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CITATION:

Yamamoto, Kokichi. On the Physiology of the Peritoneal Melanophore of the Frog Tadpole. Memoirs of the College of Science, Kyoto Imperial University. Ser. B 1937, 12(2): 175-186

ISSUE DATE:

1937-03-31

URL:

http://hdl.handle.net/2433/257854

MEMOIRS OF THE COLLEGE OF SCIENCE, KYOTO IMPERIAL UNIVERSITY, SERIES B,
VOL. XII, No. 2, ART. 8, 1937.

On the Physiology of the Peritoneal Melanophore of the Frog Tadpole

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With Plate XII

(Received December, 4, 1936)

Introduction

Much has been written concerning the colour changes of the frog tadpoles, and the behavior of their dermal chromatophores responding to various sorts of stimulation or in varying situations. Almost nothing, however, refers to such internal chromatophores as the peritoneal melanophores or the melanophores lying within the deeper layers of the musculatures.

There are two kinds of melanophores in the skin of the tadpole of the Japanese frog, *Rana rugosa*. The first is small in size and great in number, being situated more or less shallower in the skin, so that it may properly be called "epidermal melanophore." The second one is larger but more scarce than the first one, takes its position deeper in the skin, and can be termed "dermal melanophore." The latter are distributed so that a number of them often form a group surrounding the mucous gland. Therefore, as far as the abdominal wall of *Rana rugosa* is concerned, there are three layers of melanophores, namely: the epidermal which lies quite superficially, the dermal which lies somewhat deeper in the skin, and the peritoneal which lies in the peritoneal membrane lining the body cavity.

When the epidermal melanophore completely contracts,* its size is reduced to that of a simple dot. When it slightly expands,* it

* By the term "contraction" is meant the concentration of pigment granules to the central portion of the cell. By "expansion" is meant the dispersion of the granules all over the cell.

assumes a spindle shape. Fully expanded, all melanophores make a continuous network, each one being connected with its neighbours by means of its radiating processes. The dermal melanophores are similar in form to the foregoing but generally larger. The peritoneal melanophores are arranged in parallel rows along the course of ribs, or perpendicular to the long axis of the animal. They are very numerous and usually cover both the internal and the external surface of the peritoneal membrane. Accordingly, if they expand sufficiently, the peritoneum appears as a black film. On the contrary, if contracted to a certain degree, the peritoneum shows a light grey tint to the naked eye.

In the peritoneal membrane of a minnow, *Acheilognathus intermedia*, it was found by the author (1931) that some of the melanophores behave as a group, smaller cells surrounding a larger central cell and contracting and expanding simultaneously with this. In the peritoneal membrane of the present tadpole such a phenomenon has never been detected.

Each kind of these melanophores is distinguished by characteristic responses to the various sorts of stimulation. In the present study, however, the main object is to investigate the behavior of the peritoneal melanophore, so that the other two kinds of melanophore will be referred to only when the comparison between them and the peritoneal melanophores is necessary.

Materials

Rana rugosa is one of the common frogs in Central Japan. Its egg is hatched in late summer and its tadpole passes the winter on the bottom of shallow pools or weedy ponds. It is not difficult to catch many tadpoles of 30–40 mm. body length during the season from autumn to early spring. This is a good time for the culture of this animal in the laboratory. Another reason why the tadpole of this species is preferable is that its development proceeds rather slowly and the individuals of the earlier stages can be easily obtained. The full-grown tadpoles of any frog are not only too large for treatment or operation but the distribution of the chromatophores are too dense and irregular for distinguishing one kind from the other. In this respect the tadpole of *Rana rugosa* is the fittest, for the distribution of the chromatophores as well as their reactions to stimulus is regular and characteristic.

Methods

There are two ways of stimulating the peritoneal melanophores of the tadpole, namely, the indirect method by which the animal as a whole is kept in a certain situation and its melanophores stimulated by nervous or other physiological processes, and the direct method in which the tissue that contains the melanophores is cut from the body and exposed to the action of various stimulations. The former is more or less natural while the latter is rather artificial.

By the indirect method, the tadpole may receive a stimulus in various ways. It may be that at a moment when the application of a certain kind of stimulation is interrupted, the pigment motor center in the central nervous system is still in an excited state or at the same time some other central parts are excited, the effect of which may be associated with that of the former. Even though it does not act directly on the condition of the peritoneal melanophores, it may act at least as an indirect cause of their behavior. That is to say, the excitation of the nervous system, the condition of circulatory system, etc., may influence the changes of excitation of the peritoneal melanophores as a consequence of the indirect stimulations.

By the direct method, stimulations are applied straight to the peritoneal melanophores. In this case all central nerve fibres are shut out and either the protoplasma of the melanophore receive the stimuli directly or a mere nerve ending of the pigment motor-fibre in this region serves as an effective receptor.

When the indirect stimulations are taking place, the whole body of the tadpole is exposed to the stimulation, but after the experiments are over it is impossible to observe the peritoneum immediately as it is inside of the body, so that the peritoneum must be cut out of the body. For the sake of maintaining the same state of the melanophore in the stimulated individual during the operation, the tadpole is fixed with a high percent of alcohol or alcoholformol liquor just after the treatment is over. After a while a piece of the peritoneum is taken of the animal. To perform this object the skin on the dorsal side is at first opened, the peritoneal wall under it can then be seen, a piece of which is cut from the body with a pair of scissors. This piece is placed on a glass slide with its internal side upwards. Then this slide is placed under a

microscope focussed to the peritoneal melanophores with a low magnification (always cir. × 100) and the features are sketched with the aid of ABBE's drawing apparatus. In most cases the melanophores of the skin of both the external and the internal sides are carefully observed.

Such are my general methods for all cases. Of the other special methods for my experiments explanations will be given in each case respectively.

Experiments and Observation

A) *Indirect photic stimulation*

1. *Dark room experiments on sound animals.*

A small number of tadpoles were kept in a glass jar in a dark room for varying periods. After such confinement in the dark, the peritoneal melanophores of the tadpole were examined according to the above-mentioned method. The effects of the reduced light upon the peritoneal melanophores was fairly constant and may be compiled as follows :—After one hour's confinement in the dark the peritoneal melanophore is slightly inclined to contract. After four hours the contraction proceeds considerably and in this state is remained for twenty-four, forty-eight, or more hours as long as the fish is in the dark. In the same condition the epidermal melanophore is also inclined to contract after one hour's confinement in the dark, but the dermal one maintains its original somewhat expanded state. Such states are unchangeable for forty-eight hours or more as long as the animal is kept in the dark.

As in the case of the peritoneal melanophores of the minnow, all these responses are reversible. If the tadpole is taken from the darkness after a confinement sufficiently long to cause its peritoneal melanophore to fall into a good contracting state, and placed in a light room, the peritoneal melanophore soon begins to relax. This process is so rapid that they become greatly expanded in only half an hour. In short, the peritoneal melanophore of the sound tadpole contracts in the dark, the epidermal one does likewise but in less degree and the dermal one shows almost no reaction.

2. *Dark room experiments on blind animals.*

The eyes of the tadpole are destroyed by means of a red-hot needle-point. The above mentioned experiments were repeated on the blind tadpole several days after the operation, when it had

recovered from the shock of the operation. The results are as follows:—

After two hours, the three kinds of melanophores are still in an expanded state. In twenty-four hours the peritoneal melanophore shows a very slight contraction. After forty-eight hours it is still as slight as in the foregoing. After sixty hours the peritoneal melanophore begins showing a more or less advanced contraction state. As for the other two kinds of melanophores, the dermal one does not show any change at all even when ninety-six hours have passed, while the epidermal one, on the contrary, begins to contract only after 3 or 4 hours and this state reaches a considerable degree of contraction in four days.

But when the tadpole is placed in the light after long confinement in the dark, the peritoneal melanophore quickly re-expands, the dermal melanophore still remains in the former state, while the epidermal one remains in a contracted state for the first hour after the exposure to the light, but in another hour they all are inclined to expand and in the third hour they contract again. In short, in the blind animal the peritoneal melanophore reacts with contraction to the diminution of light and with expansion to the increase of light, the epidermal melanophore has the same response of less intensity and the dermal melanophore shows no reaction to such stimulation.

Such simultaneous changes of the melanophores in the blind tadpoles coincide with the results of PARKER and LANCHNERS (1922) experiments on the dermal melanophore of the sound common killifish, *Fundulus heteroclitus*, but differ from the reactions ascertained by my previous experiment (1931) on that of the blind minnow, *Acheilognathus intermedia*, in which the dermal melanophore behaves just opposite to the peritoneal melanophore.

The comparison of three kinds of animals in regard to the behavior of the epidermal melanophore may be tabulated as follows.

Table 1

Reaction of epidermal melanophores to photic stimuli.

		Fundulus	Acheilognathus	Tadpole of Rana
When brought into dark room	sound	Contraction	Expansion	Indifferent
	blind	——	Expansion	Contraction
When taken from dark room	sound	First expansion then contraction	Contraction	Indifferent
	blind	——	Contraction	First expansion then contraction

Now for a discussion of the changes in the condition of the melanophores of the frog tadpole as a reaction to the diminution of light intensity. Comparing the velocity of the responses of the sound and blind animals to the diminishing light, the melanophores of the sound tadpoles are quicker than those of the blind ones. But when the dark-adapted individuals of either are brought to the light and their melanophores begin reacting to light, no differences of velocity are detectable between the two.

With regard to the melanophores of the sound tadpole, they receive a photic stimulus through the eyes till they are suddenly moved into the darkness. But the melanophores of the blind tadpole do not receive any such stimulus through the eyes during the time they are in the light as equally as they are in the dark. Now, melanophores are influenced by photic stimulations either through the nerve-endings which are inserted in order to control them, or directly by their own protoplasma through the body skin of the animal. That is, the melanophore of the sound tadpole while in the light, can receive photic stimulations both indirectly by means of the eyes and directly by the photic receptors in the cell itself, but after the animal is brought into the darkness no photic stimuli can reach the cell at all. On the other hand, the melanophore of the blind tadpole can receive the stimulus only through the cell, so that when such an animal is carried from the light to the dark the change takes place through the latter way only. Such a difference between the sound and the blind animals must be the reason of the dissimilar reactions stated above. That is, when melanophores are going to contract, the effect of the interrupted photic stimulation is intensified by reaching the cell, through two approaches, hence a quicker contraction; but when they are going to expand, the stimuli which are received either simply by the cell or both through the eyes and by the cells, may equally cause the expansion, and no contractions occur.

B) *Direct photic stimulation*

If a small piece of the peritoneal wall is cut out and exposed to the action of weak or intense electric light, the melanophores on both sides of the wall begin to contract or expand in the same direction as the epidermal or the dermal melanophores do. Hence in the present tadpole the behavior of the peritoneal melanophore against the light is similar to that of the minnow.

C) *Mechanical stimulation*

Some efforts were made to mechanically stimulate the peritoneal melanophore, but none of them were successful.

D) *Effect of narcotics (in indirect stimulation)*

When the tadpole is affected by ether, chloreton, or chloroform, all kinds of melanophores expand to a very advanced state so that the animal becomes darker in general coloration.

E) *Direct chemical stimulations of some ions.*

The melanophores of the tadpole are fairly good material for studying the ionic action of various elements. A part of the peritoneal wall with melanophores is taken out from the body of living tadpoles, cut as big as 5 mm. square and dipped into 1/10 normal solutions of a series of potassium salts, such as KI, KBr, KNO_3, KCl, and K_2SO_4. It was in the midwinter that these experiments were carried on and the tissue of the cut peritoneum remained for several days in the solution without putrifying or decomposing. After immersing this piece of the peritoneum into the solution the melanophores are repeatedly observed under a microscope at short intervals. Within fifteen minutes, they expand in all solutions. After $2\frac{1}{2}$ hours, some melanophores in the KI solution are contracted and the others incline to contraction, but in KBr solution they only incline to this contraction, and in the other three they remain in an expanded state. When one day has passed many of the melanophores in the KI solution are in strong contraction and the rest of them are somewhat contracting; and those in KBr and KNO_3 are only inclining to contract; but those in KCl and K_2SO_4 are still in a state of expansion. After three days those which were dipped into KBr and KNO_3 are in a somewhat contracted state and those in KCl, too, show a somewhat similar state, but those in K_2SO_4 only do not show any contracting state even on the third day after immersion in this solution. All these reactions were reversible and the contracted or expanded phases of the melanophore could be brought back to the former state. Judging from these results the order of the intensities of the action of these anions can be arranged as follows:—

$$I > Br,\ NO_3 > Cl > SO_4.$$

This order almost coincides with that in the case of the peritoneal melanophores of the minnow, *Acheilognathus intermedia.*

F) *Effects of some alkaloids (in direct stimulation)*

A piece of the peritoneal wall, about 5 mm. square in size, as dipped into a very dilute solution of the chlorides of pilocarpine, strychnine, nicotine, or atropine, kept on the laboratory table in room light, and the change in the state of the peritoneal melanophores was traced. The results are as follows:

In pilocarpine (0.1%) the melanophore stays in a well expanded state after 15 to 30 minutes and later on, till a slight degree of contraction occurs after 24 hours. Strychnine (0.02%) always causes a strong expansion. Nicotine (0.02%) causes moderate expansion at the beginning, strong expansion after 24 hours. Atropine (0.02%) nearly the same as nicotine but slightly less effective.

In general the effect of alkaloids can hardly be said to be clear, for the peritoneal melanophore reacts with the usual expanded state for 24 hours or more.

Consulting various investigations of previous authors we see that the reactions of the chromatophores of the vertebrate animals to the alkaloids are not uniform. With pilocarpine BARBOUR and SPAETH (1917) have seen an expansion in *Fundulus* and GILSON (1926) a contraction in larval *Fundulus*, while with atropine SPAETH and BARBOUR (1917) saw an expansion in the isolated scales of *Fundulus* and WYMAN (1924) a complete expansion with the same materials: KAHN (1922) an expansion on a hypodermically injected frog; HOGBEN and WINTON (1922) no visible effect in a frog. In our present knowledge, therefore, nothing can be said as fixed in regard to the effect of alkaloids on the chromatophores.

G) *Effects of hormones*

These were tested by two ways, namely, by leaving the tadpoles for a while swimming in an aquarium with very dilute contents of adrenalin or pituitrin (cir. 0.00005%) and by injecting a dilute solution of the same into the abdominal cavity of the tadpole. The results with adrenalin were almost negative. In the injection experiment the peritoneal as well as the dermal melanophores remained always in an expanded state, while the epidermal one contracted a small degree after 24 hours. In the aquarium test it was observed that there was almost no influence in the dark and only a weak contraction after 3 hours in the light. This effect was nothing compared with what would be expected as a reaction to adrenalin. The effect of pituitrin on the contrary was rather

distinct. In both the injection experiment and the aquarium test all kinds of melanophores remain in the expanded state, regardless of whether the aquarium was kept in the light or in the dark, that is, in spite of the absence or interference of the photic stimulus.

H) *Effects of osmotic pressure*

A small piece of the peritoneal wall is immersed in distilled water, boiled water, saturated or weaker water solution of NaCl or cane sugar and so on. The results are as follows:—

1) Peritoneal melanophore

Distilled water—no reaction in first 5 minutes, a sign of degeneration in 15 minutes, then a special feature with darker cell margin which is an indication of decomposition. Boiled water—an expansion in the beginning, the start of degeneration in 15 minutes, a typical phase in 20 minutes. NaCl—a pathological change. Sugar—a continued expansion.

2) Epidermal melanophore

Distilled water and boiled water—degeneration after 2 hours. NaCl and sugar—a continued expansion, no sign of degeneration.

3) Dermal melanophore

Distilled water—degeneration after 2 hours. Boiled water—degeneration after 40 minutes. NaCl and sugar—a continued expansion, no sign of degeneration.

In general, the peritoneal melanophore is affected much quickly than the epidermal and the dermal ones.

I) *Effects of high or low temperature*

A small basin in which the tadpoles are kept is heated or cooled so that its water temperature is gradually raised from 14°C to 33°C or depressed from 33°C to 14°C. In order to give a more rapid change of temperature the animals were scooped with a small net and transfered to another aquarium of different temperature. To the gradual change of temperature, the epidermal melanophore only reacts with distinct expansion or contraction, the dermal melanophore is very inactive, while the peritoneal melanophore exhibits only very weak responses. A sudden or extreme change of temperature, higher as well as lower, causes the temporary contraction of the epidermal melanophore and after the stimulation is over, the cell, if not ill, regains its usual slightly expanded state, which is supposed to be its most relaxed condition. How much

this phenomenon is subjected to nervous control, as was suggested by SMITH (1928) in the case of trunk melanophore of *Fundulus*, is not clear in so far as my experiments have gone.

It is queer that the peritoneal melanophore is less sensitive than the epidermal and dermal ones especially to the change of temperature, for the former is more sensitive to the other sorts of stimuli, i. e., photic, chemical and osmotic. Perhaps it is a matter of the central nervous control.

Summary

1) There are three kinds of melanophores in the tadpoles of *Rana rugosa*: the epidermal, the dermal and the peritoneal. The epidermal, as it is termed so in this paper, is nothing other than a superficially sheeted dermal melanophore. The peritoneal melanophore is situated on both sides of the peritoneum and lies in parallel rows running dorso-ventrally.

2) These melanophores show some difference in their form, distribution and also in the movement of the granules within them, which is conveniently described as the contraction and expansion of the cells.

3) The stimulation of the peritoneal melanophore was carried on either indirectly by keeping the animal in a certain situation or exposing it to the stimulant or directly cutting out a small piece of the peritoneal wall and subjecting this to experiments.

4) The peritoneal melanophore responds by contraction and expansion to the decrease and increase of light respectively. It is similar to the epidermal and dermal but generally more distinct and intense.

The reaction to the photic stimuli coincides with that of the peritoneal melanophore of *Acheilognathus*, but not with the dermal one of the latter whose two melanophores are different in behavior.

5) By narcotising the tadpole all kinds of melanophores are forced to expand considerably.

6) The peritoneal melanophore is a good material for demonstrating the ionic action of the halogenic anions.

7) The effect of alkaloids is ambiguous. The humoral effects of hormones are variable. Adrenalin does not seem to be effective, while pituitrin can keep all kinds of melanophores in an expanded state.

8) The peritoneal melanophore reacts to the change of osmotic pressure, and in this it is much more sensitive than the other two kinds of melanophores.

9) To change of temperature the peritoneal melanophore is less sensitive than the other two.

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Explanation of plate XII

All figures were sketched from the tadpole of *Rana rugosa* under a microscope with ABBE's drawing apparatus. The magnification is 100 times each.

Figs. 1, 2, 3 and 4. The peritoneal melanophores of the sound tadpoles which were kept in the dark or the light. Their characteristic arrangement into dorso-ventral rows is well represented.

Fig. 1. The peritoneal melanophores of the tadpole after one hour's confinement in a dark room, just about to concentrate the pigment and to be isolated from each other.

Fig. 2. The same, after four hours' treatment in a dark room, with well concentrated pigment.

Fig. 3. The same, after twenty-four hours' treatment in the same way.

Fig. 4. The same, after thirty minutes' treatment of the tadpole in the light. It was previously kept in a dark room for forty-eight hours. The pigment is considerably dispersed.

Figs. 5, 6 and 7. Figures are taken from an artificially blinded tadpole after ninety-six hours' confinement in a dark room.

Fig. 5. The peritoneal melanophores with well concentrated pigment.

Fig. 6. The dermal melanophores with partially concentrated pigment.

Fig. 7. The epidermal melanophores with concentrated pigment.

Figs. 8, 9 and 10. Figures are taken from a tadpole after twenty four hours' confinement in a dark room. Its swimming medium was a cir. 0.00005 % solution of pituitrin.

Fig. 8. The peritoneal melanophores with dispersed pigment.

Fig. 9. The dermal melanophores with dispersed pigment.

Fig. 10. The epidermal melanophores with dispersed pigment.

Fig. 11. The melanophores in a degenerated piece of the peritoneal wall after this was dipped into distilled water.

K. Yamamoto del.