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Effect of the Intracellular Injection of Inorganic Salts on Fish Scale Melanophore¹⁾

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(With 2 Text-figures)

With the view of analysing the mechanism of intracellular migration of melanin granules in melanophores of a fish scale, numerous investigations have hitherto been carried out by many authors (Mathews 1931, D.C. Smith 1939, Kamada and Kinoshita 1944 and H. Kinoshita 1953). In these investigations the most general method to determine the effect of chemical agents to the melanophore response was the observation of the isolated fish scale immersed into a solution of the agents. However, no doubt the chromatophore in teleost scale is under nervous control in normal condition, so that it is very difficult to ascertain whether a certain stimulant acts directly upon chromatophore or acts rather indirectly stimulating nervous elements which are left alive. Recently, Nagahama (1953) confirmed the direct effect of K ion upon melanophore of isolated scale of *Oryzias latipes* by exposing the scale partially to a solution of KCl.

The studies reported here were carried out in an attempt to analyse intracellular condition in causing granular movement within the melanophore by means of the micro-injection.

Material and methods

The isolated scale of *Oryzias latipes* was used as a material. The scales were picked off with a fine pincette from the dorso-lateral part of the fish and immersed into M/7.5 physiological salts solution²⁾ for about 30 minutes before each experiment.

The apparatus in which the isolated scale was mounted was essentially the same as that described by H. Kinoshita (1953). As shown in Fig. 1, the isolated scale was placed on two pieces of thin glass which were fixed on the side walls of the glass trough separately 1 mm apart from each other. The scale was covered with deck glass. In this preparation we were able to move the scale with the microneedle to find out melanophores which is convenient for the purpose of the micro-injection.

The micro-injection was performed with a micropipette of Pyrex glass operated by a micromanipulator. The aperture at the tip of the micropipette was

1) Contributions from the Department of Biology, Faculty of Science, Kyushu University, No. 61.

2) A mixture of M/7.5 NaCl, M/7.5 KCl and M/11 CaCl₂ in volume ratio of 100 : 2.0 : 2.1 (buffered by NaHCO₃)

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approximately 2 micra across. The pipettes were bent by a microflame at about right angle in three parts.

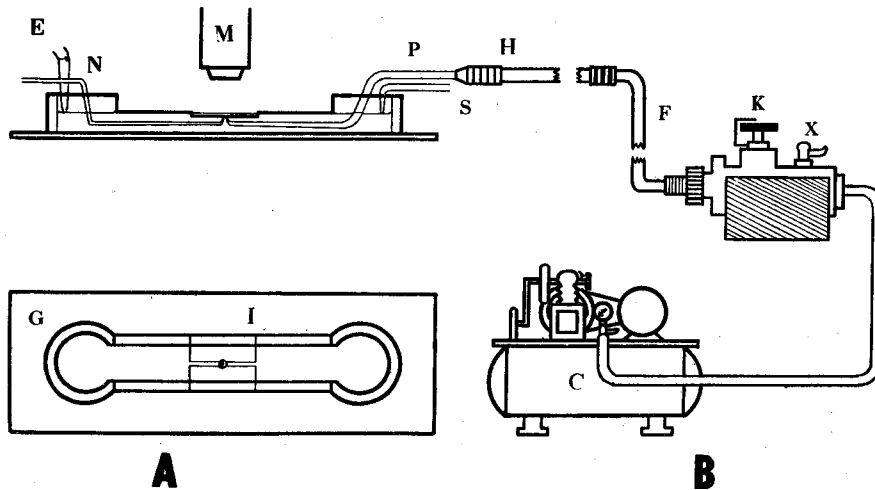


Fig. 1. Diagrams A, of the glass trough in which the isolated scale mounted, and B, of the injecting arrangements. Not drawn to scale. E: Part of funnel where external solution is poured. S: suction pipette operated by stream pump. G: glass trough from dorsal side. I: isolated scale placed. M: objective. N: microneedle mounted in micromanipulator. P: micropipette. H: pipette holder mounted in micromanipulator. F: flexible pipe made by plastic. K: stopcock. X: exhaust cock. C: air compressor.

As technique for the discharge of the injecting solution the Chambers' type was adopted. As for the compressor, however, an air compressor joined with a flexible pipe was employed instead of an injection syringe. In order to ascertain the discharging of the solution from the tip of micropipette, test injections were made into the liquid paraffin placed under the microscope and then the injected solution could be observed clearly as a vacuole. The solution in the micropipette was replaced by a fresh one just before each injection. The amount of solution injected was about 30 cubic micra. The size of the melanophores used for this experiments was 25-40 micra in diameter of centrosphere and about 9 micra thick. The micropipette was thrust into the centrosphere of the melanophore vertically from the under surface. The solution needed to immersed melanophore in the experiment was poured continually into the trough at one end, and at the other end of the trough it was sucked up by a suction pipette operated by a stream pump. Thus the melanophore was kept immersed in the continually flowing solution.

The average room temperature was about 17°C. The observations were carried out under the microscope (magnification : $\times 600$).

Experiments

When the solution was injected into the centrosphere of the melanophore, the melanin granules were driven away temporarily from the site of injection and returned again within a few seconds. This was a good evidence that the injected solution had really entered into the melanophore. It may be assumed that the injected solution mixed immediately with the protoplasm of the melanophore.

Since the external media of M/7.5 NaCl solution (PH 7.2 by NaHCO₃) and M/7.5 physiological salt solution (PH. 7.3 by NaHCO₃) were used, each injection was performed on the melanophore in dispersed state. In this state, the Brownian movement of the melanin granules was usually observed.

1) Injection of NaCl and KCl solutions

1M or M/7.5 NaCl solution (non-buffered, PH 6.8) and 1 M or M/7.5 KCl solution (non-buffered, PH 6.6) were used as the injection fluid. None of these solutions induced clear displacement of the melanin granules. It was observed that after the injection the melanophore maintained the dispersed state in the same way as that of controls, and the Brownian movement of the melanin granules was normally observed.

2) Injection of CaCl₂ solution

1 M or M/11 CaCl₂ solution (non-buffered, PH 6.6) was used for injection. The injection of CaCl₂ solution also did not give rise to particular migration of the melanin granules. However it was observed by the injection of 1M CaCl₂ solution that the aggregation of the melanin granules occurred in the vicinity of the site of the injection and they wrapped up the nucleus of the melanophore. The melanin granules in other part of the melanophore remained unaffected, continuing Brownian movement as before the injection.

3) Injection of Na-oxalate and Na₂CO₃ solutions

M/11 Na-oxalate solution (pH 7.2) and M/11 Na₂CO₃ solution (pH 11) were used in this experiment. When Na-oxalate solution was injected into the melanophore, the aggregation of the melanin granules appears first locally at the site of injection and then spread such local aggregations all over the melanophore. Following the spreading of the local aggregations the centripetal migration of these aggregates was observed. After that, the concentrated granules began to disperse again before they took punctate state. The concentration and dispersion of the granules continued for about two hours till at last the granules ceased in a slightly dispersed state. In this case the centrosphere of the melanophore was wrapped in many aggregates of the melanin granules in whole regions of the melanophore and the Brownian movement of granules ceased. The rhythmical response was observed in all three melanophores located on the same scale, which were injected with this solution. However, the other melanophores on the same

scale (uninjected controls) did not any response. The response is similar to the pulsation which is observed when the scale is immersed into Na-oxalate solution. Fig. 2 represents the outline of this response.

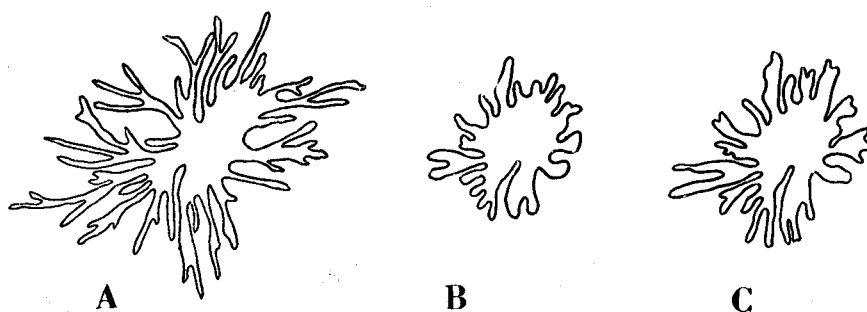


Fig. 2. A: initial state. B: concentrated state in rhythmical response. C: dispersed state in rhythmical response.

On the other hand, the injection of Na_2CO_3 caused the "contraction" of the melanophore. Soon after injection, the melanin granules migrated to the centrosphere and they formed a compact mass. The response was observed only when NaCl solution was employed as an external medium.

4) Injection of distilled water

No displacement of the melanin granules was induced by the injection of distilled water (non-buffered pH 6.6) and the Brownian movement of the granules was also remained just as before the injection.

5) Injection of NaHCO_3 and NaOH solutions

N/10 NaHCO_3 solution (pH 8.9) and N/10 NaOH solution (pH 13) were used as injection fluids. In order to test the effect of OH ion the injection of the solu-

Table 1.

Injected solution	External medium		Remarks
	M/7.5 P.S.S	M/7.5 NaCl	
M/7.5 NaCl		-	Pulsation Concentration
1M NaCl		-	
M/7.5 KCl		-	
1M KCl	-	-	
M/11 CaCl_2		-	
1M CaCl_2	-	-	
M/11 Na-oxalate		+	
M/11 Na_2CO_3	-	+	
N/10 NaOH	-	-	
N/10 NaHCO_3	-	-	
dist. water	-	-	

tion of these kinds were carried out. Injection of the solution of these kinds caused no displacement of the melanin granules. When, however, N/10 NaHCO₃ solution was injected, a definite boundary appeared around the drop of solution injected and temporarily offered a vacuole-like form.

The results obtained in the experiments 1-5 are summarised in Tab. 1. In the table the sign "—" shows the case of none migration, and "+" the case of migration of granules respectively after injection of corresponding solution.

Discussion

Kamada (1938) showed in his micro-injection experiments on *Paramecium caudatum* that the intracellular application of KCl or NaCl solution did not cause the reversal of the direction of ciliary beat of *Paramecium*. However, it is well known that both of the ions, Na and K, cause the reversal if they are applied externally. It is clear, in this case, that the injection of the Na-salts whose anions are calcium-precipitants, caused the ciliary reversal. He assumed from these results that the diminution of the intracellular calcium ions may be involved in the mechanism underlying this phenomenon. It may be assumed that if external K ion concentrations are increased, the internal Ca ions are partly replaced by K ions. This may result in the diminution of the internal Ca ions. It is interesting in this respect that also in the present writer's experiment no response of the melanophore was observed when KCl solution was injected into melanophore. In fact the melanophore shows the concentration response when it is immersed into KCl solution, but the writer's results indicate that the response of the melanophore is not caused directly by the increase of the internal concentration of K ions. Since the melanophore is buried in the epithelial tissue of the scale, it is not exposed to the external medium. But, if it is assumed that the protoplasmic membrane of the melanophore is of cation permeable as well as that of *Paramecium*, an exchange of intracellular cation such as calcium ions with the external potassium ions can be expected to occur, when the scale is immersed into the KCl solution. From the results of the injection of calcium precipitants we can assume also in this case the decrease of the intracellular concentration of Ca ion to be the cause of the response of the melanophore.

According to T. Yamamoto (1933), Na-oxalate solution is the most effective among various agents in causing the melanophore pulsation. When the isolated scale is immersed into the solution of Na-oxalate, nearly all the melanophores involved begin clear pulsation and continue this pulsation until they stop, after a few minutes in the state of dispersion. It was remarkable, however, in this micro-injection that the melanophores into which Na-oxalate solution was injected maintained their pulsation for more than two hours. Yamamoto reported also that the Na-salts containing no Ca-precipitant anion also caused the melanophore pulsation, but in those cases generally the percentage of the melanophores showing pulsations was generally far lower than in the case of Ca-precipitants. The mel-

nophore pulsation was also induced by the change of hydrogen ion concentration of the medium into which the melanophore was immersed (D.C. Smith, 1930).

Parker and Pumphrey (1936) observed that the pulsations could be caused in the melanophores of *Fundulus* from which all traces of nervous elements have been removed by a degenerative operation, and they assumed that the pulsation of the melanophore was caused directly by the effect of the stimulating agents. Also in this experiment it was observed that the melanophore pulsation was induced directly by the intracellular injection of the Na-oxalate solution.

According to Parker and Pumphrey the extent of the migration of melanin granules reached from complete concentration, where the melanin granules had the punctate form, to about half or three-quarters of full dispersion. However, in pulsation observed in this micro-injection experiments the extent of the migration of granules reached only from about one-third of full dispersion where the melanin granules had the stellate form to about half of full dispersion. This type of pulsation, with such small amplitude, had not been observed, hitherto, in immersion experiments.

Summary

1. The intracellular injection of some inorganic salts was performed on the isolated scale melanophores of *Oryzias latipes* to analyse the intracellular condition in causing the migration of melanin granules within the melanophore.
2. The injection of the NaCl, KCl and CaCl₂ solution as well as that of distilled water caused no migration of melanin granules.
3. The Na-oxalate and Na₂CO₃ solution clearly caused the migration of melanin granules.
4. The diminution of intracellular calcium ions may be involved in the mechanism of the migration of melanin granules within the melanophore.

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