution of the colouring matter which I have described in full in the paper referred to.

Cœlenterata.—The discovery of polyperythrin (which I have shown to be identical with hæmatoporphyrin) in many Cœlenterates by Prof. Moseleyl,¶ led me to hope that in the brown pigment of *Chrysaora* I might see a banded spectrum, but I could not see any bands whatever, and my further results have confirmed exactly those of Prof. McKendrick,** who examined this jelly-fish.

In the beautiful little *Corynactis viridis,* when it has a red colour,

* Jl. Physiol., vol. vii, No. 3; see also vol. viii, No. 6.

† See Proc. Roy. Soc., vol. xxxv (1883), p. 370, *et seq.;* also Philos. Trans., Pt. i, 1886.

‡ Jl. Physiol., vol. vi, No. 6.

§ Vergl. physiol. Stud., II Reihe, 3 Abth., 1882, S. 53.

‖ Proc. Zool. Soc., Dec., 1884, p. 563.

¶ Quart. Journ. Mic. Sci., vol. xvii, N.S., 1877.

** Journ. Anat. and Physiol., vol. xv.

I find actiniohæmatin present, differing in no essential respect from that colouring matter as I have described it elsewhere.*

In *Tubularia indivisa* the bright red colour of the polyp heads was found to be due to a lipochrome.

Vermes.—In *Arenicola,* as in *Lumbricus terrestris,* the glandular tissue surrounding the intestine was found to contain a lipochrome, as well as the integument. From the latter situation the black pigment could be extracted by caustic potash, but in solution it gave no bands. Hæmoglobin is present in this worm, as is known by Prof. Ray Lankester's researches.

In *Terebella,* besides the hæmoglobin (Lankester), to which, and not to tetronerythrin the colour of the tentacles is due, the integument contains a lipochrome. In *Cirratulus* the tentacles also owe their colour to oxyhæmoglobin, but they also yield a lipochrome to solvents, as does the integument.

In *Nereis* a lipochrome is also present besides the hæmoglobin. The blood of *Nereis Dumerilii* showed one broad band like that of reduced hæmoglobin. In some parts of the worm only one band like the first one of oxyhæmoglobin was seen. An aqueous solution of the blood, however, gave two bands, but on adding ammonium sulphide they disappeared, and did not seem to be replaced by the single band of reduced hæmoglobin.

In *Pontobdella* an undescribed colouring matter bearing a remote resemblance to chlorophyll can be extracted from the integument by absolute alcohol. This comes from the large green-coloured corpuscles situated in the deeper parts of the integument.† The green solution showed no red fluorescence, nor did it show all the chlorophyll bands. With hydrochloric acid it became a deeper blue colour, but did not show the phyllocyanin spectrum.

In *Polynoe* a phosphorescent area was noticed surrounding the head ganglion, which latter was of a red colour, and gave a band situated approximately in the same part of the spectrum as that of reduced hæmoglobin, but in no part of the worm could I see the spectrum of oxyhæmoglobin. In one specimen the cerebral ganglion showed one band like the first of oxyhæmoglobin, and in another this ganglion was yellow and showed no band.

I have examined the green colouring matter of *Chætopterus insignis* (Baird) and can confirm Professor Lankester's statement that it is chlorophyll. The alcohol solution possesses a fine red fluorescence and gives all the chlorophyll band and yields " modified " and " acid " chlorophyll as well as phyllocyanin by suitable treatment.

* Philos. Trans., Pt. ii, 1885.

† See A. Gibbs Bourne's paper, Quart. Journ. Mic. Sci., July, 1884.

I could detect no hæmoglobin in *Nemertes Neesii*, nor did the integument yield hæmatoporphyrin to acidulated alcohol.

In *Phyllodoce viridis* a special green pigment occurs which is not chlorophyll. This pigment is described at length in the complete paper. It may be remembered that P. Geddes* exposed this green polychæte Annelid to sunlight and failed to get any evolution of oxygen. Of course the above result explains why.

Tunicates : In *Styela grossularia* the brilliant red pigment surrounding the exhalent and inhalent orifices was found to be a red lipochrome, which by Merejkowski† would doubtless be taken for tetronerythrin, but in this, as well as in many other instances, it is more closely related to Kühne's rhodophan than to anything else. The ether solution was at first a fine red colour, but soon changed to greenish, and although it did not show a red fluorescence it gave a chlorophylloid spectrum. But the pigment present was not chlorophyll; it seemed to possess characters, however, which should class it among the lipochromes and which show that the step from one to the other is not a great one.

Botryllus violaceus yields to solvents such as alcohol and ether a yellow lipochrome, which in a deep layer of alcohol solution showed a band in red like that of chlorophyll. It is probable that in this instance also a colouring matter is present possessing some of the characters of chlorophyll and being yet a lipochrome.‡

In *Botrylloides* an allied pigment is present.

In *Clavellina lepadiformis* some bluish colouring matter occurs which showed some shading at the blue end of green and a feeble shading at D, but I was unable to examine it further.

In *Ascidia virginea* a reddish pigment was noticed which gave two shadings in the green and strongly absorbed the violet end of the spectrum, but I failed to get it into solution. It is probably, however, related to the lipochromes.

Bryozoans : The examination of *Lepralia foliacea* yielded interesting results. It contains abundance of chlorophyll mixed with a lipochrome, to which latter the fine orange of this species is due. The chlorophyll is also accompanied by a second pigment, probably chlorofucin,§ if so, the latter must be due either to symbiotic algæ or to brown marine algæ. The ether solution shows the bands of the latter pigment well marked, and has a red fluorescence, which is

* Proc. Roy. Soc. Edin., vol. xi, 1881–82.

† Bullet. de la Société zoolog. de France, 1883, p. 81 *et seq.;* cf. Dr. W. Wurm in Jahreshefte des Vereins für vaterl. Naturkunde in Württ., 1885, S. 262—265.

‡ Cf. Krukenberg, Verg. physiol. Stud., 2 Reihe, 3 Abth., 1882, Tafel v, 7.

§ Sorby, Proc. Roy. Soc., No. 146, vol. xxi, 1873; see also my paper in Quart. Journ. Mic. Soc., vol. xxvii.

not so well marked in the case of the alcohol solution. Water extracted from *Lepralia* a little reddish yellow colouring matter, showing some shading at the blue end of green, and glycerine a little yellow. The acetic acid solution was brownish yellow, and in deep layers absorbed all the spectrum except the red, while in a thin layer it showed a band at the blue end of green.*

I formerly found chlorophyll in *Flustra foliacea*, where it is evidently due to the presence of the brown bodies, the remains of the atrophied Zooids.

The only mollusc which I had time to examine roughly was *Doris*, but in this I found evidence of the presence of a hæmochromogen-like spectrum, resembling exactly that of the pigment which I have named enterohæmatin, and which Sorby found in several snails and slugs, and I found in *Patella* and *Astacus*.† This pigment is, as I have shown, connected with the histohæmatins, which have a very wide distribution throughout the animal kingdom.

Remarks.—It was evident to me that every pigment which I met with, in this somewhat rough and unfinished series of observations, could be classified under groups which have already been described by others and myself; but they are not on that account the less interesting, as the distribution of these pigments is of great importance.

To morphologists the study of animal chromatology may seem trivial, but the pigments are of great importance from a physiological point of view, and the discovery of hæmoglobin, hæmocyanin, the histohæmatins, echinochrome, and other respiratory colouring matters, has thrown much light on the respiratory processes in animals.

With regard to the lipochromes, it is difficult to understand what *rôle* they play. I cannot think that they can be of much use in respiration, as they are unaffected by oxidizing and reducing agents as are other respiratory substances. And I therefore differ from Merejkowski with regard to tetronerythrin being respiratory, as it—as I said before—has been shown to be a lipochrome. It is significant that such widely separated structures as the eye-spot of a starfish and the rods of the Vertebrate eye should each yield lipochromes; it would seem that in such cases they are concerned in the absorption of light-rays.‡ Probably their simple chemical constitution, as they all consist of only three elements, carbon, hydrogen, and oxygen, has been taken advantage of for such purposes, as they can be built up with the minimum expenditure of energy. It is interesting also to note that they have a very strong absorptive power for the violet end of the spectrum.

* Cf. Krukenberg, Verg. physiol. Stud., 2 Reihe, 3 Abth., S. 29.

† Philos. Trans., Pt. i, 1886, p. 239, and Ibid., p. 268.

‡ Cf. Sir J. Lubbock in The Senses of Animals, Internat. Sci. Series, p. 3, *et seq.*

Their very intimate association with chlorophyll, and the actual change of that substance into them, which I have sometimes observed, teach that the step from one to the other colouring matter is not a great one. When we also consider that they are widely distributed throughout the vegetable kingdom, it no longer becomes difficult to understand why chlorophyll should be built up by animals. I am quite convinced that Prof. Lankester's contention that true animal chlorophyll exists can no longer be contradicted.

Krukenberg* has shown that the lipochromes are connected closely with the lipochromoids and melanoids, and through the latter with the melanins; if so, they furnish the radicals for the construction of many black and brown pigments (such as we find in *Holothuria nigra*, whose interior is, as said before, pigmented, to an extraordinary degree, by lipochromes).

The narrow view that all or nearly all the pigments of the vertebrate body are formed from hæmoglobin, held by many human physiologists up to a very late period, is shown to be erroneous by a study of the chromatology of the lower forms. Thus there are other mother-substances such as the histohæmatins, which are of as great importance to many Invertebrates as hæmoglobin is to the higher forms, and it is only by a knowledge of this fact that we can explain the occasional occurrence of such pigments as hæmato-porphyrin in the integument of a starfish, in slugs, and in *Solecurtus strigillatus*,† as I have shown, or of biliverdin in *Actinia mesembryanthemum*, as I have also shown, or in the shells of various molluscs, as Krukenberg has pointed out.‡

These histohæmatins and others, such as the enterohæmatin of snails, slugs, the common limpet, the crayfish and *Doris*, actino-hæmatin, and Lankester's chlorocruorin, may possibly, and probably do, represent immature kinds of hæmoglobin on their way, as it were, to form that complex body, but they certainly are not meta-bolites of hæmoglobin.

The view that modified myohæmatin from pigeon's muscle is hæmochromogen, which Herr Ludwig Levy holds, and endeavours to prove in a recent paper,§ cannot be maintained by anyone who extends his observations to invertebrate animals. Levy further states that it is derived from hæmoglobin, but where is the hæmoglobin from which it comes in a bee, a wasp, a butterfly, a slug, a snail, a crayfish, or a lobster, or in many others in which not a single trace of hæmoglobin can be detected by the most careful spectroscopic

* Centralb. f. d. medic. Wissensch., 1883, S. 785—788.

† Jl. Physiol., loc. cit.

‡ Centralb. f. d. medic. Wissensch., loc. cit.

§ *Über den Farbstoff der Muskeln*, Inaug. Dissert. Strassburg, 1888.

observation ?* I am quite aware of the near relationship of these pigments to hæmatin, and I know that modified myohæmatin and enterohæmatin are very like hæmochromogen : it was for that reason that I gave them their present names; but apart from the fact that, as I have just stated, no hæmoglobin can be found in some animals where they occur, there remains the no less important one, that they do not behave chemically, as they ought to do, if they were metabolites of hæmoglobin.

* Philos. Trans., loc. cit.