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On the Physiology of the Peritoneal Melanophore of the Fish.

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With Plate XVI & XVII and 1 Text-figure

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INTRODUCTION

The dermal chromatophore of the teleost fishes has been much studied from the embryological as well as the physiological point of view. Almost nothing, however, is known about the internal chromatophore, i. e. the pigment cells in the internal structures such as the peritoneum, the pericardium, the walls of the thicker blood vessels, the membranous coatings of the central nervous system and the myolemma in the partition between the dorsal and the ventral half of the parietal muscles. The object of the present account is to report on some remarkable differences between the behavior of the peritoneal chromatophores and that of the dermal ones.

THE MORPHOLOGY OF PERITONEAL CHROMATOPHORES.

In common cyprinoid fishes there are usually two kinds of peritoneal chromatophores: the melanophore and the guanophore (or Iridocyte), but no xanthophores. As far as a small Japanese species of minnow, *Acheilognathus intermedia* (TEMMINCK et SCHLEGEL) is concerned, which I selected for the material of this study, the melanophore is common everywhere in the internal structures mentioned above. It is a minute stellate cell with numerous peripheral protoplasmic processes, rich in dark brown, often almost black, pigments. It differs from the dermal melanophore in two respects, namely

1) in shape, the peritoneal melanophore is in general rather round in contour with short stump processes, while the dermal melanophore is usually stellate with more distinct and longer peripheral ramifications. This shape of melanophore varies, of course, with the grades of expansion and contraction, which will be dealt with later in detail.

2) in distribution, the peritoneal melanophores are much more numerous and are arranged more densely than the dermal melanophores, so that in the former the relation between neighbouring cells may be compared to the plates in a mosaic floor, and they more readily fuse with each other to form a continuous black screen even when expanded in a moderate degree. Other internal melanophores within or around the other internal organs, such as the blood vessels or the spinal cord, seem to be similar in form and distribution to the peritoneal melanophores, rather than to the dermal ones. The peritoneal guanophore is an elliptical, oval or polyhedral thin plate, composed of numerous long rhomboidal platelets arranged side by side. It is so distributed in the deeper layer of the peritoneum as to form a continuous background below the melanophore, seen from the interior of the body cavity.

The period when the peritoneal chromatophores make their appearance is very early. Since the fish here concerned is the commonest species around Kyôto, especially in Lake Biwa, all the year round, I had no difficulty in obtaining any of the younger stages of it. A few melanophores are seen around the visceral cavity in the embryonic stage as early as when the fish-fry is still provided with an umbilical sac. The guanophore seems to be established still earlier, although I was unable to trace back the process.

It does not appear to me that the two kinds of peritoneal chromatophores increase or decrease according to the season.

THE BEHAVIOR OF THE PERITONEAL CHROMATOPHORE.

I. Methods, of Study.

For examination of the responses of the peritoneal chromatophores to mechanical, chemical and other stimuli, the younger fish of ca. 40 mm. in body length, presumably a little more than one year old, were preferable, since in the fullgrown fish of ca. 55 mm. in length there is so dense and thick pigmentation in the peritoneum that the contour of the individual melanophores is invisible.

The belly of the fish being dissected along its median line, the sidewall of the body-cavity was cut out in square pieces of a convenient size. Then as much as possible of the external part of this piece with the skin and the parietal musculature was removed, but so carefully that the internal part composed of the peritoneum and the adjoining tissues was kept intact as much as possible, if not completely. Frequently it was unnecessary to remove the outer half of the tissue of the piece, since the results of the experiments were satisfactorily distinct even in thick materials with the reflecting light under the microscope. These pieces were placed, immediately or after various forms of necessary treatment, on the stage of microscope, the peritoneal surface upwards, and examined by low magnifications.

After some experiments, I was convinced that the responses of the chromatophore may vary with the age of the fish, so that it was desirable to use fishes of the same age and size as the material of the experiment. This was a rather easy matter, since the young fish are usually found in Lake Biwa prowling in large swarms, when caught,

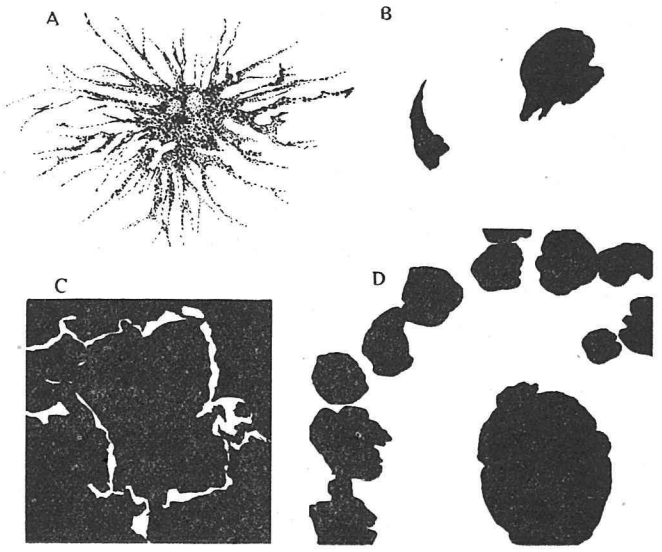

Fig. 1.

Dermal and peritoneal melanophores of *Acheilognathus intermedia*. A. Dermal melanophore in a well-expanded state. B. The same in a highly contracted state. C. Peritoneal melanophore almost fully expanded; neighbouring cells are nearly fused together. D. The same in a considerable degree of contraction; the typical grouping of the cells is very distinct.

and can be kept in a small aquarium in an apparently healthy condition for several months. Strictly speaking, there may be a slight difference in nutrition between the wild and the tame individuals, but practically, there has been noticed no disagreement between them so far as the responses of the chromatophores are concerned.

The degree of expansion or contraction of the chromatophore thus observed under the microscope, was sketched by means of ABBE's drawing apparatus. To check any changes of state, i. e. further expansion or contraction of the chromatophore during this treatment, it was found advisable to dip the pieces in 70%–90% alcohol, which fixes the cells quite satisfactorily.

II. Dark-room Experiments

Experiments, series I.

In order to study the responses to photic stimulation in the fish in the natural or healthy condition, about half a dozen individual fish were kept on a desk in a dark room for several days, then dissected as quickly as possible and then the conditions of their peritoneal melanophores were tested carefully. In the fish thus kept in the dark for even three hours the melanophore shows signs of contraction (fig. 1). After four hours the contraction is in general very conspicuous (fig. 2), each cell showing the figure of a small black ball without any processes, and the contraction is complete in fish kept in the dark for 48 hours and no further marked change is seen.

If the aquarium is brought out of the dark-room so that the fish are again exposed to daylight, the peritoneal melanophores begin to expand immediately at a rapid rate. When they are examined after 30 minutes (figs. 9, 10) signs of expansion are already visible and after 1 hour (figs. 11, 12) all the melanophores in the same group*, are completely, or nearly so, fused into a single black mass. In 24 hours after the fish are brought out of the dark-room, the peritoneal wall is uniformly black, all the peritoneal melanophores being so completely expanded as to fuse with each other to a continuous sheet.

Such a change in the state of contraction in the peritoneal melanophores differs from that of the dermal melanophores in two respects, namely : (1) the change occurs in a reciprocal manner, and (2) the change is much more active in the dermal melanophores.

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* Of this group a detailed explanation is given in the next paragraph.

The dermal melanophore expands when the fish has been kept in the dark and contracts when it is kept in a light place, while the peritoneal melanophore contracts when kept in the dark and expands when kept in the light. Here must be added a short reference to a difference between the behavior of the dermal melanophores of the present Japanese minnow and that of the American Fundulus, studied by PARKER and LANCHNER (1922). In *Fundulus* the dermal melanophores contract when the fish is put before a white background, expand before a black background and contract again when kept in a dark room. If in the last experiment the fish is exposed to light the melanophores expand a little for a short time and then begin to contract. In the case of our fish the dermal melanophore behaves in just the same way as given above when put before a white or black background, but expands, instead of contracting, when the fish is kept in complete darkness. Therefore, a wide survey of the various groups of fishes seems to show that there are two types; (1) that in which the dermal melanophore contracts when kept in a dark room, (2) that in which it expands in such conditions.

It may be objected that the melanophore when brought into a dark-room expands first and then contracts and remains permanently in that state, so that the result obtained by PARKER and LANCHNER is the final state while the expansion detected in my experiment is merely a temporary condition. This, however, can hardly be accepted, since I ascertained that the expansion lasted more than 144 hours and no signs of contraction were shown in any specimen.

It is remarkable that the peritoneal melanophore behaves reciprocally to the dermal melanophore. This special behavior of the peritoneal melanophore must have some important physiological or oecological significance. It may be true that the peritoneal melanophore plays a rôle in protecting the visceral organs from possible ill-effects of the penetrating radiation, although I hesitate at present to offer any definite opinion on this point.

In the next place it is a general rule that the change is much more active in the dermal melanophore. While the peritoneal melanophore shows a moderate degree of contraction after the fish has been confined for 3 days in the dark room, the dermal melanophore, in a shorter time, expands so strikingly that the skin of the fish looks darker than before the confinement.

Using a considerable number of the fish and killing them one after the other during the time when they were kept in the dark room

or exposed to bright light alternately, I found that this change in the peritoneal melanophore was perfectly reversible.

Experiment, series II.

A similar series of experiments was carried out with fish, whose eyes had been practically destroyed by injury or by burning with a hot needle. The results were quite the same as in series I. Thus it is clear that the visual sense has very little to do with the reflex-arc for the expansion and contraction of the peritoneal melanophores. According to previous authors, the dermal melanophores in blind fish may sometimes fail to react to photic stimuli or, even though they respond positively, the change is usually very slow. In this respect, therefore, the peritoneal melanophores are very unlike the dermal melanophores in their behavior.

The modes of the contraction and expansion may be described here. In two cases illustrated in figs. 4 and 7 can be seen a much advanced condition in the contraction of the peritoneal melanophores. In such cases the peritoneum, when taken out and inspected with the naked eye, looks pale grey, the melanophores being contracted to a punctate form and the silver-white guanophores being well visible through an opaque membrane. There are of course some individual differences, different grades of contraction occuring in different individuals from the same aquarium. These differences are more conspicuous in the earlier part of the process of contraction or expansion, five or six hours after the beginning of contraction and some thirty minutes after the start of expansion. As shown in figs. 3, 5, 6 and 8, a small quantity of melanin granules may remain in a pellucid suburval space which was formerly dark, occupied by the expanded pigments.

The contraction of the melanophore seems to begin all over the peritoneal wall of the visceral cavity at the same time. It is very interesting and deserves special notice that there can be recognized a grouping of the melanophores, that is to say, a larger central melanophore is surrounded by ca. 10–30 somewhat smaller peripheral ones, as is very distinctly shown in figs. 4, 7, 18 and 19. In figs. 15, 16 and 17, where the contraction is not so great as in the foregoing cases, the peripheral ones are so fused with each other to form an irregular ring, but the central one is still separated by a narrow free space from the peripheral ones. If the expansion of the peritoneal melanophores advances some degrees further all the members of the group unite into a black spot. At that time this spot is separated by

a free space from the neighbouring spots, or rather black masses belonging to the neighbouring groups, as is shown in figs. 10, 12, 13 and 14. When the expansion goes still further all the spots fuse together and the peritoneum is uniformly black without any part left uncovered by the pigment cells.

As to the nature of this grouping of 10–30 peritoneal melanophores nothing can be said at present. But it is highly probable that the central and peripheral melanophores of the same group are connected with each other by means of some minute pseudopodial structures or may be innervated with one and the same branch of nerve fibre, so that they can contract or expand synchronously in a well coordinated manner. This is extraordinarily interesting since it reminds us of the behavior of the complex chromatophores in both Crustacea and Cephalopoda, though there is of course a great difference in histological structure.

III. Direct Responses of Peritoneal Melanophores to Various Sorts of Stimuli.

(I) MECHANICAL STIMULATION.

A piece of the peritoneum with contracted melanophores cut out from a fish that had been kept a long time in the dark-room was laid on a slide glass and a continuous mechanical pressure was imposed on the piece from its upper surface by means of a thin glass rod. At that spot the black figure of the melanophore became thicker and wider and in one hour reached the state illustrated in fig. 20. This made a striking contrast with an unpressed portion of the piece, where no expansion of the melanophores took place. The same experiments were carried out with a piece of the preitoneum cut out from a light-adapted fish. In that preparation the melanophores were naturally in an expanded state. No change was seen in either the mechanically pressed or the untouched portions. Such results do not coinside with those observed by SPAETH (1913a) in the dermal melanophores of *Fundulus*.

(II) ELECTRICAL STIMULATION.

A small piece of the peritoneal wall taken out from both dark-adapted and light-adapted fishes was stimulated by a single induction shock or an interrupted tetanizing current of considerable intensity, but no reactions of the melanophore were detected at all, though the muscles contracted very strongly.

(III) CHANGE OF OSMOTIC PRESSURE.

Exp. Series I. An isotonic physiological saline solution ($\frac{N}{8}$NaCl, KCl etc.) was first used as a medium. When pieces of peritoneum were dipped in this solution and kept partly in the dark and partly in the light, the results were as follows :

(a) Expanded melanophores from a light-adapted fish kept in the dark . . . . . no change.

(b) Expanded melanophores from a light-adapted fish kept in the light . . . . . no change.

(c) Contracted melanophore from a dark-adapted fish kept in the dark . . . . . no marked change, but a slight expansion.

(d) Contracted melanophore from a dark-adapted fish kept in the light . . . . . the same as in Experiment c.

Thus it is clear that the isotonic saline solution was practically inactive, having neither stimulating nor poisonous effects.

Exp. Series II. Then similar pieces were tested with saturated NaCl-solution, saturated succarose solution, distilled water, and tap water.

(e). *NaCl.* An expanded melanophore, kept in the light, is inclined to contract, and 1 hour later is still in a more or less contracted state.

NaCl. Expanded melanophores kept in the dark show no contraction.

NaCl. Contracted melanophores kept in the light expand slightly but not very conspicuously.

NaCl. Contracted melanophores kept in the dark expanded a little more conspicuously than in the foregoing case.

(f) *Sugar solution.* No marked change, the expanded melanophore remaining in the same state even after three days. A contracted melanophore may show a slight expansion after 1 hour but no further change was ever observed.

(g) *Distilled Water.* Expanded melanophores take a stellate form and finally decompose. Contracted melanophores also take a stellate form after a little longer time. These processes are to be seen both in dark and in light backgrounds.

(h) *Tap Water.* Expanded melanophores in the light showed no change after 15 minutes; after 1 hour the radiating processes of the melanophophore became slender; after 24 hours such an irregular contour as shown in fig. 21 was seen. This is no doubt a sign of decomposition.

(IV) CHEMICAL STIMULATION.

A. Ionic actions.

A piece of the peritoneal wall taken out from a fish in the way explained on page 190 was cut into many smaller pieces and these were dipped into 0.2 normal solutions of various pure salts, such as KCN, KI, KNO_3, KBr, KCl, K_2SO_4, Na_2SO_4, $(NH_4)_2SO_4$. Then the state of the melanophore was examined after 5 minutes, 15 minutes, 45 minutes, 4 hours, 24 hours, 30 hours, and 48 hours respectively.

(a) *Action of Anions.*

In all cases the action of KCN and KI was remarkable, the contraction beginning after 15 minutes (fig. 22) and reaching the maximum after 48 hours.

The action of KNO_3, KBr and KCl is a little weaker than this, the reaction taking place after about 4 hours.

That of K_2SO_4 is somewhat similar though a little later. From such experiments it is to be concluded that in the order of the intensity of their actions these anions are to be arranged as follows:—

$$SO_4 < Cl < Br < NO_3 < I < CN.$$

(b) *Action of Cathions.*

To Na_2SO_4 the melanophore seems to be rather indifferent, the contraction being indistinct even after 48 hours.

$(NH_4)_2SO_4$ is somewhat more effective, the contraction being visible to the naked eye.

K_2SO_4 is the most effective of the three salts, inducing the start of contraction earlier, though the state of the melanophore after 48 hours (fig. 23) is almost the same as that produced by the other two.

These experiments are sufficient to suggest that the ionic action of various anions and cathions is unequal and falls in the well-known lyotropic series. This is the same result as that reached by SPAETH (1913a) and LOWE (1917) with the dermal melanophores of fishes.

In short, the peritoneal melanophore reacts to chemical stimuli in the same manner as the dermal melanophore, notwithstanding that its reaction to photic stimuli is opposite.

(c) *Direct responses of the peritoneal melanophore when photic stimuli are combined with chemical stimuli.*

Pieces of the peritoneal wall were cut out from fishes that had been kept in the dark-room for at least 70 hours and dipped into 0.2 normal solutions of various salts in a room provided with rather intense

light. The results seen under microscope after 15 minutes, 1 hour, 6 hours, and 24 hours are as follows :—

(1) KI. The contraction of the peritoneal melanophore is sustained, no trace of expansion being visible.

(2) KBr. There is a considerable degree of contraction after 1 hour (fig. 24) then a queer dendritic figure with accompanying dots is seen (fig. 25). This latter phenomenon is not a normal process but presumably a symptom of decomposition of the cell.

(3) KCl. A similar course of change, but the decomposing effect, when inspected after 24 hours seemed somewhat weaker (fig. 26).

(4) NaCl. Almost complete retardation, the expansion being indistinct even after 24 hours (figs. 27–30).

(5) Na_2SO_4. The expansion began after only 15 minutes (fig. 31), and was pretty marked after 1–6 hours (figs. 32, 33). Then contraction occurred again after 24 hours (fig. 34) but not so much as to suggest decomposition of the cell.

(6) $(NH_4)_2SO_4$. Expansion of slight degree after 1 hour, that state continuing for more than 24 hours (figs. 35–38). This effect is the same as that with KCl and KBr in that the retarding effect diminishes after 1 hour or two, but differs from these in that the second contraction does not occur even after 24 hours.

Judging from these data we can conclude that (i) the expansion of the peritoneal melanophores, which should start as soon as they are brought into the daylight, seems to be hindered by the effects of various salts in various ways and (ii) of the 6 salts tested in the experiments, KI is the most effective, the retardation being almost complete and permanent; Na_2SO_4 is least so, being unable to retard the expansion for more than 15 minutes; KBr and KCl rank midway between these two, being able to stop the expansion for more than 1 hour. $(NH_4)_2SO_4$ is equally effective in retarding the expansion but not so much so as to damage the cell. NaCl is extraordinarily strong in the retarding effect.

B. Effects of chemicals that change the irritability of the nervous system.

Strychnine and atropine were selected as representative drugs to heighten or lower the irritability of the nervous system. 0.05 c.c. of either 1 percent or 0.5 percent solutions of each were injected into the body cavity of the fish. Soon after the fish had died the abdominal wall was cut out and the state of the peritoneal melanophore was examined.

It was found that the peritoneal melanophore was always in a contracted state under the influence of strychnine and in an expanded condition owing to the action of atropine. This is just as was to be expected, but the effect was always much weaker than that shown by the dermal melanophore in parallel experiments.

C. Effect of water lacking in oxygen.

A piece of the peritoneal wall of the fish was dipped into water which had been carefully boiled and cooled to room temperature. The melanophores in the piece showed a tendency to contract after 1 hour, and became greatly shrunken after 3 hours, staying in that state permanently. This is a similar result to that obtained by many previous authors with the dermal melanophores.

If a fish is kept in a small quantity of water destitute of oxygen until it dies from asphyxia after ca. 6 hours, it can be easily seen that the peritoneal melanophores have undergone the same change.

(V) PHOTIC STIMULATION.

Some dark or pale pieces of peritoneal wall, with expanded or contracted melanophores, cut out from a fish preliminarily kept in the light or the dark, were dipped into an isotonic physiological saline solution and laid before a white or black background. While a similar piece of peritoneal wall is stimulated by intense light, such as arc lamps, or gas light, the melanophores in this experiment showed no response at all.

(VI) THERMAL STIMULATION.

Dark or pale pieces of peritoneal wall prepared as above were dipped into warm isotonic physiological saline solution. The temperature of the solution was 23°, 35°, 40° and 50°C. From none of these experiments, however, were any positive results obtained, the grade of the expansion and contraction being almost the same from the beginning till 30 minutes or more after.

IV. Effects of Decerebration and Post-mortal Change.

Experiment I. *Decerebration.*

The anterior portion of the brain was cut off in a light-adapted fish which was then kept in a tank in a light or a dark place. After three or four hours the peritoneal melanophore was inspected. The results were as follows :—

(1) Light-adapted fish, kept in the light after operation : . . . . . some expansion, but not so much that all the cells were fused together into a continuous sheet (Fig. 39).

(2) Dark-adapted fish, kept in the light after operation : . . . . . no contraction, rather expansion as in the foregoing Fig. 39.

(3) Light-adapted fish, kept in the dark after operation : . . . . . rather inclined to contract in reaction to darkness.

(4) Dark-adapted fish, kept in the dark after operation : . . . . . a contracted state was sustained (Fig. 40).

In short, the behavior of the peritoneal melanophore is similar in both the intact and the decerebrated fishes.

Experiment II. *Post-mortal Change.*

One lateral half of the abdominal wall of the body cavity and the viscera therein were removed so that the other half of the peritoneal wall was exposed. The surface of this was kept wet by covering with a filter paper moistened with physiological saline solution. The state of the peritoneal melanophore was observed. Then the fish was killed by destroying the entire brain. The change in the state of the melanophore was observed just as it was or when the spinal cord was stimulated electrically. There occurred no change in the state of the peritoneal melanophore in any of the cases. This is just the contrary of the result obtained by SPAETH (1913a) in the dermal melanophore, which always responded with a rapid breaching.

These two experiments show that the peritoneal melanophores are controlled by the central nervous system very weakly.

SUMMARY.

The peritoneal melanophore of a small cyprinoid fish, *Acheilognatus intermedia* (T. & SCHL.) differs in shape and distribution from the dermal melanophore of the same fish.

A remarkable contrast between the peritoneal and the dermal melanophores in their responses to a photic stimulus is that when illuminated the former generally contracts while the latter expands as a rule. The physiological or the ecological significance of this is not clear at present, but it is probable that the peritoneal melanophores serve as a screen to protect the visceral organs from certain ill-effects of the penetrating light.

A common phenomenon in the peritoneal melanophore is that more than twelve smaller cells surround a single larger central one,

all behaving as just one group in expansion as well as in contraction.

The peritoneal melanophores react positively to mechanical but not to electric stimuli. The reaction to change in the osmotic pressure is not distinct.

Direct ionic action of many chemicals is to be seen, the responses being just like those of the dermal melanophore.

Alkaloids that change the irritability of the nervous system may cause expansion or contraction of the peritoneal melanophores, but always in a small degree.

Damage to the brain does not change the behavior of the peritoneal melanophore. The post-mortal change in the melanophore is not distinct.

The innervation of the peritoneal melanophore by the central nervous system is, if any, not so distinct as in the case of the dermal melanophore.

Chronologically measured the responses of the peritoneal melanophore is in general slower than that of the dermal melanophore.

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Explanation of the Plates I and II.

All the figures show the peritoneal melanophores of *Acheilognathus intermedia* in various degrees of expansion or contraction, taken from nature with a microscope and an ABBE'S drawing apparatus.

Fig. 1. After 3 hours' confinement of the fish in a dark room. The peritoneal melanophores are going to contract and to be separated from each other. ×250.

Fig. 2. After 4 hours' treatment in the same way. ×200.

Fig. 3. After 5 hours' treatment in the same way. ×330.

Figs. 4, 5 and 6. After 24 hours' treatment in the same way. Thin threads radiating from the rough margin of the cell are well visible in figs. 5 and 6. That the threads are composed of minute melanin granules is sometimes very clearly seen, but quite invisible at other times. (all) ×200.

Fig. 7. After 48 hours' treatment in the same way. ×330.

Fig. 8. After 48 hours' treatment in the same way. The cells are almost fully contracted. ×200.

Figs. 9 and 10. After 48 hours' confinement in a dark room, the fish was taken out to the light and left ca. 30 minutes. Then cut and examined. The peritoneal melanophores are already commencing to expand. (both) ×330.

Fig. 11. After 48 hours in darkness the fish was exposed to light for 1 hour. ×200.

Fig. 12. The same. The cells are going to fuse with each other to form a complete black screen. ×400.

Fig. 13. The same after 2 hours' exposure of the fish to moderately intense light. ×330.

Fig. 14. The same after 24 hours' exposure to room light. The peritoneal melanophores have recovered their normal state. ×200.

Fig. 15. After 48 hours in the dark room, and then surrounded by a white background for 3 hours. × 50.

Fig. 16. After 2 hours' confinement of a blind fish. ×200.

Fig. 17. After 4 hours' confinement of a blind fish. ×330.

Fig. 18. After 17 hours' confinement of a blind fish. × 50.

Fig. 19. After 21 hours' confinement of a blind fish. A typical contraction of the peritoneal melanophore. × 50.

Fig. 20. The peritoneum of a dark-adapted fish with contracted melanophores was taken and its surface was pressed with a glass rod for 1 hour. × 50.

Fig. 21. A piece of the peritoneum of a light-adapted fish with expanded melanophores was dipped into tap water and left there for 48 hours. The individual melanophore has an irregular shape. × 33.

Fig. 22. A piece of a similar peritoneum dipped into 0.2N solution of KI and left for 15 minutes. The contraction has already commenced. × 50.

Fig. 23. After 48 hours in 0.2N solution of K_2SO_4. The melanophores are highly contracted. × 50.

Fig. 24. After 1 hour in 0.2N solution of KBr. The contraction is commencing. × 50.

Fig. 25. After 24 hours in the same solution. The queer dendritic shape of the melanophore is presumably a sign of decomposition. × 50.

Fig. 26. After 24 hours in 0.2N solution of KCl. × 25.

Figs. 27, 28, 29 and 30. After 15 minutes, 1 hour, 6 hours and 24 hours respectively in the same solution. The process of expansion is indistinct. (all) × 33.

Fig. 31. After 15 minutes in 0.2N solution of Na_2SO_4. A tendency to expand is visible. × 50.

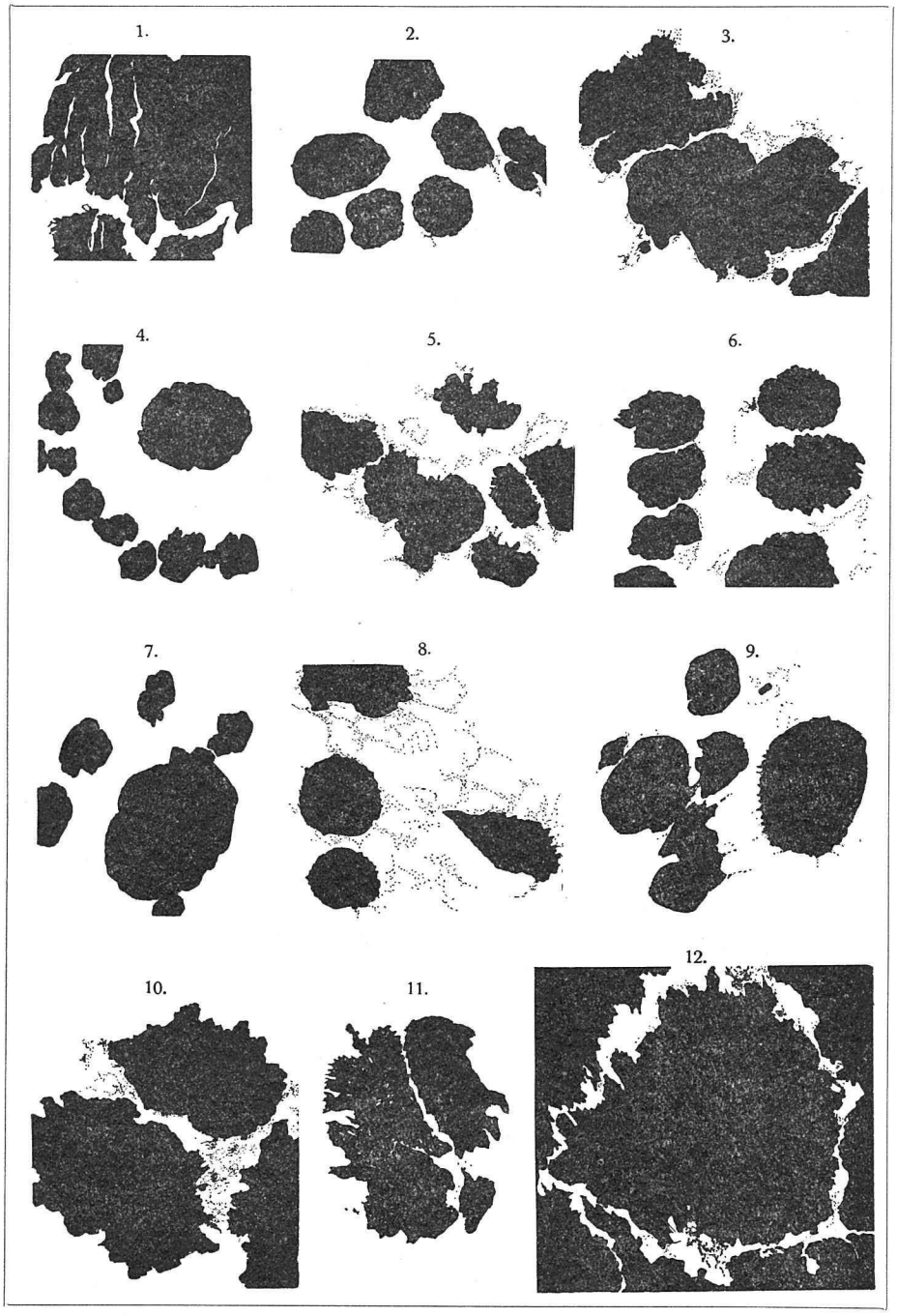
Figs. 32 and 33. After 1 and 6 hours respectively in the same solution. Expansion of a slight degree is seen. (both) × 50.

Fig. 34. After 24 hours in the same solution. The melanophores are inclined to contract again and decompose by and by. × 50.

Figs. 35, 36, 37 and 38. A piece of the peritoneum with melanophores in a contracted state was dipped into 0.2N solution of $(NH_4)_2SO_4$ and left 15 minutes, 1 hour, 6 hours and 24 hours respectively. The expansion of the melanophores is occurring distinctly. (all) × 33.

Fig. 39. A light-adapted fish was injured in the central nervous system and kept in the light. The expansion of the melanophore is commencing. × 33.

Fig. 40. A dark-adapted fish was injured and kept in darkness. The contracted state of the melanophore continued. × 33.



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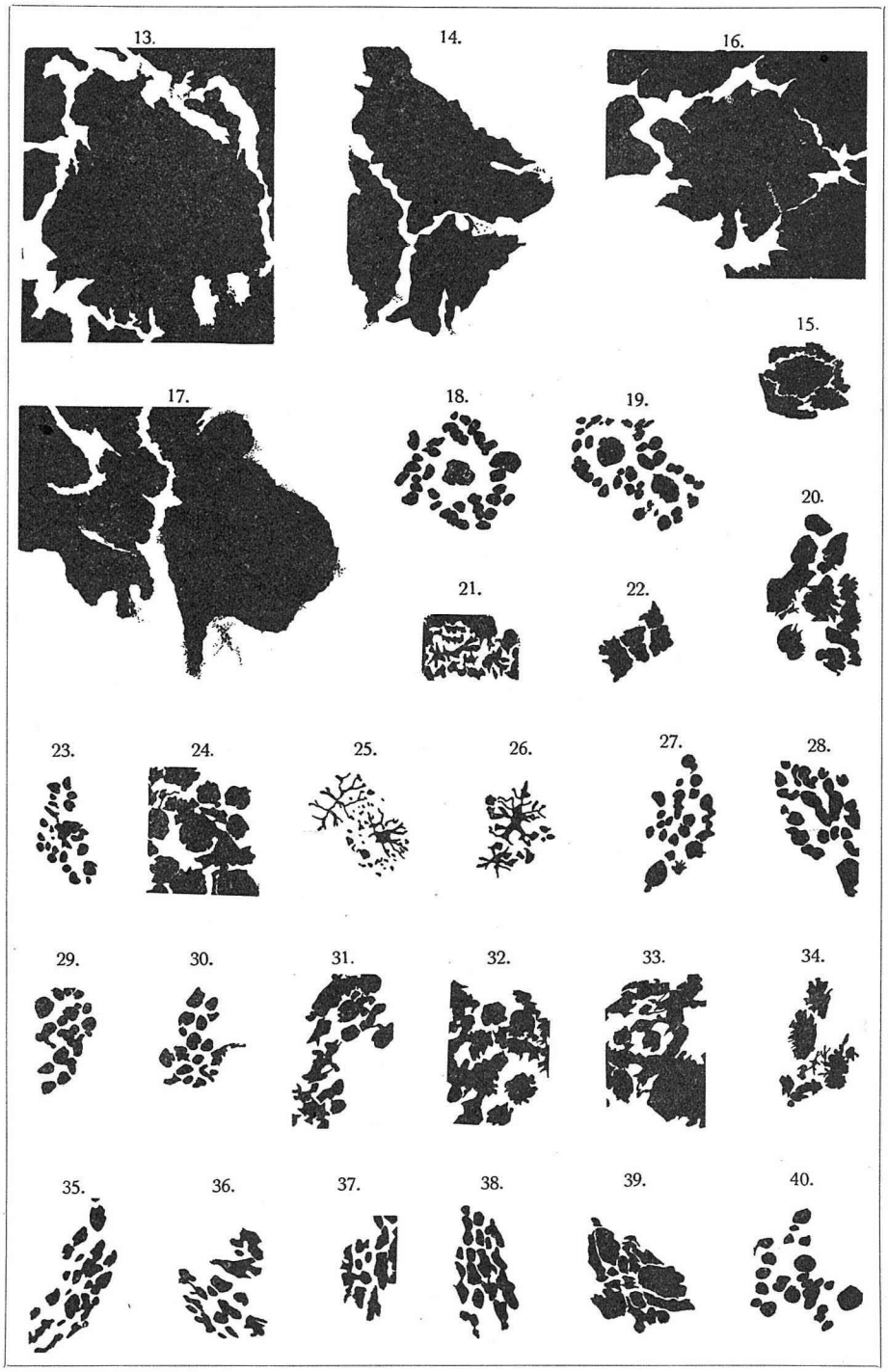

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