XI. *Observations on the Chromatology of Actiniæ.*

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[Plates 69, 70.]

Very few observations have been made on the Chromatology of the Actiniæ, the reason probably being that observers have been led to believe that since their colouring matters cannot be got into solution with the ordinary solvents, the study of their spectra could hardly be attended with fruitful results.

Krukenberg,* who has added much to our knowledge of animal Chromatology generally, has attempted to enlarge the knowledge of this subject by his study of the pigments of *Anthea viridis* and *A. cereus*.

Professor Moseley† published a short paper in 1873 on the colouring matter found by him in *Bunodes crassicornis*, which he called Actiniochrome, and in a subsequent paper,‡ in which an immense number of new animal pigments were brought to light, he describes a dark red colouring matter in *Anthea* which absorbed the blue end of the spectrum from before E onwards, and another in *Adamsia* which was pink, and in the fresh state gave a band between D and E. The filaments emitted from the pores of the body wall also gave two bands in green.

Heider§ examined *Cerianthus membranaceus*, var. *fusca* and var. *violacea*, and the colouring matter obtained, which Krukenberg calls *Purpuridin*, was extracted by ammoniacal water. It appears to resemble in some respects (*e.g.*, colour-changes) one of the pigments which I have found in *Sagartia parasitica*, to be referred to again.

O. and R. Hertwig‖ have tried to show that the so-called "yellow cells" or "pigment

* 'Vergleichend-physiologische Studien,' 1ste Reihe, 5te Abth., 1881, pp. 38–42, and, ditto, 2te Reihe, 3te Abth., 1882, pp. 72–87.

† "On Actiniochrome, a Colouring Matter of Actiniæ." Quart. Journ. Micro. Soc., vol. xii., N.S., 1873, p. 143.

‡ "On the Colouring Matters of various Animals, and especially of Deep Sea Forms dredged by H.M.S. Challenger." Quart. Journ. Micro. Soc., vol. xvii., N.S., 1877, pp. 1–23. Polyperythrin was also found in some Actiniæ by Prof. Moseley.

§ "*Cerianthus membranaceus*." (Haime.) "Ein Beitrag zur Anatomie der Actinien." Sonderabdr. aus den Sitzb. d. kk. Acad. der Wiss. zu Wien. Bd. lxxix., I. Abth., 1879, S. 7; also "Ueber Sagartia troglodytes." Sitzungsb. der Wien. Akad., 1877.

‖ "Die Actinien." 'Jenaische Zeitschrift für Naturwiss.' Bd. xiii., 1879, S. 495–500.

bodies" of certain Actiniæ are parasitic or rather symbiotic algæ, a view which P. GEDDES* by his remarkable experiments has endeavoured to support.

I had made some preliminary observations† on the occurrence of a band in *Actinia mesembryanthemum*, which I believed to be due to MOSELEY's Actiniochrome, but since I have been able to extract the pigment by means of glycerin I find that the band of Actiniochrome is not coincident with that seen in *Actinia mesembryanthemum*, as the former is nearer the red end of the spectrum, and the latter belongs to a colouring matter which, as I will shortly endeavour to show, yields certain decomposition products having a most remarkable resemblance to those obtainable from hæmoglobin. No purpuridin is found in the last-mentioned Actinia (although supposed to be present by KRUKENBERG).

Method of Examination.—The solid portions of Actiniæ are first examined by means of the microspectroscope, and they are then treated with various solvents. The solid portions are examined in the "compressorium," which enables any desired thickness to be obtained; they have to be well illuminated,‡ and for this purpose a substage condenser is used. The solutions are examined first with the microspectroscope, and then their bands measured with the large spectroscope, obtained by means of the grant allowed me by the Royal Society. Wave-lengths calculated by means of the microspectroscope cannot be sufficiently relied upon owing to the shortness of the spectrum, but owing to its superior definition the microspectroscope is indispensable, without it faint bands would be missed, and a comparison of a chlorophyll solution in both instruments shows the presence of bands with the microspectroscope which are almost invisible with the chemical spectroscope.

Actiniæ examined.—I have examined the following Actiniæ: *Actinia mesembryanthemum, Bunodes crassicornis, B. ballii, Sagartia bellis, S. dianthus, S. parasitica, S. viduata, S. troglodytes*, and *Anthea cereus*. I have not been able to obtain other species, and my results would have been more valuable if I had had a greater abundance of material; this dearth of material has been the cause of my not having been able to attempt the complete isolation of some of the pigments to be described, and of my not being able to generalise as fully as I might otherwise have done. I trust on these grounds too much will not be expected from these preliminary observations.

* "Further Researches on Animals containing Chlorophyll." 'Nature,' Jan. 26, 1882, pp. 303–5. And "On the Nature and Functions of the Yellow Cells of Radiolarians and Cœlenterates." Proc. Roy. Soc. Edinburgh, vol. xi., 1881–82, pp. 377–96. *Cf.* also Dr. BRANDT, 'Sitzungsbericht der Gesellschaft naturforsch. Freunde zu Berlin,' No. 9, 1881; and Professor LANKESTER, Quart. Journ. Micro. Soc., vol. xxii., pp. 229 *et seq.*; *vide* also 'Nature' for 1882, for letters of Professors MOSELEY and E. P. WRIGHT.

† Proc. Birm. Philos. Soc., vol. iii., p. 374.

‡ The source of light is an Argand gas-burner, or sometimes a "9-candle-power" SWAN lamp. The fanciful curves, recently published in Germany, obtained by means of a heliostat and chemical spectroscope, cannot be accurate, and show more imagination on the part of the observer than most people possess. The flat and other shaped summits of the bands are very remarkable.

*Actinia mesembryanthemum.**—A tolerably uniform result is obtained by the examination of all red-coloured specimens of this species. When the solid portions from the ectoderm, endoderm, and tentacles (in most cases) are examined in the manner described, a band which closely resembles that of reduced hæmoglobin is seen, accompanied generally by two other bands nearer the violet end of the spectrum, Chart I., spectrum 1. The extreme edges of the shading of the band extend from λ 600 to λ 560, while its darkest part is from λ 580 to λ 563. These measurements vary, however, according to the colour of the specimen, for in brown specimens the dominant band is nearer the violet, and in some a band is also present before D. As subsequent observations showed, the latter spectrum belongs to modifications of the same colouring matter, for the same decomposition products are obtained in both cases. The best way to show this variation of the band according to the colour of the specimen is by drawing a number of spectra from different cases, which accordingly I have done in the accompanying Chart I., spectrum 1 to 8. The blue chromatophores (="eye-spots") show always a spectrum in which the band is nearer the red than in the ectoderm and endoderm, and has a likeness to that of indigo-blue. This spectrum is shown in Chart I., spectrum 9. In purely brown specimens the spectrum is tolerably constant, as they all showed a band between D and E, and generally one at D, spectra 5 and 6, Chart I. They also—as I have stated—give the same decomposition products as the red specimens, and this remark applies to greenish specimens. The spectrum of brown specimens has a close resemblance to that of the pigments to which I have given the name histohæmatins, and a spectrum even more closely related to these is seen in *Sagartia troglodytes.*

I tried by the use of alcohol, ether, chloroform, bisulphide of carbon, and other solvents, to get this colouring matter out of the different parts of *Actina mesembryanthemum,* but failed. I succeeded in getting it out changed by boiling with rectified spirit and caustic potash or caustic soda, also by digesting in the cold with the same solutions, but at last I found that it could be extracted with glycerin.

When boiled with caustic potash and rectified spirit, or slowly extracted in the cold, a reddish solution was always obtained, which showed a band at D, spectrum 10, Chart I., generally extending from λ 625 to λ 589, which recalls to mind the spectrum of alkaline hæmatin; when sulphide of ammonium was added to this, the band at D disappeared, to be replaced in every case by two well-defined bands, which are undistinguishable from hæmochromogen, spectrum 11, Chart I. The first extended from λ 564·5 to λ 553, the second from λ 537 to λ 521·5. Their peculiarities of shading and their position are those of hæmochromogen. I then observed that all the red colouring-matter in the Actiniæ gave after this treatment in the solid state the spectrum of hæmochromogen. Now Hoppe-Seyler† found that if solutions of hæmoglobin are treated with caustic alkalies

* In every case the finely divided portions of the Actiniæ were well washed in distilled water before examination.

† "Weitere Mittheilungen über die Eigenschaften des Blutfarbstoffs." Zeitschrift f. physiol. Chem., vol. i., p. 138, and Physiol. Chemie, also Professor Gamgee's 'Physiological Chemistry,' vol. i., 1880.

in an apparatus from which the air is carefully excluded, the hæmoglobin becomes changed into hæmochromogen. In the solid tissues of the Actinia, a similar reaction occurs, but in the solution used to extract the pigment the hæmatin becomes oxidised as it comes out of the tissue and shows the alkaline hæmatin spectrum, which, however, can be reconverted into hæmochromogen by the addition of ammonium sulphide.

I could not, with certainty, obtain acid hæmatin, but I did succeed in converting the colouring matter into hæmatoporphyrin. By digesting portions of an Actinia in sulphuric acid, and filtering through asbestos, a purple-red solution was obtained which showed bands like those of acid hæmatoporphyrin, spectrum 12, Chart I., a little rectified spirit being added to the acid solution, but the band nearer the violet is not placed exactly in the same position as the corresponding band of hæmatoporphyrin obtained from hæmoglobin. The first band (in one experiment) extended from λ 605 to λ 595, and second from λ 563 to λ 551, but owing to the presence of biliverdin and proteids these measurements may not be quite reliable, still they possess a certain value when the results are compared with other cases. If this spectrum be that of a kind of hæmatoporphyrin, it ought to be changeable into alkaline hæmatoporphyrin, and such is the case. The solution was largely diluted with water, and ammonia added to cause the precipitation of the pigment. A flocculent precipitate fell; on filtering, an ochry-coloured precipitate was left, which, on being dissolved in alcohol and ammonia, gave a red solution, showing the spectrum of alkaline hæmatoporphyrin, as shown in spectrum 13, Chart I. The solution was too dilute to enable me to take the wave-lengths of its bands in the large spectroscope, but in the microspectroscope every band could be measured easily.

Hence there can no longer be a doubt that in *Actinia mesembryanthemum* a colouring matter is present which can be changed into hæmochromogen and hæmatoporphyrin.* But a more remarkable likeness to the higher animals as regards its pigments is shown by this Actinia, as I find that it contains a pigment which cannot in any way be distinguished from biliverdin. I could not believe this at first, but there is now no doubt that such is the case, my conclusions being based on a great number of experiments.

Beneath the ectoderm of many specimens, and also in the base (of attachment), a green coloration is perceptible. I found that if such portions of an Actinia were put into strong sulphuric acid they immediately assumed a vivid green colour, but on examining such portions spectroscopically they were found free from absorption bands, but absorbed some of the red end of the spectrum and transmitted the green intensified. I then found that if portions having the green colour were put into a mixture of alcohol and sulphuric acid, and even in alcohol alone, the solution soon

* The hæmatin of the bile of pulmonate mollusks and of the crayfish is not changeable into alkaline hæmatin, nor into hæmatoporphyrin, so far as one can judge; it is an immature kind of hæmatin, as Sorby has shown. Krukenberg's name, helicorubin, is not appropriate, as this pigment is met with in other animals. *Enterohæmatin* is the name I now propose for it for obvious reasons.

acquired a green colour. If red portions of Actiniæ were removed with the green ones, then I could sometimes see hæmatoporphyrin-like bands in the solution obtained by the action of alcohol and sulphuric acid upon them, but if the green coloured parts alone were treated in this manner the resulting solution showed no bands. On treating the solution so prepared on a white dish with nitric acid the blue, violet, red, and yellow colours of GMELIN'S reaction were successively and distinctly seen; and if the solution was placed in a glass-vessel and its spectrum observed, the very characteristic bands which accompany the colour-changes of this reaction were distinctly visible. But a purer solution of biliverdin is obtained by extracting the integument after the above treatment with alcohol; this purely green solution now absorbs more of the red end and transmits green intensified, as shown in the map, spectrum 14, Chart I., which is quite free from absorption bands, except a very feeble shading before F. Treated on a white dish with nitric acid the green, blue, violet, red, and yellow stages of GMELIN'S reaction are clearly seen. If placed in a test tube and examined before the slit of the spectroscope, and nitric acid added, as the colour changes a band appears before D and one at F, then a band also appears after D, the last fades away, then that before D fades away, and finally that at F. No other pigment but biliverdin behaves in this manner, as I have previously shown.*

It is no easy matter to read the bands in the case of GMELIN'S reaction, they disappear so soon, but I believe the following are fairly accurate, for the above solution:—the 1st band is about λ 623 to λ 593, the second faded too quickly to measure it, and the third extended from λ 511·5 to λ 488,† spectrum 15, Chart I. *Compared with an alcohol solution of biliverdin obtained from bile, these bands corresponded exactly.* These experiments were repeated several times, and the result was always the same. Hence *Actinia mesembryanthemum* contains in its mesoderm and elsewhere a colouring matter undistinguishable from biliverdin. From the fact that dull green parts of *Actinia mesembryanthemum* assume a vivid green under the influence of the acidulated alcohol, it is probable that a portion at least of the biliverdin is present in the condition of a chromogen.

For a long time I could not exactly determine whether the band in red specimens of *Actinia mesembryanthemum*, occupying the position nearly of that of reduced hæmoglobin, was not the same as that of Actiniochrome, because Professor MOSELEY'S drawing of the spectrum was lost, but on examining *Bunodes crassicornis*, in which Actiniochrome occurs, I was able to decide this point. The band of Actiniochrome is nearer the red, and is represented in Chart I., spectrum 16.

The next point to be determined was whether the same decomposition products could be obtained from the respective colouring matters, but as long as I dealt with solid tissues I got conflicting results; at length, however, I found that I could get Actinio-

* 'Spectroscope in Medicine,' 1880, and Proc. Roy. Soc., No. 208, 1880, No. 226, 1883, and Journ. Physiol., vol. vi., pp. 22–39.

† The reading of the violet edge of the last band may not be quite accurate.

chrome into solution to a slight extent by extracting parts in which it was seen to be present—by preliminary spectroscopic examination—with glycerin, which also extracts —to a slight extent—the hæmatin-yielding pigment. But from the glycerin it could not be obtained in sufficient quantity in the solid state to be thoroughly studied. Knowing that various ferments can be precipitated out of glycerin extracts of organs by means of absolute alcohol I tried to precipitate these pigments in the same way, but failed, owing to dearth of material and the presence of mucus, &c. I shall, however, attempt this again when I have an opportunity. Examination of glycerin extracts, however, enabled me to decide the question referred to above, namely: whether MOSELEY's Actiniochrome yields hæmochromogen or not.

Every specimen of *Actinia mesembryanthemum*, whether its colour was red, reddish-brown, brown, or greenish-brown, gave to the glycerin, after some days' extraction, a certain amount of colouring matter, *which in every case could be made to change into hæmochromogen, while Actiniochrome never could be changed into it; hence the respective pigments are very different. One is a respiratory colouring matter, the other is an ornamental one.*

If a glycerin extract is made of Actiniochrome, a band may be seen from about λ 593 to λ 566 (centre at λ 579). On adding to this caustic soda the band is moved slightly nearer violet, and then on adding sulphide of ammonium it is only *darkened.*

If a glycerin extract be made of the ectoderm of an anemone yielding the broad band of actiniohæmatin, referred to before, it gives a band at D and one between D and E, spectrum 17, Chart I. If now sulphide of ammonium be added, no change, or only darkening, of the second band takes place; but if caustic soda or caustic potash be added previously, and afterwards sulphide of ammonium, the spectrum is changed into that of hæmochromogen, spectrum 18, Chart I.

The caustic soda in this case does not, however, cause the spectrum of alkaline hæmatin to appear, but removes the band at D altogether.

In such glycerin solutions of *Actinia mesembryanthemum* the first band reads from λ 613 to λ 576, the second from λ 566 to λ 545·5, and generally a third from λ 479 to λ 458·5. After caustic soda the first band disappears and the second is made very faint; on adding ammonium sulphide the hæmochromogen bands read: first from λ 564·5 to λ 554·5, and the second from λ 537 to λ 524·5. I reserve for the end of this paper the conclusions which one may draw as to the functions of the hæmatin-yielding pigment. This Actinia, without doubt, contains a pigment yielding hæmatin and hæmatoporphyrin, and perhaps it may save confusion to name the mother-substance provisionally *Actiniohæmatin.* In none of the specimens of this Actinia examined could I find any "yellow cells." It appears that this hæmatin-yielding pigment does not give the same spectrum in brown specimens as in red; but the spectrum of the glycerin extract of red Actiniæ has a close resemblance to that of the spectrum of the solid ectoderm and other parts of brown specimens. This does not

show that the pigment has been altered by extraction with glycerin, but its molecular condition may be altered. It is well known that the spectrum of a pigment may differ in the solid and liquid state without any necessary change in its composition (VOGEL and KUNDT).

Bunodes crassicornis.—As already stated, this species has been examined by Professor MOSELEY,* who found that the parts scraped off from the general body wall and tentacles gave no bands, while those from the circumoral disc did give a band, nearly coinciding with the less refrangible band of oxy-hæmoglobin; he also found that most of the western species were entirely green, and in about one in ten the tips of the gonidial tubercles retained a bright red colouring. In two specimens the tentacles were a beautiful rose colour, and this colour was found to be due to Actiniochrome, though it was absent from the entire remainder of the body. I have found that the colour and spectra of this species differ considerably in different cases. In some obtained from Weymouth the specimens were mostly a dull brownish-green, mottled irregularly with red, and the base was also mottled with red. In some a green layer was present beneath the ectoderm. (The colour of other specimens will be referred to further on.) In the ectoderm of these a band, occupying the same position as that in *Actinia mesembryanthemum*, was present in the red parts. The tentacles were colourless at the apices, and those parts of the tentacles which had a yellowish-red colour gave spectrum 1, Chart II, which belonged to the ectodermal layer. No Actiniochrome could be detected. The red parts of the ectoderm gave spectrum 2, Chart II. In none of the tentacles of this variety could "yellow cells" be detected. On cutting out the red portions of the ectoderm and digesting them in rectified spirit and caustic potash a reddish-yellow solution was obtained, giving an ill-defined band before D, and absorption of the violet end of the spectrum, spectrum 3, Chart II. On adding ammonium sulphide the hæmochromogen bands appeared, the solution becoming redder; the first band extended from λ 564·5 to λ 554·5, and the second from λ 537 to λ 524·5. An extraction of the tentacles with alcohol was without result.

The colours of other specimens from the same locality were a dirty green in the ectoderm which was mottled with light green spots and with a bright red circumoral ring, the tentacles being yellowish with a red zone belonging to the ectodermal layer.† The red ectoderm and the brown-red endodermal parts gave the same spectrum as the former specimens. The ectodermal and endodermal parts, and also the tentacles, contained actiniohæmatin, as proved by the action of rectified spirit and caustic potash, sulphide of ammonium, &c., the same decomposition products having been obtained as in former experiments. No Actiniochrome could be detected with certainty.

* *Loc. cit.*

† The spectrum of these tentacles in the yellowish parts is shown in Chart II., spectrum 1. They had a bluish-white base, a red median zone, and almost colourless points. The red zone, as said *supra*, gave the same band as the ectoderm, &c., and yielded hæmochromogen.

In other specimens of *Bunodes crassicornis*, from Llandudno, the ectoderm had a dark purple-red colour, and the tentacles were purple-red, with more lightly tinted apices; here the tentacles gave MOSELEY'S Actiniochrome spectrum with great distinctness, spectrum 16, Chart I. and 5, II., and on squeezing them in the compressorium two other bands nearer the violet end of the spectrum. The red colouring matter (Actiniochrome) was confined to the ectodermal layer of the tentacles. Its band read from λ 600 to λ 560, including the feeble shadings on each side of it. The ectoderm of the body wall gave a band somewhat like the last specimens, but nearer violet, which evidently resembles the band of actiniohæmatin in former cases. In the endodermal parts the same band could be seen, but less distinctly. Beneath the ectoderm (mesoderm) a greenish layer was seen showing no bands.

On digesting the tentacles for three days in glycerin a violet-red solution was obtained, and this gave spectrum 5, Chart II. This band of Actiniochrome in glycerin extended from λ 596·5 to λ 563,* centre at λ 579. The band in violet of the same spectrum from λ 477 to λ 458·5 (?). On treatment with caustic soda the former band remained *unchanged*, and no change—except very slight shifting towards violet—was produced by adding ammonium sulphide. On treatment with acetic acid (of a glycerin extract), the colour and spectrum became less distinct; sulphuric acid also made the band fainter. On treating some tentacles with caustic potash and spirit, and subsequently ammonium sulphide, no evidence of the presence of hæmochromogen could be obtained. Now the red colouring matter of the ectoderm, when treated in the same way, yielded a solution which, with caustic soda and subsequently ammonium sulphide, gave the hæmochromogen bands. Other portions of ectoderms were extracted with rectified spirit and caustic potash, and yielded a red solution, showing an ill-defined band before D (as in other cases), and on treatment with ammonium sulphide the bands of hæmochromogen appeared, the first, from λ 563 to λ 554·5, and the second from λ 540 to λ 524·5. The solid parts of such ectoderms after this treatment with caustic potash and alcohol—as in all cases when actiniohæmatin is present—showed two well-marked hæmochromogen bands, which on measurement were found to have the following wave-lengths, the first, from λ 567·5 to λ 556, and the second, from λ 537 to λ 521·5. On comparison with the spectrum of an alcoholic or glycerin solution of hæmochromogen, a discrepancy in the measurements is noticeable, the reason is simply this: that in one case the pigment is in the solid state, in the other in solution (spectrum 6, Chart II.). The endodermal parts also contained actiniohæmatin, as proved by the same treatment.

Just as in *Actinia mesembryanthemum* so also in *Bunodes crassicornis*, I succeeded in getting hæmatoporphyrin from the actiniohæmatin. Portions of ectoderm, in which the pigments had been converted into hæmochromogen by treatment with rectified spirit and caustic potash, were acted upon with sulphuric acid, and the resulting purple-

* Its darkest part was from λ 589 to λ 569.

red solution filtered through asbestos; in this solution the acid hæmatoporphyrin spectrum was plainly seen, much more plainly than in the case of other Actiniæ, so that readings could be easily taken, the first band extended from λ 613 to λ 596·5, and the second from λ 566 to λ 551; here a third feeble shading from λ 524·5 to λ 501 was also seen. On comparing these readings with those got in former cases the agreement is tolerably close, especially when we take into consideration the fact that in the case of *Actinia mesembryanthemum* the bands of a solution diluted with rectified spirit were measured, while here the pigment was dissolved in pure sulphuric acid. In the latter case the pigment could also be converted into alkaline hæmatoporphyrin.

If a *Bunodes crassicornis* be taken whose tentacles show the actiniohæmatin band, and if the tentacles be extracted with glycerin, the solution shows a much broader band between D and E than that of Actiniochrome; then on adding caustic soda the band is made fainter, and on adding sulphide of ammonium the hæmochromogen bands are seen with distinctness, but if those tentacles which show Professor MOSELEY'S Actiniochrome are treated in the same way a negative result is obtained.

Biliverdin appears to be present in the mesoderm and in other green parts.

In some specimens I found actiniohæmatin and actiniochrome mixed together, and in the glycerin extract of some tentacles two bands nearer the violet, as shown in spectrum 7, Chart II. I do not think these bands belong to a lipochrome (= lutein) as they are not found in solutions which dissolve the lipochromes.* Similar bands were seen in the case of other anemones, as will be seen by referring to the spectra figured.

Hence in *Bunodes crassicornis* we find actiniohæmatin with tolerable constancy, occasionally actiniochrome and also biliverdin, besides the lutein-like pigments. In the ectoderm as well as in the endoderm, and sometimes in the tentacles, actiniohæmatin is present. In none of the specimens were "yellow cells" present, and by no other solvents except glycerin, and alkaline and acid alcoholic solutions, could any pigments be got into solution.

Bunodes ballii (large variety).—The specimens which I have examined came from Weymouth. The body wall was dotted with red, the tentacles and body at their base were green, and the tentacles presented a contrast in their opacity to the Actiniæ, examined *supra*. In other specimens the body wall (ectodermal layer) was red or brownish-red, the upper fifth or so greenish, with rows of white dots running from base to disc.

The tentacles gave a number of bands, (see spectrum 10, Chart II.,) which showed

* "Lipochromes" is a name proposed by KRUKENBERG for a class of pigments some of which were formerly known as luteins. See his 'Vergleichend-physiologische Studien,' 1880–1882, and 'Grundzüge einer vergleichenden Physiologie der Farbstoffe und der Farben,' 1884. I hope to have more to say on this point in a future paper.

that a chlorophyll-like* pigment was present in them. The interior parts—mesenteries, &c., gave the same spectrum. The ectoderm in the redder parts gave a faint band in green, as shown in spectrum 11, Chart II., while in its green parts—with few exceptions—the same spectrum as that of the tentacles could be detected. On extracting the tentacles with absolute alcohol for a couple of days and filtering, an orange-coloured solution, with a distinct red fluorescence, was obtained, and this gave spectrum 12, Chart II. These bands gave the following readings: 1st band, λ 675, to λ 657, 2nd band, λ 642·5 to λ 629, and 3rd band, λ 595 to λ 579, the light being completely absorbed at λ 543. On treatment with one drop of nitric acid the spectrum changed completely, a darker band appeared placed over a lighter one, the whole compound band extended from λ 675 to λ 647, and its dark part from λ 675 to λ 665; another also before D from λ 623 to λ 596·5, light being completely absorbed at λ 521·5. Spectrum 13, Chart II. A feeble band also was seen after D, not shown in the map. The first alcohol extract left the tentacles a vivid green, but on repeated extraction with alcohol the colour became much diminished, and the second alcohol extract did not differ from the first.

On extracting the interior parts of this anemone with absolute alcohol and filtering, an orange-coloured solution was obtained, and this gave a very faint red fluorescence and a faint spectrum, not differing from that of the alcohol solution of the tentacles, after allowing for the smaller amount of pigment present in the solution. In a thin layer of the solution a feeble band was seen extending approximately from λ 509 to λ 484·5.

On extracting the ectoderm with absolute alcohol and filtering, an orange-coloured solution was also obtained, giving the same spectrum as the parts referred to, spectrum 14, Chart II., and having a red fluorescence. These bands read as follows: 1st from λ 675 to λ 657, 2nd from λ 645 to λ 629, and 3rd from λ 595 to λ 580·5, the spectrum being extinguished at λ 551.

On treating this last alcoholic solution† with caustic potash *the spectrum changed completely*, as shown in spectrum 15, Chart II. The result obtained by extracting the ectoderm with rectified spirit and caustic potash and filtering was no less striking; the resulting solution was distinctly green, and gave the spectrum shown in spectrum 16, Chart II. The first and second band read: 1st from λ 649 to λ 627, 2nd λ 609 to λ 585. In a thin layer a third became detached at λ 492·5 to λ 475. No hæmochromogen bands could be obtained. At the same time I believe the red portions contain a small amount of actiniohæmatin, its presence being masked by

* In the list of chlorophyll-containing animals, enumerated by Professor LANKESTER, in the second English edition of SACHS's 'Botany,' the word *chlorofucin* is placed after *Anthea cereus*. I have accordingly introduced the spectrum of chlorofucin from *Fucus serratus* (Linn.), and there is seen a remarkable likeness, if not an identity, between it and the pigments of the above, *S. bellis* and *A. cereus*. Spectra 8 and 9, Chart II. SORBY was the first who showed the presence of *chlorofucin* in *A. cereus*. Proc. Royal Soc., No. 146, vol. xxi., 1873, p. 454.

† Or that of the tentacles.

the chlorophyll-like pigment. On microscopic examination the tentacles were found packed with "yellow cells," lodged in part in their endodermal lining. They differed in no respect from the "yellow cells" of *Sagartia bellis* and *Anthea cereus*, to be described further on.* SCHULZE'S fluid developed a distinct cellulose reaction in their outer wall, and evidence of starch was obtained by treatment with iodine in iodide of potassium, especially after maceration for some time in a weak solution of caustic potash and neutralizing with acetic acid (GEDDES). Further, it would seem that these "yellow cells" had taken the place of the red colouring matter of other species, to a great extent, since the latter was present in mere traces.

On examining the *small variety* of *Bunodes ballii* from the same locality no "yellow cells" could be detected in the tentacles or elsewhere. Their tentacles were mostly coloured pink in the inner row, and colourless in the outer row, and the former gave a well-marked actiniochrome-like spectrum, spectrum 17, Chart II. The ectoderm was in places lake-red (and the base dotted with red), and gave spectrum 18, Chart II. This spectrum, on comparison with the tentacles, shows the presence of a band near the violet, while the other band is well marked. In some specimens the lining of the body cavity (endoderm) was pink, and in such cases gave spectrum 1, Chart III.; the second band of which is remarkably dark, in other cases it was brownish-yellow, and gave no well-marked band. I failed to procure hæmochromogen, however, from these anemones, which is very remarkable. Digestion of the different parts in rectified spirit and caustic potash gave a negative result after repeated trials. Still the fact is interesting that the colouring matter of the ectoderm resembles, with regard to the first band of its spectrum, that of *Actinia mesembryanthemum*. It may have been a pigment which is intermediate between actiniochrome and actiniohæmatin. But this I hope to decide when I have an opportunity. The replacement of this pigment by the colouring matter of the "yellow cells" in the large variety is of great interest, and teaches that the presence of the colouring matter has something to do with the absence of "yellow cells" in the small variety. It would be premature to say that this pigment is peculiar to the small variety, but it may possibly be a special one like that of *Sagartia parasitica*.

Sagartia dianthus.—Specimens of this Actinia were procured from Weymouth. Some were yellow-brown externally, others white. The tentacles generally flesh-coloured; the lining of the body cavity generally reddish. The ectoderm of the yellow-brown specimens showed a very badly marked band before D, and a feeble shading in green, which recalled to mind the spectrum of brown specimens of *Actinia mesembryanthemum;* the ectoderm of white specimens showed no band. The endodermal parts showed only a faint shading in green; and all these spectra were too indistinct to be mapped.

In a small orange coloured *Sagartia dianthus* a bit of ectoderm and the orange brown-red endodermal parts gave spectrum 2, Chart III.

* These yellow cells measured $\frac{1}{2000}$th inch in diameter, others $\frac{1}{3000}$th; they were mostly $\frac{1}{2400}$th.

But although the examination of the solid parts of this species is so unsatisfactory, I got distinct evidence of the presence of a hæmatin-yielding pigment. On digesting portion of the ectoderm of brown specimens in rectified spirit and caustic potash a reddish-yellow solution was obtained which gave a band at D, and a shading at the blue end of green, the former from λ 611 to λ 579. And then, on adding ammonium sulphide, this band *did not disappear*, but a faint band resembling the first reduced hæmatin band was distinctly seen, spectrum 3, Chart III. On digesting the lining of the body cavity of the same specimens in the same solution, a very feeble band at D was detected, and on adding ammonium sulphide the result was the same as in the case of the ectoderm. The tentacles were digested in absolute alcohol, and the almost colourless solution gave a feeble band from λ 505 to λ 484·5, unchanged by caustic potash. The examination of the endodermal parts of white specimens gave the same result as above.

In a large brown *Sagartia dianthus* I also got distinct evidence of the presence of actiniohæmatin. The reddish-yellow filtered extract, obtained by digesting the ectoderm in rectified spirit and caustic potash, showed a band from λ 623 to λ 585, and on adding sulphide of ammonium two hæmochromogen bands were seen, of which the first (approximately) measured from λ 564·5 to λ 554·5. The same ectoderm yielded nothing to absolute alcohol, nor did the same part of an orange *S. dianthus* to the same solvent.

Sagartia viduata.—The few specimens which I had an opportunity of examining were striped with brown and white, and no well-marked band could be detected either in the ectoderm or endoderm. A faint shading in green was the only noticeable appearance.

On extracting the ectoderm for twenty-four hours with rectified spirit and caustic potash a yellowish solution was obtained, and this gave spectrum 4, Chart III. In this spectrum the first band read from λ 657 to λ 631, and the second from λ 611 to λ 582 (?). If this result is compared with the result of the examination of *Bunodes ballii* a resemblance is noticed. Faint traces of hæmochromogen were detected on adding ammonium sulphide.*

Sargatia parasitica.—In some specimens of this species—of a brown colour, externally striped with yellow and being internally a reddish-yellow colour,—no distinct band could be seen. In the brownish-red base no band could be detected, but both by the ectoderm and by the latter the violet end of the spectrum was strongly absorbed. The tentacles were white, but here and there dotted with a purplish pigment which showed no band. In some specimens the circumoral part had a purple-red colour, which gave an ill-defined band between D and E. Microscopically two kinds of pigment could be seen in them—brown and purple-red. Corresponding to these appearances was the result of extracting the various parts with solvents, as by their

* I believe the above chlorophyll-like spectrum was due to presence of "yellow cells," but I failed to detect them in the specimens examined.

use the presence of *two* pigments was proved. One of them was the familiar actinio-hæmatin, the other a pigment different from any I had examined before.

On digesting the interior parts in rectified spirit and caustic potash an orange-coloured solution was obtained, and on treatment with ammonium sulphide two bands like those of reduced hæmatin appeared, as shown in spectrum 5, Chart III.,* of which the first read from λ 563 to λ 554·5, the second was not distinct enough to be read.

A different result was obtained on examining the solutions obtained from the ectoderm.

As usual, I digested the ectoderm in rectified spirit and caustic potash, and after filtering obtained a red solution, and at the end of six weeks a similar solution retained a fine carmine-red colour. A deep layer transmitted red and a little green, and in a thinner one a broad band with ill-defined shading occupied the middle of the spectrum; both are shown in spectra 6 and 7, Chart III. Measurement showed that a deep layer absorbed the spectrum from λ 557 onwards to the violet, and the broad band extended from λ 540 to λ 467. On treatment with ammonium sulphide the red colour was replaced by a brownish-yellow, and now a broad shading covered D and extended towards E (spectrum 8, Chart III.). But it is not necessary to use an alkali for extracting this colouring matter, as it goes into solution when the ectoderm is digested in absolute alcohol. Such a solution is red in deep layers and yellow in thin, and in the former gives a band at D, broad and ill-defined, and absorbs strongly the whole of the spectrum from close before E onwards towards violet,† spectrum 9, Chart III., while in a thin layer a double band became detached, as shown in the spectrum 10, Chart III. The broad band at D extended from λ 640 to λ 576, and the second about λ 535 to λ 511·5, while the third was from λ 505 to λ 484·5 (?). On adding sulphide of ammonium the solution became a dark purple colour, changing rapidly to yellowish-red, which appeared a fine red by gaslight. A curious appearance was here noted, showing that this colouring matter is capable of existing in two states of oxidation; a dark purplish zone was noticed on the surface of the liquid in contact with the air, which momentarily disappeared on inverting the test tube, but at once reappeared when left to rest. The reduced solution gave spectrum 11, Chart III. The purple colour is due to the action of alkali, and the yellowish-red to the action of the reducing agent; and the change from the alkaline oxidised state to the alkaline reduced state, could be brought about as often as one wished. The band of the reduced alkaline solution extended from λ 623 to λ 572. The band of the purple solution (oxidised state) is broader and darker than the latter. This solution, after being kept for four months, preserved a blue colour, the H_2S having gradually escaped.

* They are slightly nearer violet than in other cases, as can be seen by comparison with former spectra.

† The spectrum of this body is not unlike KÜHNE's "rhodophan," also tetronerythrin, but it does not belong to one or the other, as its colour-changes show.

On treating an alcoholic solution with a little nitric acid the band at D disappeared, the solution changed to gamboge-yellow (gaslight), and a band from λ 535 to λ 509 was seen. The abrupt absorption of the violet end of the spectrum was not removed by the acid.

On treating some alcoholic solutions with caustic potash the colour became redder (gaslight) and the band at D was no longer seen, and in a thin layer a band could be seen from about λ 548·5 to λ 509, but in a deeper layer it extended up to λ 458·5. On treatment of the latter solutions with ammonium sulphide a faint bluish tinge appeared and quickly disappeared, and now some shading at D appeared.

If an alcoholic solution be treated with ammonia alone a splendid purple-blue solution is obtained, giving a dark band from λ 660 up to λ 516, and another from λ 498·5 to λ 475. The first broad band was strongly shaded at first on the redward side, but it eventually changed to that shown in spectrum 12, Chart III. On diluting this solution with more alcohol it became a deep blue, and in the more dilute solution a band was seen from λ 645 to λ 548·5 with its darkest part from λ 627 to λ 582. On neutralising this solution with acetic acid it became yellow, and the whole of the blue and violet part of the spectrum was now strongly absorbed, and in a thin layer the same kind of double band as that of the original alcoholic solution was seen.

The same colouring matter occurs in the tentacles, as I have proved, and they contain no hæmatin-yielding substance, this being confined to the endodermal parts of the body from which I could not extract any of the above-mentioned pigments. I do not think, in the present state of knowledge of the Chromatology of Actiniæ, that one is justified in calling this pigment by a new name, but so far as my experience goes it is peculiar to this species. In its colour-changes with acids it has a very remote resemblance to the purple pentacrinin of Professor MOSELEY,* also to the colouring matter of *Aplysia*,† but differs in spectrum and in some colour-changes. It has also a slight *resemblance* in character of spectrum and colour-changes to the colouring matter obtainable from the petals of some red flowers, *e.g.*, scarlet geranium, red rose, &c., but the respective spectra are not the same.

Out of *Cerianthus membranaceus*, according to HEIDER,‡ by means of ammoniacal water, a colouring matter can be extracted which KRUKENBERG§ calls *purpuridin*, but he says it gives no absorption bands, and as the above pigment does give bands, they cannot be the same.

I failed to find any yellow cells in *Sagartia parasitica;* its colouring matter appears to me to be capable of uniting with oxygen and of giving it up again, and is, therefore, probably of respiratory use.

* *Loc. cit.*

† Professor MOSELEY, *loc. cit.*, also myself in Proc. Birm. Phil. Soc., vol. iii., 1883, p. 392.

‡ *Loc. cit.*

§ "Vergleichend-physiologische Studien," 2te Reihe, 3te Abth., 1882, p. 72.

Sagartia troglodytes.—The solid tissues of this species—at least in the specimens examined by me—are poor in colouring matters. They were mostly a pale flesh colour, or yellow-white, or almost white, externally. Internally almost colourless or yellowish-white, the tentacles slightly green or almost colourless. A dirty brown tint was sometimes present in the circumoral part (externally), and in the same place some greenish spots, which on microscopic examination appeared to be due to the presence of some foreign substance, but not "yellow cells."

In the solid ectoderm a spectrum is detectable which is of great interest, as it indicates the presence of a colouring matter which is related to hæmochromogen, and in some parts the second band of that pigment can be seen. In the stomach wall of some invertebrate animals, *e.g.*, *Uraster rubens*, *Asterias*, &c., a similar band or bands can be seen. These belong to a *histo-hæmatin*, and I have no doubt that such is present in *S. troglodytes*. I have only shown the single band in spectrum 13, Chart III. In the interior portion a feeble band before D is also present. I could not see a band in the tentacles or in the brown part of the ectoderm referred to.

Although the lining of the body cavity was so pale, a solution obtained by digesting it in rectified spirit and caustic potash, on the addition of ammonium sulphide, gave the bands of reduced hæmatin. Traces of the same were found in a similar solution of the ectoderm.

The tentacles on extraction with absolute alcohol yielded only an almost colourless solution, showing no bands.

Sagartia bellis.—Some were yellowish externally, with a greenish zone around the mouth (externally); internally, brownish; the tentacles were quite opaque, and when squeezed out between glasses brownish in colour, and under the microscope they were found packed with "yellow cells." Other specimens were of a dirty yellow, with white stripes running from the tentacles to the base, their interior was orange and the tentacles dirty green; round the bases of the latter a ring of greenish-brown colouring matter was seen, and this was also present in the others. In some, however, a pale reddish-yellow zone encircled the *Sagartia*.

The first specimens referred to on spectroscopic examination gave in the ectoderm some uncertain shading in green, while in a few I saw a narrow band in green like that of *S. troglodytes*, and perhaps a second one. In the lining of the body cavity no distinct bands were seen.

In others the results were the same. In striking contrast to these results was the examination of the tentacles, as in every instance they showed a banded spectrum reminding of chlorophyll.* This spectrum belongs to the mass of "yellow cells" which were confined to the interior of the tentacles, and probably embedded in their endodermal linings. This spectrum is shown in spectrum 1, Chart IV. On digesting the ectoderm in rectified spirit and caustic potash, a pale yellow solution was obtained, giving spectrum 2, Chart IV. On treatment with sulphide of ammonium no reduced

* Or rather *chlorofucin*.

hæmatin bands appeared. The solution evidently contained a trace of the pigments belonging to the yellow cells which are present in the ectoderm. A similar solution of the interior parts was faintly greenish-yellow, and its spectrum was the same as that of the last solution; it contained no hæmatin; the band in red was rather doubtful.

The examination of solutions of the tentacles, however, furnished more interesting results.

An absolute alcohol solution of tentacles after filtering was deep yellow in colour, with a tinge of green, and had a red fluorescence; a deep layer, showed spectrum 3, Chart IV., while in a thinner layer two other bands were present. The whole series of bands read as follows:—1st, λ 675 to λ 660; 2nd, λ 642·5 to λ 629; 3rd, λ 593 to λ 577·5; 4th, λ 505 to λ 481; and 5th (about) λ 458·5 to λ 445. On adding a drop of nitric acid the colour did not appear much changed, but the bands were changed, as shown in spectrum 4, Chart IV., and the first two bands read from λ 669 to λ 649 and λ 613 to λ 593 (?).

Although very little, if any, effect was produced by ammonia, *the spectrum was completely altered by caustic potash.*

The result of treatment with this reagent is shown in spectrum 5, Chart IV., and the wave-lengths of these bands are as follows:—The shading on the red side came up to λ 636, next band λ 613 to λ 589, next λ 574 to λ 553, and a shading from λ 532 to λ 513, and another λ 496·5 to λ 473·5 (?). *This reaction distinguishes the colouring matter of the "yellow cells" from enterochlorophyll, other animal chlorophylls and plant chlorophyll, and teaches that it is quite useless to saponify this pigment, for if decomposed by caustic alkali in the cold it would become further decomposed by boiling with it.* (The change is the same as in *Bunodes ballii* and *Anthea cereus.*)

By the tests adopted in other cases the yellow cells were found to have a cellulose wall and to contain starch, but they had to be treated with a weak solution of caustic potash, then a little acetic acid, before the reactions with iodine in iodide of potassium and "SCHULZE'S fluid" could be distinctly seen.

The small amount of other pigments present is very noticeable, especially when we compare these results with those obtained in the case of *Bunodes ballii* and *Anthea cereus.* It would appear that the presence of the "yellow cells" has something to do with the absence or suppression of respiratory pigments.

Anthea cereus.—In some specimens the ectoderm was a pale red, also the base, and the tentacles a pale green, tipped with violet. In the violet apices of the tentacles actiniochrome was detected (see spectrum 6, Chart IV.). The rest of the tentacles gave a spectrum resembling that of chlorophyll,* and those of the tentacles of *Bunodes ballii* and *Sagartia bellis,* spectrum 8, Chart IV. If the contents of a tentacle be squeezed out, an entirely different spectrum is obtained from the empty tentacle, spectrum 7, Chart IV. It is probably the pigment to which these bands belong that is found in

* Chlorofucin.

glycerin extracts of some tentacles to be referred to further on. The base in some specimens had a pale reddish colour which showed a faint band like that of actiniohæmatin. *The "yellow cells" of Anthea are not confined to the tentacles*, for they could be detected in several places in the ectoderm by the aid of the microscope, and they seemed disposed in rows. They were also found in the lining of the body cavity. In other specimens examined the ectoderm was brownish, with lighter-coloured stripes running from above downwards, the base tinged orange-red, and the tentacles bluish-white, without any violet coloration. In both tentacles and ectoderm there was a great abundance of "yellow cells." On extracting the tentacles with absolute alcohol, and filtering, an orange coloured solution having a red fluorescence was obtained, which showed spectrum 9, Chart IV., the bands of which read: 1st, from λ 675 to λ 657; 2nd, from λ 645 to λ 629; 3rd, λ 595 to λ 579. On adding nitric acid in small quantity a violet and a blue ring formed in contact with the acid, and on mixing acid and solution a decided change took place, the solution giving spectrum 10, Chart IV., and then acquired a pale yellow colour. On adding a little more nitric acid a curious change ensued, as spectrum 11, Chart IV., now appeared. After some time the band in red could not be seen; this reaction distinguishes the present colouring matter from enterochlorophyll, other animal chlorophylls, and plant chlorophyll, but a more striking difference is apparent when this alcoholic solution is treated with caustic soda.* On treatment with this reagent the fluid assumed a redder colour (gaslight), and the spectrum changed to that shown in spectrum 12, Chart IV.; these bands gave the following wave-lengths: 1st, λ 609 to λ 589; 2nd, λ 569 to λ 554·5, and also a shading between green and blue which were too faint to be measured.

On digesting some of the reddish parts of *Anthea cereus* in rectified spirit and caustic potash, a greenish solution was obtained which had a red fluorescence, this solution gave spectrum 13, Chart IV., the more prominent bands of which read: λ 649 to λ 633·5, and λ 607 to λ 587. And there was also a band between green and blue visible in a thin layer from λ 498·5 to λ 477. With ammonium sulphide alone a change was brought about in an alcoholic solution as shown in Chart IV., spectrum 14.

The "yellow cells," on treatment with SCHULZE'S fluid, gave a distinct cellulose reaction, and in their interior the presence of starch was detected with iodine in iodide of potassium, but, as in former cases, the best results were obtained by previously extracting the tentacles with alcohol, and then macerating them in a weak solution of caustic potash, and neutralising with acetic acid. In young specimens of *Anthea cereus* I found much fewer cells than in the larger ones, and in these the starch and cellulose reactions could be more easily obtained.

Since caustic potash and caustic soda change this colouring matter completely, it is useless to attempt to saponify it, and this very fact distinguishes, as I said before, the colouring matter of the "yellow cells" from enterochlorophyll, other animal chloro-

* KRUKENBERG noticed this change with caustic soda, *loc. cit.*, but failed in seeing that this test distinguished the pigment from what he called hepatochromates (= enterochlorophyll).

phylls, and plant chlorophyll. I cannot, on these grounds, agree with KRUKENBERG* that it resembles the hepatochromates, which is his name for the pigment named by me enterochlorophyll,† nor does he by any means succeed in proving that it is a purely animal pigment.

The other violet pigment referred to by KRUKENBERG is probably the actiniochrome of Professor MOSELEY. In some specimens the apices of the tentacles are tinged of a violet colour, which showed the actiniochrome spectrum well marked, spectrum 6, Chart IV. The glycerin extract of the tentacles of some specimens of *Anthea cereus*, after some days' extraction, gives a solution which, although only yellowish-red, possesses a magnificent emerald-green fluorescence. I have no doubt that this is the pigment whose bands are seen in the almost colourless tentacle after removal of the yellow cells, as it gives a band in blue and in the violet, spectrum 15, Chart IV., besides the first. They read: 1st, λ 582 to λ 560; 2nd, λ 520·5 to λ 506; 3rd, λ 494·5 to λ 475. The first band probably belongs to a trace of actiniohæmatin. That this fluorescent pigment was not a so-called "lipochrome" is shown by the fact that, on adding caustic soda, one of the bands near the violet end disappeared, while with acetic acid the second and third bands appeared merged into one.

Summary and Remarks.

The above observations show that a respiratory colouring matter is present in *Actinia mesembryanthemum*, *Bunodes crassicornis*, and other Actiniæ. That it must be respiratory is shown by the fact that one of its decomposition products is capable of existing in a state of oxidation and reduction. That it is closely related to hæmoglobin is shown by the results attending my attempts to convert it into hæmochromogen and hæmatoporphyrin, as the pigments corresponding to these, obtained from the above Actiniæ, are undistinguishable from those obtained from hæmoglobin.

The occurrence of biliverdin in such lowly-organised animals is of great interest; it may probably be looked upon as excretory, and, as I said above, a part of it seems to be present in the state of a chromogen, as proved by the action of acids upon it.

The discovery of biliverdin in the shells of certain mollusks by KRUKENBERG‡ acquires an additional interest from these observations; but it is premature to conclude, as KRUKENBERG does, that here it is independent of the presence of hæmoglobin, because it is possible that the pharyngeal muscles of the animals in which he found biliverdin may contain hæmoglobin as Professor LANKESTER has shown to be the case in other mollusks; or their tissues may contain the colouring matters which I find widely

* *Loc. cit.* KRUKENBERG did *not* show that enterochlorophyll and plant chlorophyll give the same spectrum; he figures spectra of liver-extracts, but in no case is the fully-developed spectrum shown. The position of the FRAUNHOFER lines in most of his spectra is not quite correct.

† Proc. Roy. Soc., No. 226, 1883

‡ "Zur Kenntniss der Genese der Gallenfarbstoffe und der Melanine." Sep. Abdr. a. d. Centralblatt für die med. Wiss., 1883, No. 44.

distributed in the animal kingdom, and which I have called *histohæmatins* or tissue hæmatins, so that the biliverdin may in that case also be looked upon as excretory, and at the same time useful for decorative purposes.

A similar instance of a used-up pigment being got rid of in the integument is the occurrence of hæmatoporphyrin in the integument of starfishes and slugs, as I have proved.* This colouring matter can be extracted by digesting the integument in the cold for some hours in alcohol acidulated with sulphuric acid, and it can be easily shown that it is present in the state of hæmatoporphyrin *as such*. The presence again of enterohæmatin in the "bile" of pulmonate mollusks, in that of the crayfish, and in that of *Patella vulgaris*, as I have lately found, and of histohæmatins in various parts of their bodies, is a parallel instance of the presence of an immature respiratory pigment or pigments taking the place of the hæmatin-yielding pigment in the Actiniæ. The latter cannot, however, be looked upon as a colouring matter intended to *carry* oxygen, but rather to *keep* it in combination until it is wanted by the cells for purposes of metabolism. As it is distributed all over the surface of some Actiniæ, the whole body of such an animal may, in a physiological as well as in a morphological sense, be considered comparable to a single organ of a higher animal, so far, at least, as *internal*† respiration is concerned.

In *Sagartia parasitica* the hæmatin-yielding pigment is replaced by a special one,‡ as already referred to, and in every species of Actiniæ, even in those almost destitute of colour, the presence of respiratory pigments has been detected.

In *Anthea cereus*, *Sagartia bellis*, and *Bunodes ballii* the same colouring matter is present not only in the tentacles but in other parts, and from the observations recorded in this paper, it is quite clear that all the chlorophylloid colouring matter in these three species is entirely due to the presence of "yellow cells." It is not within the scope of this paper to enter on a discussion of the nature of these "yellow cells," it will be sufficient to call attention to the facts that whenever present they have been found to possess a cellulose wall and to contain starch. This is in favour of the view held by GEDDES,§ BRANDT, the HERTWIGS, and others, that they are of a vegetable nature, and are symbiotic algæ. Another point in favour of this view is the behaviour of their solutions with caustic potash and soda, which, as I have already stated, distinguishes them from animal chlorophyll and ordinary chlorophyll (of green land plants). The colouring matter itself, as Dr. SORBY and Professor LANKESTER‖ show

* Proc. Birm. Philos. Soc., vol. iii., pp. 378 *et seq.* Another very suggestive connexion between biliverdin and hæmatoporphyrin is furnished by the fact discovered by SORBY, namely, that these pigments occur in birds' eggs. I have also found hæmatoporphyrin in the integument of the earth-worm.

† *I.e.*, tissue respiration. ‡ In the ectoderm, see above.

§ See 'Nature,' January, 1882, for GEDDES's paper and the subsequent letters of Professors MOSELEY and E. P. WRIGHT as to BRANDT'S priority, also P. GEDDES's reply.

‖ See list of chlorophyll containing animals drawn up by Professor LANKESTER, for 2nd English edition of SACHS's 'Botany;' also his paper "On Chlorophyll Corpuscles and Amyloid Deposits of Spongilla and Hydra," Quart. Journ. Micro. Soc., vol. xxii., p. 229, &c.; also note to page 650, *supra*.

(in the case of *Anthea cereus*), is probably identical with chlorofucin. To see if this is the case I have figured the spectrum of chlorofucin in the natural condition in *Fucus serratus* and in alcoholic solution, and on comparison of these spectra with those of the above-mentioned Actiniæ a remarkable likeness is apparent. (See spectra 8 and 9, Chart II.)

As I have already shown, the conclusion of KRUKENBERG* that the colouring matter of the yellow cells of *Anthea cereus* is identical with that of the hepatochromates (= enterochlorophyll) is not borne out by this fact; viz., that in the case of enterochlorophyll no such change is produced by caustic alkalies as is produced in solutions of the colouring matter of the "yellow cells" with these reagents.

Another very remarkable fact noticed should go to support the view of the "yellow cells" being symbiotic algæ, namely, that they appear to cause a suppression of those pigments which in other Actiniæ appear to discharge a respiratory function. In most, if not in all cases, this fact impressed itself strongly on my attention, and I believe the observation is correct. At the same time we must remember that the "yellow cells"—if they are symbiotic algæ—only give oxygen up to the tissues of the animal, which would still require to be *fixed* in the tissues by a combination with something else, such as actiniohæmatin or other pigments; so that too much importance ought not to be attached to the apparent absence or presence of such pigments in the ectoderm, endoderm, or other parts. Their absence too ought not to be concluded from the mere fact that they cannot be detected in certain solutions of these parts, as there is no doubt that they cannot always be got into such solutions even when present.

Besides these pigments of a direct respiratory use, there are others which appear to be of use for decorative purposes, and to this class Professor MOSELEY'S actiniochrome belongs. I always found (with one or two doubtful exceptions) that this is a pigment confined to the tentacles, and, as already stated, it cannot be changed into anything capable of being oxidised and reduced; but whether it is intended for a protective purpose or as a means of attracting prey, further research may decide.

Another kind of pigment is that found in the "eye-spot" of *Actinia mesembryanthemum* (spectrum 9, Chart I.). It is a noteworthy fact that in the eye of *Musca domestica* † a red colouring matter occurs which gives a band covering D. It is also found in other insects' eyes. Possibly, the band in *Actinia mesembryanthemum* denotes that the pigment is capable of absorbing certain rays of light, so as to enable the animal to distinguish light from darkness. The presence of other light-absorbing pigments, and their possible use in some obscure photo-chemical processes in the bodies of Actiniæ, ought not to be overlooked.

The preceding observations have brought to light the following facts among others:—

(1.) That *Actinia mesembryanthemum* contains a colouring matter which can be

* *Loc. cit.*

† *Cf.* KRUKENBERG, *loc. cit.*, he did not notice the band referred to.

changed into hæmochromogen and hæmatoporphyrin, and that it is present in other Actiniæ. It is named *actiniohæmatin.*

(2.) It is not actiniochrome, which is a colouring matter found widely distributed in Actiniæ, which is not changeable into other pigments capable of oxidation and reduction, and is mostly confined to the tentacles.

(3.) A special colouring matter not identical with either is found in *Sagartia parasitica,* which is capable of existing in the oxidised and reduced state.

(4.) In the mesoderm of *Actinia mesembryanthemum,* in other parts of the same Actinia, and in other Actiniæ a green pigment occurs which gives all the reactions of biliverdin.

(5.) *Anthea cereus* (as already known), *Bunodes ballii,* and *Sagartia bellis* yield to solvents a colouring matter resembling chlorofucin, and all the colouring matter which shows this spectrum is derived from the "yellow cells" which are abundantly present in their tentacles and elsewhere. By its behaviour with reagents this pigment can be shown to be quite different from enterochlorophyll, plant chlorophyll, and the chlorophyll of *Spongilla,* or other animal chlorophyll.

(6.) When "yellow cells" are present there is probably a suppression of those colouring matters, which in other Actiniæ appear to be of respiratory use.

(7.) Other colouring matters which give bands in the blue and violet, and spectroscopically resemble lutein, are found especially in the tentacles, but they are not generally soluble in those solvents which dissolve the luteins.

(8.) Pigments whose nature is yet uncertain are also met with, which have been described in this paper.

Explanation of the Spectrum Charts.

CHART I. Plate 69.

Sp. 1. Spectrum of the ectoderm of a brownish-red *Actinia mesembryanthemum.*
Sp. 2. Spectrum of a red *Actinia mesembryanthemum* (ectoderm).
Sp. 3. Spectrum of a reddish-brown *Actinia mesembryanthemum* (ectoderm).
Sp. 4. Spectrum of a red *Actinia mesembryanthemum* (ectoderm).
Sp. 5. Spectrum of a brown *Actinia mesembryanthemum* (ectoderm).
Sp. 6. Spectrum of a brown *Actinia mesembryanthemum* (ectoderm).
Sp. 7. Spectrum of a brownish-red *Actinia mesembryanthemum* (ectoderm).
Sp. 8. Spectrum of a reddish-brown *Actinia mesembryanthemum* (ectoderm).
Sp. 9. Blue "eye-spot" of *Actinia mesembryanthemum.*
Sp. 10. Spectrum like that of alkaline hæmatin, from a rectified spirit and caustic potash extract of the ectoderm of *Actinia mesembryanthemum.*

Sp. 11. The same with ammonium sulphide (hæmochromogen).
Sp. 12. Spectrum of acid hæmatoporphyrin from a solution obtained as described, from *Actinia mesembryanthemum.*
Sp. 13. Spectrum of alkaline hæmatoporphyrin, from the same.
Sp. 14. Biliverdin in alcohol solution from the mesoderm of *Actinia mesembryanthemum.*
Sp. 15. The same solution with nitric acid, early stage of reaction.
Sp. 16. Spectrum of Actiniochrome and another pigment, from tentacles of *Bunodes crassicornis,* &c.
Sp. 17. Glycerin extract of the ectoderm of *Actinia mesembryanthemum.*
Sp. 18. The same after treatment with caustic soda or caustic potash and afterwards ammonium sulphide (hæmochromogen).

CHART II. Plate 69.

Sp. 1. Yellowish-red tentacles of *Bunodes crassicornis.*
Sp. 2. Red ectoderm of *Bunodes crassicornis,* showing the spectrum of the hæmatin-yielding pigment.
Sp. 3. Rectified spirit and caustic potash solution of the same.
Sp. 4. The same on addition of ammonium sulphide (hæmochromogen).
Sp. 5. Glycerin extract of tentacles of *Bunodes crassicornis,* showing spectrum of actiniochrome.
Sp. 6. Solid ectoderm of *Bunodes crassicornis* after digestion in rectified spirit and caustic potash.
Sp. 7. Glycerin extract of the tentacles of some specimens of *Bunodes crassicornis,* showing spectrum of actiniochrome and another pigment.
Sp. 8. Spectrum of *Fucus serratus* in solid state.
Sp. 9. Alcohol solution of the colouring matter of same, showing chlorofucin bands for comparison with those of the pigment of "yellow cells."
Sp. 10. Spectrum of "yellow cells," *in situ,* in tentacles of *Bunodes ballii. Cf.* 8.
Sp. 11. Ectoderm of *Bunodes ballii,* showing presence of a trace of the hæmatin-yielding pigment.
Sp. 12. Absolute alcohol extract of tentacles of *Bunodes ballii. Cf.* 9.
Sp. 13. The same with nitric acid.
Sp. 14. Absolute alcohol extract of ectoderm of *Bunodes ballii.*
Sp. 15. Absolute alcohol extract of tentacles of *Bunodes ballii,* with caustic potash.
Sp. 16. Rectified spirit and caustic potash extract of the ectoderm of *Bunodes ballii,* showing that the spectrum of the pigment of the "yellow cells" is changed by KHO.
Sp. 17. Tentacles of small variety of *Bunodes ballii,* showing actiniochrome.
Sp. 18. Ectoderm of the same.

CHART III. Plate 70.

Sp. 1. Spectrum of endoderm (of body cavity) of a small *Bunodes ballii.*
Sp. 2. Ectoderm of small orange *Sagartia dianthus.*
Sp. 3. Rectified spirit and caustic potash extract of ectoderm of a brown *Sagartia dianthus* (trace of hæmochromogen ?).
Sp. 4. Rectified spirit and caustic potash extract of ectoderm of *Sagartia viduata.*
Sp. 5. Endodermal parts of *Sagartia parasitica* in rectified spirit and caustic potash after addition of ammonium sulphide.
Sp. 6. Rectified spirit and caustic potash extract of ectoderm of *Sagartia parasitica.*
Sp. 7. Thinner layer of the same.
Sp. 8. The same solution with ammonium sulphide.
Sp. 9. Absolute alcohol extract of ectoderm of *Sagartia parasitica.*
Sp. 10. Thinner layer of same.
Sp. 11. The same with sulphide of ammonium.
Sp. 12. Alcoholic solution of this colouring matter with ammonia.
Sp. 13. Spectrum obtained from ectoderm of *Sagartia troglodytes.*
In other parts a second band nearer violet is seen.

CHART IV. Plate 70.

Sp. 1. Spectrum due to "yellow cells" in tentacles of *Sagartia bellis.* *Cf.* Chart II., spectra 8 and 10, and also 8, *infra.*
Sp. 2. Rectified spirit and caustic potash extract of ectoderm of same.
Sp. 3. Absolute alcohol solution of the tentacles of same. *Cf.* spectra 9 and 12, also 9, *infra.*
Sp. 4. The same with nitric acid, early stage of reaction.
Sp. 5. Change of spectrum of an alcoholic solution of tentacles with caustic potash.
Sp. 6. Actiniochrome in the tentacles of *Anthea cereus.*
Sp. 7. An empty tentacle of *Anthea cereus* (*i.e.*, free from yellow cells).
Sp. 8. A tentacle full of "yellow cells." *Cf.* 1, *supra,* &c.
Sp. 9. Absolute alcohol extract of tentacles of *Anthea cereus.*
Sp. 10. The same with nitric acid, early stage of reaction.
Sp. 11. The same with nitric acid, later stage of reaction.
Sp. 12. An alcoholic solution of the tentacles of *Anthea cereus* with *caustic soda.* *Cf.* also spectrum 5, *supra,* also Chart II., 15.
Sp. 13. Rectified spirit and caustic potash extract of ectoderm, &c.
Sp. 14. An alcoholic solution of the same pigment with ammonium sulphide.
Sp. 15. Glycerin extract of the tentacles of a specimen of *Anthea cereus;* it showed a splendid green fluorescence, but this pigment does not occur in every specimen of *Anthea cereus.*

CHART II.

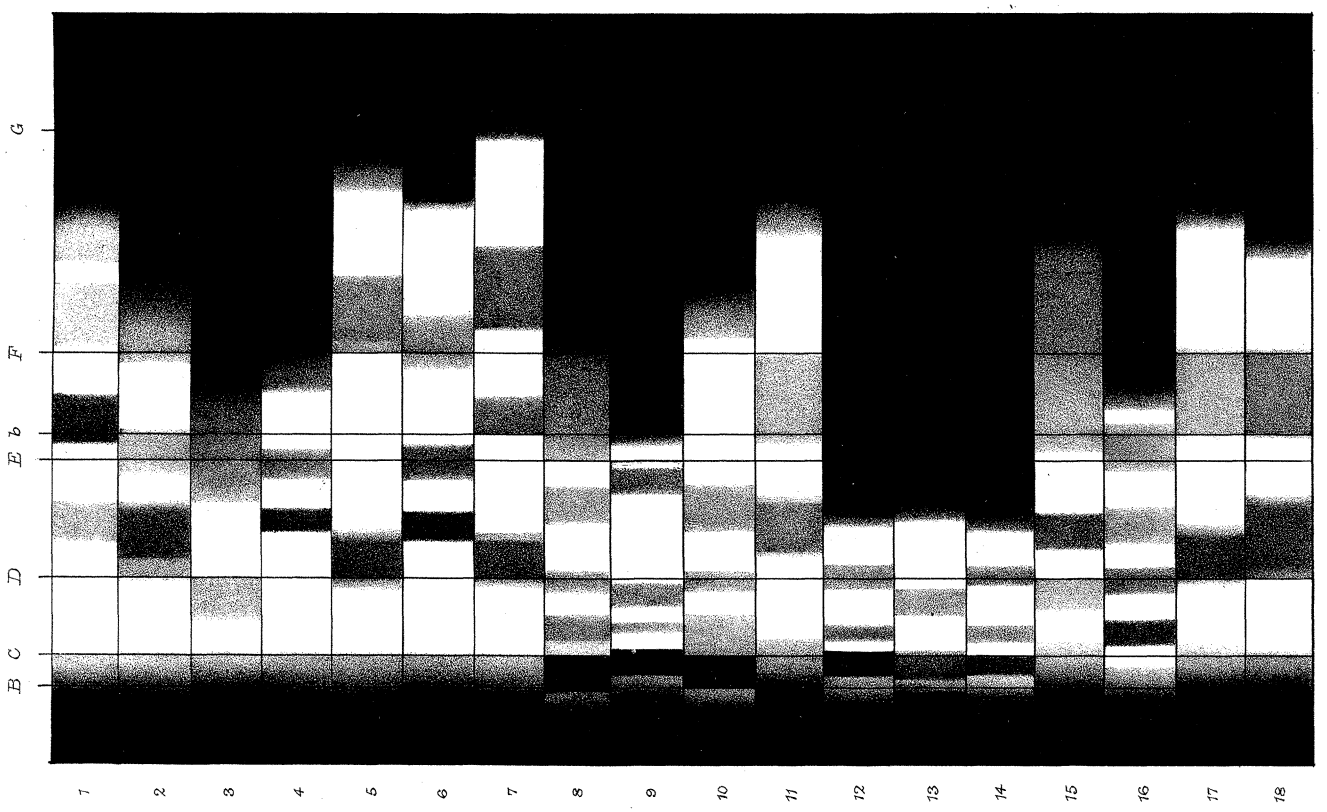
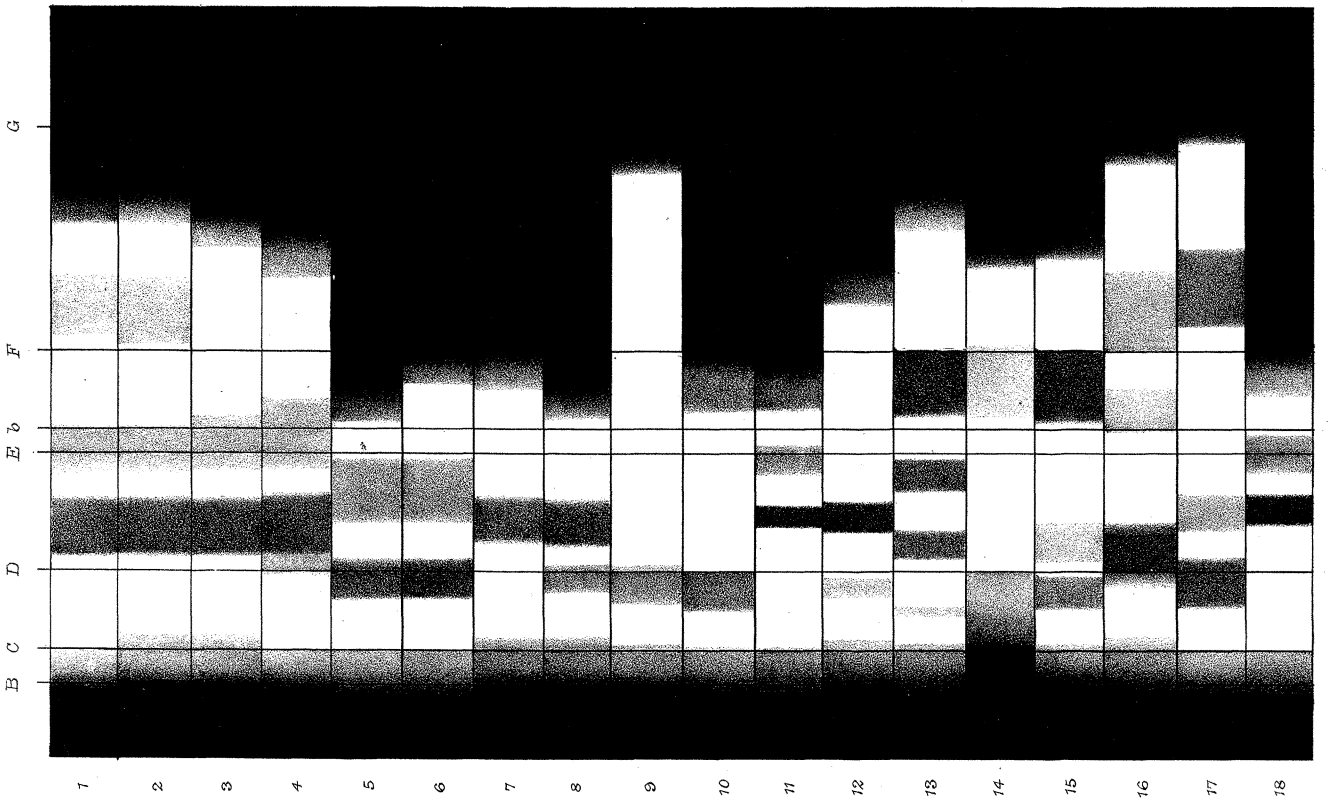


CHART I.



West, Newman & Co. lith.

CHART IV.

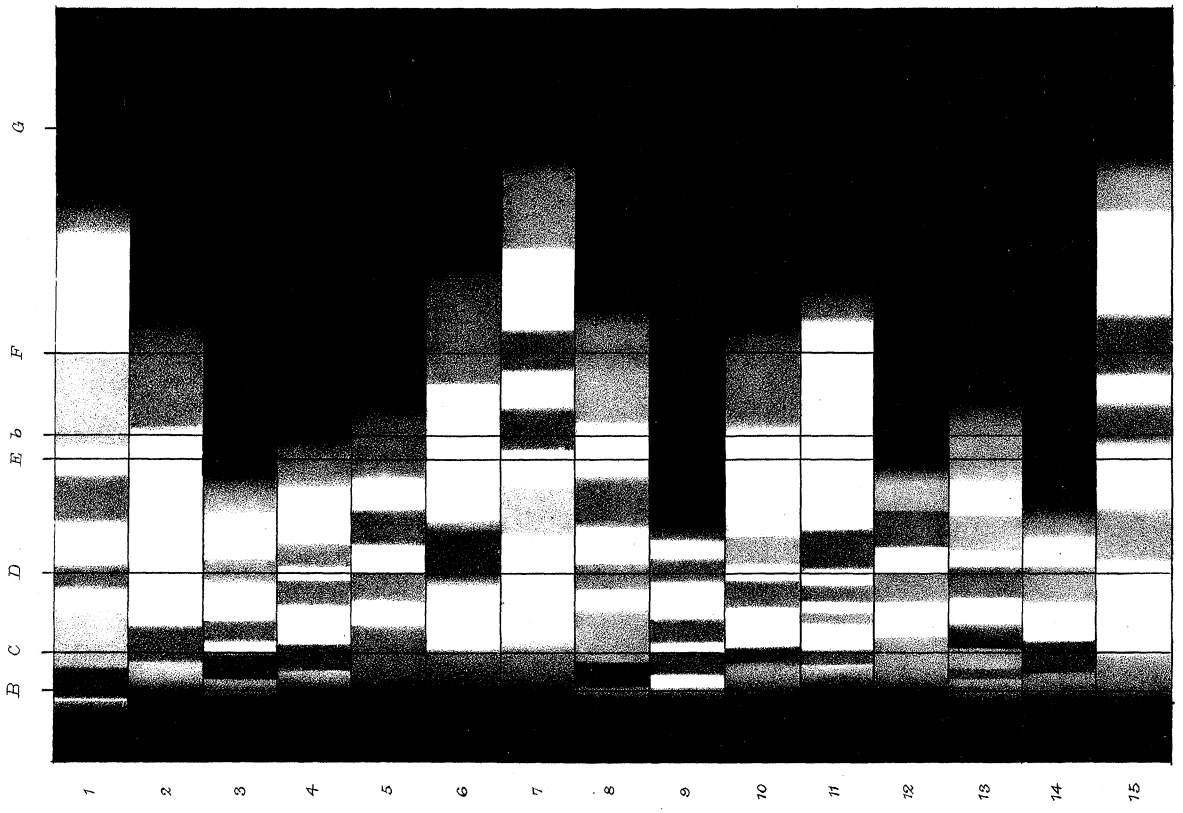
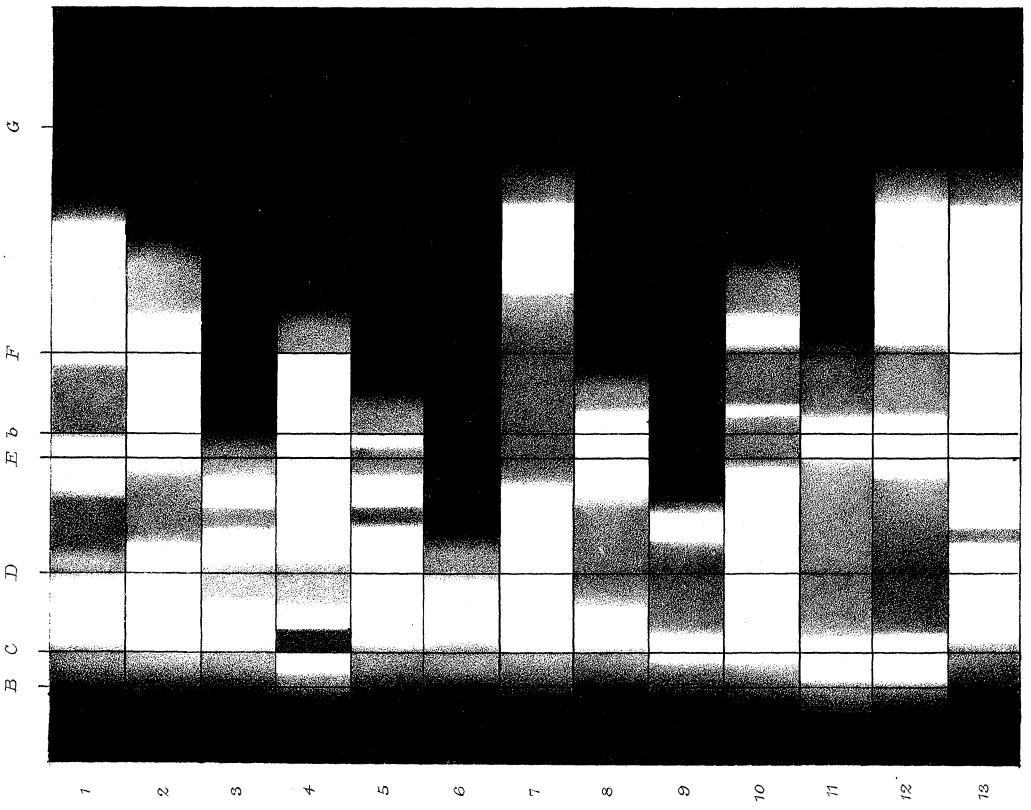


CHART III



West, Newman & Co. lith.