On the Green Pigment of the Intestinal Wall of the Annelid Chætopterus.

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With Plates 34—37.

I. Introduction.

In the year 1864 I obtained specimens of Chætopterus variopedatus, Renier (at that time called C. insignis by Baird), when collecting at Herm in the Channel Islands.

My specimens preserved in alcohol gave to the spirit a strong blackish-brown coloration, and the fluid was observed to have a deep red fluorescence. I showed the coloured fluid to Professor Stokes, of Cambridge, where in 1864 I was an undergraduate, and he rapidly examined it with a direct-vision spectroscope. He pointed out to me the remarkable absorption bands which the fluid caused in the spectrum of light passed through it, and expressed the opinion that these were similar to if not identical with those caused by some solutions of chlorophyll. In view of the fact that the colouring matter was soluble in alcohol and caused a red fluorescence, as well as a banded absorption spectrum resembling that of chlorophyll, Professor Stokes was of the opinion that the source of the colour was probably to be found in chlorophyll swallowed by the marine worm which had been immersed in the spirit.

A year or two later I acquired a Sorby-Browning spectroscope of my own, and became familiar with the absorption spectra of chlorophyll, of hæmoglobin, of turacin, and many

other organic pigments. I satisfied myself that the pigment derived from Chætopterus was in no way connected with the lodgment of particles of green vegetable matter in its alimentary canal, but was due to a strong and abundant blackish-green substance formed in the actual walls of the middle portion of the alimentary tract of the Chætopterus. I supposed that this substance, on account of its solubility, fluorescence, and banded absorption spectrum, must be considered as a 'variety' or 'species' of "chlorophyll."

In 1872 I obtained at Naples specimens of the Gephyrean Bonellia viridis, and was extremely interested to find that they—like Chætopterus—imparted a strong greenish colour to the alcohol in which they were placed, accompanied by fluorescence.

This coloured solution I found also gave a very powerful series of absorption bands when examined with the spectroscope, and I erroneously concluded that this colouring matter too—from Bonellia—must be considered as a "chlorophylloid" substance.

In the first and second editions of the English translation of Sach's 'Botany' (Oxford, second edition, 1882, p. 767) a note is published in which, on my authority, it is stated that "a chlorophylloid substance" occurs in the intestinal wall of Chætopterus and in the integument of Bonellia.

I had observed that the absorption spectrum of the colouring matter of Bonellia, though resembling that of chlorophyll, yet differed considerably from it, and I proposed to myself to make a more careful examination both of it and of the green pigment from Chætopterus, with the use of adequate measuring apparatus and scale in fixing the position of the bands in their absorption spectra.

I was prevented from carrying out my purpose by the loss of my Chætopterus material and the difficulty of obtaining more, and by the pressure of other pieces of work.

It fortunately occurred to me in 1875 to ask my friend Mr. H. C. Sorby, F.R.S., to undertake the investigation of the colouring matter of Bonellia, of which I had a certain quantity.

The result was the interesting and important paper by Mr. Sorby published in this Journal in 1875, wherein he describes the acid, neutral, and alkaline conditions of this pigment, characterises it spectroscopically, and gives to it the name "Bonellein." I shall take the liberty of changing that name in the present paper to "Bonellin."

Bonellin was subsequently studied by Krukenberg ('Vergl. Physiol. Studien,' ii, 1882), and by Schenck (Sitzb. k. Akad. Wiss. Wien,' 72, ii), who did not add anything material to the observations published by Sorby. Krukenberg, indeed, fell into some errors on the subject.

After Sorby's demonstration of the independent and peculiar nature of Bonellin, I was more than ever anxious to re-examine the pigment of Chætopterus, but more than twenty years have elapsed before I have carried out my intention.

In the meantime my friend and fellow-student Moseley provided himself with a micro-spectroscope as part of his equipment when he served as naturalist on the "Challenger" Expedition, and he published on his return in this Journal (vol. xvii, 1877) a very interesting account of a number of colouring matters obtained from marine animals and examined by him with the spectroscope. One of the most remarkable of these was a pigment from the integument of species of Pentacrinus, which occurs in these animals in such abundance that the spirit in which they are first preserved becomes deeply impregnated with it, and will yield the pigment as a powder on evaporation.

Moseley gave the name Purple Pentacrinin to this substance. He showed that its solution in alcohol was fluorescent, and, like Bonellin, could be changed in colour accordingly as the fluid was rendered alkaline or acid. It gave a very definite set of three absorption bands, the position of which were fixed and recorded in a spectrum map by Moseley. The colour transmitted by the acid solution is "an intense pink," that by the alkaline is bluish green, whilst the fluorescence is red. A second equally striking colouring matter was called Antedonin by Moseley, and found by him in deep-sea species of Antedon

(not in the European forms), and also in a Holothurian. This pigment also shows an acid and an alkaline condition, and can be converted from one to the other an indefinite number of times. It is, however, soluble in distilled water, as well as in alcohol.[1]

The Bonellin of Sorby and the Pentacrinin of Moseley indicate the existence of a group of tegumentary pigments in marine animals, which whilst they resemble chlorophyll in their solubility in alcohol, non-solubility in water, in the fluorescence of their solutions, and in their banded absorption spectra, yet differ from chlorophyll or any known derivative of that substance in the exact position and number of their absorption bands, and in their relative stability when exposed to sunlight, but most characteristically in the fact that they undergo a striking change of colour and in the position of their absorption bands, accordingly as the solution is rendered acid or alkaline, whilst the change from the acid to the alkaline state and back again can be effected an indefinite number of times without destruction of the pigment.

I may state at once that the pigment from the intestine of Chætopterus appears to be one of this class of bodies, and I propose to speak of it as Chætopterin.

Whilst I do not propose on the present occasion to attempt a review or classification of animal pigments, I think it appropriate to point out that there are other green tegumentary pigments among Gephyrea, Chætopoda, and Arthropoda which have properties very different from those of the Bonellin group.

I shall only refer to three of these, and but briefly. In the present number of this Journal Professor Herdman describes a new green-coloured Thalassema, which he has been kind enough to dedicate to me. At first one would naturally be inclined to suppose that the green pigment of Professor Herdman's Th. Lankesteri must be identical in nature with

[1] The Holothuria nigra of the Cornish coast imparts a magnificent flame colour to the alcohol in which it is placed, and the solution has a brilliant green fluorescence. The absorption spectrum has not been studied but it gives, I believe, no detached bands.

that of the allied Bonellia viridis. It is, however, quite unlike it. In actual tint Th. Lankesteri is of a much brighter green than Bonellia viridis—tending to what is called apple-green, whilst Bonellia is rather to be described as chrome-green. The colour of Th. Lankesteri is exactly the same as that of Hamingia arctica. I am able to state this as I am, I believe, the only person who has seen and recorded by a coloured drawing this Gephyræan in a living condition. I dredged it in company with the Rev. Dr. Norman, F.R.S., and Professor Bourne, F.R.S., of Madras, at the mouth of Lervik Harbour, Stordoe, Norway, in 1882. Moreover, the green pigment of both Thalassema Lankesteri and Hamingia arctica differs from that of Bonellia in that it is not soluble in alcohol.[1] According to Professor Herdman's observations, the pigment of Th. Lankesteri is slightly soluble in formol. Whether this signifies more than that the water holding the formaldehyde in solution takes up the pigment, and would do so even were the formaldehyde not present, seems doubtful. I have no observation as to solubility in water in reference to the green pigment of Hamingia, but it appears to me highly probable that it is identical with that of Thalassema Lankesteri. In addition to their want of solubility in alcohol, these two pigments differ from that of Bonellia in not yielding a series of detached absorption bands. I determined this in the case of Hamingia, and Professor Herdman has done so for his new Thalassema. Thalassemin does not change its colour when acted on by dilute acids, whilst Bonellin is changed to a rich violet tint.

A second instance of green tegumentary pigments differing from the Bonellin group is presented by Idotea viridis, the Isopod crustacean. The pigment is in this case insoluble in water, alcohol, or benzine. It is of a brilliant grass-green colour, and is not improbably similar in character and origin to the green pigment situated in the skin of some Lepidopterous larvæ, and other adult leaf-frequenting insects. It is time

[1] See Professor Herdman's paper, and accompanying notes by Professors Sherrington and Noël Paton, and Miss Newbigin.

that an effort was made to arrive at a further knowledge of these insoluble pigments.

A third case to which I wish to allude is the green coloration of the blood of some Lepidopterous larvæ. My friend and colleague Professor Poulton has shown ('Proc. Roy. Soc.,' 1885, and vol. liv, 1893) strong reason to suppose that the green colour of the blood in these larvæ is determined by the presence of chlorophyll or of etiolin in the food consumed by them. He has shown that the blood gives an acid reaction; the suggestion made by him is that the green chlorophyll or its immediate antecedent passes through the wall of the alimentary canal from the digested food into the blood in a modified state, which he calls metachlorophyll. Such a passage seems, on the other hand, to be impossible without a very radical change in the chlorophyll, which is itself neither diffusible nor soluble in watery media. It is most desirable that the study of the green pigment in the blood and skin of Lepidopterous larvæ should be carried further. One circumstance which induces me to allude to it here is that Professor Baldwin Spencer, of Melbourne, in his fine memoir on Pentastoma published in this Journal in 1893, vol. xxxiv, p. 31, made a suggestion similar to that made by Professor Poulton in regard to the passage of chlorophyll through the intestinal wall. Professor Spencer found that the perivisceral fluid of Pentastoma was coloured blood-red by hæmoglobin, and he supposes that the hæmoglobin has passed from the cavity of the gut of the Pentastoma through its wall into the perivisceral fluid. Some of the Nematoid parasites of birds have a blood-red perivisceral fluid which has not been examined spectroscopically. Possibly it also is due to hæmoglobin, and might throw further light on the question. It is a noteworthy fact that the suggestion should be made from two separate sources, that non-diffusible substances like chlorophyll and hæmoglobin pass through the wall of the alimentary canal into the blood fluid unchanged or but little changed. There is evidently something here worthy of further investigation. With regard to the supposed occurrence of chlorophyll in the blood of Lepidopterous larvæ, Professor Poulton's spectro-

scopic observations seem to prove conclusively that the green pigment present in the blood is not chlorophyll. In order to prove that it is a direct derivative of the chlorophyll taken in with food into the alimentary canal, it seems to be necessary to study the derivatives of chlorophyll, and to show that by chemical processes a substance can be produced from chlorophyll having the absorption spectrum of Poulton's metachlorophyll, which it has not; having the power of resisting the destructive action of light, which it has not; capable of diffusing through a living membrane, and of existing in solution in an acid albuminous fluid, which it is not; and lastly of changing to an opaque blackish brown pigment when simply exposed to oxygen gas, which it is not.

II. Description of Chætopterin: its Mode of Occurrence and Optical Properties.

Mode of Occurrence.—Dr. Blaxland Benham has kindly furnished me with an account of the mode of occurrence of the intestinal pigment of Chætopterus variopedatus from observations made by him at my suggestion in Mr. Hornell's laboratory in Jersey in the summer of 1896, and on material preserved by him in formol and brought to Oxford. The drawings in Plate 34 are by Dr. Benham, who has also recorded the spectra of Chætopterin and has carried out similar observations on Bonellin in my laboratory at Oxford. I am greatly indebted to him for his assistance in preparing this account of the two pigments.

The body of this strange-looking Chætopod is divided into three regions, as shown in fig. 1. The dark green, almost black-looking pigment is confined to the intestinal epithelium of the middle region. It gives the whole of the inner surface a black appearance, and can be seen through the transparent tissues of the body-wall.

In a transverse section its disposition is seen to coincide with that of the entire epithelial layer of the intestine, as shown in fig. 2. In order to observe satisfactorily its natural

position, the use of alcohol must be avoided since the pigment is dissolved by that preservative. It is, however, insoluble in formol.

Claparède, in his 'Annélides Sedentaires,' has described the pigmentation of the epithelium of this region of the intestine. A careful examination, by teazing fresh material and also by sections of material preserved in formol, shows, according to Benham's observations, that the pigment occurs solely in the form of spherical corpuscles varying in size (fig. 4), and embedded in the protoplasm of the epithelial cells (fig. 3). These granules are not dissolved by alcohol entirely, but a colourless, oily-looking stroma, quite structureless and translucent, of the same shape as the original coloured granule, is left in the cell-body.

Claparède speaks of the pigment in the intestinal wall as "hepatic" pigment. Joyeux-Laffuie ('Archives de Zoologie Expérim.,' 1890) gives a detailed account of the distribution of the pigment-bearing cells in the intestinal wall; he figures the cells and terms them "cellules biliaires." Dr. Benham distinguishes the elongated ciliated cells which contain the green granules from other associated "gland-cells," of which there appear to be two varieties.

It is evident that the terms "hepatic pigment" and "bile-cells" are not open to the same objection when applied to these cells of the enteric epithelium, as when applied, according to the custom of writers of forty years ago, to the brown-coloured tunic of the earthworm's intestine, now often called the "chloragogenous" tunic or cells. The pigmented cells of the intestine of Chætopterus are really of enteric origin, as is the hepatic gland in Vertebrates, whilst on the other hand the chloragogenous tunic is part of the cœlomic epithelium.

It is impossible to suppose, in view of the fact that Chætopterus lives buried in the sand in a large parchment-like tube, that the intestinal pigment can have any function as pigment. On the other hand, it is not unlikely that it may eventually be shown that this green fluorescent "Chætopterin" is really representative of the biliverdin of Vertebrate bile.

An extremely interesting comparison suggests itself with the "entero-chlorophyll" described by Dr. MacMunn as occurring in corpuscles in the livers (gastric glands) of Mollusca and in other Invertebrata ('Proc. Roy. Soc.,' vol. xxxv, p. 370). Dr. MacMunn was probably ill-advised in using the term "chlorophyll" in connection with the substance discovered by him. I have no doubt that he was led by my own erroneous classification[1] of several green pigments in animals under the chlorophyll group. It is not possible to come to a conclusion from a comparison of the absorption spectrum assigned by Dr. MacMunn to his entero-chlorophyll with that of Chætopterin, since Dr. MacMunn's pigment is very difficult to obtain free from admixture with other substances. On the other hand, Chætopterin can be obtained in a fairly pure condition, so far as the admixture of other pigments is concerned, by isolating the mid-region of the body of Chætopterus and preparing the alcoholic solution from that region only. It is true that even so the solution contains fatty matters and other impurities, and that we have not yet obtained Chætopterin either as a pure thoroughly cleansed powder or in the crystalline condition.

An investigation of the chemical properties of Chætopterin has been undertaken at my request by Miss Newbigin in Professor Noël Paton's laboratory, and there is reason to hope that before long we shall obtain this body in a chemically pure state, and learn something as to its chemical constitution and properties, which cannot fail to throw light on its physiological significance and possible relationship to MacMunn's entero-chlorophyll.

The determination of the characters of a body occurring in such definite form in the enteric epithelium of one of the simpler forms of animal life cannot but lead to a better understanding of the physiology of the alimentary canal, and of the

[1] This error of course did not include the green pigments of Spongilla, Hydra, and such ciliate Protozoa as Stentor. In them there is no doubt that the green pigment is chlorophyll, and that it occurs, as in plants, in self-propagating corpuscles.

internal chemical activities of the cells, upon a knowledge of which a true physiology must be based.

Colour and Absorption Spectra of Chætopterin.—The freshly-prepared alcoholic solution of Chætopterin (as obtained from a fresh specimen of the mid-region of the animal's body) is of a blackish green colour by transmitted light (see Pl. 35, fig. 6), and shows a powerful red fluorescence, resembling in colour that of an alcoholic solution of chlorophyll. This solution is found to be neutral in reaction. When examined with the spectroscope it shows four detached absorption bands, the position and intensity of which are represented in Dr. Benham's drawing (Pl. 34, fig. 5, uppermost spectrum), but are more exactly shown in the valuable observations kindly made for me by Professor Engelmann, and recorded in the two charts on Pl. 36.

When the neutral solution of Chætopterin is rendered acid by a very slight addition of HCl, it assumes a fine indigo-blue colour, as shown in Pl. 35, fig. 7. The absorption spectrum is still four-banded, but the position of all the bands is shifted, notably of the two in the blue. Dr. Benham's drawing in Pl. 34, fig. 5, shows this; the exact position and intensity of the absorption is shown by the two dotted lines in Professor Engelmann's chart, Pl. 36. Professor Engelmann finds in a sufficiently thin layer of the coloured liquid a faint "fifth" band at wave length 500, indicated by a dip and rapid rise in the curve traced by the upper dotted line of his chart. In a layer of greater thickness (recorded by the lower dotted line) the differentiation of this band from the absorption on either side of it is (as in Dr. Benham's drawing) inappreciable, excepting by the most careful measurement and comparison.

The acidulated solution may now be rendered alkaline by addition of KHO or NaHO, when it assumes a bright lemon-green colour (Pl. 35, fig. 8). The alkaline solution still exhibits four detached absorption bands, but they are very much in the same position as those of the neutral solution. The difference in the colour of the neutral and the alkaline solution is due, as is shown very clearly by Professor Engel-

mann's chart (Pl. 36), not to a difference in the position of the points of maximum absorption, but to a difference in the position of the points of maximum luminosity, and consequently of the area and graduation of the absorption around its maxima. This is very difficult to represent or record by shaded drawings, but is given with absolute precision by Professor Engelmann's beautiful method of observation and record, of which I will give some explanation below. The alkaline solution can be rendered again neutral or acid, and the process reversed and repeated indefinitely.

III. Colour and Absorption Spectra of Bonellin.

I had been anxious to compare the absorption spectra of Bonellin and Chætopterin for myself, and after I obtained a supply of the latter was unable for some time to procure the former.

I heard, however, in 1896 that Bonellia was flourishing in the beautiful healthy tanks of the Laboratoire Arago, erected and directed by Professor Henri de Lacaze Duthiers at Banyuls-sur-Mer, near Perpignan. I accordingly wrote to that distinguished zoologist, stating my desire to examine living specimens of Bonellia in Oxford. With a kindness and courtesy for which he is universally known and beloved, Professor de Lacaze Duthiers sent to me from Banyuls, by express parcels-service, two bottles of sea water, containing each a magnificent specimen of Bonellia viridis, which arrived in Oxford in a perfect condition of living vigour. I was thus able to examine again the pigment Bonellin, and to satisfy myself as to the position in which it occurs in the body of Bonellia. My best thanks are due to Professor de Lacaze Duthiers, and are here recorded, for his great kindness.

Greef has already correctly described the mode of occurrence of the green pigment of Bonellia. It is distributed in the superficial ectodermic epithelium in the form of very fine granules which give the ectodermic cells a grass-green appearance. It also occurs as fine granules in clusters of subepidermic cells apparently belonging to the connective tissue.

From the specimens received in Oxford, after examination of the histological relations of the pigment, Dr. Benham prepared an alcoholic solution which we proceeded to study by means of the spectroscope, and the application of acids and alkalies. A portion of the pigment in alcoholic solution was sent by me in the spring of 1897 to Professor Engelmann, together with the solution of Chætopterin. I am thus able to give here a very accurate record of the absorption spectra of Bonellin (Plate 37), for the purpose of comparison with those of Chætopterin. It will be seen at once that the two bodies differ entirely from one another in colour and absorption phenomena, whilst agreeing in solubility, fluorescence, and in the exhibition of neutral, acid, and alkaline conditions.

The appearance of the absorption bands of alkaline and acidulated alcoholic solutions of Bonellin, as seen with the Sorby-Browning micro-spectroscope, are drawn by Dr. Benham in Plate 35, fig. 5. The freshly prepared solution of Bonellin is alkaline, of a deep chrome-green colour. It is in this condition that the pigment appears to exist in the skin of the animal (Plate 35, fig. 11). When neutralised the solution assumes a greyish-blue colour (Plate 35, fig. 9). The addition of a small quantity of acid to the neutral solution, changes the colour to a splendid violet (Plate 35, fig. 10). The absorption spectra of these three conditions of Bonellin have been described by Sorby and after him by Krukenberg and by Schenck (who erroneously regarded Bonellin as a form of chlorophyll).

It will be found that the statements of these authors (cited on p. 449) are at variance in minor details with one another, and also with what I now place on record as the result of the observations of Professor Engelmann.

The carefully neutralised alcoholic solution of Bonellin exhibits but four marked or isolated maxima of absorption (absorption bands), as shown by Professor Engelmann's chart (Plate 37). These are as different in position as they well can be from the four bands of Chætopterin. There is no need to refer any further to a possible relationship between these two bodies.

The acid Bonellin also exhibits four, and only four absorption bands, but these do not coincide with any of the four bands of the neutral solution. The alkaline solution presents a six-banded spectrum, and of these six it is remarkable that the strongest, viz. the first and the sixth, coincide in position with those of neutral Bonellin; whilst the remaining four are similar to those of acid Bonellin, but all shifted a little towards the red end of the spectrum.

I am not prepared to discuss here either Sorby's slight divergences from Engelmann's record or Krukenberg's theory of Bonellin and Bonellidin. My principal object is to show how widely Bonellin differs from Chætopterin (though resembling it in general characters), and to present an accurate record of the absorption phenomena of neutral, acid, and alkaline alcoholic solutions of the pigment as obtained from fresh specimens of Bonellia.

It now only remains for me to give some explanation of the method of observation and record of absorption spectra—introduced by Professor Engelmann,—without which the reader will not properly understand the value of Plates 36 and 37.

IV. Measurements of the Absorption Spectra of Chætopterin and Bonellin by Professor Engelmann.

Professor Engelmann kindly offered last year (1896), when I was on a visit to Utrecht, to apply his beautiful instrument for the measurement of the absorption of the luminous spectrum by coloured media to Chætopterin and Bonellin. I was very glad to avail myself of his kind offer, in order to procure a more accurate record of the position and intensity of the absorption bands given by those pigments than is possible with the ordinary micro-spectroscope. The charts forwarded to me by Professor Engelmann as a result of his examination of the solutions which I sent to him are reproduced in Plates 36 and 37.

The instrument used by Professor Engelmann is described by him in the 'Archives de Microscopie.' It is applied to the body of an ordinary microscope, and consists in an arrange-

ment by which the light from a powerful incandescent lamp is passed through two parallel slits, A and B, giving in the field of view of the eye-piece spectroscope two spectra exactly parallel to one another, and of exactly equal intensity of light. A diaphragm is made to traverse the field by the turning of a screw, so as to present for observation a narrow band only of the juxtaposed spectra. The exact wave length of this strip is given by a scale introduced. Thus a width of the spectrum corresponding to a range of only some two or three millionths of a millimetre in wave length and of measured position in the spectrum can be examined, whilst there is on either side absolute darkness. The portion of the strip of light thus studied belonging to the light coming through slit A can be compared, as to the amount of light present, with the identical representative portion of the spectrum belonging to slit B. Under the conditions so far stated, the intensity of illumination (amount of light) of each half of the strip (that belonging to spectrum of slit A, and that belonging to spectrum of slit B) are exactly and sensibly equal. If now there be placed in front of slit A a coloured transparent body, some of the light passing through that slit will be stopped. Suppose the travelling eye-piece diaphragm is adjusted so as to present to the observer a strip of each spectrum corresponding to a wave length which is partially absorbed by the coloured medium introduced before slit A, then the portion of the strip belonging to slit A will be sensibly dimmer than that belonging to slit B.

Now by a micrometer screw Engelmann can reduce the width of slit B until the amount of light coming through that slit is no greater than the amount coming through slit A, obscured as it is by the coloured medium. The amount of movement of the screw required to bring the light of slit B down to the intensity of that of slit A furnishes the measurement of the absorption due to the coloured medium for the wave length under observation (isolated by the travelling eye-piece diaphragm slit).

The micrometer screw is standardised so as to give readings in

percentages of the total amount of light passing through the slit when not obscured, and having an aperture of ·2 millimetre.

The percentage is then written off on the chart by a dot corresponding to the wave-length (right or left of vertical lines of the chart), the percentage itself being given by the higher or lower position of the dot in relation to the horizontal lines.

The following is the report kindly furnished to me by Prof. Engelmann, together with the table of measurements and the charts given in Plates 36 and 37. It will be seen that by the present method it is easy to calculate the form of absorption curve for a greater thickness of solution from the observation of that of a less, and vice versâ, and that this has enabled Prof. Engelmann to apply a satisfactory test to the accuracy of his observations, which it must be remembered depend upon a very delicate comparative judgment of the light intensity of the two adjacent strips of spectrum, one of which is gradually darkened by the turning of the micrometer screw until it is judged to be exactly equal in light intensity to the other.

UTRECHT; 25th April, 1897.

. . . At last I am able to send you the results of the quantitative colour-analysis of your Chætopterin and Bonellin. You will find them in the accompanying tables, and in graphic form on the four charts of curves.

The measurements were carried out with my micro-spectrophotometer ('Zeitschr. f. wissen. Mikroskopie,' Sept., 1888; 'Archives de Microsc.,' xxiii, 1888, p. 82, pl. iv; 'Onderzochingen gedann in het physiol. laborat. des Utrechtsche Hoogeschool.,' 3, xi, 1889, pp. 39—49) with the use of an Auer's incandescent lamp as the source of light. The slit width of the spectrum apparatus was in all cases 0·2 mm. The light intensity thus given without absorption is taken as = 100 for all wave lengths. Measurements were made of the intensity of the light for a large number of λ, after passage through a plane-parallel layer of coloured solution of 5 and also of 2 mm. in thickness.

The measure of this intensity was in every case the width of

the comparison slit, by which, for a given wave length, an apparently equal luminosity in the absorbed and in the comparison slits was given. The apparatus allows one easily to read off a slit-width as small as 0·0005 mm. Every measurement was five times repeated. The number set down on the records is in each case the mean. Their probable error is in general barely more than 1 per cent. of the measured value; only in the outer red and violet, and with very powerful absorption, is the error greater on account of the diminished intensity of light. The course of the absorption curve (as drawn on the charts) will then in all essentials faithfully represent the fact.

An objective test for the criticism of the trustworthiness of the measurement results is afforded by the comparison of the numbers arrived at when two different thicknesses of the absorbing layer are used. If for any wave length the intensity of the original light is weakened to $\frac{100}{x}$ per cent, by passage through a layer of the thickness 1, then for the same wave length the intensity ($= i$) after passage through a layer of the thickness n is given by the formula $i_n = \frac{100}{x^n}$. Accordingly, if we have measured the course of the absorption for a known thickness of layer, we can reckon it also for every other possible thickness of layer. I have carried out the calculation for neutral Bonellin and neutral Chætopterin, and inserted the calculated values in thick type in the tables. The agreement between calculation and observation is amply sufficient, especially when we consider that important alterations in the light intensity value must be brought about by minute changes in the position and breadth of the spectrum strip, the average light intensity of which is being determined, in those parts of the spectrum where there are sharp alterations in the absorption. The greatest care was given on this account to the exact position and borders of the spectrum strip, and every time it was carefully determined whether the wave length scale was exactly in its proper position. The breadth of the spectrum strips, separately

examined as to light intensity, was on the average equal to $\frac{1}{2}$·001 μ wave length. For example, for determining the intensity at $\lambda = 600$ $\mu\mu$, the spectrum strip lying between wave lengths 0·597 μ and about 0·630 μ was isolated by means of the ocular screw diaphragm slit (accordingly the rest of the spectrum shut off). In the outermost red, where the dispersion is too small, spectrum strips of 0·01—0·02 breadth were isolated.

The coloured solutions were examined in small glass chambers of known height, which I had prepared for the purpose by Zeiss. They are to be recommended also for merely qualitative spectroscopic observations on coloured solutions, since one can work with a very small quantity of fluid (a few cubic millimetres). I intend soon to describe them and explain their use more fully. You can get them from Zeiss.

As you will observe, I have analysed a neutral as well as an acid and alkaline solution. All three show characteristic differences, and indeed the colours also appear different to the eye. It is a pity that neither Bonellin nor Chætopterin have been prepared in a chemically pure state, and perhaps cannot be. If they were, one could make exact determinations upon the (clearly very great) influence of the solvent upon the concentration of the solution.

CHÆTOPTERIN.

λ = Wave lengths in $\mu\mu$ ($1 = 0{\cdot}001\ \mu$).

i_n = Intensity of the perpendicularly falling light passing through a plane-parallel layer of n mm. thickness, in percentages of the original light intensity.

a. Neutral alcoholic solution.					*b* = *a*, made acid by HCl.				*c* = *b*, made alkaline by NaHO.			
λ.	i_5.	i_2.	(i_2 calculated from i_5).		λ.	i_5.	i_2.		λ.	i_5.	i_2.	
700	62·5	87·7	(**83·0**)		700	50·4	79·0		700	57·0	75·0	
680	44·2	73·5	(**72·0**)		680	45·0	74·0		680	39·0	65·0	
670	22·5	54·5	(**54·0**)		670	30·0	65·0		670	18·0	52·0	
655	2·4	18·7	(**23·0**)	I. Min.	650	3·5	28·0	I. Min.	655	2·0	19·0	I. Min.
640	11·2	42·0	(**41·5**)		640	7·0	34·0		640	15·0	43·0	
625	30·6	57·5	(**61·5**)		625	29·0	59·0		620	23·0	54·5	
600	14·5	47·3	(**46·0**)	II. Min.	620	27·5	54·0		610	21·5	52·0	
									600	15·0	45·0	II. Min.
580	20·1	52·0	(**52·0**)		615	23·0	51·0		590	18·0	50·0	
570	21·0	53·7	(**53·0**)		597	15·0	44·5	II. Min.	575	17·5	47·5	
560	17·6	50·7	(**49·6**)		575	18·5	50·0		560	14·0	42·2	
535	12·8	43·2	(**44·0**)	III. Min.	560	14·0	44·0	III. Min.	550	13·5	41·0	
					550	16·0	46·0					
520	15·8	48·2	(**48·0**)		540	17·0	48·0		540	11·0	40·0	III. Min.
500	10·9	42·2	(**41·4**)	IV. Min.	533	15·0	44·0	IV. Min.	520	13·5	44·5	
					520	17·0	46·0					
480	14·3	45·4	(**45·7**)		510	17·5	48·5		510	8·5	37·5	
					500	16·5	47·5	V.? Min.				
460	9·8	39·2	(**39·6**)		490	17·0	50·0		500	5·5	32·5	IV. Min.
440	1·6	15·5	(**19·0**)		480	16·0	49·0		480	9·0	36·5	
					470	12·0	—					
420	0·7	9·5	(**12·0**)		460	9·5	45·0		460	4·5	22·0	
					450	3·5	—					
					440	?	24·0		440	2·0	13·0	
					(too little light)							
					420	?	15·0		420	?	5·0	

BONELLIN.

λ = Wave lengths in $\mu\mu$ (1 = 0·001 μ).

i_n = Intensity of the perpendicularly falling light passing through a plane-parallel layer of n mm. thickness, in percentages of the original light intensity.

a. Neutral alcoholic solution.				*b* = *a*, made acid by HCl.			*c* = *b*, made alkaline by NaHO.		
λ.	i_5.	i_2.	(i_2 calculated from i_5.)	λ.	i_5.		λ.	i_5.	
700	74·2	89·8	(**88·8**)	700	80·5		700	77·7	
680	69·6	86·5	(**86·5**)	680	73·5		680	69·7	
655	60·8	83·2	(**82·0**)	645	64·9		650	49·7	
635	1·7	18·0	(**19·6**)	I. Min.	630	48·9		635	10·6	I. Min.
615	47·5	72·2	(**74·2**)		620	16·7		625	23·3	
605	50·4	75·2	(**76 0**)		613	6·5	I. Min.	614	20·3	II. Min.
595	53·7	78·2	(**78·0**)		600	36·9		605	35·2	
585	50·9	75·8	(**76·3**)	II. Min.	590	43·4		595	40·5	
560	62·3	80·3	(**82·9**)		570	38·2	II. Min.	585	37·0	III. Min.
540	53·9	78·0	(**78·2**)		552	39·3		565	39·0	
520	50·5	75·8	(**76·1**)	III. Min.	545	37·3	III. Min.	550	37·3	IV. Min.
510	52·0	77·7	(**77·0**)		535	39·8		540	37·7	
490	23·1	56·8	(**55·6**)	IV. Min.	515	35·6	IV. Min.	530	36·0	
470	45·8	71·0	(**73·2**)		500	38·3		520	33·8	V. Min.
					480	49·9				
450	49·6	73·0	(**75·5**)		460	52·6		510	36·7	
					440	52·1				
430	50·2	73·5	(**75·8**)		433	47·1		490	30·7	VI. Min.
					430	43·3				
					425	40·7		470	45·8	
					420	37·7	V. Min.	450	55·0	

EXPLANATION OF PLATES 34—37,

Illustrating Professor Ray Lankester's Memoir on "The Green Pigment of the Intestinal Wall of the Annelid Chætopterus."

PLATE 34.

FIG. 1.—Chætopterus variopedatus, drawn of the natural size, as seen when removed from its tube. *A*. Anterior or cephalic region. *B*. Mid-region (in which the dark pigment occurs). *C*. Posterior region. *a*. Pinnule (modified notopodium of the 11th segment). *b*. Median sucker formed by modification of the pair of notopodia of the 12th segment. *c*. "Fans" formed by fusion of right and left notopodia in segments 13, 14, 15. *d*. Neuropodia of segments 12, 13, 14, 15. *e*. Right nephridiopore of the 15th segment. *f*. Notopodial cirrhi of posterior segments. *g*. Neuropodia of posterior segments. *h*. Right phosphorescent gland at the base of the right pinnule. *int*. Pigment of the intestinal wall showing through the integument.

FIG. 2.—Transverse section of the body of Chætopterus variopedatus taken at the point marked *int*. in fig. 1. (From a drawing by Dr. Benham.) *a*. Dorsal musculature forming a median crest or ridge. *b*. Transparent integument. *c*. Connective tissue holding the gut-wall to the body-wall. *d*. Cavity of the gut. *e*. Green pigmented epithelium of the gut. *f*. A nephridium in section. *g*. Ventral musculature. *h*. Nerve-cords.

FIG. 3.—Epithelial cells of the gut to show the position of the green granules. From a section made from a specimen preserved in formol so as to avoid the solution of the green granules which occurs when alcohol is employed. *a*. Free surface of the epithelium. *b*. Branched base of an epithelial cell. *c*. Oval nucleus.

FIG. 4.—Some of the green granules detached from the epithelial cells and more highly magnified, showing their varied size and spherical form. (Drawn by Dr. Benham.)

FIG. 5.—Absorption spectra of Chætopterin and Bonellin as seen with Sorby's micro-spectroscope. (Drawn by Dr. Benham.) Besides the shading or absorption in the form of bands of greater or less breadth, the position of the chief Frauenhoffer lines is indicated, and the whole spectrum is divided into thirty-five spaces, the divisions between which correspond to wave lengths ranging from 400 millionths of a millimetre on the right (blue end) to 750

millionths of a millimetre on the left (red end)—a division line being ruled at every point corresponding to the position of a difference in wave length of 10 millionths of a millimetre. In this drawing the dispersion of the spectrum as actually seen is represented, the intervals corresponding to 10 millionths of a millimetre of wave-length becoming increasingly larger as we pass from the red (wave lengths of 750—650 millionths) to the blue and violet (wave lengths of 500 to 400 millionths). But in the charts given in the next two plates, for which I am indebted to Professor Engelmann, the intervals occupied by wave lengths differing by ten millionths of a millimetre are laid down without reference to dispersion at equal distances from one another. The charts in fact correspond to a pure spectrum, whilst the drawing, fig. 5, represents the appearance given by a prism of small dispersion.

PLATE 35.

FIGS. 6—8.—Representation of the colour of the neutral, acid, and alkaline alcoholic solutions of Chætopterin, as seen by transmitted light.

FIGS. 9—11.—Representation of the colour of the neutral, acid, and alkaline alcoholic solutions of Bonellin, as seen by transmitted light.

PLATE 36.

Charts prepared by Professor Engelmann showing the intensity of absorption in different parts of the spectrum of acidulated, alkaline, and neutral alcoholic solutions of Chætopterin. The horizontal lines in the charts—numbered in ten groups of ten—correspond to 100 units of light intensity. The round dots indicate the successive parts of the spectrum observed and measured. The position of the dot in vertical displacement records the percentage of light transmitted. Thus the highest horizontal = 100 per cent., the lowest 0 per cent. or complete absorption. The successive "dot points" of observation are joined by oblique lines, giving thus a continuous but irregular curve of absorption. The vertical lines as shown by lettering on the chart correspond to millionths of a millimetre of wave length. The position of the chief solar lines is also indicated by strong vertical lines on the charts. The upper chart has the record of four distinct solutions. The two records in dotted lines are those relating to experiments with an acidulated solution—in the one case the light was passed through a thickness of the solution amounting to 5 millimetres, in the other case only 2 millimetres were used (of the same solution). [It is not possible in our present knowledge of Chætopterin to say what percentage of pure Chætopterin was present in the alcoholic solution.] The unbroken black lines are the records of similar experiments with an alkaline alcoholic solution of Chætopterin. In

the lower charts two records are given of the absorption of two different thicknesses (respectively 2 millimetres and 5 millimetres) of a carefully neutralised alcoholic solution of Chætopterin. (For further details and the comparison of the observed absorption of the smaller thickness of solution with the theoretical value calculated from that given by the greater thickness, the reader is referred to the text.)

PLATE 37.

Charts prepared by Dr. Engelmann of the observed absorption of the spectrum by acidulated, alkaline, and neutralised alcoholic solutions of Bonellin. Two thicknesses of each solution were made use of, and their absorption recorded. See explanation of Plate 36 and the fuller statements in the text.

Fig. 1.

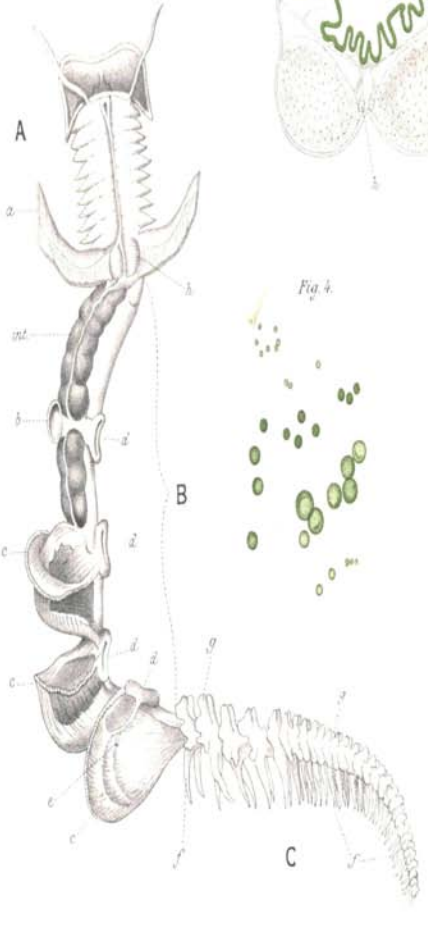


Fig. 2.

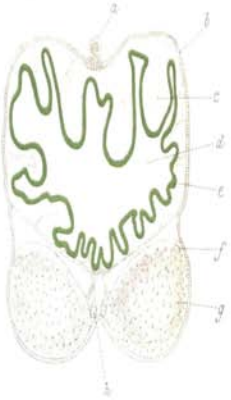


Fig. 3.

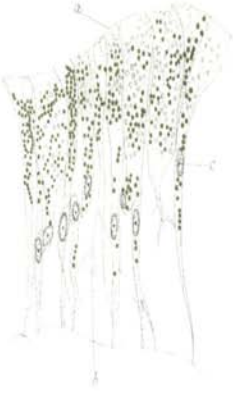


Fig. 4.

Fig. 5.

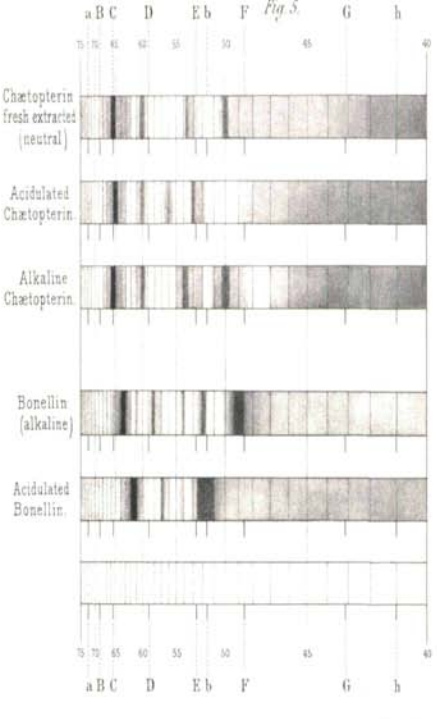


Fig. 6.

Neutral
CHÆTOPTERIN.
(Normal)

Fig. 7.

Acid
CHÆTOPTERIN.

Fig. 8.

Alkaline
CHÆTOPTERIN.

Fig. 9.

Neutral
BONELLIN.

Fig. 10.

Acid
BONELLIN.

Fig. 11.

Alkaline
BONELLIN.
(Normal)

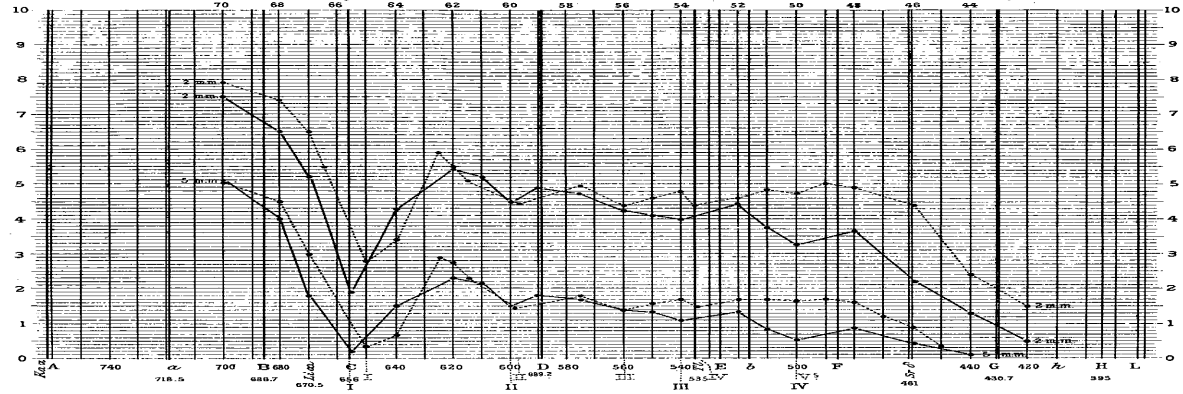
E. R. L. pinxit.

F. Huth, Lith^r Edin^r

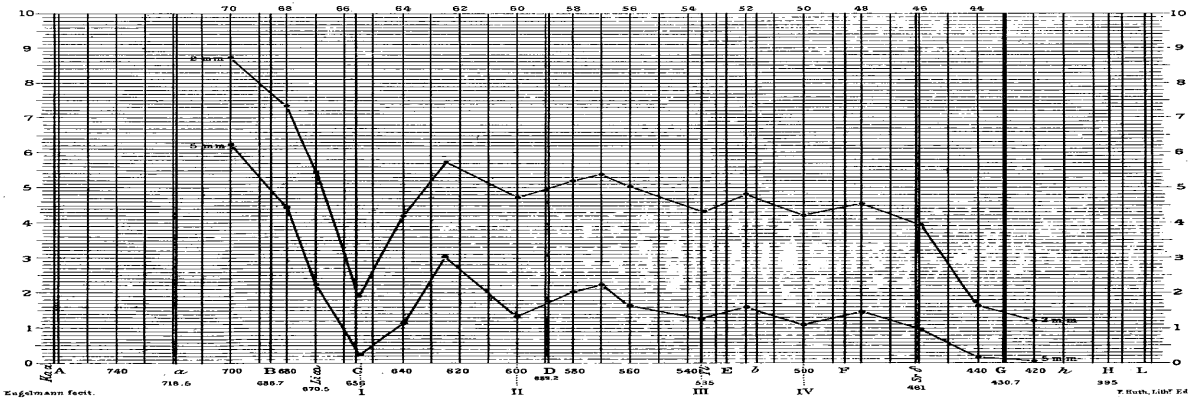
Quart. Journ. Micr. Sci. Vol. 40, N.S. Pl. 36.

CHAETOPTERIN
in Alcohol 96%

acidulated by H Cl. — alkaline by Na OH.



CHAETOPTERIN
in neutral alcoholic Solution.



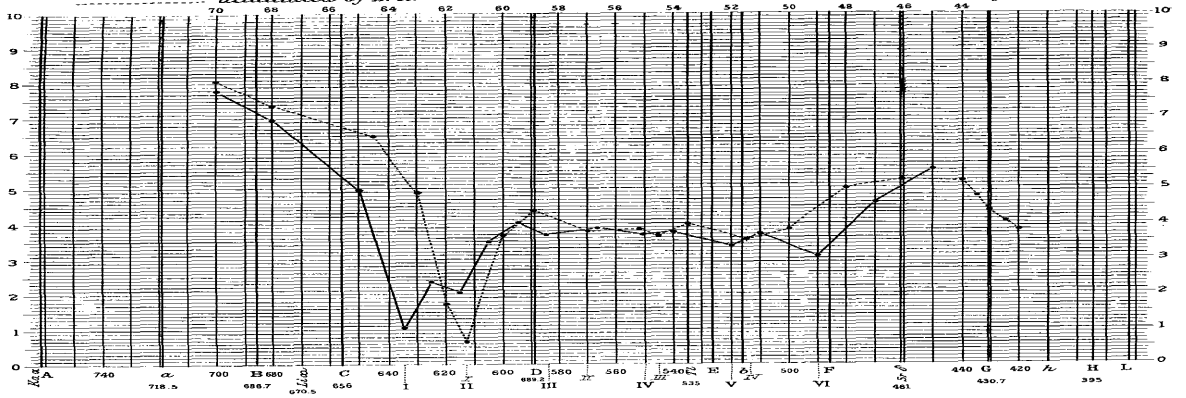
Engelmann fecit.

T. Huth, Libl. Edinr.

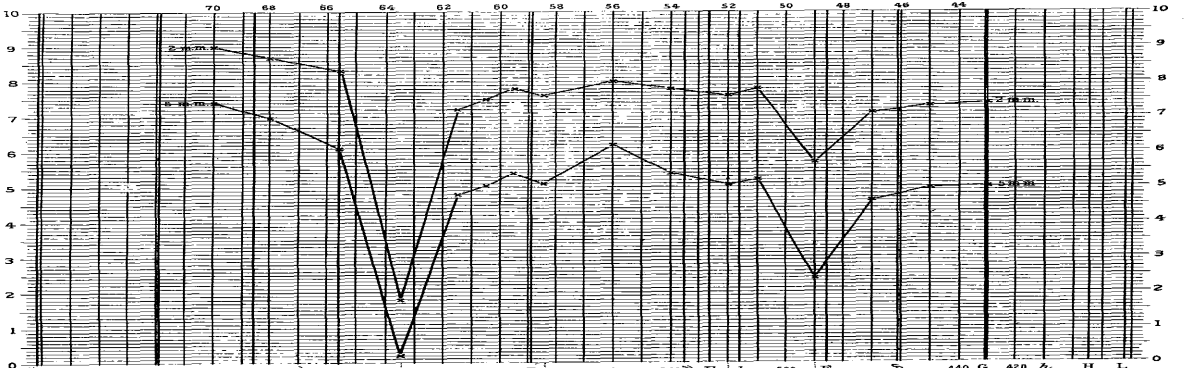
Quart. Journ. Micr. Sci. Vol. 40, N.S. Pl. 37.

BONELLIN
in Alcohol 96%.

acidulated by H. Cl. ---------- alkaline by Na. OH. ——————



BONELLIN
in neutral alcoholic Solution.



Engelmann fecit. Anth. Lith. Edin.