PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Studies in Colour Changes of Fish. Part II, An Analysis of the Colour Patterns of the Dab; Part III, The Action of Nicotin and Adrenalin in the Dab; Part IV, The Action of Caffeine in the Dab, and a Theory of the Control of Colour Changes in Fish

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III. *Studies in Colour Changes of Fish.**

Part II.—An Analysis of the Colour Patterns of the Dab.

Part III.—The Action of Nicotin and Adrenalin in the Dab.

Part IV.—The Action of Caffeine in the Dab, and a Theory of the Control of Colour Changes in Fish.

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[Plates 13–15.]

Contents.

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PART II.

1. Introduction.

In the course of investigations on the action of certain drugs on the colour of the dab, it became increasingly evident that not only should a microscopic examination be made of the results, but that apart from a complete analysis of the colour pattern of the fish, such an examination would be unintelligible. I am not aware that any such analysis has been made of so complicated a colour scheme. Abolin (see List of Papers, p. 198) has analysed the colour pattern of the minnow, and in a previous paper I have pointed out that the barring on the minnow is partly due to physiological differences and partly to morphological variations. Sumner has also given a description of the patterns on certain flat-fish, but his observations were purely macroscopic.

The main problem which has presented itself in this field is this :—Do all the chromatophores on the skin expand and contract synchronously and to the same degree ? In other words : Are the various colour phases and manifestations of markings due to morphological differences alone ? Sumner, as the result of researches carried out on flat-fish, has come to the conclusion that the many variations of colour, pattern and shade cannot be altogether explained by the assumption of a synchronous reaction of all the chromatophores, and that there must be some differential reaction of the chromatophores situated on the markings. Hogben is opposed to this view and thinks that, as " the extent of melanophore expansion in response to the intensity of a uniform background displays a very subtle gradation, it is not unlikely that the effect of a variegated background is simply to reproduce throughout the body a state of melanophore response, which renders more or less apparent a pattern dependent wholly on the numerical distribution of the melanophores in different areas." In the present research this problem has been approached by a study of the reactions of the dab to uniform backgrounds, to ascertain the extent of the " subtle gradation " and to supply the necessary data for the further discussion of this problem.

The general hue of the dab when removed from the trawl was a dark brown. This was relieved by orange and black spots, darker patches, and pale and even white spots. This general pattern can be divided into types of markings. These types, as will be seen, act almost as separate systems. It has been found necessary to apply certain terms, for the most part descriptive, to these types, which will now be defined.

The most prominent are the *orange and black spots*. They are present all over the right side, some being quite constant in position. A typical arrangement of the most stable is given in text-fig. 1.

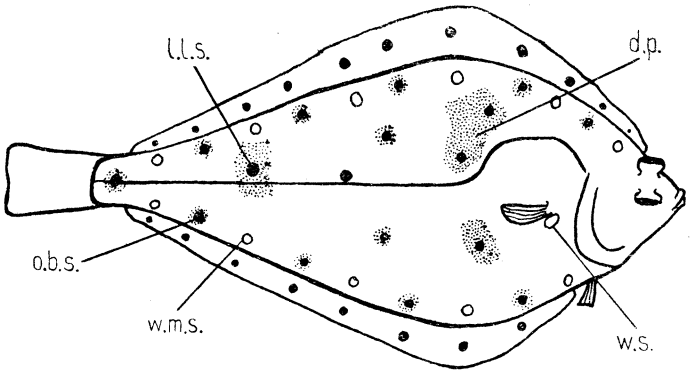
Associated with these are the *dark patches*. These areas are of considerable extent and occur around the stable orange and black spots. Two of these patches (*l.l.s.* and *d.p.*, text-fig. 1.) are very marked and occur in all the common Pleuronectidæ.

The *pale spots* are scattered all over the body. Their only macroscopic characteristic is that they appear paler than the general shade on certain backgrounds.

Alternating with the orange and black spots on the fin-ray muscles are the *white marginal spots*. They are distinctly white in colour, when visible. A large spot of this character occurs at the base of the pectoral fin on the right side, and very small ones are sometimes found near the lateral line.

The general ground-work has been divided into the *normal area* and the *yellowish spots*, depending on microscopical differences.

In calculating the average number of chromatophores in a unit area, the unit of area has always been the same, namely 0·16 sq. mm., whether in the young or in the adult. This means that if there are the same number of chromatophores per unit area in the young as in the adult, then a large increase in numbers has taken place during the growth of the fish as a whole. Measurements of the size of the various chromatophores are expressed as diameters of the contracted bodies in millimetres. Throughout, the terms "expanded" and "contracted" have been used irrespective of the mechanism of the movements of the granules.



TEXT-FIG. 1.—Dab showing the various colour markings. *o.b.s.* (in black), the orange and black spots; *l.l.s.*, the lateral line orange and black spot; *d.p.* (dotted), the dark patches; *w.m.s.* (ringed), the white marginal spots; *w.s.*, the white spot at the base of the right pectoral fin.

It was found that 40 per cent. formol was the only efficient fixative. Lower concentrations did not penetrate sufficiently quickly to give a fixed picture akin to the living state, expansion of the melanophores having commenced. After an hour in 40 per cent. formol the fish were transferred to 4 per cent. formol, to complete the hardening, for another two hours. The skin from the right side was then removed and washed in running tap-water for half an hour; taken through 50 per cent. and pure glycerine, and selected portions mounted in Brady's glycerine jelly. After ringing with Canada balsam, these preparations will keep well without fading.

The material was collected and the experiments performed at the Marine Biological Association's Laboratories at Plymouth. Most of the microscopical data were worked out in the Zoological Laboratories of the Imperial College of Science and Technology. The author wishes to tender his thanks to Prof. MACBRIDE, under whose direction the work was carried out, and also to Dr. CANNON and the members of the staff at Plymouth.

2. The Chromatophores.

Four main types of chromatophores are present in the dab :—melanophores, erythrophores, xanthophores and iridophores. Examination of the reactions indicate that all are important in the colour changes.

(*a*) *Melanophores.*

The melanophores are present in three layers, one in the epidermis and two in the dermis. The two latter are situated above and below the scales respectively. The upper dermal layer is by far the most important of the three in the colour changes.

(i) *Epidermal melanophores.*—These cells are found only on the orange and black spots and even then their distribution is irregular. Cunningham and McMunn found that the flounder had epidermal melanophores all over the right side, although the dab had only a few here and there. I have found that epidermal melanophores can be produced all over the body of the dab (except on the pale spots) by keeping small dabs (about 4 cms. long) on a black background for two or three weeks. This is suggestive that the distribution of these melanophores is a function of the environment.

Their shape differs considerably from that of the other two types of melanophores. There are only a few branches from the main cell body, which divide almost at right angles in all three dimensions. These characteristics may well be due to the type of tissue in which the cells lie, for any flat expanse in one plane such as that occupied by the dermal melanophores would be difficult to find among the tightly compressed spherical epidermal cells (see Plate 13, fig. 4).

Two theories of their origin have been propounded. Firstly, that they arise from the dermal layers by migration outwards of the dermal melanophores. Secondly, that they arise *de novo* from the epidermal cells by deposition of melanin within them.

In several instances cells were found in the epidermis which had a small quantity of melanin and appeared to be intermediate stages in the formation of epidermal melanophores. But indications of a degenerative process were found in sections, and Kuntz attributes the presence of these cells to this process.

On the other hand, no increase in the numbers of upper dermal melanophores was found after exposure to black background, as previously described. According to Babak, such an increase should occur. There were, however, many more epidermal ones. This points to a migration outwards of the upper dermal melanophores. The author is inclined to this latter view of the origin of the epidermal melanophores.

The size of these melanophores corresponds to that of the upper dermal melanophores immediately below them. In the young dabs (4 cms.) they are 0·011 mm. in diameter. In the adult they have increased up to 0·022 mm. on the lateral line spot and 0·015 mm. on smaller spots which have arisen later (Plate 13, figs. 1*b* and 2*b*).

(ii) *Upper dermal melanophores.*—These melanophores are illustrated in figs. 1, 2 and 3. In distribution they may be found all over the right side of the body in greater or less numbers. The actual number present varies both with age and position relative to

the markings. In the adults: on the normal area there are 104 per unit area, on the lateral line spots (orange and black spots) there are only 89 on an average, and on the pale spots there is a slight increase averaging 111 per unit area. In the young dabs (4 cms.) there are 99 per unit area on the normal area and 141 per unit area on the orange and black spots. This last figure is even more striking when it is realised that it includes counts from fish in a transitional stage. In the smallest dab I examined (3 cms.), the average was 113 on the normal area and 286 on the orange and black spots. There was a rapid change in the number per unit area on these latter spots when the fish were passing through the five- and six-centimetre stages.

Differences in size were also noticeable. In the adult the average diameter of the melanophores on the normal area is 0·011 mm. and on the orange and black spots 0·022 mm. In the young little difference is noticeable, the average for the normal area being 0·010 mm. and on the orange and black spots 0·015 mm. Again, the latter figure includes some in the transitional stage.

The density of the pigment varies considerably. This is important when considering the appearance of the different markings macroscopically. The following are rough estimates which I have made from careful examination of a large number of preparations. Comparisons are made with the melanophores on the normal area.

1. On the orange and black spots, particularly on the lateral line spots and the other major spots, the melanin is much denser.
2. On the pale spots and on the white marginal spots the melanin is less dense.
3. On the yellowish spots the melanin is slightly denser.

The number of processes emanating from the central cell body is large. The branching takes place at acute angles and lies in one plane (fig. 3).

(iii) *Lower dermal melanophores.*—These are present all over the body on the right side. No differentiation in relation to markings has been seen. They are few in number per unit area but have a large total expanse. There are a few main branches which are, however, thick at the base but taper rapidly. One or two processes may extend far out beyond the rest (fig. 5). Even when fully expanded they do not cover the whole area. At the edge of each scale this layer (below the scale) appears to be continuous with the upper dermal layer of the underlying scale. This can be seen in section in fig. 22 (Plate 15). The characteristics enumerated above fall off as they approach the upper dermal layer proper, and these melanophores assume the appearance of the upper dermal ones.

(*b*) *Erythrophores.*

The erythrophores are present only in the upper dermal layer. Within this layer they are confined to the normal area, the orange and black spots, and they may be present in small numbers in the white marginal spots. They are entirely absent from the pale spots. Even when the fish had been kept on an orange background for two or three weeks and the number of erythrophores had increased elsewhere, none were found on these spots.

They vary in number per unit area according to the marking on which they are situated. In the normal area there are about 73 per unit area in both young and adult dabs. On the orange and black spots in the adults the average is about 174. In the young fish such areas are too small to give an accurate count, but it is evident that there is an increase in numbers on these spots over that on the normal area.

The number can be influenced by background. After a week on a white background the average number per unit area had been decreased to 63. Following a further two or three weeks on an orange background the average was increased to 83.

The actual size of these cells is difficult to estimate. The processes are broad and flat and when expanded appear to be fused at the base. Measurements of the contracted masses give 0·009 mm. as an average diameter for all erythrophores. This figure, however, is much smaller than appears in the expanded condition; it probably represents the mass of red pigment in the centre of the cell body only. Around the pale spots they are apparently smaller in size, but this in all probability shows a diminution in the amount of pigment present. Figures of the erythrophores in various stages are given in fig. 12 (Plate 13).

(*c*) *Xanthophores.*

The xanthophores are present in both dermal layers.

(i) *Upper dermal xanthophores.*—They occur only on the yellowish spots. They are comparable in size and shape with the erythrophores, to which they are doubtless closely related. (At the base of most scales the erythrophores show a slight tinge of yellow.) They appear slightly fewer in numbers, although no counts were made owing to the small size of the spots (fig. 11).

(ii) *Lower dermal xanthophores.*—They occur all over the right side. Their morphological form is similar to the lower dermal melanophores, with which they are closely associated in distribution. They are few in numbers, but larger in total expanse than the upper dermal xanthophores (fig. 9).

(*d*) *Iridophores.*

The iridophores to be dealt with here are those on the right side of the body, associated with the other chromatophores. They are generally absent from the black area of the orange and black spots but are numerous on the orange area. The average number per unit area on the normal area is 180. This is slightly increased on the pale spots and considerably so on the white marginal spots. The number can be increased by keeping on a white background. Small dabs kept in this way for two to three weeks showed an increase in numbers from 190 to 275 per unit area.

In size and shape they vary considerably, but they appear to be incapable of expansion and contraction. Examination under a high power shows that the iridophores are composed of bundles of crystalline needles, lying in a rather nondescript way (fig. 8). This means that as reflecting surfaces they are efficient whatever may be the direction of

the incident light. This arrangement is particularly noticeable under crossed Nicols. The central pale area, which is free from crystals, is presumably the nucleus.

3. The Types of Markings.

Below I have assembled all the morphological features of the various markings. Some of this has necessarily been mentioned under the description of the chromatophores, but particular stress is here laid on the complicated associations of the chromatophores.

(*a*) *The Normal Area.* (Fig. 13.)

The chromatophores in this area are melanophores, erythrophores and iridophores. The average numbers of each kind per unit area are 104, 73 and 180, respectively. The melanophores have a diameter of 0·011 mm. and the erythrophores one of 0·009 mm. when in the contracted condition. The iridophores are to some extent orientated around the erythrophores (fig. 10), so that when the latter are in the expanded phase they screen the iridophores.

(*b*) *The Yellowish Spots.*

These are very small spots lying in the normal area and possessing xanthophores instead of erythrophores. Further, the melanophores present are slightly larger and fewer in number than on the normal area. No accurate counts could be made, owing to the small size of the spots.

(*c*) *The Orange and Black Spots.*

In these spots the orange part is usually distinct from the black and may appear as a patch at the side or as a ring enclosing a black centre. On the orange spot, melanophores, erythrophores and iridophores are present. The melanophores are few in number and in the adult larger than normal. The erythrophores are of normal size, but occur in large numbers, averaging 174 per unit area. The iridophores appear normal in every way.

On the black spot melanophores and a few erythrophores are present. In the young large numbers of melanophores of normal size are found varying from 141 to 286 per unit area. As they grow older multiplication appears to cease and growth in size takes place, so that the adult melanophores have double the diameter of the normal. Decrease in numbers per unit area is thus compensated for by an increase in size, and the general tone of the black patch is maintained (figs. 14 and 15).

Epidermal melanophores are present and add to the general darkness of tone.

(*d*) *The Dark Patches.*

The position of these spots has already been described. Despite careful examination of the skin in these regions, no morphological differences among the chromatophores in any particular has been noticed, other than those attributable to one or other of the other types of markings. Their appearance on pale fish is due entirely to the expansion

(partial) of the melanophores in these areas. The evidence points to their being nervous in origin, for fish kept on a white background for several weeks will display this marking, although handling or excitement will cause rapid paling. Individual differences in reaction are great.

(*e*) *The Pale Spots.*

These spots are distinguished by the absence of erythrophores and xanthophores. Only melanophores and iridophores are present; the former average 111 per unit area, slightly higher than the normal. Their total expanse is also larger but the pigment less dense than on normal areas. In some few cases near the lateral line there is a tendency for the iridophores to become grouped round the melanophores. The morphological stability of these spots has already been remarked upon in connection with the erythrophores and the epidermal melanophores.

(*f*) *The White Marginal Spots.*

Melanophores and iridophores and sometimes erythrophores occur in these spots. The iridophores are always grouped round the melanophores (fig. 7), and in extreme cases the iridophores become so numerous that they form a platework of reflecting tissue. This arrangement results in an obscuring of the iridophores by the expanded melanophores.

4. Reactions to Background.

In the following analysis of the reactions to background the behaviour of the chromatophores on the pale spots and on the white marginal spots has been particularly investigated. The estimates of the various phases have been made by eye, but the chromatophores of certain markings have been treated numerically. This has been done by measuring the diameters of the total expanse of a number of chromatophores, and from the average of these the area covered by one chromatophore has been calculated, assuming the extent to be on the average circular in nature. The average number per unit area gives a further figure, so that in the data given the figures show the area covered by one particular type of chromatophore in one unit of area (0·16 sq. mm.) under the given conditions.

(*a*) *White Background.*

Macroscopically the fish were uniformly pale, with the exception of the orange and black spots and occasionally the dark patches. In some the white marginal spots appeared slightly darker than the general pallor; this was more marked in the posterior region (fig. 23*a*).

Microscopically the most marked features considering the reaction as a whole were :—1, the incomplete contraction of the melanophores; 2, the incomplete contraction of the xanthophores; and 3, the complete contraction of the erythrophores.

The reactions of the melanophores varied according to the markings to which they belonged :—On the *normal area* they were "stellate"; on the *yellowish spots* there

was a tendency to be " slightly stellate " ; on the *pale spots* (fig. 16) and *white marginal spots* they were " fully expanded."

Measurements showed that the area covered :—

On the *normal area* :—
By melanophores was 0·08 sq. mm.
By erythrophores was 0·01 sq. mm.
On the *pale spots* :—
By melanophores was 0·12 sq. mm.
On the *white marginal spots* :—
By melanophores was 0·13 sq. mm.

(*b*) *Orange Background.*

Macroscopically the fish presented a fairly even orange tone, although the pale spots were slightly in evidence (fig. 23*b*).

Microscopically the main features were :—1, the complete expansion of the erythrophores and xanthophores ; and 2, the incomplete contraction of the melanophores. In the latter there was again differentiation according to the markings. On the *normal area* they were " slightly expanded " or " stellate " ; on the *yellowish spots* they were only " stellate " or " slightly stellate " ; on the *pale spots* " fully expanded " ; and on the *white marginal spots* they varied, mostly " stellate," but a few individuals showed " expanded " or " fully expanded " phases.

Measurements showed that the area covered :—

On the *normal area* :—
By melanophores was 0·08 sq. mm.
By erythrophores was 0·04 sq. mm.
On the *pale spots* :—
By melanophores was 0·12 sq. mm.
On the *white marginal spots* :—
By melanophores was 0·11 sq. mm.

The chief points to be noticed in comparison with the reaction to white background are :—1, the increase in the area covered by the erythrophores ; 2, the constancy of the area covered by the melanophores on the pale spots ; and 3, the decrease of the area covered by the melanophores on the white marginal spots.

(*c*) *Black Background.*

Macroscopically the fish were very dark but not of an even tone ; for the pale spots stood out vividly as well as the white marginal spots which appear white in the majority of cases (fig. 23*c*).

Microscopically the general features were :—1, the complete expansion of the melanophores ; and 2, the incomplete expansion of the erythrophores and xanthophores.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

On the *white marginal spots* the melanophores were nearly always "stellate," some few individuals showing the "expanded" phase (fig. 17). The area covered :—

On the *normal area* :—
By melanophores was 0·11 sq. mm.
By erythrophores was 0·03 sq. mm.
On the *pale spots* :—
By melanophores was 0·12 sq. mm.
On the *white marginal spots* :—
By melanophores was 0·07 sq. mm.

The chief points to be noticed in comparison with the two previous reactions are :— 1, the increase of the area covered by the melanophores on the normal area ; 2 the decrease of the area covered by the erythrophores ; 3, the constancy of the area covered by the melanophores on the pale spots ; and 4, the further decrease of the area covered by the melanophores on the white marginal spots.

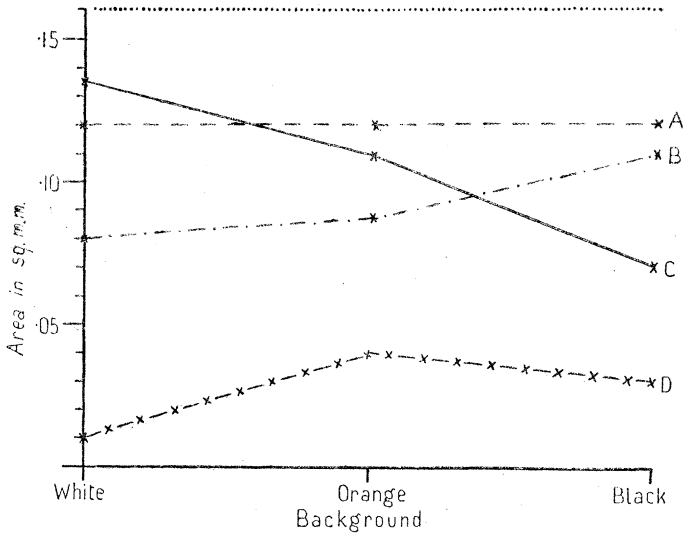
5. Conclusions.

Mast and Kuntz have worked on the adaptation to shades, colours and patterns in the flounders *Paralichthys* and *Ancylopsetta*. They found a differential expansion and contraction between the melanophores and xanthophores (erythrophores + xanthophores). Kuntz states that on a dark red background the orange xanthophores (erythrophores) are expanded although the yellow xanthophores (xanthophores) are markedly contracted. This may be compared with the dab on a white background, where almost the reverse is the case—contracted erythrophores and partially expanded xanthophores.

Text-fig. 2 gives some idea graphically of the entire lack of co-ordination in the reactions of the chromatophores. Not only is differentiation in reaction found between the different types of chromatophore, but even between the melanophores belonging to one marking and those of another. Explanations of differences between erythrophores, xanthophores and melanophores can be given, based on the possible differences in the direct effect of differently coloured lights on the various pigments, although no such differentiation has been found as yet—in fact, Mast is convinced that the fish are possessed of colour-vision and thus respond to the different colours.

But in the case of melanophores possessing only slight variations in size, etc., there can be little room for doubt in this direction. Three prominent instances of this occur in the dab—the pale spots, the white marginal spots and the dark patches. Such phenomena can only be interpreted on the basis of some sort of physiological differentiation. The locality of this differentiation may be either in the chromatophores, on which the nervous stimuli or possibly hormones can work to produce reactions determined only by the nature of the end-organ, or it may exist in a complicated nervous control. It remains to investigate and discuss the relative values of these two hypotheses.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



TEXT-FIG. 2.—Graph to show the lack of co-ordination in the reactions of the various chromatophores; A, Melanophores on the pale spots; B, melanophores on the normal area; C, melanophores on the white marginal spots; D, erythrophores on the normal area.

PART III.

1. INTRODUCTION.

In a previous paper the question of the point of action of adrenalin was left in abeyance until further work had been done with other drugs of a sympathomimetic nature. All that was known was that the seat of its action was peripheral. It was decided to investigate the action of nicotin. Firstly, because WYMAN had shown that peripherally applied it caused an expansion of denervated melanophores. This would assist in localising the action of adrenalin. Secondly, the actions of nicotin have been fully worked out by LANGLEY, and his results suggest that in fish it might also have an action on the sympathetic chain ganglia.

The nicotin employed in all the critical experiments was obtained from Hopkin and Williams, where it had been freshly distilled. It was described as "pure medicinal" and underwent little degeneration during the course of the experiments. The adrenalin used was of the "Soloid" brand, of Burroughs Wellcome and Co. Solutions were made up freshly for each set of experiments in 50 per cent. artificial sea-water, which is approximately isotonic with the blood of the dab. Where the nicotin was applied externally, the solutions were in undiluted artificial sea-water.

During the experiments in which nicotin was employed, notes were made of the fluctuations in movements and breathing synchronous with the changes of colour. These observations were used as criteria for the stage of the action of the nicotin.

Urethane was used as an anæsthetic in those operations greater than superficial venesection. The fish were immersed in a 0·5 per cent. solution, and as soon as all movements had ceased (except breathing) they were removed to clean sea-water for the rest of

the experiment. Recovery from the anæsthetic usually took about 30 minutes, so that the experiments were completely finished before the normal reactions had begun to reappear.

Previous to their being used, the dabs were kept in white porcelain tanks with a circulation of sea-water. Unless otherwise stated, in all these experiments, the fish were kept on a white background. During the operations they were placed in a tank with a sloping bottom, so that they were enabled to breathe fresh sea-water and at the same time the operation could be carried out above water.

2. Section of Nerves.

Some preliminary experiments were performed by sectioning various nerves in order to ascertain the degree of expansion and the general nature of such reactions in the dab. These experiments constitute practically a repetition of part of Pouchet's and von Frisch's work.

(*a*) *Section of the Sympathetic Chain.*

The operation can only be carried out together with a partial or complete section of the hæmal canal, so that, although the effect of anæmia is to cause a contraction of the melanophores, the expansion which has been observed as the regular result of this operation is not necessarily due to the nerve section alone, but may be due to the lack of stimuli in the presence of unoxygenated blood.

In the present case, however, microscopical examination of the melanophores in the tail and in the dorsal and ventral fins was carried out twelve hours after the operation, and at the same time observations of the conditions of the blood-supply were made. In several instances it was found that the blood-supply was fairly normal and that the melanophores were expanded.

The following is an example :—

May 12th. Section of sympathetic cord and hæmal canal in the caudal region under urethane.

Replaced in white tank.

After recovery from anæsthetic—darkening posterior to point of section.

May 13th. Macroscopically—no change, skin apparently healthy and motor activity unimpaired.

Microscopically—melanophores expanded in the dark area and circulation of the blood well marked.

May 14th. Macroscopically—no change.

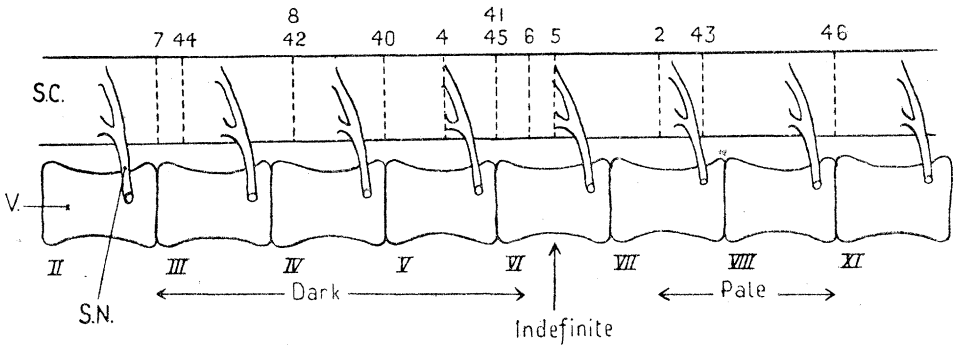
Microscopically—melanophores expanded as before ; circulation of blood excellent in both dorsal and ventral fins, but not in tail, which had become injured.

Thus it is fairly certain that section of the chain alone can produce expansion of the melanophores.

(*b*) *Section of the Spinal Cord.*

Pouchet found that no change in colour was produced by section of the spinal cord, but von Frisch observed that if the cord was cut at a high level darkening of the whole animal occurred, while at lower levels no change was evoked. This was confirmed in the present research to a certain extent.

Altogether thirteen operations were successfully carried out; the fish were afterwards examined and the exact position of the section ascertained. A diagram showing points of section and results obtained is given in text-fig. 3. It was found that the nerves



Text-Fig. 3.—Diagram to show sections of C.N.S. S.C., spinal cord; S.N., spinal nerve; V., vertebræ. The Roman numerals are the numbers of the vertebræ. The plain numerals refer to the index number of the fish used for the particular section indicated by the dotted line.

controlling the colour changes left the spinal cord in the spinal nerve corresponding to the sixth vertebra. Nine sections anterior to this point were made, and all caused a complete darkening of the whole fish. Three sections posteriorly placed caused no change in the colour. One was found to be on the point itself, where the roots entered the cord and gave a mottled effect, due probably to a partial section of the rami. Motor paralysis occurred in all cases posterior to the point of section. The only point of difference between this and von Frisch's results in the minnow is that the nerves concerned leave the spinal cord much higher up. This is possibly to be accounted for by the considerable foreshortening of the trunk region in the dab.

(*c*) *Section of the Trigeminal.*

In the dab it is just possible to section the trigeminal peripheral to its receiving a branch from the sympathetic, without interfering with the blood-supply other than by removing its nervous control.

A large number of these operations was carried out, as it was rare that the fish lived longer than three or four days after the operation. Only the mandibular branch of this nerve was sectioned.

After recovery from anæsthetic the mandibular region was seen to be darker than the rest of the body. About twelve hours later the mandibular region was extremely pale, showing a pallor in excess of that of the rest of the body, although the fish was kept on

a white background. If the fish were removed and placed on a black background the whole of the rest of the body would assume the normal dark hue, but the lower jaw would remain pale. Microscopical examination of this phase showed that the melanophores in that region were intensely contracted (fig. 21). As a rule this pallor on the lower jaw persisted until death, a day or two later; but in some cases, after a further lapse of twenty-four hours, this region would regain its darker tone and maintain this condition until the fish were killed for examination, ten days after the operation. Then it was seen that the melanophores were fully expanded.

This reversal of reaction may be explained on the basis of blood-supply, for in most of the fatal cases where the pale phase persisted, the blood-vessel had been injured and internal bleeding had taken place to a considerable extent. This would result in anæmia, causing the contraction of the melanophores. Subsequent restoration of the blood-supply and a complete recovery of the animal would again reverse the reaction to its original phase of expansion. On the other hand, it is difficult to see why this effect of anæmia does not appear sooner after the operation if this is a true explanation. Further, the line of demarcation between the normal area and the secondarily paled area is very sharp and suggests nervous action rather than that of blood (fig. 21).

3. The Action of Nicotin and Adrenalin.

(*a*) *Nicotin Painted on to the Sympathetic Chain Ganglia.*

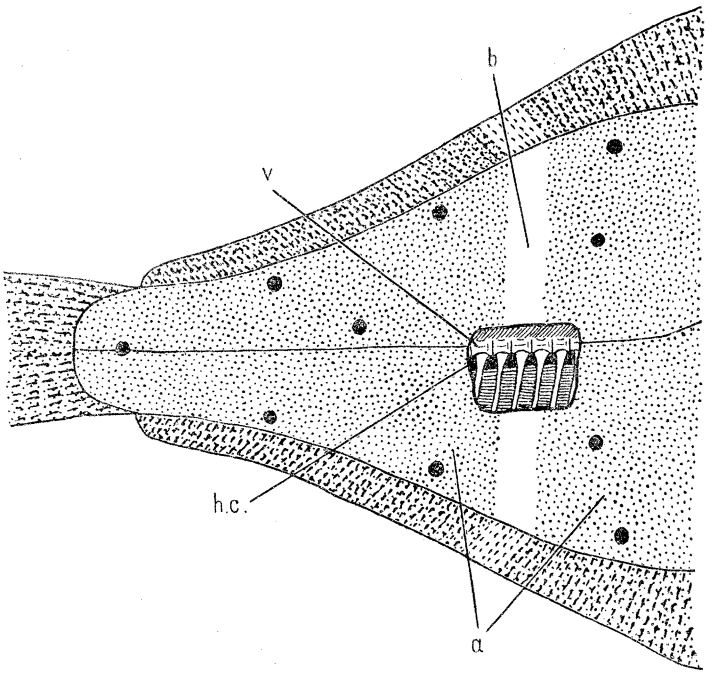
The fish were placed under anæsthetic. This had the effect of darkening the whole animal so that only paling effects could be produced and observed. The sympathetic chain was exposed for a short distance in the caudal region and 1 per cent. nicotin solution painted on to it. Excess of nicotin was removed by a wad to prevent spreading. Pallor appeared almost immediately in the segments served by the ganglia treated. It was strictly limited and appeared as a streak running dorso-ventrally (text-fig. 4). After about one minute this pallor gradually disappeared and the fish recovered its dark hue all over. The fish were then killed before recovery from anæsthetic.

(*b*) *Nicotin on a Cotton Wad in the Peritoneum.*

Again the whole experiment was carried out under anæsthetic, so that paling only was noticeable. A small incision was made in the peritoneal wall in a dorso-ventral direction, in order not to sever any of the spinal nerves lying in the wall. A small wad of sterilised cotton wool, soaked in 1 per cent. nicotin solution, was inserted into the peritoneum and the wad pushed dorsally until it lay as near to the sympathetic chain as possible. The incision was then quickly sewn up by a single stitch and the fish replaced in a large amount of fresh sea-water. The whole operation did not take more than two minutes.

Soon pallor began to appear, at first in the segments in which the wad was lying and then anterior to the wad in the head region. From here it spread backwards until the whole body was excessively pale. Usually by the time that the tail region had become

pale the head region had begun to show signs of darkening again. Accompanying this pallor there were muscular convulsions. Subsequently, as the fish regained their dark colour, they became inert as before the application of nicotin. The fish were then killed



TEXT-FIG. 4.—Showing the effect of painting the sympathetic chain ganglia with nicotin: *v*, vertebræ; *h.c.*, hæmal canal on which lie the ganglia; *a*, darkening due to anæsthetic; *b*, segmental pallor due to the action of nicotin.

before recovery from anæsthetic. Post-mortem examination showed that in the majority of cases the wads did not lie closely opposed to the sympathetic chain, but some little way off.

(*c*) *Nicotin applied Externally to the Skin.*

Nicotin was applied by means of a brush, or by a piece of filter paper soaked in the nicotin solution placed on the skin. The fish were kept on a white background so that they were in the pale phase. The effect of so treating the skin was to cause a darkening of the area painted. The darkening was strictly limited to the area treated, the line of demarcation being extremely sharp. The nicotin solution employed was usually 1 per cent., but 0·1 per cent. will evoke the same response. A 0·01 per cent. solution has no effect. When using a 1 per cent. solution, if the paper is applied for a considerable time, the fish becomes intensely pale, with convulsions, leaving only the initial dark patch. This is followed by paralysis and general darkening, in which the local dark area is indistinguishable (*cf.* (*d*)).

(*d*) *Nicotin Injected Intramuscularly.*

No anæsthetics were used in these experiments. The fish were injected dorsal to the lateral line above the peritoneum on the right side; the nicotin entered, therefore, at a

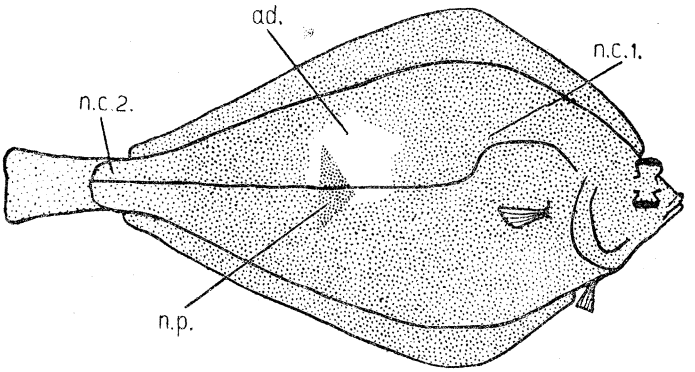
considerable distance from the sympathetic chain. 0·5 mgm. nicotin made up to 0·5 c.c. in 50 per cent. artificial sea-water was used. Such an injection produced an intense pallor in the immediate segments, which rapidly spread over the head and then posteriorly over the whole body. This was accompanied by muscular convulsions, which usually preceded the pallor in the various regions by a few seconds. Following this phase, a period of darkening and muscular paralysis set in. This usually proceeded from the anterior end backwards, but frequently one segment was affected before another and a striped effect produced before the darkening became general. Both the pallor and the darkening showed unmistakable signs of being segmental in origin (*cf.* (*a*) and (*b*)).

(*e*) *Nicotin by Injection followed by Adrenalin* (injected).

The nicotin was injected as described in (*d*), and the fish passed through the various phases. When they had become completely dark all over the body, adrenalin was injected in sufficient quantity to produce only a localised patch of pallor in the normal animal (0·1 mgm.). This had an immediate result, causing a local pale patch to become evident against the general darkening.

(*f*) *Nicotin applied Externally followed by Adrenalin* (injected).

The nicotin was applied externally (*c*) and the adrenalin injected near by. The results depended upon the concentrations of nicotin and adrenalin employed. If 1 per cent. nicotin solution was used, then doses of 0·05 mgm., 0·06 mgm. and 0·1 mgm. of adrenalin



Text-Fig. 5.—Dab showing actions of nicotin and adrenalin: *n.p.*, darkening due to peripheral action of nicotin; *n.c.*1, darkening following central stimulation by nicotin; *n.c.*2, posterior region still showing signs of the stimulation; *a.d.*, pallor caused by adrenalin superimposed on *n.c.*1, but not removing the local darkening (*n.p.*).

The actual tones of *n.p.* and *n.c.*1 are identical.

had no effect in removing the dark patch; 0·2 mgm., however, frequently caused a paling of the darkening, and sometimes removed it altogether. Similarly if 0·1 per cent. nicotin solution was applied, then 0·03 mgm. of adrenalin sometimes failed to remove the darkening, while 0·06 mgm. always paled the dark patch (text-fig. 5).

The reaction to the painting of the sympathetic chain ganglia with nicotin proves conclusively that one action of nicotin is *to stimulate the cells giving rise to the post-ganglionic fibres in these ganglia*. This action is also apparent in the experiments described in (*b*).

A second action of nicotin is demonstrated by the external application of nicotin, which causes a darkening of the skin. This reaction differs radically from the first in that it causes a peripheral paralysis. It will be seen that this action can be overcome by adrenalin, but only when the latter is in sufficient quantity. It is probable, therefore, that they act upon the same point, and since WYMAN has shown that they will act (separately) on the denervated melanophores, I suggest that they both act on some structure comparable to the myo-neural junction.

The effects produced by the injection of nicotin remain to be explained. I think that the initial pallor which is produced (and which shows signs of a segmental origin) may be attributed to the first action of nicotin enumerated above. Considerations of time also indicate this. The secondary darkening is not of the same nature as that produced by the peripheral action of nicotin, for it is destroyed by a minimal dose of adrenalin. As it also appears to be segmental in nature, LANGLEY'S work suggests that it is probably due to a secondary paralysis of ganglion cells following the primary stimulation. This cannot be stated definitely, however, as the anæsthetic obscures the reaction in the crucial experiments detailed in (*a*).

4. Reactions of the Chromatophores and Conclusions.

So far only the general nature of these actions has been examined. It is now necessary to investigate the minute nature of their effects on the various types of chromatophore. In Part II of this series of papers stress was laid upon the appearance of differentiation in the reactions of the chromatophores in the normal conditions. It is interesting, therefore, to examine the actions of nicotin and adrenalin, to see whether by any differentiation among the chromatophores (end-organs) a differential expansion has been produced in one or other of the different markings.

If a piece of skin paled by the action of nicotin on the sympathetic ganglia be examined, it is found that all the chromatophores are completely contracted. Melanophores, erythrophores and xanthophores, irrespective of their positions with regard to the markings, are all in the contracted phase. Similarly, if skin paled by adrenalin be taken, the same phenomenon is observed.

Turning to the dark phases, skin darkened by the external application of nicotin is found to have all the chromatophores completely expanded. And, supposing the secondary darkening produced by an injection of nicotin to be a true action of nicotin on the sympathetic chain ganglia, it is found that this action calls forth a similar response, namely, complete expansion of all the chromatophores. Hence it will be seen that *complete uniformity is the most prominent feature of these reactions*, and that *there does*

not appear to be any differentiation among the chromatophores to account for the appearance produced in the normal life of the fish and noted in Part II.

It has been urged that adrenalin is possibly responsible for pallor in fish. The following facts, brought to light in the present paper and in a previous one, militate against this :— 1. Adrenalin causes no such differential contraction between xanthophores and erythrophores as has been observed in the dabs on white backgrounds. 2. Even the minimal dose of adrenalin administered intramuscularly causes a *complete* contraction of *all* the chromatophores—no gradation is seen.

Having found no differential mechanism in the peripheral structures, it is now necessary to investigate the brain, in which a " centre " connected in some way with the control of colour changes is known to exist.

PART IV.

1. Introduction.

The choice of caffeine to investigate the colour-change centre in the brain of fish was more or less arbitrary. Caffeine affects the so-called psychic centres of the brain, and since many of the rapid changes in colour in certain fish seem to be evoked by the presence of food or by sexual activity, it was deemed the most likely agent to produce some differential results if a mechanism such as that postulated existed.

The action of caffeine is very selective, so that failure to obtain differential action would not necessarily disprove the theory suggested, for it might well be that whatever differential mechanism may exist is not acted upon by this drug. In the dab there are at least three types of markings which seem to require some differential mechanism to explain their reactions—the dark patches, the pale spots and the white marginal spots—so that a fair opportunity presents itself. For the same reason identical reactions in all individuals would not be necessary, for individual differences are important.

In the present research fairly small dabs were used, about twelve or thirteen centimetres in length. They are sexually distinguishable and do not differ in respect of colour from the larger specimens. " Tabloid " hypodermic caffeine sodio-salicylate was employed throughout. After a few preliminary experiments a uniform dosage of 2·5 mgm. was used. This was made up in 50 per cent. artificial sea-water and injected intramuscularly. This limitation of dosage was considered advisable, having regard to the large variation in reaction, due to individual differences, which was found within the range of one dosage. All the fish used were apparently quite healthy, and were kept on a white background for at least two days before and during the experiments.

2. The Action of Caffeine.

The immediate result of an injection of 2·5 mgm. of caffeine intramuscularly was to cause a local muscular paralysis. This was accompanied by a local darkening, which spread fairly rapidly, so that in five minutes it had reached its maximum. Frequently

when the fish was rather small (11 cms.) this darkening would extend over a considerable area of the right dorsal side. The muscular paralysis passed off soon and did not extend to the same degree as the darkening. The latter may be spread over wider areas by the application of larger doses and is probably the same reaction as that described by Lowe and Wyman, when they produced a darkening over the whole body. Intravenous injections produced a uniform darkening, but without the paralysis. Intraperitoneal injections had no effect.

Following the local darkening, after intramuscular injections there appeared in many instances a general darkening, which became evident as the tone of the rest of the body was gradually raised up to that of the locally darkened patch. This darkening did not appear segmentally nor as an extension of the local patch. It usually reached its maximum ten to twelve minutes after the injection. Accompanying this there became evident, on many occasions, the pale spots. It has been pointed out that, in the normal reactions of the dab, as the general tone of the fish darkens, the pale spots become evident; but the appearance of these spots after an injection of caffeine was frequently extremely vivid, and not wholly to be accounted for by the general darkening of tone. Often the white marginal spots would also become very prominent.

After the appearance of these spots or, if they did not become evident, after the lapse of ample time over and above the normal period for such an appearance, the fish were killed and quickly fixed, and preparations were made of the skin in both the locally darkened and generally darkened areas.

Controls were made by injecting 50 per cent. artificial sea-water into dabs kept on a white background. Twelve such injections were made, and neither the local nor the general darkening appeared.

It was impossible to localise definitely the seat of action, causing the general darkening, in the brain, as section of the spinal cord injured the fish to such an extent that they did not live long enough to be in a state for further experiments with caffeine. Considerations of time and the known actions of caffeine suggest the brain as the point of action.

3. The Reactions of the Chromatophores.

Examination of the minute reactions of the chromatophores must now be made. In the case of the local darkening the reactions are simple. All the chromatophores are fully expanded without regard to their situation in the markings.

Turning now to the general darkening, the results which were obtained seem to be extraordinarily confusing. The accompanying table sets them forth, in the order in which they were obtained in the course of the experiments.

Certain definite conclusions may be arrived at, however, without obtaining a full explanation. Firstly, that the melanophores on the pale spots can be contracted without a contraction of the other melanophores on the normal area. Secondly, that the melanophores on the white marginal spots can be induced to contract on a white background, although the melanophores on the normal area may be more or less expanded. In

many of these individuals, it will be seen that the melanophores on the normal area are more expanded than usual. This definitely shows that there is a certain independence of the various markings. In fact the discordance in these results as a whole goes to support this.

TABLE TO SHOW ACTION OF CAFFEINE.

No. of animal.	Normal area.	Pale spots.	White marginal spots.
115	Slightly expanded	Fully expanded	Fully expanded.
118	Expanded	Fully expanded	Fully expanded.
139	Slightly expanded	Expanded	Stellate.
140	Expanded	Contracted—slightly stellate	Slightly expanded.
141	Expanded	Slightly stellate—stellate	Stellate.
142	Expanded	Contracted—slightly stellate	Contracted.
143	Stellate	Stellate	Fully expanded.
144	Slightly expanded	Slightly expanded	—
145	Stellate	Slightly stellate	Stellate.
146	Stellate	Slightly expanded	Stellate.
147	Expanded	Fully expanded	Fully expanded.
148	Stellate	Slightly stellate—stellate	Contracted—slightly stellate.
149	Expanded	Contracted—slightly stellate	Slightly expanded.
150	Expanded	Stellate—slightly expanded	Expanded.
151	Slightly expanded	Expanded	Contracted.
152	Slightly expanded	Contracted—slightly stellate	Contracted.
153	Expanded	Slightly stellate—stellate	Slightly expanded.
154	Slightly expanded	Contracted—slightly stellate	Slightly stellate—stellate.
155	Slightly expanded	Fully expanded	Fully expanded.
156	Stellate—slightly expanded	Slightly stellate	Expanded.

The erythrophores and xanthophores remain as in the controls—the former contracted, the latter stellate. Again independent reaction is seen. *The action of caffeine, therefore* (apart from its peripheral one), *may be stated generally as a differential one.*

These results lend support to the suggestion of the presence of a discriminating centre (hypothesised after an analysis of the normal reactions) which, if the seat of action of caffeine be accepted as the brain, may possibly coincide with that found by VON FRISCH. Such an explanation seems to me to be the most likely.

4. THE CONTROL OF COLOUR CHANGES.

We are now in a position to form some concrete idea of an internal system for the control of colour changes in fish. Since only the outlines are known it is but a skeleton system which can be proposed, while its complexities, though based on observed phenomena, are but hypothetical.

The amount of research bearing on this problem is immense, and much of it seems to be irrelevant, or at any rate unimportant. Further, a great deal has been done without precautions which subsequent work has shown to be necessary, and a repetition of

much of this work is essential before it can be seriously considered. I have tried to lay down a system which, although simple in itself, is capable of complexity along certain lines. I have also attempted to indicate those lines.

The results of the present researches tend to place the responsibility of colour control on the central nervous system, and in particular on the brain, rather than in the end-organs and substances directly affecting them. The evidence which I have given, though not conclusive, is very suggestive; for until it is demonstrated that there is a differential reaction in the chromatophores to the action of some one or other drug, there is no other adequate explanation of the differential reactions which occur under normal conditions.

MAST, realising the necessity for a discriminating centre, suggests the brain as the "natural" region for such an organ, and is convinced that the fish he investigated were possessed of colour vision and by that means were able to adjust the relations between the erythrophores, xanthophores and melanophores. MAST further says:—"*The size of the light and dark areas on the background and the relative amount of surface covered by them have a profound effect on the pattern produced in the skin, but the form and arrangement of these areas have, at least within rather wide limits, none.*" (The italics are his.) SUMNER earlier made similar statements.

The analysis of the colour pattern of the dab which I have given in Part II suggests a basis for these phenomena, namely, that the types of markings exist for the most part as morphological entities, and that the background, on which the fish is placed, induces the nearest approximation that is possible for the fish concerned. Naturally the fish with the largest variety of types of markings to select from are those which show the greatest adaptation to the largest number of varying backgrounds. (The only type in the dab which seems to break through the limits of this suggestion is that of the dark patches, but from my observations I think the distribution of the nerves is the limiting morphological factor.) This, then, would necessitate a power of discrimination for *the degree of subdivision of backgrounds as well as for tone and for colour*. These are lines along which complexity may be found.

To summarise:—There exists in fish a central control, for colour changes, in the brain. This is complicated in nature, for the discrimination of colours, shades, etc., of patterns on the background is here translated into terms of the morphological types of markings, which exist as the effecters in the skin. Impulses leaving the brain do not alter in character. The chromatophores are capable of variation in size, distribution and associations, but not in reactions to similar nervous impulses. Such variations are more or less permanent in nature. These complications are paralleled by corresponding ones in the controlling centre in the brain.

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EXPLANATION OF PLATES.

Plate 13.

Fig. 1.—To show the relative sizes of the melanophores in the adult fish. × 630. *a*, Upper dermal melanophore from the normal area. *b*, Epidermal melanophore from an orange and black spot. *c*, Upper dermal malanophore from an orange and black spot.

Fig. 2.—To show the relative sizes of the melanophores in the young fish (*cf.* fig. 1). × 630. *a*, Upper dermal melanophore from the normal area. *b*, Epidermal melanophore from an orange and black spot. *c*, Upper dermal melanophore from an orange and black spot.

Fig. 3.—Upper dermal melanophore to show size when expanded, density of pigment and form. × 630.

Fig. 4.—Epidermal melanophore from same fish as fig. 3. To show the form and size when expanded. Several of the shorter processes lie in a plane vertical to the paper. × 630.

Fig. 5.—Lower dermal melanophore expanded. To show size and form. × 630.

Fig. 6.—*a*, Melanophore from a pale spot. × 630. *b*, Melanophore from the normal area. × 630. The fish had been kept on a white background.

Fig. 7.—Melanophores and iridophores from a white marginal spot. × 630. Note the grouping of the iridophores around the melanophores. *a*, Melanophore slightly stellate disclosing the iridophores. *b*, Melanophore expanded, obscuring the iridophores. The melanophores have been treated diagrammatically in black so that the dotted area refers only to the iridophores.

Fig. 8.—To show the internal structure of the iridophores. Note the haphazard arrangement of the crystals of guanin (drawn as lines only). × 600.

Fig. 9.—Lower dermal xanthophore in the expanded phase. Note the similarity of form to the melanophores in the same region (fig. 5) and dissimilarity to the other xanthophores (fig. 11). × 630.

Fig. 10.—To show the distribution of the iridophores on the normal area. Note the grouping of the iridophores round the erythrophores rather than around the melanophores. × 630.

Fig. 11.—Upper dermal xanthophores to show size and shape in various phases. × 630.

Fig. 12.—Erythrophores to show size and shape in various phases. × 630.

Plate 14.

Fig. 13.—To show the distribution of the melanophores and erythrophores in the normal area. The iridophores are omitted. × 200.

Fig. 14.—To show the distribution of the melanophores and erythrophores on the orange and black spots. The drawing is made at the transitional point between the orange patch and the black. The former is towards the bottom of the drawing and the latter above it. Note the large number of erythrophores and the large size of the melanophores. The iridophores are omitted. × 200.

Fig. 15.—To show the distribution and size of the melanophores in the young fish. The erythrophores and the iridophores are omitted. At the bottom there is an orange and black spot. Note the large number of melanophores at this point; also their normal size. × 200.

Fig. 16.—Portions of the normal area (with erythrophores) and of a pale spot from a dab kept on a white background. Note the incomplete contraction of the melanophores on the normal area where the erythrophores are present; also the complete expansion of the melanophores on the pale spot. (For details of these melanophores, see fig. 6.) The iridophores are omitted. × 200.

Fig. 17.—Portions of the normal area (with expanded erythrophores) and of a white marginal spot from a dab kept on a black background. Note the complete expansion of the melanophores on the normal area; also the contracted form of the melanophores on the white marginal spot. Erythrophores may be present on the spot as shown; they are contracted. The iridophores are omitted. × 200.

Fig. 18.—To illustrate the action of caffeine. Portions of the normal area (with erythrophores) and of a pale spot after the injection of 2·5 mgm. of caffeine into a dab on a white background. Note the expanded melanophores on the normal area and the contracted ones on the pale spot (*cf.* fig. 16). × 200.

Plate 15.

Fig. 19.—Photomicrograph (× 220 approx.) showing the contraction of the melanophores during the primary or stimulatory phase of the action of nicotin on the sympathetic chain ganglia.

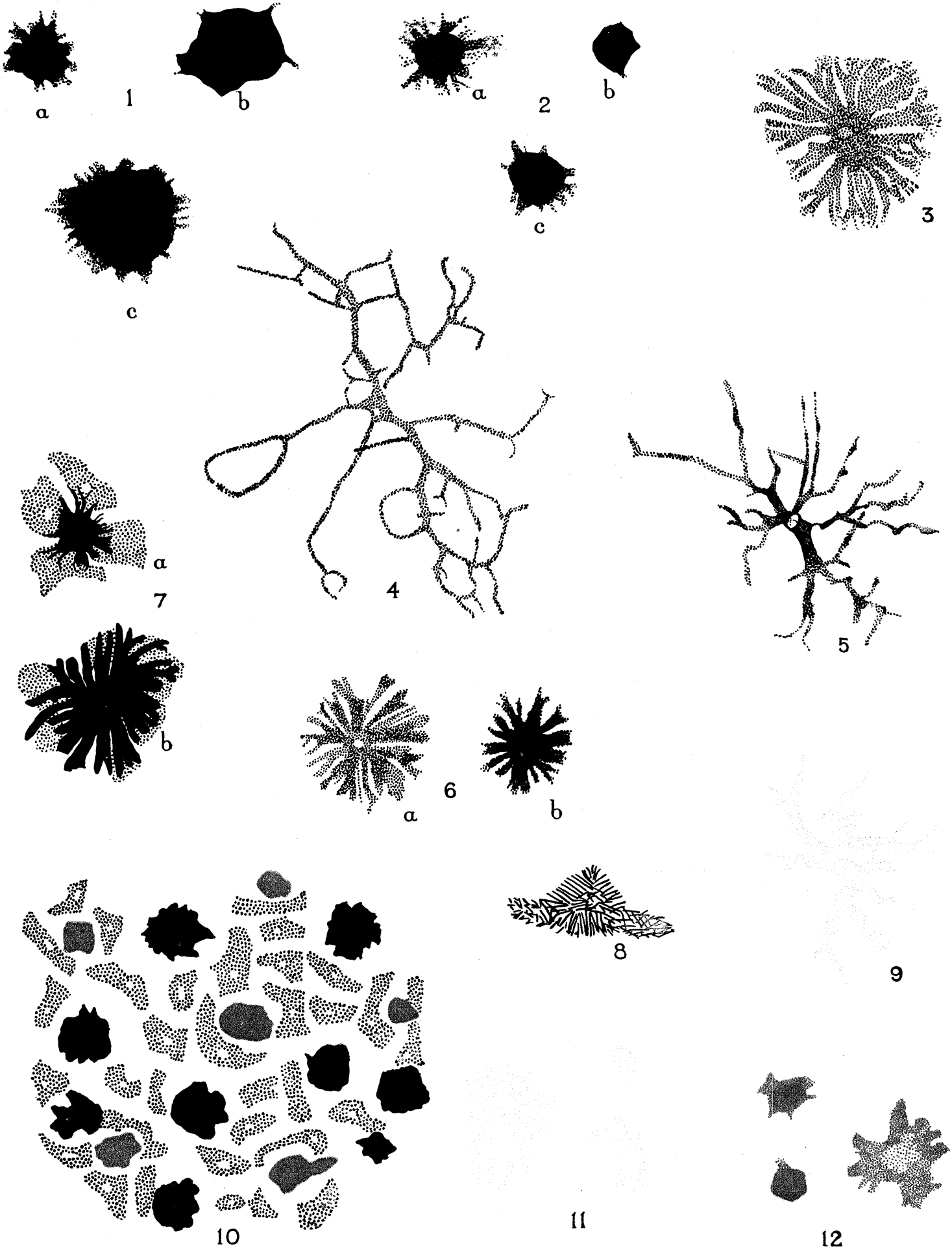
Fig. 20.—Photomicrograph (× 220 approx.) showing the expansion of the melanophores following the initial contraction after an injection of nicotin.

Fig. 21.—Photomicrograph (× 220 approx.) to show the reversal of the reaction after section of the trigeminal. The fish was killed one day after the operation. The skin was removed and fixed slowly so that the melanophores in the unaffected area had all expanded before fixation. Those in the region served by the trigeminal remained contracted as before. The photograph gives some idea of the sharp line of demarcation suggesting nervous origin of this reversal.

Fig. 22.—Photomicrograph (× 220 approx.) to show the distribution of the dermal layers of melanophores. Note particularly the continuity of the lower dermal melanophores (*l*) of the scale with the upper dermal melanophores (*u*) of the underlying scale (not shown). The large vacuities are probably due to contraction of the muscular tissue below the skin during fixation.

Fig. 23.—To show the relative tones and patterns seen macroscopically on dabs on various backgrounds. The photographs are of preparations and were taken at one exposure and printed in one, without retouching, save to block out the slides on which the preparations were mounted. ×1·5. The fish had been kept under the various conditions for two weeks. *a*, On a white background. Note the absence of the pale spots and the general even tone. The orange and black spot are evident as dark dots. The white marginal spots (*w.m.s.*) are also visible posteriorly as slightly darker patches. *b*, On an orange background. Note the slight increase in general tone and the appearance of pale spots to a slight degree. White marginal spots mostly invisible. *c*, On a black background. Note the mottled appearance. The pale spots are prominent all over the body. White marginal spots (*w.m.s.*) are very pale and obvious. The orange and black spots are still prominent.

(For the accurate identification of the principal spots, compare with text-fig. 1.)



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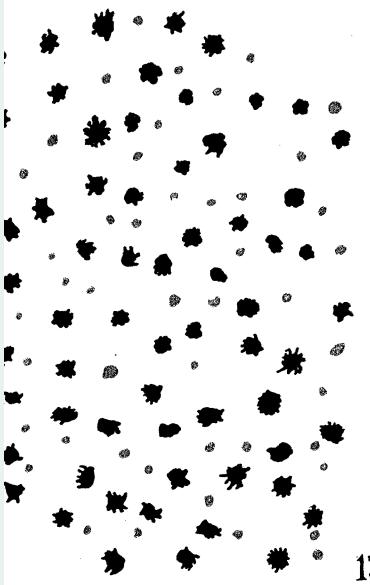

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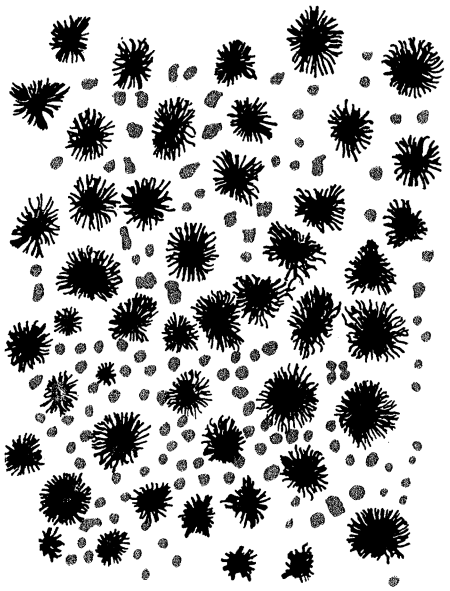

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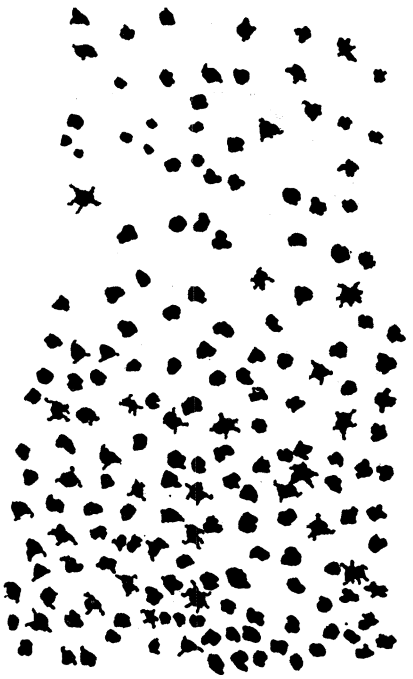

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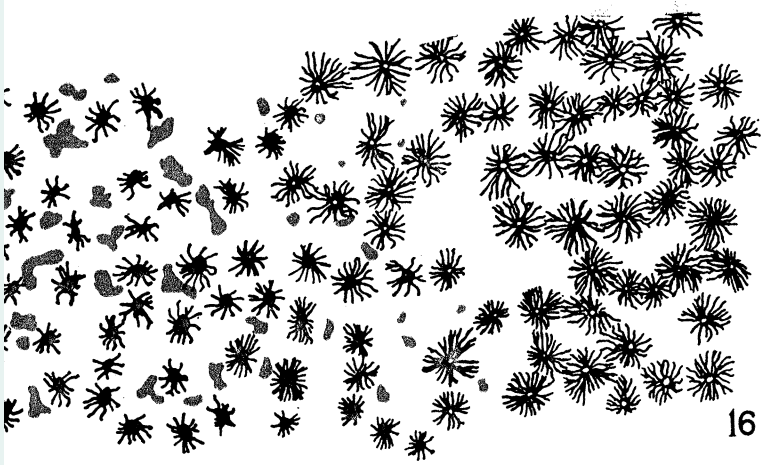

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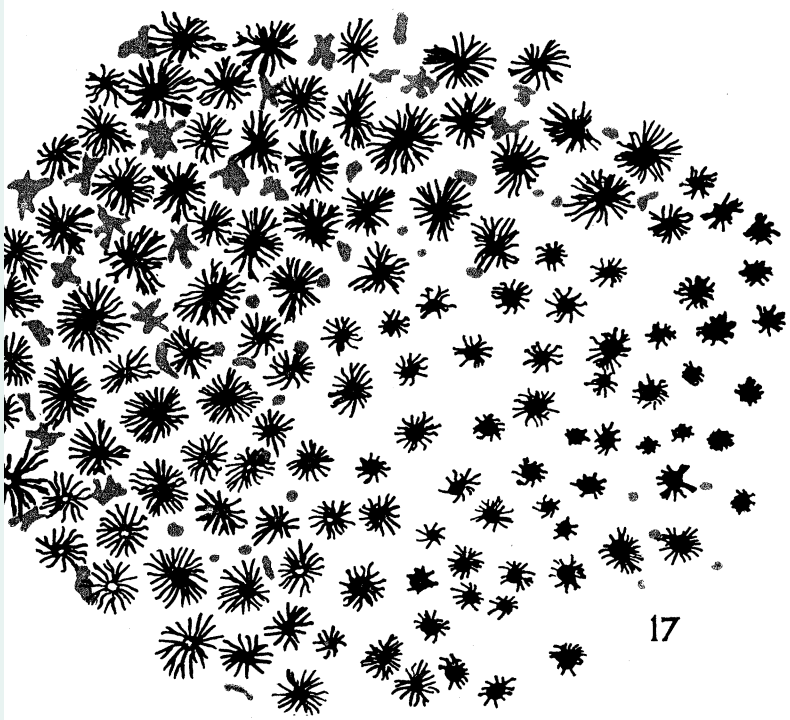

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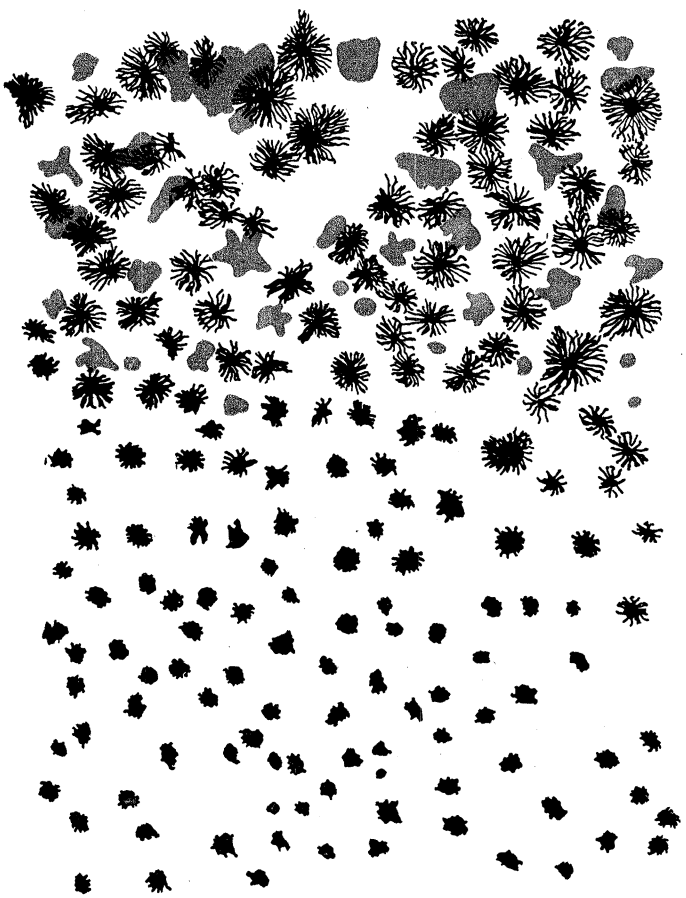

18

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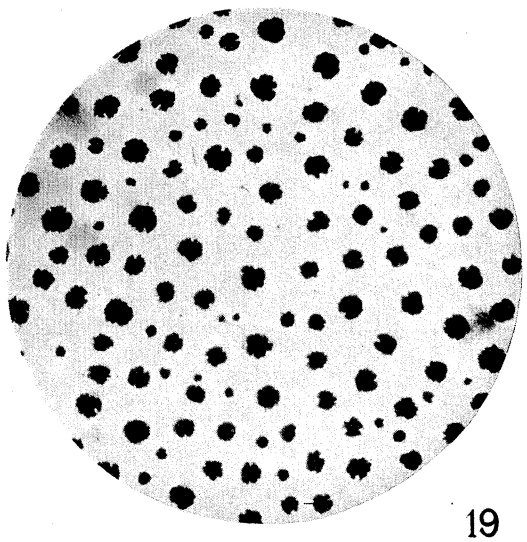

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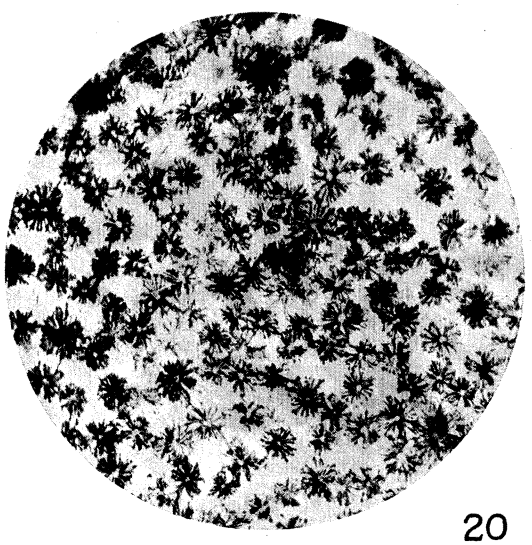

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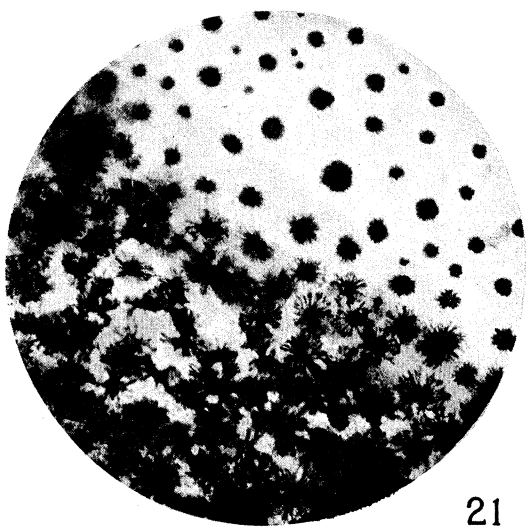

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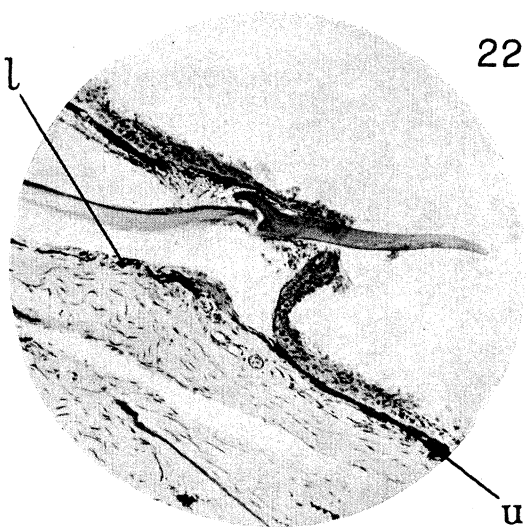

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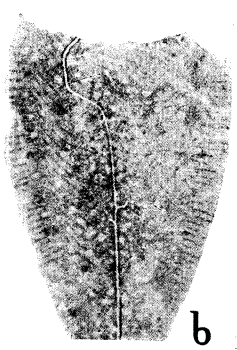

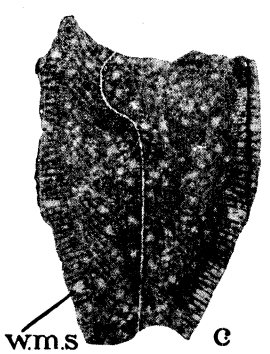

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PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

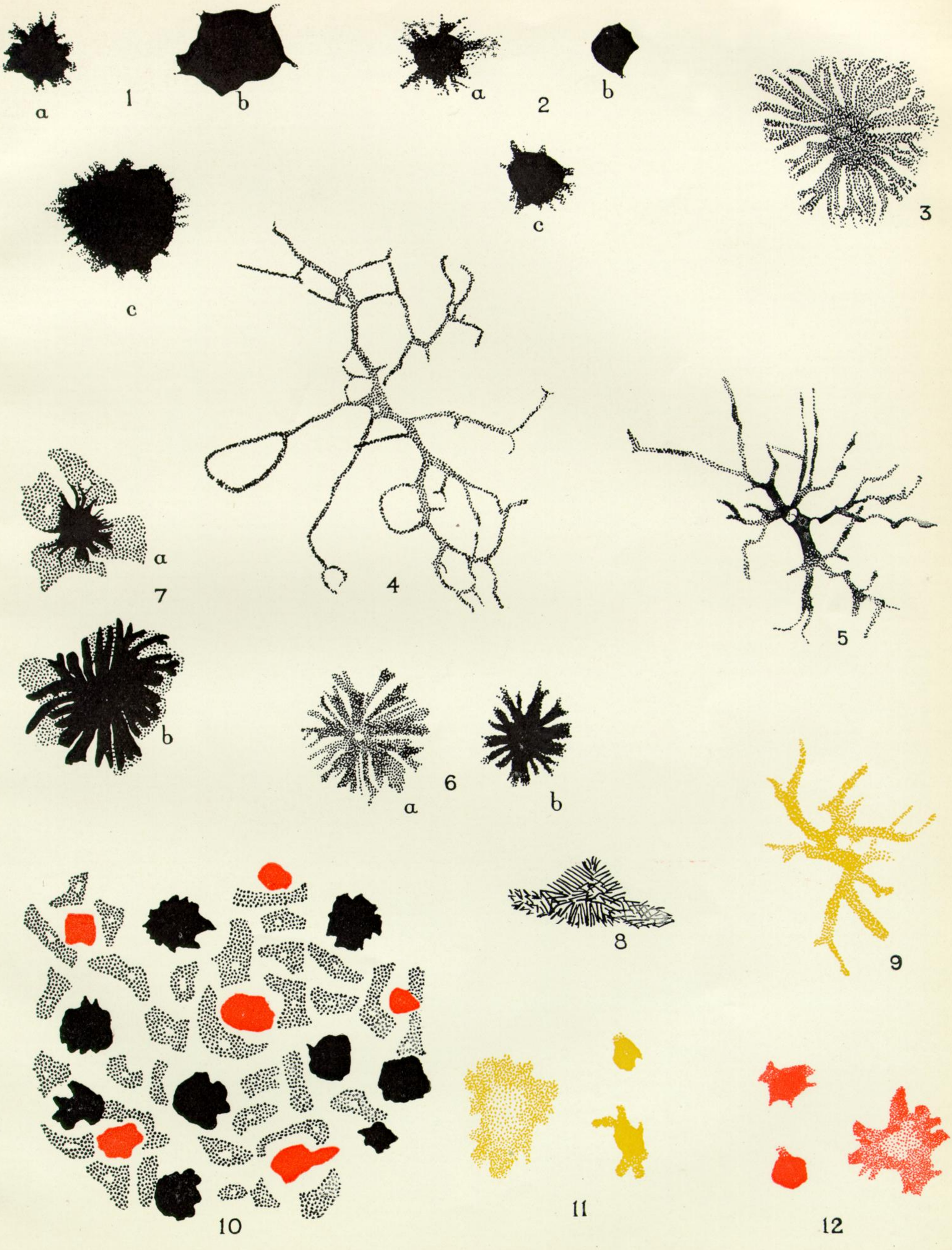


PLATE 13.

FIG. 1.—To show the relative sizes of the melanophores in the adult fish. × 630. *a*, Upper dermal melanophore from the normal area. *b*, Epidermal melanophore from an orange and black spot. *c*, Upper dermal melanophore from an orange and black spot.

FIG. 2.—To show the relative sizes of the melanophores in the young fish (*cf.* fig. 1). × 630. *a*, Upper dermal melanophore from the normal area. *b*, Epidermal melanophore from an orange and black spot. *c*, Upper dermal melanophore from an orange and black spot.

FIG. 3.—Upper dermal melanophore to show size when expanded, density of pigment and form. × 630.

FIG. 4.—Epidermal melanophore from same fish as fig. 3. To show the form and size when expanded. Several of the shorter processes lie in a plane vertical to the paper. × 630.

FIG. 5.—Lower dermal melanophore expanded. To show size and form. × 630.

FIG. 6.—*a*, Melanophore from a pale spot. × 630. *b*, Melanophore from the normal area. × 630. The fish had been kept on a white background.

FIG. 7.—Melanophores and iridophores from a white marginal spot. × 630. Note the grouping of the iridophores around the melanophores. *a*, Melanophore slightly stellate disclosing the iridophores. *b*, Melanophore expanded, obscuring the iridophores. The melanophores have been treated diagrammatically in black so that the dotted area refers only to the iridophores.

FIG. 8.—To show the internal structure of the iridophores. Note the haphazard arrangement of the crystals of guanin (drawn as lines only). × 600.

FIG. 9.—Lower dermal xanthophore in the expanded phase. Note the similarity of form to the melanophores in the same region (fig. 5) and dissimilarity to the other xanthophores (fig. 11). × 630.

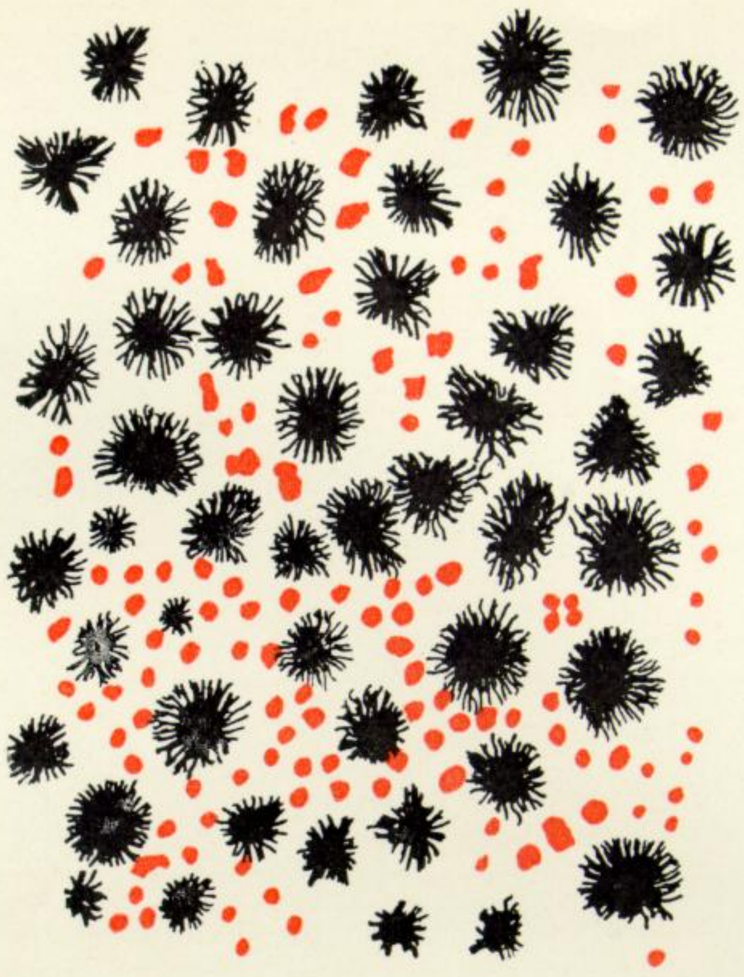
FIG. 10.—To show the distribution of the iridophores on the normal area. Note the grouping of the iridophores round the erythrophores rather than around the melanophores. × 630.

FIG. 11.—Upper dermal xanthophores to show size and shape in various phases. × 630.

FIG. 12.—Erythrophores to show size and shape in various phases. × 630.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

13



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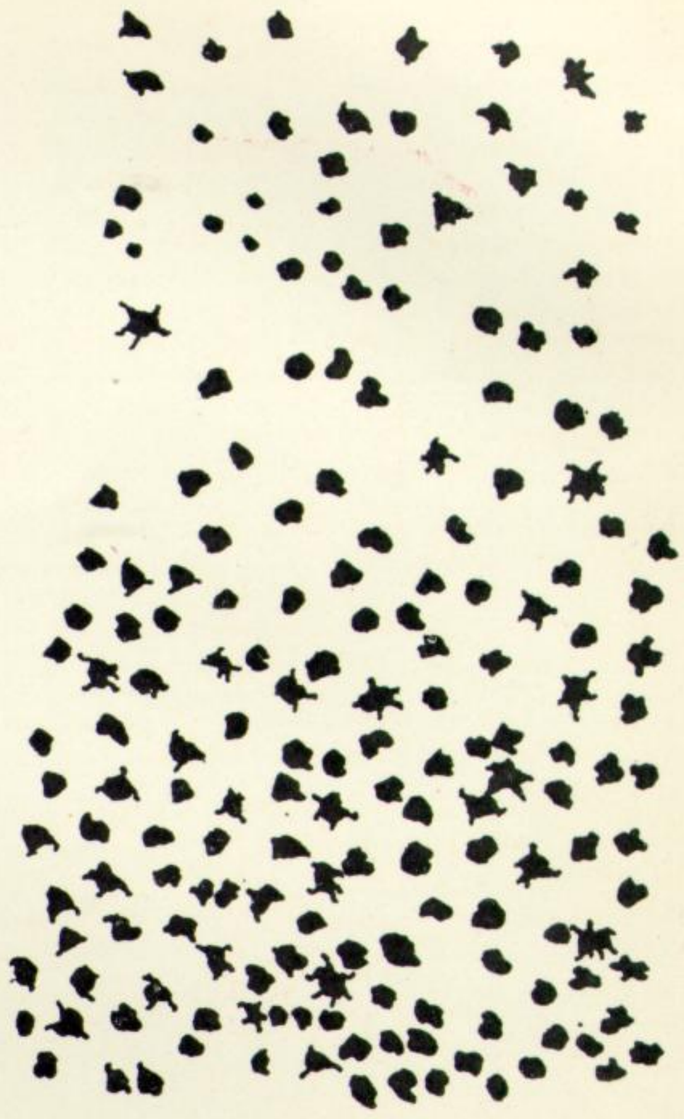

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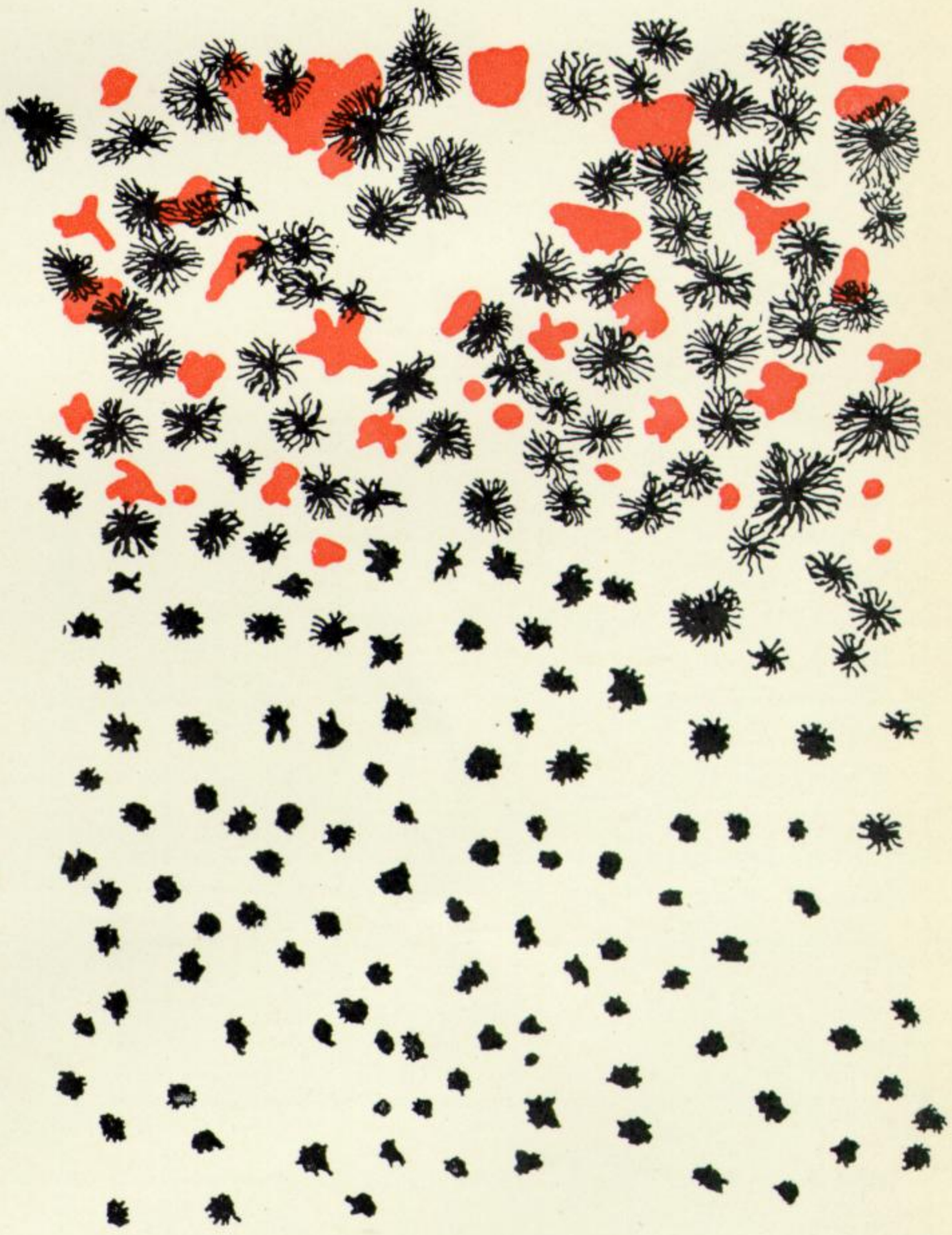


18

PLATE 14.

FIG. 13.—To show the distribution of the melanophores and erythrophores in the normal area. The iridophores are omitted. × 200.

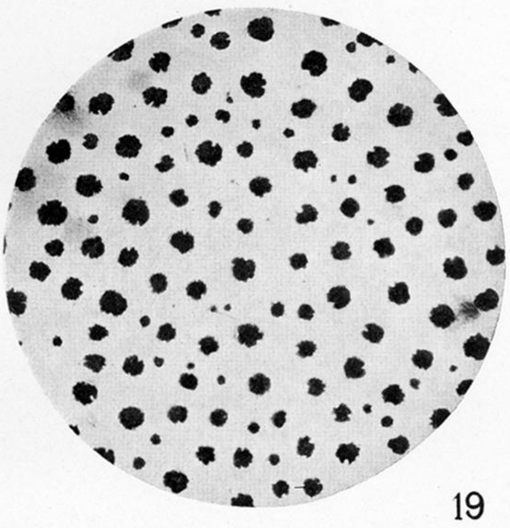
FIG. 14.—To show the distribution of the melanophores and erythrophores on the orange and black spots. The drawing is made at the transitional point between the orange patch and the black. The former is towards the bottom of the drawing and the latter above it. Note the large number of erythrophores and the large size of the melanophores. The iridophores are omitted. × 200.

FIG. 15.—To show the distribution and size of the melanophores in the young fish. The erythrophores and the iridophores are omitted. At the bottom there is an orange and black spot. Note the large number of melanophores at this point; also their normal size. × 200.

FIG. 16.—Portions of the normal area (with erythrophores) and of a pale spot from a dab kept on a white background. Note the incomplete contraction of the melanophores on the normal area where the erythrophores are present; also the complete expansion of the melanophores on the pale spot. (For details of these melanophores, see fig. 6.) The iridophores are omitted. × 200.

FIG. 17.—Portions of the normal area (with expanded erythrophores) and of a white marginal spot from a dab kept on a black background. Note the complete expansion of the melanophores on the normal area; also the contracted form of the melanophores on the white marginal spot. Erythrophores may be present on the spot as shown; they are contracted. The iridophores are omitted. × 200.

FIG. 18.—To illustrate the action of caffeine. Portions of the normal area (with erythrophores) and of a pale spot after the injection of 2·5 mgm. of caffeine into a dab on a white background. Note the expanded melanophores on the normal area and the contracted ones on the pale spot (*cf.* fig. 16). × 200.



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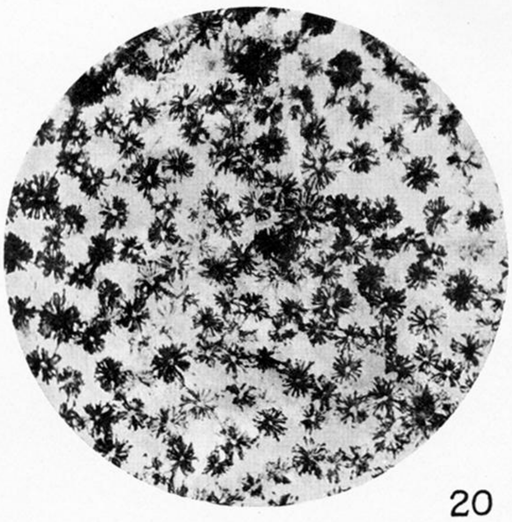

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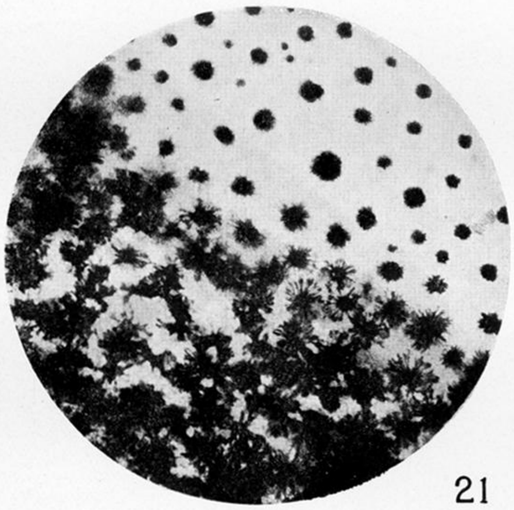

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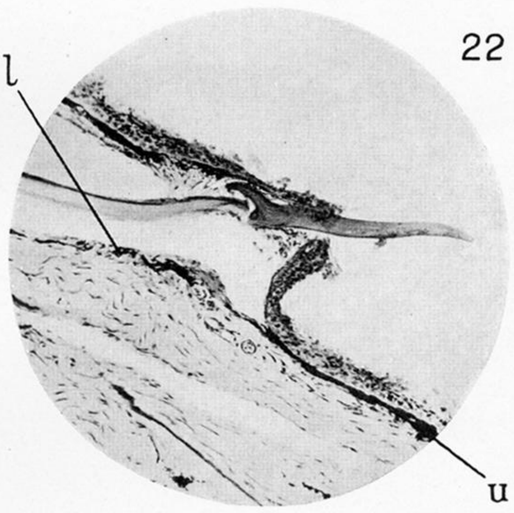

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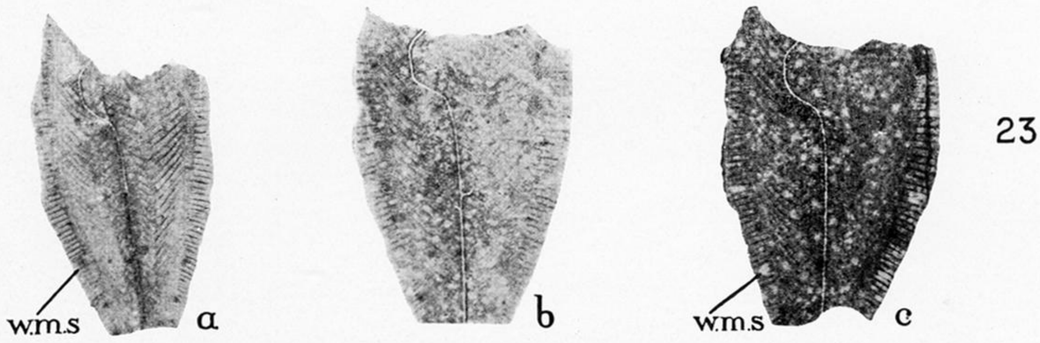

23

PLATE 15.

FIG. 19.—Photomicrograph (× 220 approx.) showing the contraction of the melanophores during the primary or stimulatory phase of the action of nicotin on the sympathetic chain ganglia.

FIG. 20.—Photomicrograph (× 220 approx.) showing the expansion of the melanophores following the initial contraction after an injection of nicotin.

FIG. 21.—Photomicrograph (× 220 approx.) to show the reversal of the reaction after section of the trigeminal. The fish was killed one day after the operation. The skin was removed and fixed slowly so that the melanophores in the unaffected area had all expanded before fixation. Those in the region served by the trigeminal remained contracted as before. The photograph gives some idea of the sharp line of demarcation suggesting nervous origin of this reversal.

FIG. 22.—Photomicrograph (× 220 approx.) to show the distribution of the dermal layers of melanophores. Note particularly the continuity of the lower dermal melanophores (*l*) of the scale with the upper dermal melanophores (*u*) of the underlying scale (not shown). The large vacuities are probably due to contraction of the muscular tissue below the skin during fixation.

FIG. 23.—To show the relative tones and patterns seen macroscopically on dabs on various backgrounds. The photographs are of preparations and were taken at one exposure and printed in one, without retouching, save to block out the slides on which the preparations were mounted. ×1·5. The fish had been kept under the various conditions for two weeks. *a*, On a white background. Note the absence of the pale spots and the general even tone. The orange and black spot are evident as dark dots. The white marginal spots (*w.m.s.*) are also visible posteriorly as slightly darker patches. *b*, On an orange background. Note the slight increase in general tone and the appearance of pale spots to a slight degree. White marginal spots mostly invisible. *c*, On a black background. Note the mottled appearance. The pale spots are prominent all over the body. White marginal spots (*w.m.s.*) are very pale and obvious. The orange and black spots are still prominent.

(For the accurate identification of the principal spots, compare with text-fig. 1.)

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