X. *On the Coloration of the Skins of Fishes, especially of Pleuronectidæ.*

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[Plates 53–55.]

The investigations which form the subject of this paper were originally commenced with the object of obtaining some reliable definite evidence on the inevitable question, why is the lower side of a Flat Fish white and the upper side coloured? The answer given to the question necessarily involves an opinion on a great many others, and hitherto the terms of the answer have depended rather on the general views held by those who gave it than on a special investigation of the phenomenon. Conversely it was therefore to be expected that a special investigation of the question would have some importance as a test of the truth of conflicting general views and theories. In order to ascertain how far the action of light was capable of producing pigment on the lower sides of Flat Fishes, a series of experiments has been carried out by Mr. Cunningham, in continuation of one of which an account was published by him in 1891.* But a review of the morphological and chemical relations of the elements and substances of the coloration being necessary for the discussion of these experiments and their results, we have thought it advisable to place this part of the subject first. For Sections III. and IV. of the memoir Mr. Cunningham is alone responsible, and Section II. is almost entirely the work of Dr. MacMunn. Both authors have contributed to Section I. The subjects of the various sections are the following:—

I. Structure and Relations of the Tissue-elements, to which Coloration is due—
  - *a.* Comparative Anatomy,
  - *b.* Histology,
  - *c.* Development.

II. Chemical and Physical Properties of the Pigments and Reflecting Substance.
III. The Artificial Production of Pigment on the Lower Sides of Flounders.
IV. Abnormalities of Coloration in Pleuronectidæ occurring in Nature.

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I.—STRUCTURE AND RELATIONS OF THE MORPHOLOGICAL ELEMENTS OF COLORATION.

(a.) *Comparative Anatomy.*

The various elements in the skins of Fishes which affect the coloration have not hitherto, so far as we have been able to discover, been completely and systematically described or analyzed. Pigment-cells or chromatophores have been frequently investigated, but other elements have received less attention. The most important description of the coloration elements in Fish-skins hitherto published seems to be that of G. POUCHET, in his memoir "On the Changes of Coloration which take place under the Influence of Nerves," published in 1876. POUCHET describes the colour cells in the skin of Pleuronectids and other Fishes under the name of chromoblasts, to distinguish these cells which are characterized by amœboid movements from the pigment cells of Cephalopods, to which he confines the term chromatophores. In this respect we shall not follow him, but shall call the pigment cells of Fishes chromatophores. POUCHET also describes other polygonal cells, which form a layer near the surface of the skin under the convenient name of iridocytes.

He mentions the dense opaque subcutaneous layer to which the silvery brilliance of many Fishes is due, under the name of "l'argenture," and states that it is composed of "lames minces étalées à plat," and states that the same particles distributed confusedly in a tissue act in the manner of transparent substances reduced to a powder and produce a sort of dull silveriness completely opaque. This description applies to the argenteum of Flat Fishes described below, although POUCHET does not mention it in this connection. He recognizes also that the properties of the iridocytes and of the lamellæ of the argenteum are due to the same organic substance, which he says appears to be always of cellular origin, but he does not refer to the fact that this substance is the definite chemical compound guanin, making no mention of the researches of BARRESWIL and VOIT quoted in our next section.

Our own observations have been directed principally to the coloration of the skins of Flat Fishes or Pleuronectidæ, and we shall describe the skins of these in detail, referring to a few other species for the sake of comparison. If a thin superficial slice of the upper side of a Flounder or Plaice is examined under a low power, elements of two kinds are seen, namely chromatophores and iridocytes, forming a beautiful mosaic. The appearance, as seen in the Turbot, has been fairly well figured by POUCHET in the memoir above cited, and the appearance seen in a piece of the skin of the Flounder is represented in Plate 54, fig. 3. The ordinary tissues of the skin of the

Fish, both of the derma and the epidermis, are colourless and translucent in the living condition, and when a superficial slice of the skin is examined in the fresh state these two kinds of elements are extremely conspicuous on account of their colour and opacity, and seem to be set like spangles in a homogeneous translucent material. The chromatophores are distinguished by their colour and irregularity of outline, but the black chromatophores differ in some respects from the yellowish. The chief features of the iridocytes are their regularity of outline, and their great reflecting power; little light passes through them, but when the piece of skin is examined by reflected light they have a very bright spangle-like appearance, although in Flat Fishes they are not very markedly iridescent. Examination of the skin of the Flounder, of fresh specimens and spirit specimens, shows that there is one layer of iridocytes situated outside of the scales, and that the chromatophores are abundant at the same level, scarce in the central layers of the skin. But another deeply pigmented layer is found on the inner surface of the skin, between the skin and the muscles, so that in dissecting off the skin this layer sometimes adheres to it, sometimes remains attached to the muscles. When this layer is examined it is seen to consist of chromatophores exactly similar to those of the superficial layer, and of iridocytes which resemble those of the latter with slight differences. These internal iridocytes are less opaque, and, therefore, have less definite outlines by transmitted light than the others, and they are also much larger. They are also less uniformly distributed than the others, being more closely crowded together in some places than in others; they are very strongly reflective, and more iridescent than the superficial iridocytes.

Examination of the skin of the lower side of the normal Flounder shows that the chromatophores are entirely absent, but the external layer of iridocytes is present and closely similar to that of the upper side of the Fish (Plate 52, figs. 4 and 5). In place of the internal pigmented layer of the upper side there are neither chromatophores nor iridocytes, but a thin perfectly opaque layer of material giving a dead-white reflection. Examined with the microscope this layer presents only a minutely granular structure, and is everywhere uniform and continuous. This layer in other Fishes reflects light in a different manner, so as to produce a silvery appearance, and we shall call it the argenteum. The same distribution of coloration elements is found in the Plaice, *Pleuronectes platessa*, but the argenteum of the lower side is here not so thick and opaque. In the Dab, *P. limanda*, the argenteum on the lower side is still less developed; in specimens about 24 centims. in length it is developed only over the anterior and central regions of the lower side, absent at the peripheral and posterior regions. Moreover, where it is present it is thin, and instead of appearing in a continuous sheet, it is seen to consist of a mosaic of polygonal plates with their edges in contact. The condition of the internal pigmented layer of the skin of the upper side in this species corresponds to that of the argenteum of the lower side, the pigment cells are few and scattered, and the iridocytes still more so. The condition of the argenteum in this species suggests that that layer not only belongs to the same category as the iridocytes, but that it is actually produced by the gradual enlargement and coalescence of separate iridocytes, and we shall show in the next section that the development of the argenteum fully confirms this suggestion. In *Pleuronectes microcephalus* at a length of 28 centims., the condition is much the same as in *limanda*, the internal surface of the upper side contains only scattered chromatophores and iridocytes, while the argenteum of the lower side does not extend over the peripheral region, and is distinctly seen in some preparations to be composed of polygonal plates.

In the Sole (*Solea vulgaris*) the condition is much the same as in *Pleuronectes;* the inner pigmented layer of the upper side is very feebly developed, and accordingly contains but few iridocytes, while the argenteum of the lower side is in large specimens continuous over the whole side, and under the microscope reveals no separate constituent elements. In a large specimen of the Sole which had been for some time in spirit, we found that the skin of the lower side could be stripped off entire, without disturbing the argenteum, which remained attached to the flesh.

In *Arnoglossus megastoma* the colour of the upper side is a light brown, while the lower white side is not an opaque chalk-white, as in the Flounder or Turbot, but translucent. On examination it is found that the outer layer of skin of the upper side outside the scales contains black chromatophores, not very closely crowded, and somewhat thin and translucent when expanded; also orange-yellow pigment in chromatophores, and rounded iridocytes. On the inner surface of the skin on this side there are also streaks and spots of colour composed of black and yellow chromatophores, and scattered iridocytes more brilliantly iridescent than those of the outer layer. On the lower side the outer layer outside the scales contains thin separate iridocytes closely arranged to form a mosaic; these are rather opaque to transmitted light, but by reflected light appear dull white, not silvery or iridescent. On the inner surface of the skin of the lower side we have been unable to detect a trace of argenteum or of iridocytes. The skin of this Fish is remarkable for the large number of prismatic crystals which it contains. They are transparent and have no effect on the coloration; they are situated at various depths in the skin and are not soluble in fresh water.

Before considering more minutely the chromatic elements as they occur in Flat Fishes, it will be convenient to ascertain how far those found in the skins of other Fishes agree with them in general character and distribution. The Gadidæ are supposed to be the nearest allies of the Pleuronectidæ, and we may therefore begin by examining the Whiting, *Gadus merlangus*. In this species the sides have a beautiful iridescence and silvery lustre, the belly is dead white, with scarcely any metallic brilliancy, the back is a dark bluish-grey. Examination of the entire Fish, and with the unaided eye, shows that the chromatophores are densest over the back, less closely crowded over the sides, absent on the belly. If the portion containing the scales is sliced off with a razor and examined under the microscope, it is found that the irisdescent silvery surface is due entirely to a layer of iridocytes on the external sides of the scales. But these iridocytes are very different in form and arrangement from those of the extralepidar portion of *Pleuronectes.* Instead of being polygonal plates distributed at a certain distance from one another, they are short narrow rods or spindles, placed close together side by side, so as to form an uninterrupted sheet. Lying upon them are black stellate chromatophores of the usual kind, and beautiful deep yellow chromatophores, which also exhibit a radiate dendritic form, their branches extending far from the centres. Here, then, we have structures closely corresponding to those found in *Pleuronectes*, only the shape of the iridocytes is very different, and their metallic lustre and iridescence vastly greater. When the superficial layer of the belly is examined these iridescent iridocytes are not seen, their place is taken by a thin layer of granules arranged in parallel wavy lines having little opacity and no iridescence. On the other hand, when the corresponding layer of the back is examined, closely crowded black chromatophores are seen intermingled with yellow, but here also the iridocytes are absent, represented if at all only by minute granules discerned with difficulty between the chromatophores.

After the superficial layer containing the scales has been removed with the razor, the part of the skin left behind is still opaque and silvery, and contains scattered chromatophores. If this portion is sliced off and examined with the microscope, the silvery reflection and opacity is easily seen to be due to a thick layer on the inner side of the skin, next to the muscles. This layer is so thick and opaque that it is difficult at first to make out any structure in it with the microscope, but where it has been crushed a little or broken, it is seen to consist of rods like the iridocytes, but considerably smaller: where the layer is thus made thinner some iridescence appears, but in the normal state there is no iridescence, only silvery reflection. The chromatophores are similar to those of the outer layer. On the belly the argenteum is well developed and continuous, but there are no chromatophores on the inner surface of the skin. On the back the inner chromatophores are well developed, but there is not a trace of argenteum or of iridocytes on the inner face of the skin in the dorsal region.

In *Trigla lyra* examination of the entire Fish shows that the sides are slightly though not very brilliantly silvery, while the belly is white and the back red. When the outer layer containing the scales from the lateral region is examined under the microscope, the silvery layer is seen to consist of strands of opaque tissue, arranged not in separate iridocytes, but in a dense net-work ; the strands themselves consist of rod-shaped minute granules, very much smaller than the rods in the Whiting above described. Over the silvery layer are brick-red chromatophores, for the most part in the form of small round spots, and showing little tendency to branch. On the back the silvery layer is evanescent, represented only by a thin layer of rounded granules, which is by no means opaque to transmitted light, and by reflected light gives only a faint silvery shimmer reminding one of pearl-dust. Besides the red there are large dendritic black chromatophores and patches of diffused light yellow pigment, which contrast strongly with the other two pigments. The skin of the Gurnard is very thick, and, in the dead Fish, more opaque than usual; yet, on the inner side in the lateral region, we find a dense opaque argenteum with a somewhat silvery lustre, which contrasts strongly with the leathery skin. On the back the argenteum is wanting; on the inner side of the skin are found black dendritic chromatophores and patches of diffused yellow pigment, and here and there a thin iridocyte or patch of argenteous matter, of no constant or regular shape, and having a greenish yellow reflection.

On the belly the chromatophores disappear, and the layer of opaque tissue becomes considerably thicker, losing its property of silvery reflection and becoming chalk-white.

In *Trigla cuculus* the coloration elements are closely similar, but the silveriness of the lateral regions is much less marked than in *T. lyra.* The inner argenteum is well developed on the lateral region and the belly, and somewhat more developed on the back than in *T. lyra.* The outer pigmented layer of the pectoral fin in *T. cucul u* is represented in Plate 55, fig. 5, as it appears in the fresh state under the microscope. In this species black chromatophores are scarce in the lateral region, and none came within the part of the field represented. The minute iridocytes have a slightly bluish tint when examined by transmitted light. These minute iridocytes in *T. cuculus* correspond to the granules which compose the strands of tissue in the same region of *T. lyra.*

In *T. gurnardus* the same general distribution of elements is found. The cause of the difference of colour between this and most other species of Gurnard, the former being grey and the latter red, is simply the greater abundance of black chromatophores in the former, and of red in the latter. The sides are slightly silvery, and the elements producing the reflection are somewhat larger than in *cuculus*, while they become smaller on the back. The appearance of the dorsal region above the lateral line in *Trigla hirundo* is shown in Plate 55, fig. 6.

The Gurnards and *Cottus* are placed in the same family, Cottidæ, by most systematists, but they do not resemble one another in their coloration. In the common *Cottus bubalis* there is no argenteum, except on the under surface of the head, and thence backwards over the ventral side in the abdominal region. In the fresh state, if the skin is dissected off in this region it separates easily and the argenteum is left behind, forming a thin silvery film over the muscles. Under the microscope this argenteum is seen to consist of thin irregularly-shaped plates irregularly distributed, in some places contiguous with one another on all sides, in others leaving considerable intervals unoccupied. Here and there a little diffused yellow pigment is seen among the plates of reflecting substance. In *Cottus* there are no scales, except in the lateral line, but, nevertheless, the coloration elements are situated in the superficial layer of the skin. They consist of large black stellate chromatophores, much smaller red chromatophores, which in some parts of the skin are separate and distinct, while in others they form an inextricable network, of a diffused yellow pigment, and of a network of reflecting substance, which is closely associated in most parts with the large black chromatophores. It goes without saying that the different colours of different parts, patches, or spots of the skin of the *Cottus* are produced by the preponderance or exclusive presence of one or other of these elements. The opaque white spots are due to a thick mass of the reflecting substance, the black to an excess of black chromatophores, red and yellow parts to excess of red or yellow pigment. The general colour of the specimens found on the shore or in shallow water is dull grey or brown, but specimens are not unfrequently obtained from deeper water which are bright red or crimson nearly all over the dorsal region. The difference in the latter case is entirely due to the greater abundance of the red pigment which forms a network of lines, and the diminution in the number of black chromatophores. These differences are shown in figs. 1 and 2, Plate 55.

In the fishes of different families thus far examined, we find that the coloration elements are chiefly deposited in two layers in the skin, outside the scales, and on the inner surface of the skin; that where the chromatophores are most developed, namely, in the dorsal region of the body, the reflecting tissue is, to a greater or less degree, evanescent, and *vice versâ;* and, lastly, that where iridescence is developed it is due to the outer reflecting layer, while the inner layer presents either a chalk-white opaque surface or an evenly bright silvery surface. In examining representatives of a few other families, we shall not find these generalizations contradicted except in trifling details.

The distribution of the elements in the Mackerel, for instance, is somewhat different from that in the Fishes previously mentioned. The scales are very deciduous in the Mackerel, and of course when the scales separate, the epidermis and the part of the cutis which covers the scales externally are lost too. A Mackerel obtained as fresh as possible on the fish-quay has lost all its scales except a few loose ones at the base of the fins, and those of the lateral line, which remain firmly attached in the skin. But the brilliancy of coloration of the skin is not apparently diminished, the sides and the belly gleam with the most vivid iridescence, changing from silver to yellow or red gold according to the angle at which the light is reflected to the eye, and the back is marked with the familiar wavy bands of black and green alternately. It is evident, therefore, that the colours of the Mackerel are not principally situated in the outer layer of the skin which contains the scales. Now, the skin of the Mackerel in this condition can be easily separated in large sheets from the body, and it comes off as a transparent tough membrane with very little colour, leaving the colour and iridescence of the Fish more brilliant than ever. Thus the chromatophores on the inner surface of the skin and the argenteum in the same position produce the coloration of the Mackerel, with scarcely any appreciable aid from the outer layer. If we examine the translucent skin stripped off, however, we find that it contains a few chromatophores.

The sockets of the scales are left on the surface of the skin in the dorsal region, and for some distance below the lateral line, as somewhat rhomboidal areas bounded by thickened bands in which black and yellow chromatophores are conspicuous. These bands represent the outer layer of the skin which has been broken through by a large aperture wherever a scale has fallen out. In the central parts of the scale-scars, that is, in the skin beneath the scale, no chromatophores are to be seen. Along with the chromatophores, and especially around the black ones, a little granular reflecting tissue is to be seen, but it is insignificant in quantity. On the scales of the lateral line which remain *in situ* the black and yellow chromatophores are more abundant and uniformly distributed. Also when the few loose scales still adhering to the skin are examined their outer sides are found to contain the same black and yellow chromatophores. It is a curious fact that, although these yellow chromatophores by transmitted light are always yellow under ZEISS, A., or a higher power, and appear dim yellow when the light is cut off from below, yet there is one condition under which they appear distinctly green, namely, when seen with ABBE's substage condenser and his centre-spot diaphragm. The reason of this is probably that they are then seen against a black back-ground.

When the internal layer of the skin in the dorsal-coloured region is examined, black and yellow chromatophores are seen closely crowded together. The black bands are caused by the greater abundance of the black chromatophores and diminution of the number of yellow. In the green bands the two kinds of chromatophores are equally abundant, and the blending of the two colours produces the green. Over the dorsal and lateral regions of the Fish, where the black and green coloration extends, the reflecting layer is also present, the chromatophores lining the inner surface of the layer. But here the argenteum is so thin that the colour of the chromatophores appears through it, and its effect is to give a silvery reflection and some iridescence to the skin. Towards the belly the chromatophores disappear, and the argenteum becomes thicker and much more opaque. The iridescence as well as the silvery glitter are thus, in the Mackerel, produced by the surface of the argenteum itself.

It is interesting to compare the coloration elements in different Pipe Fishes (Syngnathidæ), and trace the ultimate causes of their differences in colour. Thus, the commonest species, *Syngnathus acus*, is of a general brown colour, marked with darker and lighter transverse bands alternately. On the belly the brown colour is absent, the skin is very light, with a golden yellow tinge becoming deeper toward the centre. The ventral surface of the tail is almost colourless, except towards the extremity, where it becomes brown, like the dorsal skin. On examination it is found that there are scarcely any coloration elements on the inner surface of the skin in this species, only a few scattered chromatophores. On the outer side of the bony scutes are the elements which give the Fish its colour. On the sides of the body there are, in this layer of the skin, black chromatophores of the usual kind, in the interspaces between them a variable quantity of orange pigment, not in definite chromatophores, but irregularly deposited in granules and globules; this pigment becomes more red or lighter yellow, according to the thickness of the deposit. The black and orange pigment together produce the brown colour, when blended, as seen by the unaided eye. Beneath the chromatophores there is a loose reticulation, or felting, of anastomosing strands composed of multitudes of minute short rods. This layer has a feeble reflecting power, and is the cause of the greyish opaque white of the skin, which is seen where the chromatophores are scarce. On the dorsal side of the body this reflecting tissue is absent, while the black chromatophores are closely crowded together. On the ventral side the reflecting tissue is well developed, and there is an abundance of the orange pigment, but the black chromatophores are almost entirely absent.

The difference in colour between *Siphonostoma typhle*, a green species of Pipe Fish which lives among the blades of the sea-grass *Zostera*, and affords a typical instance of protective resemblance, and the brown *Syngnathus acus* is, as far as we have been able to discover, entirely due to the difference between the yellowish pigments in the two Fishes. As we have seen, this pigment in *Syngnathus* is an orange yellow which, when blended with black, makes a brown, but in *Siphonostoma* the yellowish pigment is a lemon yellow with no approach to red, and this yellow, blended with black, produces an olive green (figs. 3 and 4, Plate 55). The green of the back of the Mackerel is similarly produced by the blending of black and lemon yellow.

Specimens of *Siphonostoma typhle* are usually green, but we have seen one, a male, which appeared brown. In a green female the ventral side of the tail is as green as the dorsal side, but in front of the anus the colour instead of being dark olive green, is light greenish gold. There is an outer pigmented layer outside the scutes, and, as usual, a pigmented layer on the inner surface of the skin beneath the scutes. The outer layer when shaved off with the razor and placed under a microscope, is found to contain numerous black chromatophores and a large quantity of lemon yellow pigment, filling entirely the spaces between them; the combination of these gives the green colour, and the yellow pigment itself appears green when examined by the light of the centre-spot diaphragm. On the ventral abdominal surface both black and yellow pigments are extremely scarce. No definite iridocytes or reflecting elements are discernible in this outer layer of the skin. On the inner surface of the skin there are disconnected patches of black pigment in chromatophores, with diffuse yellow pigment similar to that of the outer layer, and associated with the black chromatophores some reflecting tissue which is small in quantity, but is very remarkably iridescent, appearing for the most part a deep metallic blue by reflected light. This blue doubtless aids a little in producing the green coloration of the skin, but not much, because there is so little of it, and it occurs only in separate small patches. This inner pigmentation is most developed on the back and sides, where the pigment in the outer layer is also most abundant. There is scarcely any of it on the ventral abdominal skin, the golden colour of which is entirely due to the peritoneum, appearing through the body wall, which is there thin. The peritoneum consists of a beautifully iridescent argenteum on the free surface turned towards the body cavity, and beneath this, towards the skin, an abundance of large black chromatophores and golden yellow pigment; the yellow pigment is most external, so that when the outer side of the peritoneum is exposed, it presents a continuous yellow surface, while the inner surface is a combination of iridescent silver and black. At the dorsal side of the body cavity, when that cavity is laid open, the peritoneum is black without silvery glitter, that is to say, the black pigment is most abundant dorsally.

The Clupeidæ resemble the Mackerel in their pelagic mode of life, and in the brilliant silvery glitter and iridescence of the skin, but minute examination shows that the elements on which the coloration depends are not distributed in exactly the same way. The chief difference is the presence of a thin layer of rod-shaped iridocytes on the inner side of each scale. This layer exhibits the most brilliant iridescence; it is not very opaque because it is so thin, but when light reflected obliquely from it enters the eye, it reminds one of the iris which changes on the burnished dove. The properties are very permanent, and remain unaltered for any length of time in a scale mounted on a slide in glycerine jelly, or FARRANT'S solution. There are also chromatophores, black and yellow, as in the Mackerel, on the outer side of the scales in the coloured regions of the body, namely, the back and sides. On the inner side of the skin, as in the Mackerel, there is a thick argenteum, with a bright silvery surface on the belly and sides; it becomes evanescent towards the back, and there chromatophores, which are absent on the belly, and scarce on the sides in this part of the skin, become abundant.

Atherina is a genus widely separated from the Clupeidæ, belonging to the *Physoclisti*, and approaching somewhat the Grey Mullet in general structure. In its coloration and adornment, however, it presents a peculiar feature, not seen in any of the species yet considered, and only matched in the genus *Osmerus* among the Salmonidæ. *Atherina presbyter* is common on the South Coast of England, where it replaces the true Smelt (*Osmerus*), and is usually known under the name of the latter. In the region of the lateral line of this little Fish there is a very distinctly defined and very brilliant silvery band, about 3 millims. wide, and running the whole length of the body. Dorsal to this band the skin has no silvery brilliance, but is pigmented, the pigment marking out very distinctly the outlines of the scales. Ventral to the lateral band there is no pigment, but a slight silveriness. Microscopic examination shows that the dorsal pigment is confined to the superficial layer of the skin, beneath the epidermis; it consists of black chromatophores, with very little yellow pigment. In this part there is no reflecting tissue either in the form of iridocytes or argenteum. In the ventral region also no iridocytes are seen, the slight silveriness is due to a thin argenteum. The lateral band is due to a very peculiar arrangement of argenteum. The latter is here very thick, and on its inner side are numerous black chromatophores with some yellow pigment, but the strange thing is that the argenteum forms a flattened tube, the width of which forms the bright band. The outer wall of the tube is continuous with the ventral argenteum, and evidently is part of the skin, but in the inner wall only argenteum and chromatophores are seen, no transparent dermal connective tissue. The interior of the tube is occupied by *muscle*, which in the fresh state is seen to be red, while the other muscles of the body are colourless. When a section of the tube is examined a large nerve is seen to run down it close beneath its outer wall, evidently the nerve of the lateral line. But no structure resembling or corresponding to the ordinary tube or sense organs of the lateral line was observed.

In *Osmerus eperlanus* the similar band was found to be due to a special local thickening of the argenteum, and beneath it the muscle was brownish, but the argenteum does not form a tube enclosing a separate lateral muscle, as in *Atherina*.

(*b.*) *Histology.*

It is generally believed and stated by histologists that pigment in the skin is contained in branched connective tissue cells in all respects similar to those which occur in uncoloured parts of the skin. We are unable either to confirm or deny this, since up to the present time we have not succeeded, in spite of persistent attempts, in demonstrating the cellular nature of the chromatophores. We have cut sections of the skin of the various Fishes examined, and have frequently made preparations by the chloride of gold methods, and by the more modern method of steeping in methylene blue and then fixing with picrate of ammonia. We have so far failed to distinguish the connective tissue cells in the skin, but hope to resume this part of the investigation with more satisfactory results at some future time. The present section merely deals with the minute relations of the coloration elements as seen in sections stained or unstained.

The first fact to be mentioned is that the pigment is not exclusively confined to the derma, but occurs in some cases also in the epidermis. A typical instance of this is afforded by the Flounder where, in any section of the upper side of the skin, black pigment is seen in the epidermis. Coloured pigments are not seen in sections, because they are dissolved out by the alcohol in the process of preparation. The black pigment is not contained as a rule in the epidermic cells but between them, sometimes in masses which resemble in section the chromatophores of the derma, and look as though some of the latter had bodily migrated into the epidermis; more usually the pigment appears in granules or thin short threads, which might be sections of branches of chromatophores. In a specimen of the Fish which had soaked for some months in diluted glycerine the epidermis peeled off in patches of some size, and these, when mounted and stained, showed beautifully the condition of the pigment in this layer. It appeared in the form of somewhat injured chromatophores; there were small spots of pigment often with processes round the circumferences, and in some cases the processes were long and branched like those of normal chromatophores. In other cases the processes were interrupted and broken up into dots, but the particles still presented in their arrangement traces of their derivation from radiating branched processes. Many of the spots of pigment were smaller, mere rounded dots, with no indication of processes. The question of the origin of the pigment in the epidermis will be considered subsequently. Pigment in the epidermis occurs also in the Dab (*P. limanda*) but much less abundantly. Particles of black pigment are occasionally seen in the epidermis of other Fishes when sections are examined, but we have met with no case in which the abundance of epidermic pigment is as great as in the Flounder, and in no case is the pigment in this layer of any importance as a factor in the visible coloration, although its presence is an important fact in relation to the physiological history of the pigment in Fish and in higher Vertebrates.

Of the relation of the "coloured" pigments to connective tissue cells or to other ultimate element of the dermal tissue, we can say still less. In some cases, as for instance the Whiting, there are yellow chromatophores having a radiate dendritic structure and as definite an outline as the black chromatophores. In the Flounder and Plaice, on the contrary, the coloured pigment is in the form of a central darker orange spot surrounded by a halo of light yellow which has no definite outline but fades away gradually at a greater or less distance from the centre. The coloured chromatophores, at least in these Flat Fishes, have to some extent the power of contraction and expansion, like the black. It may be provisionally suggested that the coloured pigment is deposited in connective tissue cells as centres, and that in many cases it diffuses from these centres into the surrounding tissue for some distance. Very often the diffused pigment become continuous over a considerable area. In *Cottus bubalis*, for instance, there is a red pigment which occurs in round dots in stellate chromatopores with short processes, or in a uniform network of fine lines in which it is impossible to distinguish the centres of the several chromatophores, and there is also in addition a yellow pigment which is always diffused; it appears in streaks and areas instead of being uniformly continuous, but it is nowhere possible to distinguish chromatophores formed by it. After the Fish has been dead some time this yellow pigment is often found to have separated itself into small spots, but these do not possess radiating processes or definite outline.

The next point to be considered is the relation of the iridocytes and reflecting substances generally to the cellular elements of the dermal tissue. In sections of the skin of the Flounder from the right or upper side the iridocytes are seen to form a single row close beneath the epidermis; they are thicker than might be expected from the appearance presented by their outer surfaces. The section of each is elliptical, and nearly half as thick as it is broad. There is nothing to show that the iridocytes bear a definite relation to the cellular elements of the dermal tissue; they appear to be rather simple deposits in the interstices of the surrounding tissue. The black chromatophores embrace them closely on the outer side, sending their processes between them, and even beneath them, so that in many places the outline of the section of the iridocyte itself is entirely obscured. The internal structure of the iridocyte itself is not merely finely granular or homogeneous, but appears to consist of parallel plates passing obliquely from the outer to the inner surface.

When the iridocytes are examined in a slice of skin cut parallel to its surface, so that their outer surfaces are seen, a small circular aperture is seen in them. Sometimes it is in the centre, sometimes near the edge, sometimes it is on the edge, and then it is no longer an aperture, but an emargination. Occasionally the emargination of one iridocyte is opposite to that of a neighbouring one, as though the two had recently been formed by the division of a single parent element. All these appearances suggest the idea that the circular aperture is really a nucleus—the nucleus of the original cell in the body of which the opaque substance has been deposited. POUCHET, in fact, speaks of the appearance of the circular aperture as due to a spherical nucleus; he is describing the iridocytes in the skin of the upper side of the Turbot. In the Flounder the iridocytes measure about 10 by 20 μ in breadth and length by 5 μ in thickness.

The section of the iridocyte shows a narrow elongated central space surrounded by the opaque substance, which is transversely striated as though consisting of thin plates placed parallel to one another, and vertical to the surfaces of the element. In superficial aspect the opaque substance is seen to form groups of parallel lines, the direction of each group forming angles with those of other groups. Presumably these lines or rods are the edges of the plates seen in section. POUCHET's description of the same appearance in the iridocytes of the Turbot is as follows:—Within the cells are seen "des corps particuliers oblongs, mesurant 1 sur 2 μ environ, paraissant quelquefois renflés à leurs extrémités, placés les uns contre les autres et affectant par groupes une disposition parallèle dans l'intérieur de la cellule." The same author mentions that on the fins of the Turbot, at the extremities of the rays, the iridocytes, instead of a compact polygonal form, have a branched form, presenting broad processes, which, coming into contact with those of their neighbours, form a net-work. He considers that this structure makes still more evident the relation of the iridocytes to the "éléments du tissu conjonctif," which we presume means the connective tissue cells. The same form and arrangement of the iridocytes occurs also in the skin of the fins of the Flounder, but the processes are scarcely comparable to those of chromatophores or those of ordinary connective tissue cells, so that it is by no means certain that these stellate iridocytes are modifications of the latter. In other Fishes, *e.g.*, *Cottus bubalis*, the reticular arrangement of the reflecting substance occurs in all parts of the skin to the exclusion of separate iridocytes; but in such cases it is by no means evident that the substance is contained in connective-tissue cells. The difficulty of connecting the iridocytes with these cells is still greater in such cases as the Whiting and Herring or Sprat, where the former have the form of short rods or prisms lying side by side in uninterrupted contact. Here there is no appearance which suggests nuclei, and no question of either polygonal or branched bodies suggesting cells. The same remark applies to nearly all the Fish mentioned in this paper, except the Pleuronectidæ, of which family the polygonal regularly arranged iridocytes are characteristic.

Indications of *any* relation to cellular or other histological elements are still less to be seen in the argenteum. It is very tough, so that uninjured sections of it are difficult to obtain. No nuclei appear in it after staining. A circumstance which is probably of some importance in relation to the histology of this layer is that in the Mackerel the black chromatophores which are on its inner or deeper side are not confined to its surface, but their processes penetrate its substance, and some of the chromatophores are imbedded in it.

(*c.*) *Development.*

It is well known that chromatophores, both black and coloured, appear in the development of Teleosteans at a very early age, namely, long before the larva is hatched. The appearance of the chromatophores in the newly hatched Flounder, and up to an early stage of the metamorphosis, when the little Fish is 10·5 millims. long, is figured in my 'Treatise on the Sole,' Plate 17, figs. 3–5. Up to the stage last-mentioned, iridocytes and argenteum are wanting, in fact the reflecting substance in any form is entirely absent, both from the skin and the peritoneum; there may be some in the eye, but that organ is here excluded from consideration. On the other hand, in young Flounders, 15 millims. long, in which the metamorphosis has been completed for some time, iridocytes are present in considerable abundance both in the upper and lower sides, but the argenteum has not yet appeared. On the upper side the iridocytes at this stage are more abundant than on the lower, the converse of the relation in the adult condition; but even on the upper side they are not uniformly distributed, but are crowded together in the neighbourhood of the lateral line, and along the intermuscular septa, absent from large areas in other parts of the skin. On this side also, the iridocytes are more abundant in the region of the body cavity than elsewhere, and beneath the skin in this region plates of opaque substance can be imperfectly discerned, which probably are situated in the peritoneum. On the lower side of the body at this stage, the iridocytes are only present in the region of the fin-muscles towards the anterior end of the body, absent elsewhere; the chromatophores have not entirely disappeared from this side. In a slightly earlier stage, when the little Flounder is only 12 millims. long, but after the left eye has reached the right side of the head, there are no iridocytes on the lower side, except a very few on the ventral border of the operculum, while, on the upper side, they are fairly abundant over the head, the region of the body cavity, and the immediate neighbourhood of the latter, but absent from the rest of the body surface. The development of the iridocytes in the skin begins therefore very soon after the metamorphosis is complete, and takes place at first more rapidly on the upper side than on the lower. At the length of 15 millims. the reflecting substance begins to appear in the peritoneum. The presence of the iridocytes in the skin of the lower side causes no opacity in the appearance when the Fish are examined with the unaided eye in the living or fresh condition; the skin appears translucent, not, of course, perfectly transparent as in the larva. The iridocytes can only be detected by the microscope. As the Fish grows older and larger, a white opaque substance begins to appear, not gradually all over the lower side, but in certain regions. In the experiments described in another section of this paper, the lower sides of the living Fish, from the stage of metamorphosis onwards, were frequently noticed and examined, as seen by reflection in the mirror through the glass on which the Fish rested. The first part to become opaque was the area corresponding to the body cavity; the opacity was confined to this region until the Fish was about 2·1 centims. long. After this the opacity appeared in regions outside this limit, appearing at first along the lateral line and the intermuscular septa, whilst the intermediate areas remained opaque. The opacity then gradually extended over the lower side of the body, becoming uniform and continuous at a length of about 7·3 centims. The development of the opacity does not correspond exactly to the size of the Fish, some becoming uniformly opaque while at a slightly smaller size than others, but it is more definitely related to the size of the Fish than to its age, or any other quality. The specimens in the experiments were all collected at the same time, and differed very little in age. But in their rate of growth the individual variation was very great. For instance, on July 18, 1892, the condition was noted as seen with the unaided eye, of thirty-five specimens reared from the previous spawning season. Only one of them exhibited uniform opacity over the whole of the lower side, it was 7·3 centims. long. Another was quite as large, but the opacity was only partially developed in it chiefly along the lateral line and the septa. The smallest were 2·1 centims. long, and in them the opacity was confined to the region of the abdominal cavity, and the rest were of various intermediate sizes, and various intermediate conditions in respect of this character.

This development of opacity, with dull whiteness of surface, is entirely due to the development of the opaque reflecting substance which is deposited on the inner surface of the skin and in the peritoneum, forming the argenteum. As was stated above this substance begins to appear in the peritoneum first, namely, when the Flounder is 15 millims. long. It causes here a uniform opacity, being developed equally in all parts, not along certain lines as in the skin. Careful microscopic examination shows that for some time after the reflecting substance has appeared in the peritoneum, there is none on the internal surface of the skin; the opacity of the abdominal region at this period is due only and entirely to the argenteum of the peritoneum. The latter consists at first of distinct polygonal plates separated from one another by thin transparent lines, and reminding one of the tiles of a tesselated pavement, or the divisions of a map. These plates are much larger than the iridocytes of the skin, measuring ·04 to ·05 in their longest diameter, and ·02 to ·03 in their shortest. The outlines of these plates are very sharp and definite, but the shape is variable, no two being exactly alike. They resemble the iridocytes of the outer layer of the skin in appearance, but are more regularly arranged, and more closely approximated. They exhibit like the iridocytes, a transparent nucleus-like spot towards the centre; their structure is finely granular.

The smallest specimens of the Fish in which the argenteum of the inner layer of the skin was detected was 5·6 centims. long. Here the deposit, like that of the peritoneum just described, consisted of polygonal plates placed edge to edge, and separated by thin transparent lines. The longest diameter of the largest of these plates was ·02 to ·03 millim. while the longest diameter of the iridocytes in the outer layer of the skin did not exceed ·01 millim. The deposit was present only over the abdominal region and in the neighbourhood of the lateral line anteriorly, absent over the rest of the lower side of the Fish. In a piece of the skin examined after being mounted entire and unstained in Canada balsam the plates exhibited the same structure as the superficial iridocytes. There was a clear spot resembling a nucleus, and the rest of the plate exhibited groups of parallel lines, some in one direction, others in others. In this specimen the peritoneal argenteum still showed a division into separate plates, but the division was less distinct than in the earlier stage described above, and no clear spot could be seen in the plates; it was evident that the originally distinct plates were coalescing into a continuous deposit. In section the plates at the inner surface of the skin exhibited a structure quite similar to that of the iridocytes near the outer surface, namely, a striated structure, the striæ being in close juxtaposition and obliquely transverse to the long diameter of the section.

The first traces of the argenteum on the inner surface of the skin of the lower side are well seen in preparations from young Brill 8 and 9 centims. long. In transverse sections the similarity of structure between the iridocytes of the outer level and the inner level is well seen. The differences are these: the external bodies are smaller, more numerous, and more uniformly distributed; while the internal are larger and fewer, several of them occurring in close proximity in one place, none at all in another. The transverse striæ described in the Flounder are well marked in the Brill both in the internal and external bodies. In a piece of the skin mounted flat in Canada balsam unstained the two layers of iridocytes can be separately seen by focussing at the corresponding levels. The distribution of the outer is related to that of the scales, they are more numerous over the central regions of the exposed portions of the scales, scarce along their borders. On the other hand, the deeper iridocytes which belong to the argenteum are arranged in rows, they are elongated in shape, and touch one another by their extremities; the rows again form a loose net work with irregular meshes.

It is evident from the facts thus elucidated, that there is no difference in kind between the two structures which have been distinguished as iridocytes and argenteum. The argenteum in its primitive condition in the Flounder is a collection of iridocytes. But no case is yet known in which the superficial iridocytes develop into a continuous layer of argenteum. We cannot say at present why the reflecting substance is abundantly deposited in the skin near both surfaces, and not at all at intermediate levels, nor why the deeper layer often increases till it becomes continuous and of considerable thickness, while the more superficial layer remains always in the form of separate iridocytes.

There are interesting cases, however, in which the argenteum developed at an early period of life diminishes or disappears at a later period. It has long been known that the various species of *Motella*, which in the mature state live at the bottom and hide themselves under stones and in holes and crevices, are in the young state active pelagic little Fishes, living at or near the surface. In the pelagic state they are silvery, in the mature state dark and dull coloured. In a section of a young *Motella tricirrata* 2 centims. long, a thick deposit of reflecting substance is seen in the skin at the sides of the body, it is absent on the back and becomes thinner towards the edge of the belly. This deposit consists of superimposed flat laminæ, and to it is, of course, due the silvery brightness of the little Fish. On the other hand, in a specimen of the same species 21 centims. long not a trace of reflecting substance can be detected either in the form of iridocytes or otherwise, either near the outer surface or at the inner surface. This suggests a relation between the presence of the reflecting substance and the conditions of life of the Fish, which will be more fully considered in another section of this paper.

II.—Chemical Composition and Spectroscopic Properties of the Pigments and Reflecting Substance.

The reflecting substance which composes the iridocytes and argenteum of the skins of Fishes is guanin, the optical properties of which are very remarkable. The presence of guanin in Fishes' skins was first demonstrated by Barreswil and Voit, about 30 years ago. This important physiological fact was reached indirectly by the analysis of the peculiar substance used for the manufacture of artificial pearls. These pearls are made chiefly in Paris from material which goes under the names of "blanc d'ablette" and "essence d'Orient." *Ablette* is the French name for the Bleak, a fresh-water Fish whose specific name is *Alburnus lucidus*, and from the scales of which the substance is prepared. Barreswil* examined this substance and found that it was insoluble in water, in ammonia, and in acetic acid, soluble in sulphuric, nitric, and hydrochloric acid; that the salts from these solutions had a characteristic crystalline form; that the sulphuric combination was easily decomposed by water; that the nitric solution, on evaporation, yielded a yellow compound which was transformed by potash to a red substance, that the nitric salt was precipitated by nitrate of silver. In all these, and various other properties the substance resembled the guanin of Bodo Unger, isolated from guano in 1845.

In 1863 Voit published in Von Siebold's 'Süsswasserfischen von Mittel-Europa'

* 'Comptes Rendus,' vol. 53, p. 246. 1861.

a similar conclusion concerning the nature of this substance, called in Germany "Perlenessenz," having examined it without any knowledge of BARRESWIL'S work. VOIT deals more fully with the matter in a later communication published in 1865.* In this paper he summarizes the investigations that had previously been made into the nature of the crystals contained in cells in the scales and other organs of Fishes, and determining the metallic lustre of these parts. Besides the material obtained from the scales of the Bleak, glittering crystals from the air-bladder of *Argentina sphyræna* are also used for making artificial or "Roman" pearls. VOIT re-examined both these substances and found in the former guanin and lime; he held that the lime was not in the form of phosphate but was in combination with the guanin. He found that the substance prepared from *Argentina* consisted of guanin somewhat contaminated with fat, but containing no inorganic matter.

According to MOREAU ('Poissons de la France,' 1881) the "essence d'Orient" obtained from the Bleak is the silvery substance which clothes the internal face of the scales, and which is formed of small acicular crystals. We have not ourselves examined the Bleak, but it is evident from this description that these acicular crystals correspond in properties and in position with those of the Herring and other Clupeoids. MOREAU adds that to obtain the best product only the ventral scales are taken, because these are free from the greenish pigment which occurs in those of the back.

In 1880 KÜHNE and SEWALL† published an account of their researches upon the eyes of Fishes, especially of *Abramis brama*, the common fresh-water Bream. They found opaque reflecting granules in the retinal epithelium, which were found to consist of pure guanin; but, on the other hand, they found that the brilliant lustre of the argentea of the iris in *Abramis* and other Teleosteans consisted of guanin combined with lime (*guaninkalk*), and they found the same compound in the tapetum of Elasmobranchs, forming the granules called ophthalmoliths by DELLE CHIAJE.

The only examination in any detail of the distribution of guanin in various species of Fishes that has preceded our own is that of EWALD and KRUKENBERG.‡ These investigators state that in Fishes, as in Reptiles and Amphibia, the guanin is contained in connective tissue cells, in some cases in fine crystalline plates as a lime compound (*guaninkalk*) (Teleostei, Ganoidei, Cyclostomata), in others in the pure condition, and then it is only dead white, and has no metallic lustre (skin of Selachians). They found it, in the various Fishes examined, in the external skin, the scale-pockets, the subcutaneous connective tissue, the fasciæ of the muscles, in the air-bladder, the gall-bladder, and the peritoneum. The authors point out various peculiarities in the distribution of the guanin in various species. They mention that the ventral skin usually contains more guanin than the dorsal; but that in *Solea*

* "Ueber die in der Schuppen und der Schwimmblase von Fischen vorkommende irisirende Krystalle." 'Zeitsch. f. wiss. Zool.,' vol. 15.

† 'Untersuch. a. d. Physiol. Inst. Heidelberg,' vol. 3, p. 221.

‡ Zeitschr. f. Biologie,' vol. 19, p. 1, 1883.

platessa, which probably is a misnomer for the Plaice, the darker surface turned towards the light is much poorer in this substance than the whitish lower side. They remark that the Mackerel and *Caranx trachurus* are also exceptions to the rule, the external blackish or steel-blue parts of the skin in these Fishes containing considerable quantities of guanin. It will be seen from the description we have given of the distribution in the Mackerel that this is a mistaken conclusion, the guanin in the subcutaneous argentea being more abundant ventrally than dorsally, while the quantity in the outer layers of the skin is comparatively insignificant. The authors point out that there is no constant relation between the quantity of guanin present in the peritoneum and the quantity in the skin; in *Zeus faber* the substance is pretty equally present in both, while in *Cepola rubescens* the peritoneum is rendered brilliantly silvery, and the skin, except on the head, contains no guanin at all.

Few observations have been made on the pigments of Fishes' skins. The most remarkable of them are those of GEO. FRANCIS,* described in a letter to 'Nature' in 1875. According to these observations, which have never been confirmed, the blue-green colour of certain brilliant Fishes, living in St. Vincent's Gulf, South Australia, namely, *Odax radiatus, O. frenatus, O. Richardsonii*, and *Labrichthys Richardsonii*, is due to a pigment which is soluble in water and in sea-water, but destroyed by heat, chlorine, acetic acid, ammonia, and alcohol. Sulphuric acid precipitates it, but does not destroy it. Its spectrum resembles that of chlorophyll. Both *Odax* and *Labrichthys* belong to the family Labridæ, or Wrasses, of which nearly all the members are brilliantly and conspicuously coloured, especially the tropical forms. No observations on any species of Wrasse are included in the present paper.

KRUKENBERG† has examined the red pigment of the tail of *Luvarus imperialis*, which he found to be situated in the form of granules in the epithelial cells (epidermis), and to be tetronerythrin (or zoonerythrin), a distinctly characterized pigment belonging to the class of lipochromes, and found in diverse divisions of the animal and vegetable kingdoms. He also‡ examined the pigments of *Cyprinus auratus, C. carpio, Barbus fluviatilis, Muræna Helena, Belone rostrata, Scorpæna scrofa, Solea vulgaris*, and *Mullus barbatus*. He found tetronerythrin in *Cyprinus auratus*, the gold-fish; also in *Cyprinus carpio*, mixed with a yellow lipochrome. In the other species he found lipochromes differing from each other occasionally in minor points. He failed to isolate or even to obtain in solution the peculiar green pigment from the scales, bones, &c., of *Belone rostrata*. Some years ago Professor RAY LANKESTER§ examined *Belone*, and also the Mackerel, Wrasse, Red Mullet, &c., with a negative result as regards absorption bands.

* 'Nature,' vol. 13, p. 167.

† 'Vergl. Physiol. Studien,' 1te Reihe, 4te Abth., 1881, pp. 32–37.

‡ '*Ibid.*, 2te Reihe, 2te Abtheil., 1882, and 3te Abtheil., 1882.

§ 'Journ. Anat. and Physiol.,' 1869, p. 119.

The general results of our own observations are as follows. The only pigments found in the skin were lipochromes and melanins, and neither could be obtained pure. A striking uniformity is perceptible in the wave-length measurements of the absorption bands of solutions of the lipochromes of allied species. For instance, in the Pleuronectidæ (see Table, p. 787) with the exception of those from *Arnoglossus megastoma*, all the measurements agree closely. Even in Fishes of widely separated species a close agreement in the position of the bands is apparent from the Table. Other points of interest are brought out by the Table; thus we see that the colour is of less importance in determining identity than the position of the bands. Here it may be remarked that inspection of a skin with the naked eye, or even with the microscope, may lead one to suppose that two pigments are present, for instance, a yellow and a red, but, on spectroscopic examination, it is found that the red colour is due to greater concentration of the same pigment, which in thin layers may be yellow, and in thick, red. Sometimes, however, two pigments, a yellow and a red, may be present. It is found when such is the case, as in *Nerophis æquoreus*, *Cottus bubalis*, and *Pleuronectes flesus*, that the yellow can be separated from the red by first extracting the skin with alcohol, and then with ether, the former taking up the yellow pigment, the latter the orange or red.

The fact ascertained by KRUKENBERG that the pigment of the Gold Fish, *Carassius auratus*, is tetronerythrin was confirmed, and the same pigment was found in the fins of *Trigla cuculus*, but not in the skin of the body, which contained another red lipochrome. The residue left by evaporation of the lipochrome solutions always gave a blue or green coloration with nitric and sulphuric acids, but gave no marked reaction with iodine in potassium iodide, although sometimes the reaction was obtained by using SCHULTZE's fluid. This negative result with iodine is often noticeable in the case of animal lipochromes.

In all cases the melanins, the pigments contained in minutely granular form in the black or brown chromatophores, were found to be very refractory towards solvents. No connection could be traced between the lipochromes and the melanins, and in the integument of a young *Alligator Mississipiensis* which showed a yellow and a black pigment the yellow was found not to be a lipochrome, so that here at least the melanin could not have owed its origin to a lipochrome.

The muscles of Fishes, as is well known, contain but little hæmoglobin. But in one or two cases it was found in such a position as to have some effect on the external appearance. In *Arnoglossus megastoma* some reddish spots visible through the integument were found to be due to hæmoglobin in the underlying muscle. In the tubular lateral silvery band of *Atherina presbyter* the enclosed muscle was found to be coloured by hæmoglobin, in places replaced by myohæmatin. In *Osmerus eperlanus* the argenteum of the lateral band is not tubular, but the muscle beneath the band is coloured by myohæmatin, limited to the breadth of the band.

The beautiful metallic appearance of the iris in Fishes is due to crystals of guanin.

This so-called argentea was examined microscopically and chemically, and wherever a silvery argentea was present, crystals of guanin were accountable for the appearance, while, where a golden appearance, as in *Trigla hirundo*, was seen, a yellow or orange lipochrome was found deposited over the guanin layer (*vide* Plate 55, fig. 12). The crystals of guanin in the argentea of the eye occur as fine needles arranged in sheaves. The guanin may easily be obtained by extracting those parts which contain it with dilute hydrochloric acid. It can be obtained as crystals by adding ammonia to the solution, but the crystals are very small and easily pass through filter paper. The silvery peritoneal layer, the silvery air-bladder, the subcutaneous reflecting layer, or argenteum, the white or silvery parts of the skin, and the argentea of the eye, all owe their appearance to guanin. The iridocytes in the skin of Flat Fishes, and the iridescent prismatic crystals in that of others, are composed of guanin. If dilute hydrochloric acid be allowed to run beneath the cover-glass, the crystals melt before it, and the guanin disappears. If the solution in this acid be evaporated down, and the residue dissolved in strong nitric acid and evaporated again over the free flame to dryness, a yellow residue is obtained which, when touched with caustic soda solution, becomes red; heated, becomes purple. To prove whether calcium is present with the guanin, as has often been stated, apparently on insufficient evidence, the guanin was dissolved out of the air-bladder of the Herring with dilute hydrochloric acid. The air-bladder contains no crystals of calcium phosphate as the skin often does. The solution of guanin was evaporated to dryness, the residue incinerated, and the ash treated with sulphuric acid on a glass slide under the microscope. Not a single crystal of calcium sulphate was obtained. But it was noticed in the course of these observations that very large prismatic crystals occur in the skin of many Fishes, *e.g.*, *Pleuronectes microcephalus*, *P. limanda*, *Arnoglossus megastoma*, *Cottus bubalis*, *Solea variegata*, *Clupea harengus*, *Trigla gurnardus*, reminding one of the crystals of ammonic magnesium phosphate met with in urine. These dissolve in dilute hydrochloric acid, and by the usual tests it was proved that they are composed of calcium phosphate. In *Solea variegata* some of these large crystals measured as much as 106 μ by 29 μ, in *Arnoglossus megastoma* 264 μ by 96 μ, down to 36 μ by 12 μ.

No sufficient evidence is available on the question of the extent to which guanin deposits occur as elements of coloration in the skins of Invertebrates; EWALD and KRUKENBERG* state that the spindle-shaped crystalloids in the brilliant silvery sklera of *Sepia officinalis* and *Loligo vulgaris* do not consist of guanin, and that it is likewise absent from the iridescent skin of these and other Cephalopods (*Sepiola Rondoletii*) which owes its metallic appearance to the fine striation of the cells. We have, however, demonstrated that in one Invertebrate chalk-white markings in the skin are due to guanin. *Antiopa cristata* is a beautiful Nudibranch Mollusc with a number of dorsal papillæ. Each papilla has a conspicuous chalk-white tip, and there are similar opaque white patches on the skin of the body. Under the microscope

* 'Zeitsch. für Biologie,' vol. 19, p. 1, 1883.

these marks were found to consist of iridescent granules in the skin, and these granules were found to be soluble in caustic potash and in hydrochloric acid. The solution in the latter responded to the guanin test with nitric acid and caustic soda.

We proceed now to give the details of our observations on the skins of Fishes from the chemical and physical point of view.

Pleuronectes flesus—the Flounder. A greenish part of the skin shows, in ZEISS' microspectroscope, a shading from about λ 485 to λ 515, but it is very indistinct. Extracted with alcohol, the skin yielded a yellow solution, in which a band is dimly seen, from about λ 496 to λ 475. The yellow residue left on evaporation of this solution was soluble in chloroform, the solution showing a band from about λ 504 to λ 477; in bisulphide of carbon with a band from about λ 526 to λ 499, and in other lipochrome solvents. An ether extract of the skin was of a deeper yellow and left, on evaporation, an orange lipochrome which was soluble in the usual solvents, and gave the usual reactions in the solid state. The measurements of the bands of the solutions are given in the Table.

No crystals were observed in the skin in this species. In the argentea of the eye, which round the margin of the pupil is slightly golden, guanin occurs in needles arranged in bundles, over which an orange-red lipochrome is deposited.

Pleuronectes platessa—the Plaice. Although one would expect to find two lipochromes in the integument of this species only one could be extracted, which was of a yellow colour. The red appearance of the pigment in the centres of the chromatophores is apparently due to greater thickness of the deposit. The solutions of the lipochrome showed only one band, the measurements of which are given in the Table.

Pleuronectes limanda—the Dab. The skin contains a comparatively small amount of yellow pigment. Only one lipochrome of a yellow colour, with a greenish tinge, was extracted by solvents, and was evidently identical with, or closely related to, the orange one found in *P. flesus*, as can be seen by consulting the Table. Why a yellow pigment from one fish should appear to be identical spectroscopically with a more orange coloured one in another is not clear. It would seem that mere colour is of less importance than spectroscopic characters.

Besides the iridocytes very minute crystals of guanin are found at the surface of the skin and exceptionally a large prismatic crystal of calcium phosphate.

In *Pleuronectes microcephalus*, the Merry Sole, as it is called at Plymouth, are found in the skin large prismatic crystals which respond to the tests for calcium phosphate. The coloured pigment, or lipochrome, yields a yellow solution, of which the bands are given in the Table.

In *Solea variegata* a yellow and a red pigment are seen in the coloured chromatophores, but only one pigment could be extracted (see Table of wave-lengths). Large and small prismatic crystals occur abundantly, the former consisting of calcium phosphate (*a*, fig. 1), the latter probably of guanin.

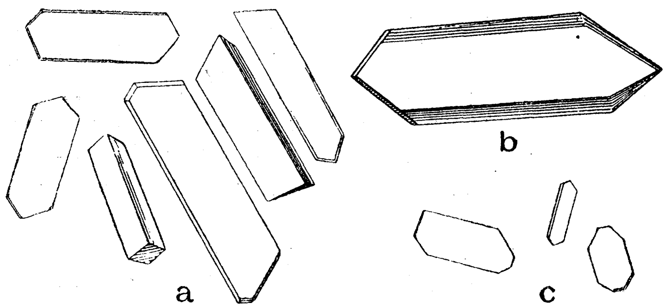
TABLE of Wave-length Measurements of Absorption Bands of Solutions of Lipochromes in Skins of Fishes.

(In millionths of a millimetre.)

	In ether.	In chloroform.	In carbon bisulphide.	Colour.
Pleuronectes flesus	494 — 471 458 — 441	504 — 477 467 — 450	520 — 494 488 — 467	Orange
Pleuronectes platessa	496 — 471	504 — 475	..	Yellow
Pleuronectes limanda	494 — 467 458 — 441	501 — 477 464 — 446	509 — 483 471 — 453	Orange yellow
Pleuronectes microcephalus	496 — 469 455 — 438	501 — 475 466 — 448	517 — 494 488 — 467	Yellow
Solea variegata	493 — 469 460 — 445	501 — 477 466 — 448	523 — 496 490 — 469	Reddish
Arnoglossus megastoma	483 — 464 451 — 435	496 — 473 462 — 444	..	Orange yellow
Trigla cuculus	481 — 460 448 — 432	490 — 469 455 — 438	509 — 485 473 — 455	Orange
Trigla hirundo	481 — 462 450 — 433	494 — 471 460 — 443	509 — 488 473 — 456	Orange
Trigla gurnardus	479 — 462 448 — 432	491 — 469 462 — 444	506 — 485 473 — 455	Yellow
Cottus bubalis	499 — 475	501 — 475	..	Yellow
Cottus bubalis	481 — 462	496 — 469 460 — 443	511 — 488 477 — 458	Reddish
Scomber scomber	477 — 458 448 — 432	488 — 469 457 — 440	504 — 481 469 — 451	Yellow
Syngnathus acus	477 — 458 448 — 432	490 — 467 458 — 442	506 — 483 473 — 455	Orange yellow
Siphonostoma typhle	479 — 460 446 — 430	492 — 469 458 — 442	506 — 485 471 — 453	Greenish yellow
Nerophis æquoreus	479 — 460 446 — 429	490 — 467 457 — 440	511 — 488 475 — 457	Reddish
Nerophis æquoreus	494 — 469	496 — 473	..	Yellow
Clupea harengus	481 — 462 450 — 435	492 — 471 460 — 443	511 — 485 473 — 453	Yellow
Atherina presbyter	479 — 460 448 — 432	490 — 469 460 — 445	509 — 485 471 — 453	Yellow
Osmerus eperlanus	492 — 467 458 — 442	501 — 477 467 — 450	523 — 496 490 — 469	Yellow
Zeus faber	479 — 460 448 — 432	491 — 469 460 — 445	509 — 485 471 — 453	Yellow
Gasterosteus spinachia	481 — 460 446 — 430	492 — 471 460 — 443	511 — 485 473 — 453	Reddish yellow
Carassius auratus	506 — 473	520 — 477	Shading at 552 517 — 483	Red

The light brown skin of *Arnoglossus megastoma* exhibits some reddish spots which were found to be due to deposits of hæmoglobin in the underlying muscle, so that here hæmoglobin plays a subordinate part in the coloration of the skin. The melanin is not abundant, an orange-yellow lipochrome is extracted by solvents. The large prismatic crystals (*b*, fig. 1), abundant in both the coloured skin of the upper side and the white of the lower, are composed of phosphate of calcium.

Fig. 1.



Large prismatic crystals of Calcium phosphate from the skins of various Fishes: *a*, from Solea variegata; *b*, from Arnoglossus megastoma; *c*, from Clupea harengus.

Trigla cuculus.—The presence of guanin in the reflecting layer, in the white skin, and in the argentea of the eye was determined by the usual tests. Spherical crystals, reminding one somewhat of leucin, which occur at the surface of the skin, consist of radiating needles of guanin. Under the microscope the red pigment in round chromatophores and the diffused yellow pigment appear very distinct. Under ZEISS' microspectroscope the reddish integument gave a band from λ 480 to λ 505. The alcohol extract of the skin was pale yellow and showed no band; on evaporation a pale yellow residue was left which was soluble in chloroform, the solution showing no noticeable band. On the other hand, an ether extract, while up to a certain depth it simply absorbed the violet end of the spectrum, to λ 520, when more concentrated showed two bands, the measurements of which, with those of the other solutions, are seen in the Table. This lipochrome responded to the usual tests and was orange-red in colour.

The fins, on the other hand, contain tetronerythrin, the ether solution of which was of a fine red colour and strongly absorbed the violet end of the spectrum. In a diluted solution a broad shading became detached in the green from about λ 517 to λ 464. The residue from the solution was of a brilliant red colour and soluble in the usual lipochrome solvents. It became dark green, when touched with a drop of SCHULTZE's liquid, dark blue with nitric acid, blue and blue-green with sulphuric acid. This tetronerythrin occurs in definite chromatophores in which granules are apparent.

Trigla hirundo.—The reflecting layer contains very small prismatic crystals of guanin—looking like bacteria. The argentea of the eye has a golden appearance, caused by the presence of guanin in needles arranged in bundles, above which an orange-coloured lipochrome occurring in granules and oil-globules can be seen in Plate 55, fig. 12. No crystals of calcium phosphate were detected.

The skin under the microspectroscope showed a band from λ 480 to λ 505, belonging to a lipochrome which readily dissolved in ether and the usual solvents, but alcohol extracted only the merest trace of pigment. This lipochrome in solution is gamboge yellow, and gave the usual reactions with nitric and sulphuric acids, but not with SCHULTZE's liquid.

Trigla gurnardus.—In addition to the usual elements large crystals are present, found to consist of phosphate of calcium. Under the microspectroscope the skin showed a band from λ 480 to λ 505, as in the preceding species. Extracted with alcohol it yielded no pigment; with ether it gave a yellow lipochrome soluble in the ordinary solvents, and giving the usual reactions with nitric and sulphuric acids, but not with SCHULTZE's liquid. For measurement of the bands see Table. Here, as in the preceding species, only one lipochrome could be detected by extraction, although under the microscope two distinct pigments seem to be present. The skin, the reflecting layer, in which the guanin occurs in small needles, and the argentea in which it is also seen as needles, all gave the guanin reaction distinctly.

Cottus bubalis.—Two lipochromes can be extracted from the skin, doubtless corresponding severally to the two pigments seen *in situ.* The spectroscopic bands of the two, one yellow in solution, the other reddish, are given in the Table. Guanin was detected in the various parts of the skin examined, and is to be referred to the network of reflecting substance. The large prismatic crystals which occur in the skin gave the reactions of phosphate of calcium.

From the skin of the Mackerel, *Scomber scomber*, a yellow lipochrome was obtained, corresponding to the yellow pigment described *in situ*, giving the bands indicated in the Table. When portions of the silvery reflecting layer or argenteum were dissolved in dilute hydrochloric acid, the solution evaporated, the residue incinerated, and the ash tested on a glass slide under the microscope with sulphuric acid, no calcium sulphate crystals could be seen. The substance consisted of guanin, not combined with calcium. In all parts of the skin, as well as in the reflecting layer, the guanin reaction was easily obtained.

The skin of *Syngnathus acus* yielded an orange-yellow lipochrome, corresponding to the orange pigment seen *in situ* (see Table). Guanin corresponding to the scanty reflecting substance was found.

The skin of *Siphonostoma typhle*, under ZEISS' microspectroscope, showed a band from about λ 480 to λ 505. The slight spectroscopic difference between the single lipochrome of this species and that of the preceding is seen in the Table. The skin contains guanin, of which, also, the peritoneal argenteum consists.

The skin of *Nerophis æquoreus*, the banded Pipe Fish, gives under the microspectroscope a band between λ 480 and λ 510. In the dorsal fin, a red lipochrome, giving a dull band in the green, occurs in chromatophores which are shown in Plate 55, fig. 7.

On extraction with ether, a lipochrome is obtained which gives nearly the same measurements as that of other Pipe Fishes. Besides this, traces of another yellow lipochrome were extracted by alcohol. Attempts were made to extract the melanin from the skin of this and other Pipe Fishes, but it was found extremely refractory towards solvents; its solution in caustic alkalies showed no absorption bands.

In the peritoneal argenteum, the guanin occurs mostly in granules which respond readily to the usual guanin reactions; besides these granules, abundance of crystals occur, whose form is best made out with a power of 800 diameters; they are triangular, with the apex of the triangle removed, and measure from 7 μ in their long diameter to 5 μ, and from 5 μ in their short diameter to $2\frac{1}{2}$ μ. Guanin was also detected in various parts of the skin.

Clupea harengus.—The greater part of the skin is free from yellow pigment, though containing abundance of oil in colourless drops. The dorsal part of the skin under the microspectroscope gave a band from λ 480 to λ 510, and it yielded to ether a yellow lipochrome soluble in the usual solvents (see Table). The argenteum and the iridocytes on the inner sides of the scales were tested and found to be guanin. Under the microscope the needles of the argenteum are seen to dissolve before hydrochloric acid, and in the resulting solution no calcium could be detected. The silvery appearance of the air-bladder is also due to a layer of guanin in long needles. When a portion of the air-bladder is placed in dilute hydrochloric acid, the silvery appearance soon disappears, more quickly if heat be applied. On evaporating such a solution, and incinerating the residue, no calcium could be detected by microchemical reactions. A mere trace can be detected when present by the formation of calcium sulphate crystals on the addition of sulphuric acid. Very fine crystals can be obtained by taking a solution of the guanin of the air-bladder in dilute hydrochloric acid, neutralizing with ammonia, and filtering off the precipitate. Examined with a $\frac{1}{12}$th immersion objective the crystals appear as in Plate 55, figs. 13 and 14, *i.e.*, in minute spherical crystals and in prisms.

Large prismatic crystals occur on the surface of the scales and in other parts of the skin, and these dissolve in dilute hydrochloric or strong acetic acid; the solution tested for calcium and for a phosphate gave a positive result.

In the dorsal region of *Atherina presbyter* occurs a yellow lipochrome, of which the bands are indicated in the Table. The reflecting substance, the peculiar arrangement of which has been described, is of course guanin. The colouring matter of the muscle enclosed in the cylinder of reflecting tissue is hæmoglobin, which is absent from the other muscles of the Fish. In some places the hæmoglobin is replaced by myohæmatin. The guanin in the argenteum is in the form of needles and prisms. When dilute hydrochloric acid is allowed to act on these the silveriness and iridescence disappear, the crystals dissolve, and others soon appear, which are larger and which give the guanin reaction; they are probably formed by partial solution of aggregates of the prisms. Guanin crystals in the form of fine needles are present in the argentea of the eye. The yellowish pigment in the dorsal skin is a lipochrome, whose absorption bands are given in the Table.

The muscle beneath the silvery band in *Osmerus eperlanus*, the true Smelt, gave the spectrum of myohæmatin instead of hæmoglobin. The lipochrome extracted from the skin was also different from that of *Atherina*, as seen in the Table. In the pigmented dorsal region of *Osmerus* crystals of guanin are present in the superficial layer of the skin (iridocytes). They are of irregular shape, and appear green by reflected, red by transmitted, gas-light.

In *Zeus faber*, the John Dorey, guanin forms the argenteum, and also occurs sparingly in the superficial layer. The lipochrome is also in small quantity.

There is an orange lipochrome in the fifteen-spined Stickleback. Its measurements are given in the table.

In the Gold Fish, *Carassius auratus*, the melanin which usually occurs in black chromatophores is absent from part or all of the skin. Iridescent prismatic crystals of guanin occur in contact with the scales; and also beneath the skin is an argenteum, which is thickest on the ventral surface. The specimens of the Fish examined were all golden-red, and no black chromatophores were found in them. Under the micro-spectroscope the red pigment *in situ*, which sometimes appears to form chromatophores, but is more usually diffused, absorbs the violet end of the spectrum, and gives a broad, ill-defined double shading in the green. All the solutions of the pigment strongly absorb the violet of the spectrum, and give ill-defined bands, whose position is indicated in the Table. The pigment on evaporation is orange red, becomes an evanescent blue with nitric acid, dark green and blue, passing into brown, with sulphuric acid, and remains unchanged with SCHULTZE's solution. The muscles contain hæmoglobin, most plentifully in the dorsal region.

III.—THE ARTIFICIAL PRODUCTION OF PIGMENT ON THE LOWER SIDES OF FLOUNDERS.

The first experiment with living Fish made in this investigation was described in the 'Zoologischer Anzeiger,' No. 354, 1891. We will briefly recapitulate the details here.

Experiment I.—About fifteen or sixteen larval Flounders, in process of metamorphosis, were used. The apparatus employed was a small glass vessel, cylindrical in shape, with a flat bottom, and about 12 inches in diameter. The sides of the vessel were covered externally with black paper, and an opaque cover was kept over the top. The water in the vessel was kept at a constant level by means of a siphon, which connected it with an overflowing bottle, clean water being supplied by a jet passed through a hole in the cover. Beneath the small aquarium was fixed a mirror, inclined at an angle of 45° to the plane of a south window, opposite which the apparatus was placed. The young Flounders, when placed in this apparatus on May 8, 1890, were

11·5 to 12·7 millims. in length, and their metamorphosis was far from complete. The new conditions to which they were subjected produced no apparent change in the course of the metamorphosis. The eyes passed into the normal adult position, and the colour disappeared at first, as far as could be seen, from the under side as usual. A specimen taken out on June 21, and carefully examined, was 2·7 centims. long and completely metamorphosed; there were no chromatophores on the lower side, except a few widely scattered on the lower side of the head. The little Fish in this experiment were not continuously exposed to the reversed conditions in relation to light which it was intended to produce, for it was observed that they frequently clung to the sides of the vessel instead of lying on the bottom, and, of course, in the former position no light could reach their lower sides.

On Aug. 27, all the Fish died from an accident to the circulation in the tank. There were thirteen of them altogether, ranging in total length from 3·2 centims. to 6·3 centims. In three of them there was a striking development of pigment in a broad band at the edges of the lower surface, the pigment being in the form of black and yellow chromatophores, exactly similar to those on the upper side. In two specimens no pigment was found on the lower side. In the remaining eight there was some pigment in one part or another of the lower surface, but only in small areas or in scattered chromatophores.

Experiment II.—The young Flounders used in Experiment I. were some of a large number, several hundred, which were received from Mevagissey between May 3 and May 8, 1890. The rest of these specimens were placed in table tanks at the bottom of which was a layer of sand, and in these they were fed regularly, and carefully reared. Excepting the confinement, these Flounders were living under normal, natural conditions, burying themselves in the sand when alarmed. On Sept. 17, 1890, four of these specimens were taken and placed in the apparatus used in Experiment I. These Fish were not actually measured, but are known, from records of the size of specimens of the same lot, to have been at that date from 7 to 9 centims. in length. They were about six months old, for, when obtained at the beginning of May, they must have been four to six weeks old. Their metamorphosis was, of course, completed in June; and, at the commencement of this experiment, they were in all respects normal specimens, with the characters of the adult. There was no pigment, no chromatophores on the left or lower side of any of the specimens; but the white, opaque layer, or argenteum, was fully developed on the whole of that side. At the commencement of this experiment the black paper was removed from the sides of the glass jar in which the Fish were placed, but the cover, which was lined with black velvet, was left on the top of the jar. In consequence of this change, light could pass through the sides of the jar as well as through the bottom from the mirror, but not through the top. The Fish now did not cling to the sides much; they seemed to have no preference for that position when the sides were no longer black and opaque. On the other hand, light passing through the sides fell obliquely on the upper sides of the Fish, so that the lower sides were not exclusively exposed to light. The mirror was arranged as before. These four specimens were kept in this apparatus until the beginning of February, 1891, when it was replaced by a shallow wooden tank with a bottom of plate glass. The dimensions of this tank were 3 feet 6 inches in length, 2 feet in width, and 10 inches in depth. The four Flounders were transferred to this tank, and a mirror of corresponding size was placed underneath it. The sides of the tank were black, and a cover was placed over the top, but this cover did not fit with sufficient accuracy to prevent light absolutely from entering above.

On March 10, it was noted that there was as yet no pigment on the lower sides of any of these four specimens, with the possible exception of the smallest. That is to say, if there was any pigment at all in the skin of the lower side of the smallest specimen, it consisted of only a few scattered chromatophores, the presence of which could not be determined with certainty without taking out the specimen and examining it with the lens or microscope—a proceeding which would have endangered the life of the specimen.

On April 26 were noticed two faint specks of pigment on the lower side of one of these specimens.

On July 1 one of the four specimens died, having got fixed between the side of the tank and one of the bricks placed inside the tank to keep the Fish in the central area of the glass bottom. This specimen had no pigment on its lower side. After they were put into the wooden tank these four Flounders followed the usual objectionable habit of clinging to the sides of the tank, instead of lying on the glass bottom with their lower sides exposed to the light. The water in the tank was kept as low as possible, but, while their breadth was small, could not be kept shallow enough to prevent the Fish doing this. As they grew larger they were compelled to abandon the habit, because, when they practised it, part of their bodies were out of water. At this date the smallest still retained this habit, but the other two remained usually horizontal. On one of these the pigment first seen on April 26 had increased a good deal in quantity, and a few specks had appeared on the others.

On August 6 the three remaining specimens were carefully examined, and the following is the entry in Mr. CUNNINGHAM's diary of that date :—"The largest of the three Flounders has pigment all over the external regions of its lower side; the second has scarcely any pigment, but has, I think, started to develop it, at least, it has slight indications of it here and there. The smallest specimen has very little. These two, until within the last few weeks, constantly clung to the side of the tank in a vertical position; the smallest often remains in this position now, but the others cannot do so because they are too large and the water is too shallow."

On September 26 one of the three remaining specimens was found dead behind one of the bricks. It was 16·7 centims. in length (6·6 inches). There was a little pigment on the posterior part of the operculum of the lower side, but nowhere else on that side

The two remaining specimens in this experiment continued to live for several months, and their lower sides were now constantly exposed to the daylight reflected upwards from the mirror, as they were now too large to cling to the sides. In November one of them, that in which pigment began to appear in April, and was first noticed on April 26, was pigmented over almost the whole of the lower side. On November 13 Miss WILLIS commenced to make a water-colour painting of this specimen from life, as it appeared by reflection in the mirror against the dark interior of the tank, and this painting is faithfully reproduced in Plate 53. The other specimen at this time was not pigmented to the same extent, nor, on the other hand, was it entirely destitute of pigment: it had three or four small spots scattered over the region of the abdominal cavity: these spots were not isolated chromatophores, but definite coloured spots, 1 to 2 millims. in diameter. The pigmented specimen figured was 22 centims. long at the time the drawing was made, the other specimen being about the same size.

During the following months the pigmentation of the lower side of these two specimens increased somewhat in area and intensity, but only very gradually. The pigment on the spotted specimen remained in separate spots, and never became continuous. On March 2, 1892, I examined the specimens to see if they were sexually ripe, as they were then 2 years old, and some specimens of the same age in the ordinary tanks were in spawning condition. I found that both were male and both ripe, ripe milt exuding from both on very slight pressure. I used some of the milt to fertilize the eggs of an ordinary Flounder of the same lot, which had been reared in an ordinary tank, but the eggs, although ripe, were not healthy, and all died.

The specimen with separate spots of pigment on the lower side died at last on July 4, 1892. The immediate cause of death was not evident, but a few days before there had been difficulties with the general salt-water circulation of the aquarium, and these two Flounders had been found several times gasping for want of oxygen. The apparatus had been provided with a constant supply of water in the main laboratory, and the Fish seemed to have recovered, but the death of this one was probably due to the after-effects of the crisis. A careful record was made of the distribution of pigment-spots on the lower side of this specimen. It was 23 centims. in length. There was pigment at the edge of the lower side, between the base of the pelvic fin and the ventral angle of the operculum; on the area covered by the pelvic fin; three spots on the area covered by the pectoral fin; seven spots, each 5 millims. in diameter, and several smaller ones, on the ventral half of the side in the neighbourhood of the posterior border of the abdominal cavity; eight minute spots on the dorsal half of the side; about eight spots close to the lateral line on both sides of it; four spots, about 5 millims. in diameter, on the lower surface of the ventral fin; a good deal of pigment over the posterior two-thirds of the lower surface of the caudal fin. The pigment on the upper or right side of the Fish was not appreciably diminished.

The other and last surviving specimen continued to live in good health; on July 23, 1892, the pigmented area as compared with that shown in the Plate was darker and more extended, reaching quite to the borders of the head anteriorly, and narrowing considerably the white band along the lateral line posteriorly; the patch on the head was also much larger.

This experiment is in some respects the most important and interesting of all those described in this paper. It proves in the most absolute and conclusive manner that normal pigmentation is produced by the incidence of daylight on the under sides of Flat Fishes which have lived for the first six months of their lives under normal conditions, and are at the end of that period perfectly normal specimens in all respects with no trace of pigment on their lower sides. All the time that the experiment was in progress a large number of specimens of the same age procured at the same time were kept in tanks of the aquarium, supplied with sea water by the same circulation, and were fed with the same food. The only difference in conditions between the two cases was that in one the Fish were living on a layer of sand and only exposed to light coming from above, while in the other the Fish were living on a glass plate beneath which was a mirror to reflect the light upwards. There were ninety specimens surviving in February and March, 1892, of those which had been kept in the ordinary tanks, and of these *only one* had a few small patches of pigment on the lower side. On the other hand, recapitulating the results obtained with the four specimens whose lower sides were exposed to light, we find that—

One specimen lived till July 1, 1891, and was exposed to light from below for $9\frac{1}{2}$ months without developing pigment on the lower side.

The second lived till September 26, 1891, and had then been exposed to light from below for a few days more than 12 months, it had only a little pigment on the operculum of the lower side.

In the third specimen pigment was not noted with absolute certainty till November, 1891, when the light had acted for 14 months. The pigment increased slowly, but surely, in quantity until July 4, 1892, when the Fish died, having been kept under the experimental conditions for 1 year and $9\frac{1}{2}$ months. The pigment when the specimen died was still in separate patches, not continuous.

In the fourth specimen pigment first appeared at the end of April, 1891, after the light had acted for a little over 7 months; by August 6, *i.e.*, in the eleventh month, there was pigment extending continuously all over the external regions of the lower side. In November the pigmentation was increased to the extent shown in the Plate, and in the following July after 1 year and 10 months, there was very little unpigmented surface left on the lower side.

Experiment III.—Only a single specimen was concerned in this experiment.

On April 4, 1891, was found in the table tank containing a number of those collected in the larval stage in May, 1890, a Flounder, 15 centims. long, in which there was a single spot of pigment on the lower side, on the area covered by the pectoral fin. This specimen was placed in the glass-bottom tank, and pigment developed on its lower side with extraordinary rapidity. It did not spread from the original pigment spot, but appeared as usual towards the centres of the halves of the surface divided by the lateral line.

On July 12, this specimen was found dead, having killed itself by falling between the bricks and the side of the tank. At the time of death it had pigment over the whole of the lower side except the head and a narrow strip along the lateral line. This specimen in fact after only 3 months' exposure exhibited more pigmentation than the specimen represented in Plate 53, that is to say pigmentation which extended over a greater area, although it was not quite so dense, and therefore not so dark in colour.

Experiment IV.—On May 21, 1891, a number (not counted) of larval Flounders in process of metamorphosis, procured from Mevagissey Harbour, were placed in the wooden tank with glass bottom previously described. The tank was divided into two equal parts by a wooden partition, and these larvæ were placed by themselves in the left-hand compartment.

On August 6, there were ten of this lot left alive, several having killed themselves by getting fixed in crevices. These ten survivors continually sought the dark corners of the tank, and remained with their lower sides in contact with the wooden sides, so that the lower sides of the Fish were by no means always exposed to the light coming through the glass bottom. Nevertheless, many of them at the date mentioned had some pigment on their lower sides.

On September 4, two of this lot of Fish died owing to an accidental stoppage of the circulation in the tank. I had placed some of the Fish in a glass bottle standing on the glass bottom of the tank in order that they might be constantly exposed to the light from below, and, when the circulation stopped, those in the bottle suffered more than those outside it, because they were in a smaller quantity of water. The larger of these two was 6·7 centims. long, and had pigment along the region of the fin-muscle on the lower side; the argenteum was developed in the peritoneum, and slightly in the skin of the abdominal region, but nowhere else. The smaller specimen was 4·3 centims. long, and had less pigment; there were in it only scattered chromatophores in the same region as in the other specimen; the argenteum was developed in the peritoneum, but not elsewhere on the lower side.

On September 10, I arranged a bell-jar over the mirror underneath the glass-bottom tank, fitted it with a constant-level circulation, and transferred to it the eight remaining Flounders of this year's brood. The reason of this was that in the wooden tank they persisted in clinging to the opaque sides. In the bell-jar, the lower sides were constantly exposed to the light. At this time, the majority of these Fish had no pigment on the lower sides, others had a little in the region of the muscles of the longitudinal fins.

In the following months, pigment developed very considerably on the lower sides of these specimens. The amount of pigment present was not definitely noted until February, 1892, although the Fish were regularly fed and tended. All this time it should be remembered the Fish were exposed to light both above and below, as no steps were taken to cover the sides of the bell-jar.

On February 13, 1892, one of the Flounders died. This death was, as usual, due to an accident. The outflow from the bell-jar was obstructed, the water overflowed at the top, and several of the Fish escaped; these were exposed for a time to the air on the floor, and one of them did not recover when restored to the water. This specimen was 8·7 centims. in length, and on its lower side pigment was strongly developed along the lateral line, and also in less quantity in the regions above and below the lateral line. There were also chromatophores along the edges of the head, and the edges of the body at the base of the tail. The argenteum was continuous over the abdominal region, absent at the bases of the fins, elsewhere developed only along the lines of the intermuscular septa. At this date, February 13, all of the seven surviving Flounders in this experiment except two, had some pigment on the lower sides.

On March 29, I removed these seven Flounders from the bell-jar, and put them into the left half of the glass-bottom tank, because I required the bell-jar in order to commence another experiment with new larval Flounders which I received on that day.

On May 12, one of the specimens died; it was one of the two in which no pigment could be seen on the lower side when it was alive in the tank. The results of an examination of the specimen were: It was 10·1 centims. long; on the lower side the argenteum was well developed, especially along the intermuscular septa. The amount of pigment on the lower side was very small; there was some at the extreme edges of the caudal portion of the body, between the longitudinal fins and the caudal fin, a small patch of chromatophores on the lower side of the caudal fin, and on the extremity of the lower pectoral.

On June 1, owing to renewed difficulty with the circulation in the glass-bottom tank, five more of the specimens in this experiment died, only one being left alive. The condition of these five was as follows:—

(1.) Length 12·4 centims., pigmented all over the lower side, except the head and extreme caudal region.

(2.) Length 11·3 centims., pigment less continuous, but still covering nearly the whole side.

(3.) 11·6 centims., same as (2), but a little less pigment.

(4.) 9·9 centims., same as (3).

(5.) 7·6 centims., pigment only in small patches.

The last surviving specimen of this experiment was pigmented at this date, and is now (September 29, 1892), about 23 centims. long, and pigmented over all the lower side except the head and caudal portion; it exhibits, in fact, more pigmentation than is shown in the figure.

The features of this experiment then briefly recapitulated are: Fish in glass-bottom tank from period of metamorphosis in May to August 6, 1891, when only ten survived, of which some had a little pigment on lower side. Two died on September 4, and showed a little pigment in the fin-muscle area. On September 10, remaining eight Fish transferred to a bell-jar, and illuminated both from above and below. On February 13, 1892, one specimen died, showing pigment considerably developed on its lower side. On March 29, the remaining seven specimens were removed to the tank with glass bottom, where the lower sides were constantly illuminated. On May 12, another specimen died; it showed scarcely any pigment on lower side. On June 1, five died; and one is still alive, all these six exhibiting a great deal of pigmentation on the lower side. Thus, pigment developed to a very marked extent in seven out of a total of eight specimens between September 10, 1891, and June 1, 1892, and I have little doubt that the specimen which died on May 12 would have developed pigment if it had lived a few months longer.

The general results, which I observe in these experiments, are the following: When, as in the first experiment, a number of young Flounders have been exposed for about four months, pigment is found to be developed on the lower side in a small proportion of them in the region of the fin-muscles, and these specimens are usually the smallest of the lot, with occasional exceptions. When the water in the apparatus becomes accidentally foul, the smaller pigmented specimens usually succumb, while the larger Fish, in which no pigment, or scarcely any, has appeared, survive. For instance, in Experiment IV., on September 4, 1891, two specimens died, both of which had pigment in the fin-muscle region, while the majority of the remaining eight had not yet developed pigment. As time goes on, the number of specimens in which pigment is present on the lower side continually increases. Thus, in Experiment IV., on February 13, 1892, one specimen died in which pigment was considerably developed, while all the rest, except two, were pigmented. On May 12, another specimen died, which had scarcely any pigment on the lower side, but all the remaining seven had developed pigment very strongly by June 1. I have noticed that where the pigment is only developed after the Fish has been exposed a long time, and after the argenteum has developed, it does not usually begin to appear, as in the younger specimens, in the fin-muscle region, but in the region of the trunk-muscles above and below the lateral line. The head and caudal region are always the last to develop pigment.

All the experiments prove clearly that the amount of pigment produced increases steadily with the duration of the exposure; but a comparison of Experiments IV. and II. shows that there is not so much difference as might have been expected between the results produced by commencing the exposure in the larval stage, and commencing it nearly four months after the metamorphosis. In the former case, pigment had appeared in some specimens in less than four months after the commencement of the exposure, and had appeared in all the surviving specimens, six out of ten, at the end of twelve months. In the latter case, pigment did not appear on any of the specimens until the exposure had lasted seven months; but it had appeared on all the specimens that lived long enough, three out of four, at the end of fourteen months. It is evident from this comparison that the development of the pigment is not connected with a particular age of the Fish, for in the latter case, although the duration of exposure was about the same as in Experiment IV., the age of the Fish was greater than the period of exposure by four months; so that whereas, in Experiment IV., pigment first appeared in specimens only about five or six months old, in Experiment II. it first appeared in specimens which were twelve months old.

One other conspicuous feature of the experiments is the great individual variation among the specimens in their susceptibility to the pigment-producing influence of light.

Having examined the anatomy and development of the elements of coloration in Pleuronectidæ and other Fishes, and the chemical and physical properties of the substances which characterize those elements, we are in a position to offer some interpretation of the experiments described, and to inquire into the causes of some of the phenomena of coloration in Fishes.

Examination of the specimens of Flounder in which coloration was produced on the lower side in these experiments, showed that the coloration was exactly of the same kind as that of the upper side in normal specimens. Wherever the skin was coloured there were both black and yellow chromatophores, not only superficially close beneath the epidermis, but also on the inner side of the skin. It was also found that, where the colour was well developed, the internal deposit of guanin, the argenteum, was scanty, as on the coloured side. We have no exact data to prove that, when colour is produced in a Fish of considerable size, in which the argenteum was fully developed before the pigmentation appeared, the guanin is absorbed, although it seems very probable. The coloration produced on the lower side further resembled that normally present on the upper in exhibiting changes of tint or intensity, being at one time very much darker than at another, under the influence of certain conditions. We have not made a special investigation of these changes, but we can assert that they are, like those of the upper side, due to dilatation and contraction of the chromatophores. Probably the condition of the latter is affected by the amount and kind of light falling upon the eyes of the Fish. Some light enters the dorsal or left eye of the Fish obliquely as it lies upon the glass bottom of the vessel, the axis of that eye being capable of being directed outward and downward; and other light enters both eyes after reflection from the top and sides of the vessel, for even black cloth does not absorb all the light that falls upon it. The amount of light thus affecting the eyes depends on the amount passing through the window, the surroundings of the Fish being constant. The exact relation between the temporary changes of coloration on the lower side and the light has not been observed, but it has been noticed that the tint of the lower side is not dark enough to harmonise with the black background against which it is seen. The most striking change which has been noticed is the extreme pallor of the colour of the lower side when the supply of water to the vessel accidentally fails for a time. The water being no longer renewed, the Fish soon suffer from a deficiency of oxygen, and are found breathing hurriedly and painfully, while the coloured parts of the lower side are a straw-coloured yellow instead of dark yellowish brown. When the circulation is restored, and the Fish recover from their semi-suffocation, the chromatophores of the lower side again dilate, and the normal colour reappears. The same pallor, but to a less degree, appears when the Fish are frightened and disturbed by a person lifting up the cover to look into the vessel from above.

Here may be mentioned some observations made in the course of these investigations which show that the chromotaphores of normal Flat Fishes are affected directly by mechanical stimulation, as, *à priori*, might be expected. When Flat Fishes are thrown into a basket as they are captured, and left to lie one upon the other for some hours until they are dead, or nearly so, it is found when they are taken out again that the upper sides are curiously marbled, areas of very irregular shape being very pale while neighbouring areas are very dark, and the boundary between the differently coloured areas being very sharp and distinct. On the other hand, if a Plaice or Flounder is taken alive from the water and allowed to die by itself with its upper side fully exposed, it invariably grows gradually darker, until when dead it is almost black. We have never observed pallor appearing as the consequence of the moribund condition in Fish dying in the usual way when taken out of the water, if they were left exposed. POUCHET * considers that general pallor, or the appearance of pale livid marks, is the consequence of the moribund condition in all cases, but as he remarks that his observations were made in the market of Concarneau, it is evident that he was referring to the curious marbling we have mentioned as appearing in Fish which have been partially covered. We at first thought that the contraction of the chromatophores in this case was due to the exclusion of light, but this seemed improbable because the exclusion of light in the living Fish usually causes the chromatophores to expand. It was observed, however, that in a piece of fresh skin partly covered by a cover-glass on a glass slide, the chromatophores under the cover-glass became contracted while those in the uncovered skin remained expanded. It was inferred from this that contact with a solid subject and slight pressure were the cause of the contraction, and this was easily proved to be true, for the course of the end of a glass rod drawn along the surface of the skin of a Plaice or Flounder became visible in a few minutes as a light streak on a dark background. In a Fish removed from the water and rapidly dying, the chromatophores remain alive after the Fish is dead, or nearly so, and so retain their contractility unaffected by influences from the nervous system. In this condition they contract if anything rests upon the skin, and when they themselves are dead they remain permanently contracted; on the other hand, if left to themselves they gradually expand as the Fish dies and as they die themselves. This is the explanation of the mottled or marbled appearance of Fish which have been packed one upon another while still alive.

* 'Changements de Coloration, etc.,' p. 33.

IV.—Abnormalities of Coloration in Pleuronectidæ occurring in Nature.

The experiments described in the previous section seem to indicate that the action of daylight determines the appearance of black and yellow chromatophores in the skin of the lower sides of Flounders, and the absorption of guanin deposits. But it must be remembered that specimens abnormally coloured are of not infrequent occurrence in various species of Flat Fishes. Every naturalist who has paid attention to the Pleuronectidæ has met with such abnormal specimens. The abnormalities are of various kinds and degrees. The commonest and least conspicuous is the mere reversal of the asymmetry which is characteristic of the species, the differences between the two sides being the same as in normal specimens, but the left side presenting the characters usually connected with the right, and *vice versâ*. According to my own experience, this variation is most frequent in the Flounder, *Pleuronectes flesus;* in the neighbourhood of Plymouth a large percentage of Flounders are sinistral instead of dextral.

It is a curious fact that in a sinistral Flat Fish, whether it is normally sinistral like the Turbot, or abnormally like a reversed Flounder, the viscera are in the same position as in a dextral specimen ; the liver is on the left side, the coils of the intestine on the right. The reversal of the relations of the two sides externally does not affect the relations of the internal organs, which remain constant.

But more important in relation to the subject of the present memoir are abnormalities of coloration, which consist in the presence of some pigment, from small patches up to complete pigmentation, on the lower side of the Fish, or the absence of pigment from part or from the whole of the ocular side.

Specimens which are pigmented all over the lower side as well as the upper, or ocular, have usually been called double, but, as this term is evidently not quite suitable, we prefer the term "ambicolorate." An abnormality of the head is frequently found in ambicolorate specimens ; in this abnormality the anterior end of the dorsal fin, instead of extending forwards towards the snout on the outer side of the dorsal eye, terminates behind the level of the eyes, and its end forms a curved hook projecting forwards, as shown in Plate 54, fig. 1.

Before referring to the descriptions of such abnormalities previously published, we shall proceed to enumerate the specimens that have come under our own notice or been described to us by other naturalists. The following specimens were collected at Plymouth :—

Pleuronectes flesus.—(1.) $7\frac{5}{8}$ inches in length. Almost entirely coloured on the lower side as well as on the upper.

Anterior end of the dorsal fin with a base forming a hook projecting over the dorsal (left) eye, which is on the edge of the head, and partly visible from the lower side.

Dorsal and ventral tubercles developed on the lower side as on the upper, but no rough scales on the lateral line on the lower side, very few on the upper.

The exact appearance of this specimen after preservation in spirit is represented in figs. 1 and 2, Plate 54, which are drawn of the natural size. The coloration is somewhat obscured by the coagulation of the tissues, due to the spirit, especially by the coagulated epidermis. It is noteworthy that the correlation between coloration and structural deformity, which we notice below in the Turbot, does not hold for the Flounder. The yellowish white part of the skin at the base of the tail is the only part of the lower side where the argenteum is normally developed, over the rest of the lower side it is almost entirely absent, as it is on the upper pigmented side in normal specimens. This is an instance of the inverse relation between pigment and guanin, which we discuss in other parts of this memoir.

(2.) Length, $13\frac{1}{4}$ inches; sex, female. Completely coloured on both sides, but the colour of the left side not quite so intense as that of the right.

Anterior end of the dorsal fin hooked, base terminating behind the left eye; left eye rather on the upper side of the head than on the edge.

Dorsal and ventral tubercles on the lower side as well as on the upper, and rough scales on lateral line on lower side also.

(3.) $11\frac{5}{8}$ inches long. Completely ambicolorate, dorsal fin hooked-shaped anteriorly. Tubercles on lower side as on upper, few rough scales on lateral line on upper side, only one or two on lower.

(4.) $8\frac{1}{4}$ inches long. Completely ambicolorate, dorsal fin hooked-shaped anteriorly. Tubercles on lower side as on upper, but few rough scales on lateral line.

(5.) $12\frac{5}{8}$ inches long. Small spots of colour irregularly scattered over lower side, dorsal fin and head normal. No tubercles on lower side, but where one of the coloured spots covered the lateral line anteriorly there were a few rough scales.

Pleuronectes microcephalus.—(1.) 13 inches long. Coloured continuously over lower side, as on upper, except an area extending a little beyond the head, which was white. No abnormality in structure. Sex, male.

(2.) $11\frac{3}{4}$ inches long. Similar to the preceding, but the outline of the anterior white region on the lower side was not so definitely marked. Sex, male.

Pleuronectes platessa.—A specimen about 10 inches long, in which the posterior two-thirds of the lower side are continuously coloured like the upper side, but the anterior third is white. The anterior end of the dorsal fin is not quite normal, the base of attachment terminating behind the eyes, although the end of the fin itself does not project into a prominent hook.

Solea vulgaris.—12 inches long. Male. Lower side white as in normal specimens, upper side almost all white also. The only pigmented parts of the ocular side are the head from the edge of the operculum forwards, the pigmentation extending behind the head only in a small spot above the base of the pelvic fin, and the distal half of the tail. The rest of the upper side is entirely destitute of pigment, and over this area the argenteum, usually rudimentary on the upper side, is as much developed as on the lower side.

The fish merchant who gave this specimen, asked if the explanation of its condition might be that it had been skinned when captured and then been thrown overboard alive and had recovered, the white upper side showing where the flayed surface had healed over! The suggestion cannot, of course, be entertained. The skin of the upper side is normal, and contains normal scales.

The following specimens were sent to Mr. CUNNINGHAM by Mr. E. W. L. HOLT, who collected them from trawlers at Grimsby.

Rhombus maximus.—(1.) $23\frac{3}{4}$ inches long. Female. The whole of the lower (right) side coloured like the upper, except part of the lower side of the head. The skin of the lower jaw was pigmented on the lower side, and the coloration extended forwards as far as the preopercular bone; the rest of this side of the head was white. Tubercles in the skin on the lower side as on the upper, but on the head they were fewer in number and smaller than on the upper side; the projecting points of these tubercles were worn down on the lower side, and slightly worn on the upper side also. Attachment of dorsal fin terminated behind the eyes and the fin projected over the right eye in the form of a hook. A little pigment in the peritoneum both of the lower side and the upper.

(2.) $20\frac{7}{8}$ inches. Female. Condition very similar to that of preceding specimen. Lower jaw and anterior end of dorsal fin pigmented, rest of head white in front of the preopercular bone. Dermal tubercles on the lower side much worn. Peritoneum slightly pigmented both on upper and lower side. Dorsal fin hook-shaped anteriorly.

(3.) $16\frac{1}{4}$ inches. Female. Condition very similar. Distal half of lower jaw on lower side pigmented, and all the rest of the lower side, except the head, in front of preopercular. Lower pectoral fin edged with white. Peritoneum slightly pigmented both above and below. Dorsal fin hook-shaped anteriorly.

(4.) 14 inches. Male. Coloured on both sides entirely. Tubercles on lower side not so numerous or so large as on upper. Dorsal eye near edge of head but not directed horizontally. Peritoneum slightly pigmented both above and below. Dorsal fin hook-shaped anteriorly.

(5.) 13 inches. Female. Coloured all over lower side except side of the head in front of preopercular and lower jaw; anterior end of dorsal fin coloured. Dorsal eye on the edge of the head, and directed horizontally. Peritoneum pigmented above and below. Dorsal fin hook-shaped anteriorly.

(6.) $11\frac{7}{8}$ inches. Similar to preceding; head on lower side unpigmented in front of preopercular, lower jaw and anterior end of dorsal fin pigmented. Dorsal fin hook-shaped anteriorly.

(7.) $19\frac{1}{2}$ inches. Female. Coloured over the whole of the lower side except head in front of preoperculum: tubercles also on lower side of body, but none on the head. Differs from the others in the absence of pigment from the lower side of the lower jaw, and from the anterior end of the dorsal fin, also in the fact that the head, eyes, and dorsal fin are *perfectly normal in structure*.

Pleuronectes platessa.—19¾ inches long. Female. The head normal, and lower side white as in normal specimens. Anterior end of upper side pigmented, and also the posterior third, but the rest of the upper side white like the lower side. On the dorsal edge of the body there was a considerable indentation, somewhat behind the head, looking like a scar resulting from the healing of a deep incised wound. This indentation was nearly in the middle of the length of the body included in the white area of the upper side, a relation which suggests the possibility that the disappearance of the pigment had been caused by the injury; this, however, is not probable.

Mr. HOLT states that such piebald Plaice are common enough in the market at Grimsby.

We are indebted to Dr. G. H. FOWLER for some notes of abnormally coloured specimens of Plaice made by Dr. BRANDT at Kiel, and communicated with his consent. They are as follows:—

(1.) Lower side entirely pigmented; head with the typical abnormalities of eye and dorsal fin.

(2.) Lower side all pigmented, upper side normal.

(3.) Lower side irregularly blotched with pigment spots.

(4.) Lower side spotted white on dark (*i.e.*, pigmented area larger than the white), very closely and evenly. Upper side irregularly blotched with white.

(5.) Lower side with two dark longitudinal streaks along the edges; upper side normal.

(6.) Lower side with a very dark posterior half, and antero-ventral patch; remainder white. Upper side normal.

(7.) Lower side quite dark, except head and pectoral fin, which are quite white, and the dorsal and ventral fins, which are mottled. Upper side normal. *Reversed.*

(8.) Lower side very slightly spotted; upper side with a circular light patch, three inches in diameter.

(9, 10.) Lower side normal. Upper side, dorsal and ventral fins quite white, except one or two pigmented patches; tail fin piebald.

(11.) Lower side spotted with pigment on body and mottled on fins. Upper side mottled with white on head and anterior region.

(12.) Lower side normal. Upper side, a white patch behind the head from pectoral fin to dorsal edge.

(13, 14.) Lower side with a few very small spots. Upper side quite white all over, except a few scattered spots of pigment.

(15.) The same as preceding, but no spots on lower side.

Among the specimens included in the above list the malformation of the head, accompanied by complete or nearly complete ambicoloration, occurs most frequently in the Turbot, namely, in six specimens. There are three specimens of the Flounder showing the same condition, and two of the Plaice, in one of which the malformation is incompletely developed. We have not yet met with, or heard of, any instances of this malformation in the Merry Sole (or Lemon Sole, *Pleuronectes microcephalus**) or in the Dab (*P. limanda*), or in the genera *Solea, Arnoglossus, Zeugopterus, Hippoglossus,* or *Hippoglossoides.*

Cases of this malformation of the head have been frequently mentioned and described by ichthyologists. The first reference to it is that of DONOVAN,† who describes an instance of it in the Turbot as type of a distinct species, *Pleuronectes cyclops.* YARRELL‡ mentions and figures the malformation in a Brill. COUCH§ describes and figures it in the Flounder, in which species, he says, it is so common in some districts "as to have raised the suspicion of its being truly a distinct species." HIGGINS|| mentions it in the Flounder, naming the specimen *Platessa melanogaster*, and it is also recorded in the same species by THOMPSON¶ and by RITZEMA BOS.** DAY states†† that, according to R. COUCH, *Zeugopterus punctatus* is "very subject to malformation of the anterior end of the dorsal fin, causing it to form an arch over the eyes;" but as he gives no reference, he was probably quoting a verbal communication, and this cannot be regarded as sufficient evidence that the malformation here considered has been observed in this species. So far as we can discover, it has not been mentioned in connection with any other species besides the Turbot, Brill, and Flounder, so that our observations of its occurrence in the Plaice is the first on record, and here it must be noted that in the specimen seen at Plymouth the malformation is not fully developed, and the only other specimen, observed at Kiel, is mentioned on the authority of Dr. FOWLER. On the other hand, DAY‡‡ states that he obtained from Brixham a double Sole, $11\frac{1}{2}$ inches in length, with the eyes placed as normally in this species. So that it would appear that, even when the lower side is coloured in the Sole, no malformation of the head occurs. The two ambicolorate specimens of *P. microcephalus* mentioned in our list had as much pigmentation on the lower side as the Turbot, in which the malformation is present, and yet there is no malformation in them.

The evidence at present available then tends to prove that the malformation of the head occurs in ambicolorate specimens of *Rhombus maximus, R. lævis, Pleuronectes flesus,* and less frequently in *P. platessa,* but not in other species. It is natural to

* Mr. HOLT informs us that he has seen the malformation partially developed in Lemon Sole.

† 'Brit. Fishes,' vol. 4, Plate 90.

‡ 'Brit. Fishes,' 1836, vol. 2, p. 242.

§ 'Brit. Fishes,' 1864, vol. 3, p. 198.

|| 'Zoologist,' 1855, vol. 13, p. 4596.

¶ 'Proc. Zool. Soc.,' 1837, p. 60.

** 'Biol. Centralbl.,' vol. 6, p. 270.

†† 'Brit. Fishes,' 1884, vol. 2, p. 19.

‡‡ *Op. cit.*, vol. 2, p. 40.

inquire whether these species differ from the rest of the family in any particulars of habit, development, or structure, which might in some way be connected with the occurrence of this malformation. With respect to development the Turbot and Brill are provided in the larval condition with an air bladder, and remain almost entirely pelagic until the metamorphosis is very nearly complete. The young of this species are found in summer swimming at the surface until the eye is at the edge of the head or even partly on the left side. In this intermediate condition they do not swim vertically, but in a slanting, oblique position, but the retention of the pelagic habit for so long a time might be supposed to be in some way connected with the frequent occurrence of the malformation, were it not that the Flounder abandons the pelagic habit as early as any member of the family, and does not resemble the Turbot in this respect at all. On the other hand, the Sole, as Mr. CUNNINGHAM has shown,* has an air bladder in its intermediate condition, although it is not often found at the very surface of the sea, and it does not exhibit this occasional malformation.

Then, again, it might be suggested that the species which pass the first part of their lives near the shore are subject to this malformation, but the young of the Sole has only been taken near the shore, and agrees in its habits with the Turbot more than does the Flounder.

Lastly, it is evident that the occurrence of the malformation does not correspond to the degree of anterior extension of the dorsal fin, characteristic of the species, for this is greater in the Sole, and much less in the Flounder than in the Turbot.

But although we are unable to discover at present a correlation between the occurrence of this malformation and any peculiarity of the species in which it occurs, there is a distinct correlation in the individual Turbot between the structural malformation and the degree of pigmentation of the lower side. We have been unable to find any evidence that a specimen of Turbot, Brill, Flounder, or Plaice has ever been observed in which the whole of the lower side was continuously pigmented, and yet the structure of the head was normal. In other words, complete ambicoloration in these species is always accompanied by the typical malformation of the head and dorsal fin. Conversely, when this structural malformation is present the lower side of the Fish is always completely or nearly completely pigmented. In the case of the Turbot it is interesting to notice the limit of this correlation between structure and coloration. Thus, of the Turbot in the above list, only one, the fourth in the list, is pigmented over the whole of the lower side, and in this, of course, the malformation occurs; in the other five in which the malformation is present, pigment is absent from the lower side of the head in front of the preoperculum, but present on the lower side of the lower jaw and of the anterior end of the dorsal fin and its neighbourhood. In the seventh specimen in the list the pigmentation has the same extent except that it is not present on the lower side of the lower jaw, nor on the anterior region of the dorsal fin; and in this specimen there is no structural malformation. Thus it would seem that if we draw

* 'Journ. Mar. Biol. Assn.,' 2, 4.

an imaginary line through the preopercular bone in the Turbot, pigmentation may extend over the whole of the lower side behind this line without any structural malformation being present, but when pigment is also present on the lower side in front of this line the characteristic structural malformation occurs also; on the other hand the structural malformation has never been observed in any specimens in which the lower side was unpigmented or pigmented to a less extent than that defined above.

Mr. HOLT has sent us a description of a Brill about ten inches long in his possession, alive, in which there is a slight notch beneath the anterior end of the dorsal fin, but the eyes are normal and there is no colour on the lower side except a very small spot above the nasal valve of the right side just behind the notch. His sketch showed that this is not a case of the so-called cyclopean malformation which we have been considering. In the latter the attachment of the dorsal fin ends anteriorly some distance behind the dorsal eye, in Mr. HOLT's Brill, the attachment is continued forwards to a point about $\frac{1}{8}$ inch in front of the anterior margin of the dorsal eye, and the free portion of the base of the fin is only $\frac{1}{4}$ inch long instead of $\frac{3}{4}$ inch or more, as in the typical case. The difference is one of degree, it is true, but it is a very great difference of degree, and does not contradict the conclusions we have formulated as to the correlation of the typical malformation with ambicoloration.

The occurrence of spots of pigment on the lower side, in a series along each border in the region of the muscles of the longitudinal fins, is very common in the Turbot and Brill. We have seen numerous specimens of the Brill exhibiting such spots, which are few in number and of considerable size, and GIARD points out that the piebald coloration of the lower side was considered by earlier French ichthyologists (DAUBENTON, BONNATERRE, LACÉPÈDE) as a specific character of *Rhombus maximus*.

It has often been stated without proof that these abnormalities of coloration are due to an abnormal exposure to light of the specimens in which they occur. But we have been unable to find any evidence in support of such statements. No reliable observations are on record of abnormal habits exhibited by these abnormal Fish, or of their existence under abnormal conditions. Mr. CUNNINGHAM has kept an abnormal specimen of the Plaice alive and under observation at the aquarium at Plymouth, from September 19, 1891, up to the present time. This specimen is the one mentioned above as coloured over three-fourths of the lower side on the same way as in the upper, the anterior fourth of the lower side being white as in normal specimens. The anterior end of the dorsal fin was not normal nor yet so abnormal as in typical "cyclopean" specimens, it terminated abruptly behind the level of the dorsal eye without projecting into a hook. This specimen was exceedingly shy and timid, and always remained completely buried in the sand at the bottom of the tank. It was never observed to swim of its own accord, and when dislodged from the sand, it swam horizontally like a normal Plaice and buried itself with violent movements of its fins as soon as possible. Moreover, it has exhibited not the slightest diminution in the coloration of the lower side since the day on which it was first brought to the aquarium, although its lower side has not since that time been exposed to the light any more than in the case of normal specimens.

Again, Professor GIARD has suggested that the malformed or monstrous ambicolorate specimens do not swim all their lives vertically, but that they remain longer in that position than normal specimens, "in any case long enough to allow the influence of the light to act efficaciously on the side ordinarily colourless." In fact, if I understand him rightly* GIARD's proposed explanation of the condition of the monstrous Turbot is this :—The Turbot has a long period of metamorphosis during which it is pelagic ; in consequence of this it is liable to have its normal metamorphosis arrested and altered, and in this case its eye does not pass completely into its proper position, and the relations of the end of the dorsal fin become abnormal. A specimen so affected remains pelagic for a longer time than usual, the right side is exposed to light for a longer time than usual, and in consequence remains pigmented instead of becoming white and unpigmented.

Now if the presence of pigment on the right side of the monstrous Turbot is to be explained in this way, all other abnormalities of coloration in Flat Fishes ought also to be explained as due to the action of light. But even GIARD himself does not suggest that the occurrence of pigmentation on the lower side, or its absence on the upper side of specimens structurally normal are due to abnormal conditions to which these specimens were exposed. We have not the slightest evidence at present that these abnormal specimens have been exposed to abnormal conditions or have had abnormal habits of life.

These abnormalities of coloration in Flat Fishes, when carefully considered, give rise to some very interesting speculations. We will take, first, cases of pigmentation on the lower side which is normally unpigmented. If the presence of the pigment is due to an unusual exposure of the lower side to light, then, of course, the phenomenon is of the same kind as that shown in Mr. CUNNINGHAM'S experiment, the pigment is an acquired character produced by the action of the environment on the individual. But suppose that it is independent of the action of light, then it is a congenital variation, independent of the action of the environment on the individual. The question then arises : is it a case of reversion or atavism, as it is generally assumed to be ? Now it is clear that ambicoloration cannot be simply reversion, because we must necessarily suppose that the symmetrical vertically swimming ancestor of the Flat Fish had an unpigmented, white or silvery ventral surface, as other symmetrical Fishes have, it could not have been uniformly pigmented all over, as a completely ambicolorate Flat Fish is. Therefore the explanation, the comprehension of this common variation, is a much more difficult and complicated matter than it seems to be at first. We may suppose that the hereditary tendency leading to reversion has been modified by a tendency of the lower side of the Fish to resemble the upper ; or we may sup-

* GIARD, "Sur la Persistance partielle de la Symétrie Bilatérale chez un Turbot, &c." 'C. R. Soc. de Biol.,' January 16, 1892, and 'Natural Science,' I., 5, p. 358.

pose that there is no reversion in the matter at all, but that there is some occult tendency, in certain individuals, of the lower side to imitate the upper. It is a curious and remarkable fact that there is no tendency shown in Flat Fishes to develop pigment on the dorsal halves of both sides whilst it is absent from the ventral halves.

An interesting fact is the distribution of pigment in the peritoneum in normal Flat Fishes. In some Fishes the colour of the peritoneum has an effect on the external appearance, because it is visible through the thin translucent body-walls. Even in Pleuronectidæ in certain species, and in certain stages of other species, the reflecting opaque tissue of the peritoneum of the lower side is visible from the exterior when there is little or no argenteum in the skin. But the fact is, that the peritoneum of the upper side in normal Pleuronectidæ is black, while that of the lower side contains little or no pigment, but contains more argenteum or more guanin than that of the upper. Now the peritoneum is of small extent compared with the whole of the lower side, and it can scarcely be maintained that the appearance of its argenteum from the exterior is of any use or advantage to the Fish, while the pigmentation of the peritoneum of the upper side adds nothing at all to the external appearance of the upper side. Fig. 8, Plate 55, shows the appearance of the peritoneum of the ovarian pouch of the upper side in the *Pleuronectes microcephalus,* a network of black chromatophores resting on a granular deposit of granules of guanin ; no "coloured" pigment was present. In the corresponding portion of the lower side there were two or three minute black chromatophores in the same area. This difference can only be explained as the effect of light falling on the upper side of the Fish, and not on the lower.

We have written of Flat Fishes in general as having no pigment on the lower sides, but the subject is still further complicated by the fact that in certain species there is normally some pigment on the lower side. We have already referred to the frequent occurrence of dorsal and ventral spots on the lower side of the Turbot and Brill ; but Mr. HOLT has kindly brought to our notice the fact that in normal specimens of the Witch or Pole Dab, *Pleuronectes cynoglossus,* the lower side is not white but grey. He sent us four specimens of this species, and we found that the superficial layer of the skin, outside the scales, contained a layer of closely crowded iridocytes, and large radiate black chromatophores uniformly distributed over the whole surface, but not very close together. There were, however, no coloured chromatophores, no lipochrome was present, although on the upper side orange chromatophores were present as usual. The internal layer of the skin on the lower side contained neither argenteum nor chromatophores. In this species the colour of the upper side is not so deep as in the Plaice, Flounder, or Turbot, and both upper and lower sides are more pigmented in larger than in smaller specimens. It should be noted that this species belongs to deeper water than the Plaice, Flounder, Turbot, and other species. Its young have not been found at less depths than 80 fathoms, and the adult has been taken at all depths up to 700 fathoms. Perhaps the peculiarity is related to the fact that light at greater depths is more dispersed than in shallow water, and therefore affects the

Fish more equally on the two sides. We do not know that the habits of the Fish expose the lower side more than usual, but any Flat Fish rises from the bottom occasionally, and in deep water, the light being more diffused, may affect the lower side more on these occasions. On the other hand, the explanation may be that this species became a Flat Fish more recently than others, and has, therefore, not advanced so far along the line of evolution of Pleuronectids, though we know of no evidence that this is so.

A species of Pleuronectid normally pigmented on the lower side has been described by JORDAN and BELLMAN ('Proc. U.S. National Museum,' vol. 12, p. 176). Several specimens of it were taken in 1887–1888 in the Pacific Ocean, off the coast of Columbia, at depths of 33 and $51\frac{1}{2}$ fathoms. It is named *Engyophrys sancti-laurentii, nov. gen., nov. sp.* It is allied to *Platophrys*, SWAINSON, and *Engyprosopon*, GÜNTHER, but differs from all related species by the peculiar coloration of the blind sides. The colour of the left or eyed side is blackish brown, with scattered white and black spots, the latter most conspicuous along the bases of the longitudinal fins: three large black blotches on the straight part of the lateral line. Blind side with five or six curved, parallel, dusky bands as wide as the eye, the first beginning on the inter-opercular, and curving across the cheeks to along the base of the dorsal; second beginning at the throat, curving along the posterior margin of the preopercular, and extending along the dorsal region parallel to first; third curving round in front of the pectoral, across the posterior part of the opercular, and extending to the base of the dorsal fin behind the middle of the body: the others behind the pectoral. All of these bands fade out behind the middle of the body, so that the posterior portion is "immaculate," which appears to mean white and unpigmented. In young examples these bands are very faint or obsolete.

DESCRIPTION OF PLATES.

PLATE 53.

Condition, drawn from life, of the lower side of a specimen of Flounder (*Pleuronectes flesus*, GOTTSCHE), on November 13, 1891; this side having been exposed to daylight since September 17, 1890. The specimen was captured in May, 1890, when about 1·2 centim. long, and was reared in the aquarium under ordinary conditions until September 17. At this time, when the experiment was commenced, there was not a trace of pigment on the lower side. The Fish is represented at its natural size.

PLATE 54.

Fig. 1.—Lower side of an abnormal specimen of a Flounder captured in the trawl at sea. Drawn of the natural size after being preserved in spirit. The dark portions are pigmented, the bluish portion is the unpigmented skin coagulated by the spirit. The white portion at the base of the tail is the only part where the argenteum is fully developed.

Fig. 2.—Anterior portion of the upper side of the same specimen.

Fig. 3.—Appearance of a superficial slice of the skin of the upper side of a freshly-killed normal Flounder as seen under ZEISS CC., Oc. 3. Drawn with ABBE'S camera lucida. The black and coloured spots are the chromatophores, the smaller grey plates are the iridocytes. Transmitted light.

Fig. 4.—Iridocytes in the superficial portion of the skin of the lower side in a freshly-killed Flounder. ZEISS CC., Oc. 3, camera. Transmitted light.

Fig. 5.—Iridocytes of the same kind more highly magnified. ZEISS E., Oc. 3.

Fig. 6.—Chromatophores and iridocytes in the superficial layer of the skin of the *lower* side of a Flounder which died in the experimental apparatus, July 4, 1892. The history of the specimen was the same as of that represented in Plate 53, but the skin was not examined until some hours after death, so that the pigment of the coloured chromatophores is somewhat faded and diffused.

PLATE 55.

Fig. 1.—Appearance of fresh skin of normal littoral specimen of *Cottus bubalis*, lateral region below posterior part of anterior dorsal fin. ZEISS A., Oc. 3.

Fig. 2.—Appearance of skin from same region of a red specimen, from deep water, of the same species.

Fig. 3.—Dorsal skin, fresh, of the brown Pipe-Fish, *Syngnathus acus*. ZEISS CC., Oc. 3. Transmitted light.

Fig. 4.—Dorsal skin, fresh, of the green Pipe-Fish, *Siphonostoma typhle*. ZEISS CC., Oc. 3. Transmitted light.

Fig 5.—Chromatophores in skin of pectoral fin of *Trigla cuculus*, the Red Gurnard. The red chromatophores contain tetronerythrin, the granules consist of guanin. Drawn some time after death. $\times$ 300.

Fig. 6.—Skin of *Trigla hirundo*, showing chromatophores and guanin granules. Drawn some time after death. $\times$ 300.

Fig. 7.—Chromatophores in skin of *Nerophis æquoreus*, the banded Pipe-Fish. Drawn some time after death.

Fig. 8.—Peritoneum of the upper side of *Pleuronectes microcephalus,* the Merry Sole or Lemon Dab. The granular layer is the reflecting tissue composed of guanin. On the lower side only this tissue is present, the network of black pigment is almost entirely absent.

Fig. 9.—Spherical masses composed of needles of guanin from skin of *Pleuronectes limanda,* the common Dab.

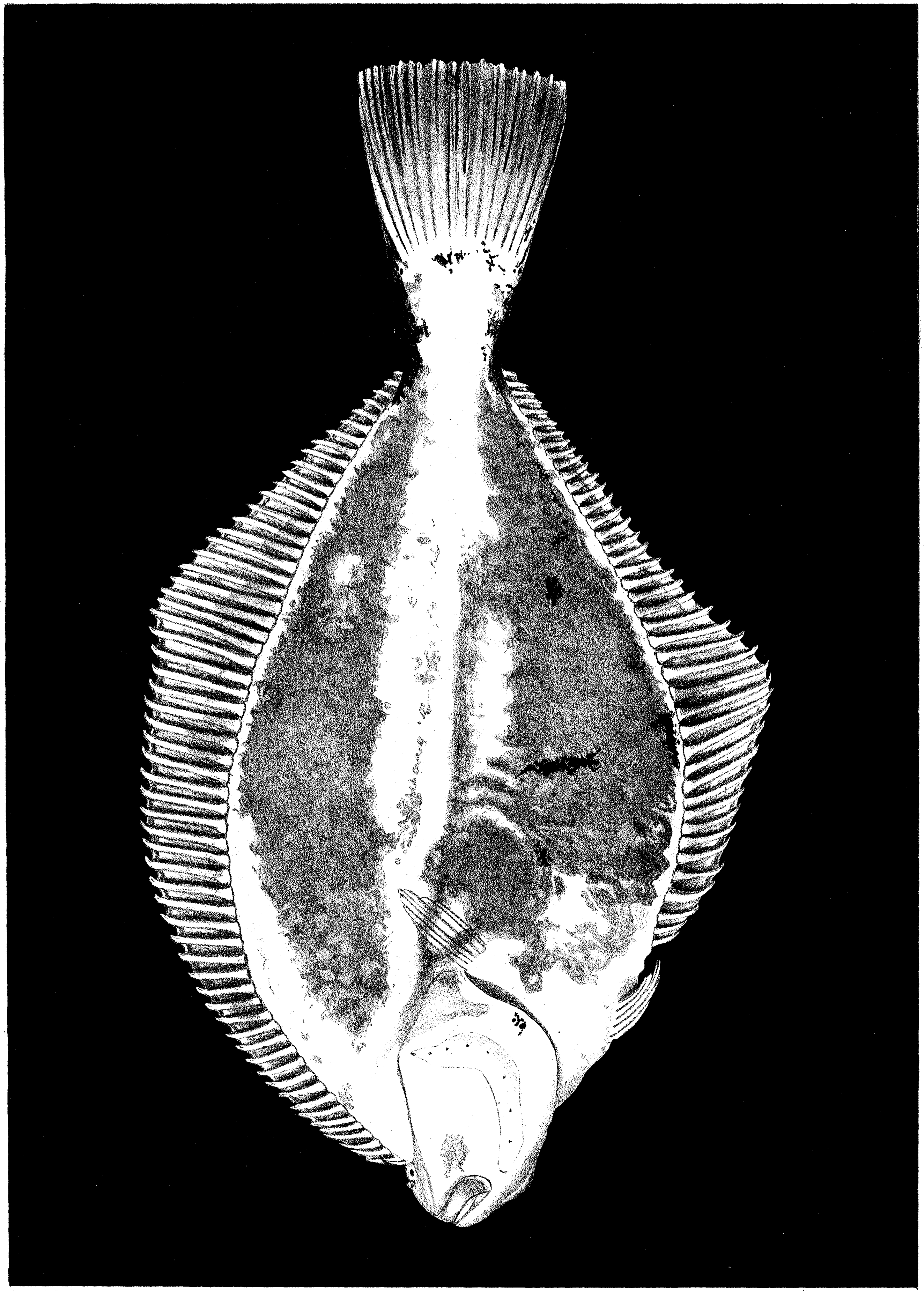
Fig. 10.—Reflecting tissue composed of bacilli from the ventral post-anal skin of *Siphonostoma typhle* outside the dermal scutes.

Fig. 11.—Rods from the silvery peritoneal layer of *Osmerus eperlanus:* guanin needles; *a,* in bundles; *b,* isolated. × 300.

Fig. 12.—Shows how the golden appearance is produced in the eye of a Fish, here from the argenteum of *Trigla hirundo.* A yellow lipochrome overlies a layer of guanin needles. × 300.

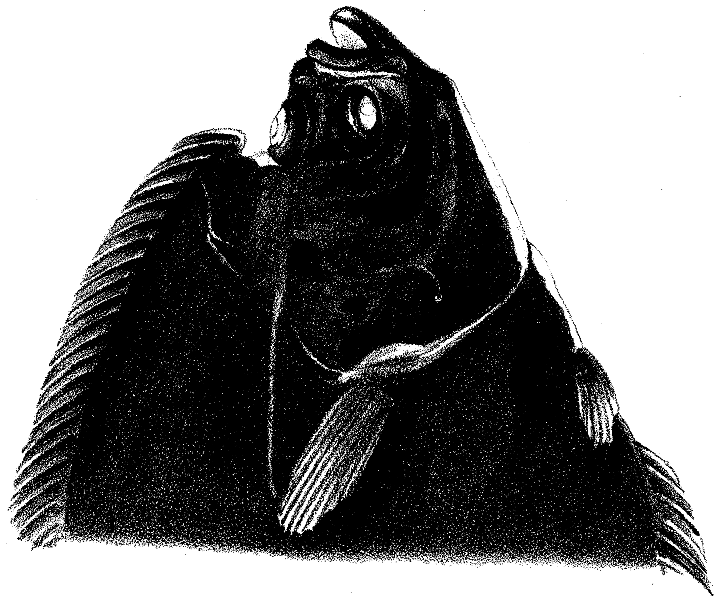
Fig. 13.—Isolated crystals of guanin from the silvery layer of the air-bladder of the Herring, prepared as described in the text. × 800.

Fig. 14.—Larger crystals from the same source. × 300.



Annie Willis pinxit. Edwin Wilson Cambridge.

PLEURONECTES FLESUS. ACQUIRED PIGMENTATION.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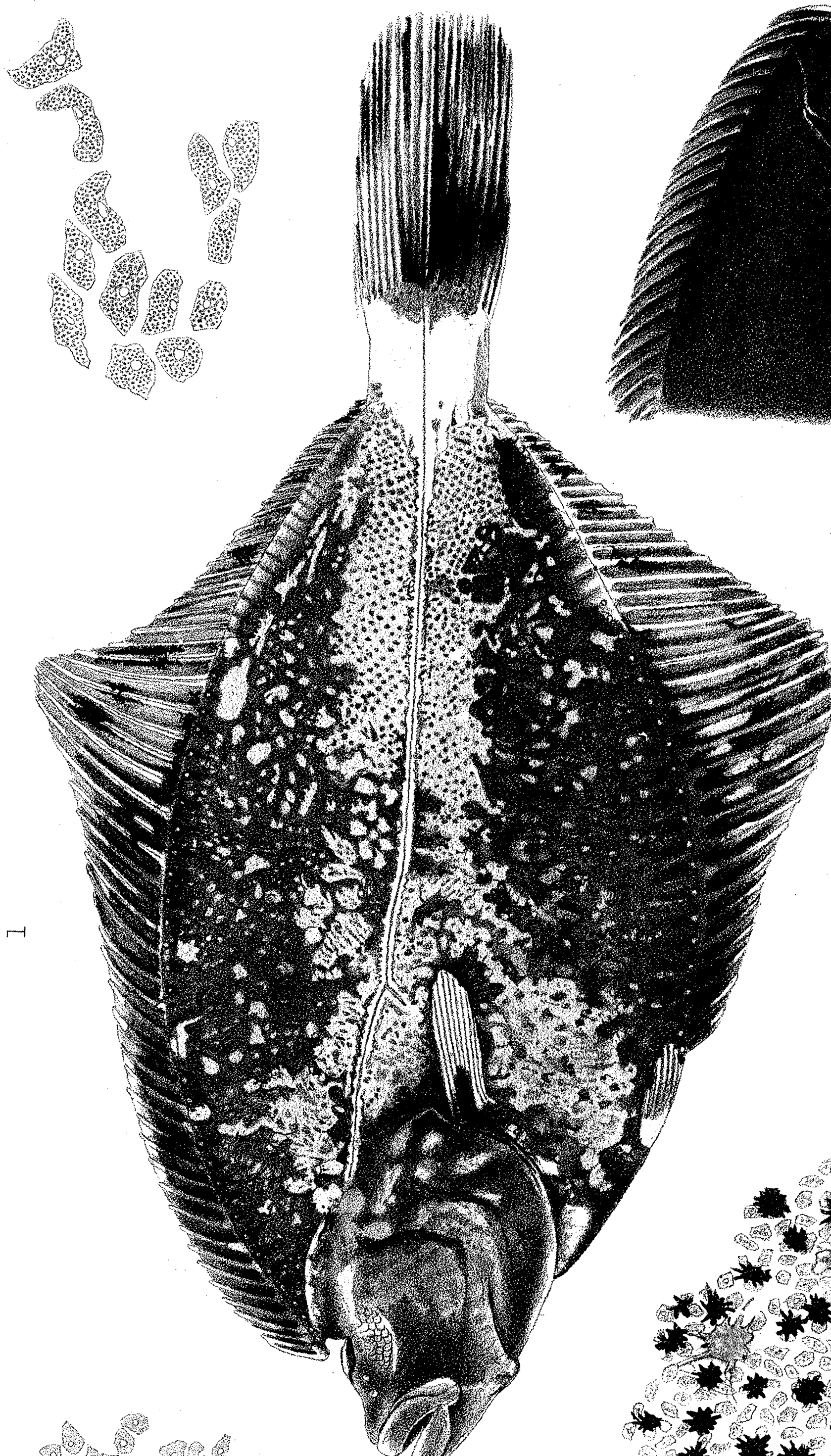
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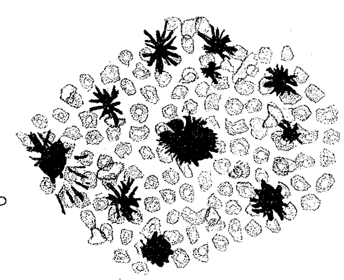
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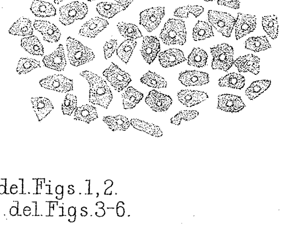
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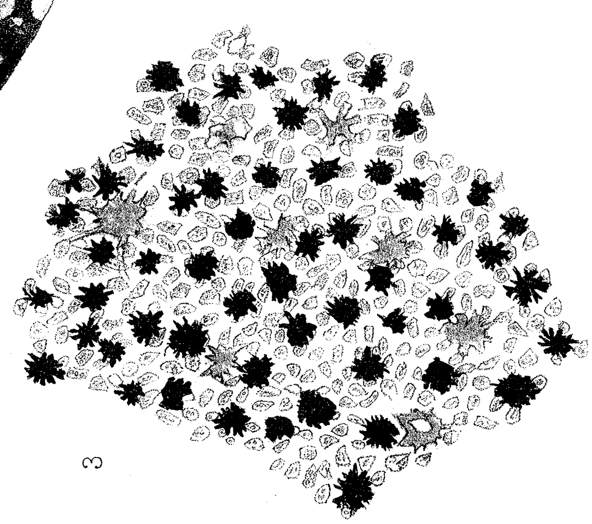
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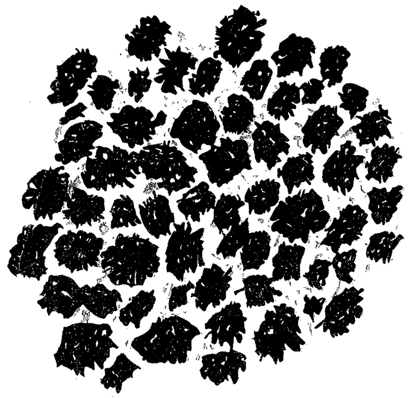
A.W. del. Figs. 1, 2.
J.T.C. del. Figs. 3-6.

Edwin Wilson. Cambridge.



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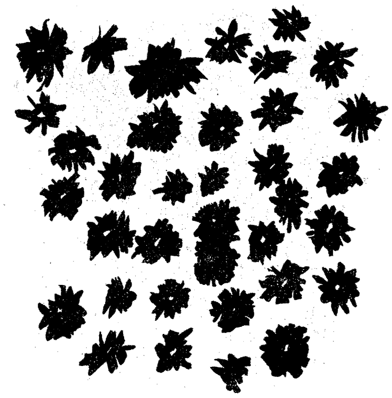

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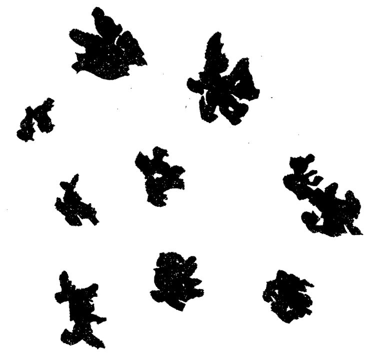

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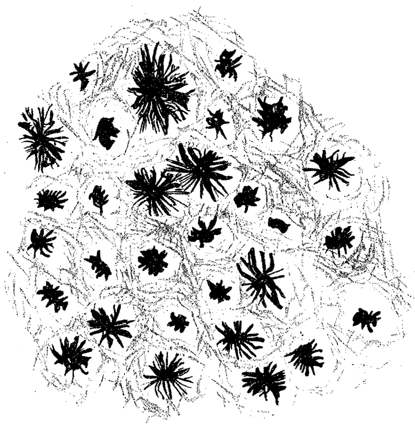

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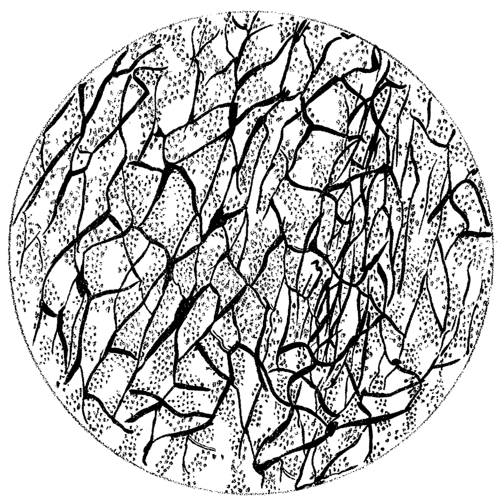

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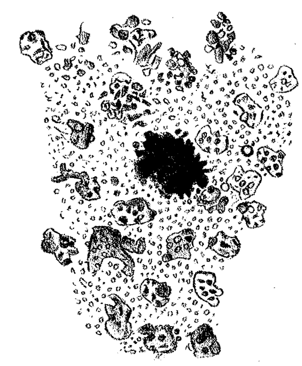

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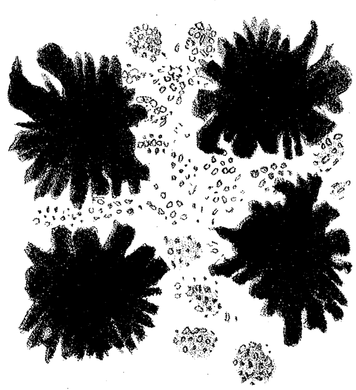

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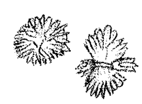

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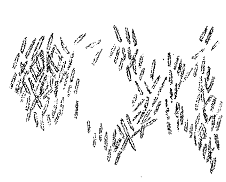

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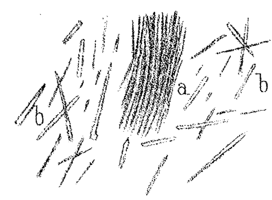

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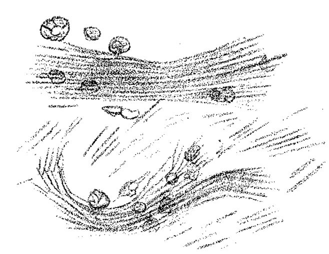

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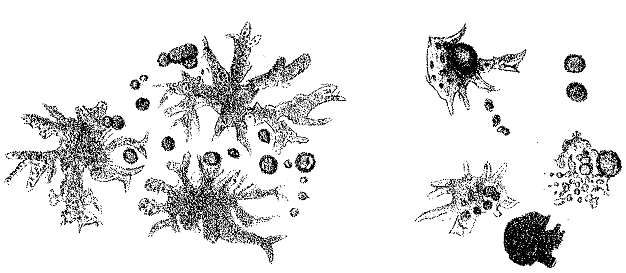

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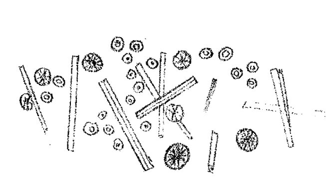

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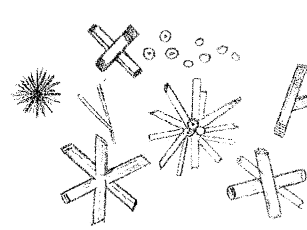

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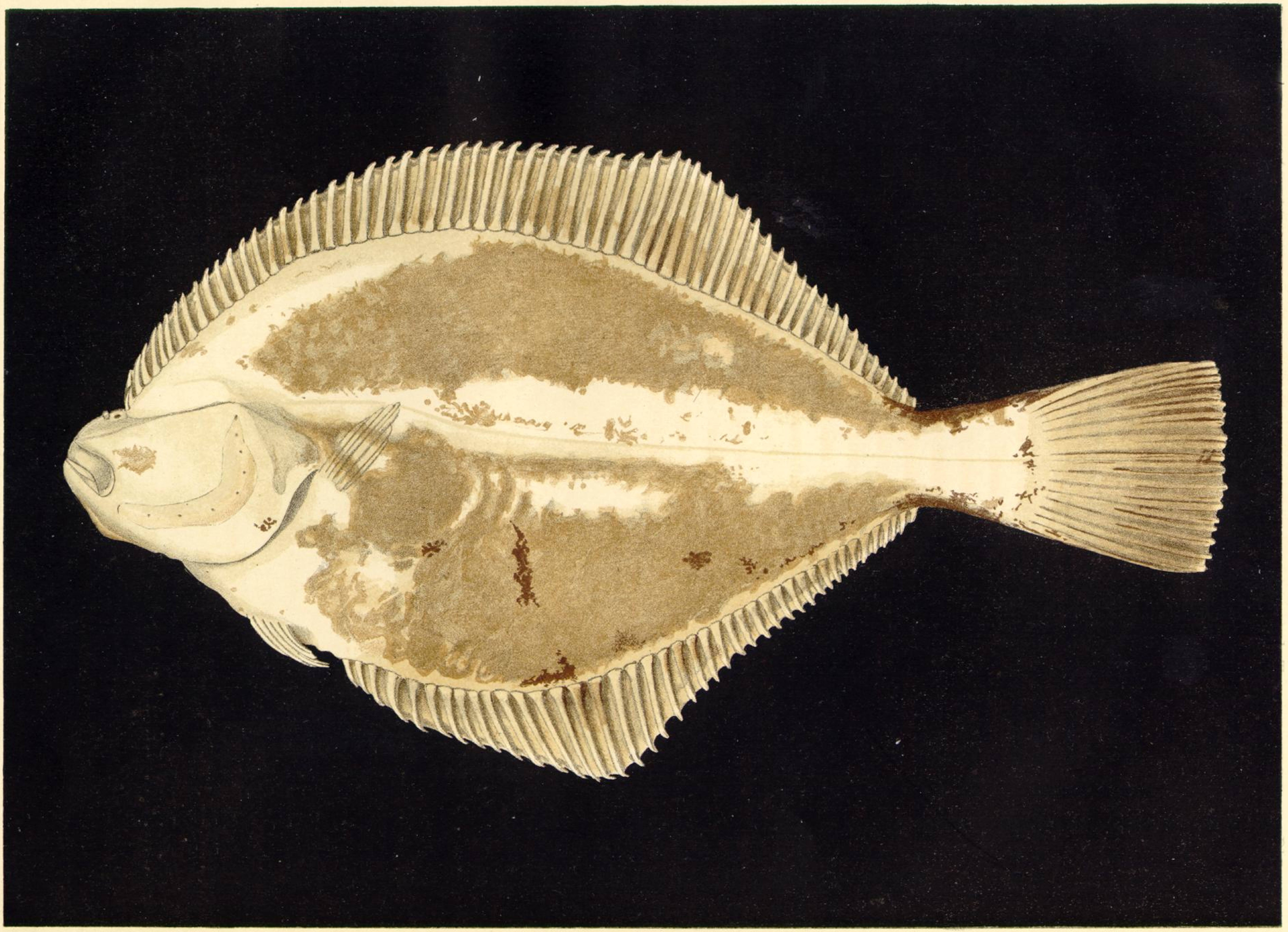
J.T.C. del. Figs 1-4, 8, 10.
C.M.M. del. Figs 5-7, 9, 11-14.

Edwin Wilson Cambridge.



PLEURONECTES FLESUS. ACQUIRED PIGMENTATION.

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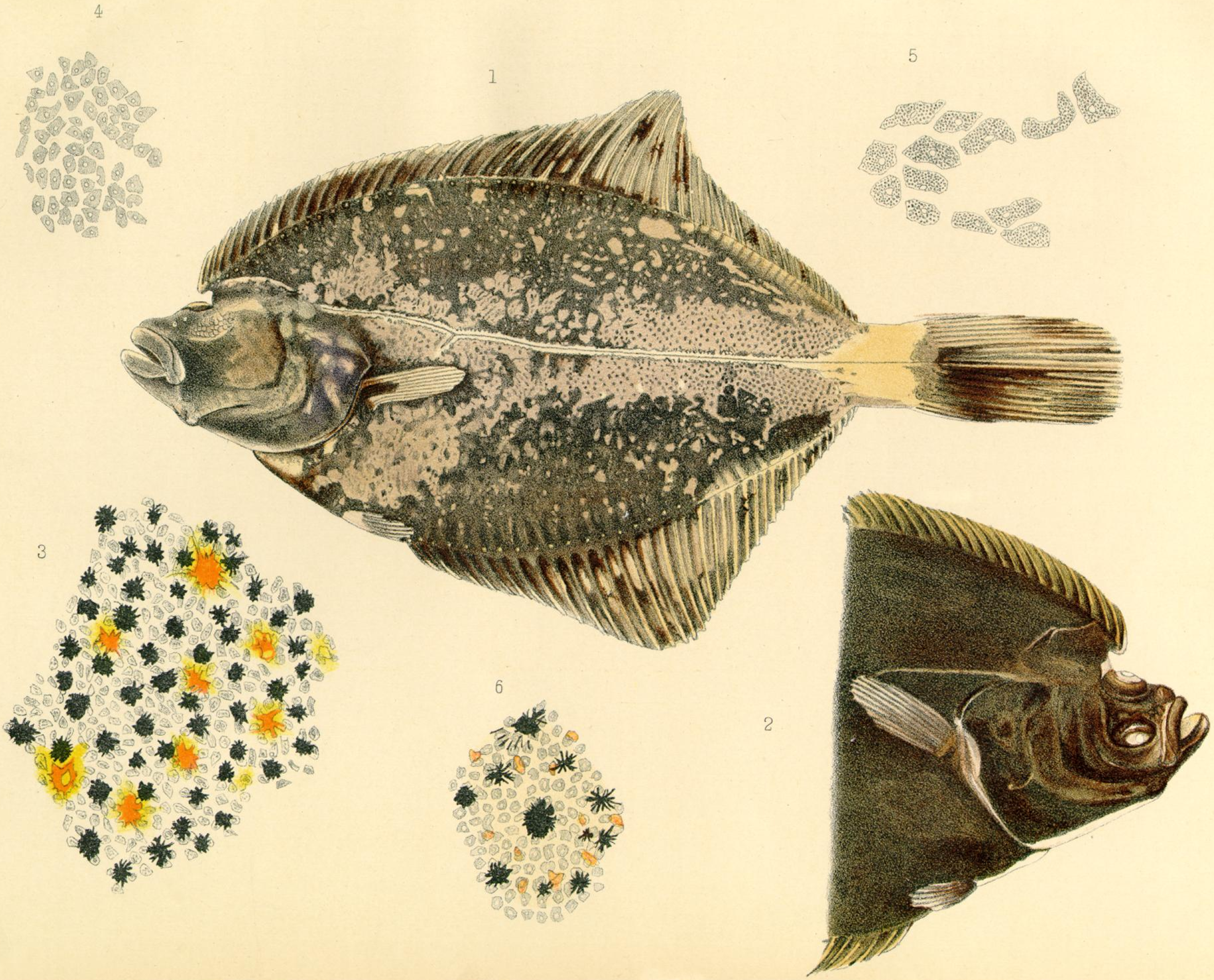

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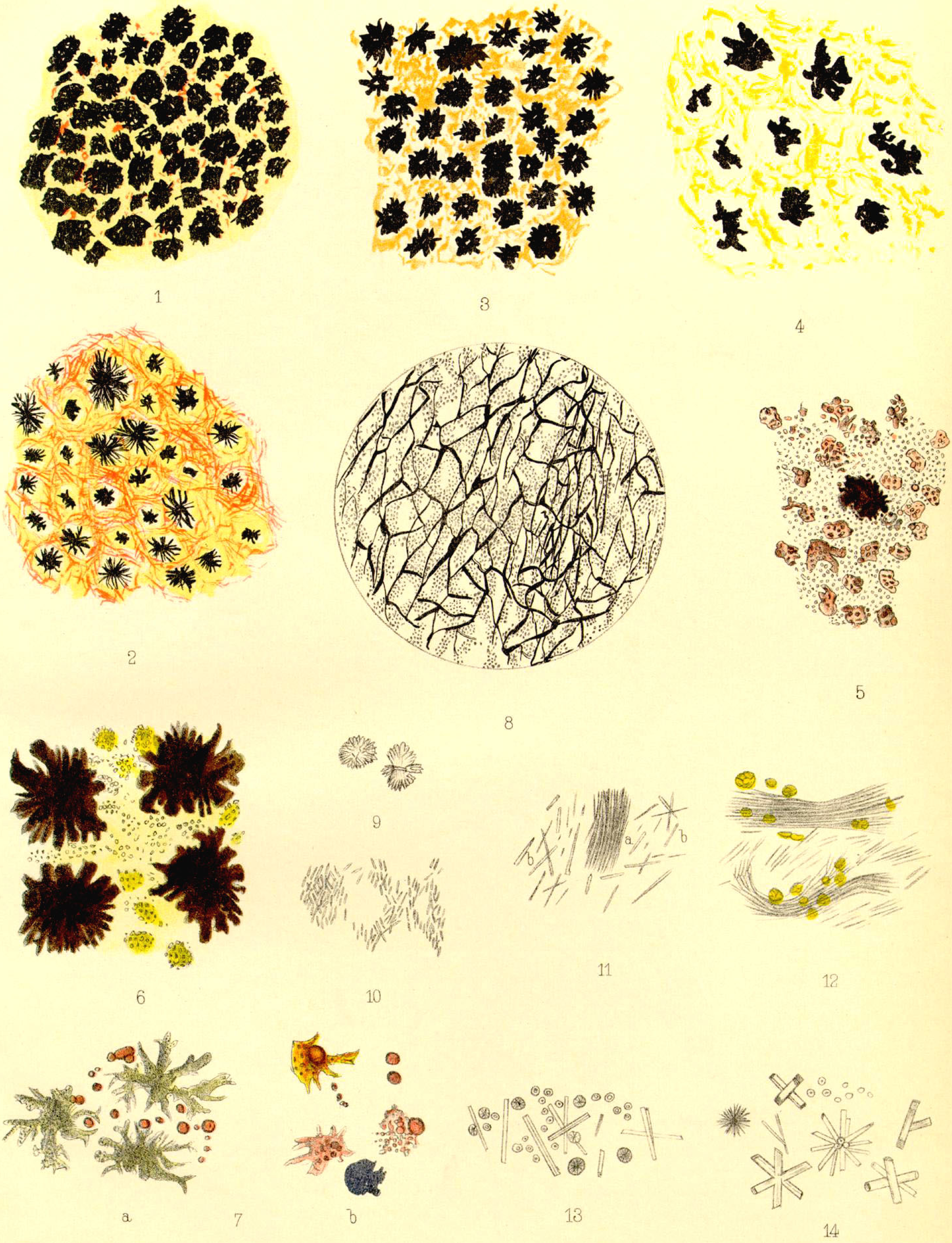

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