Physiological Studies on Fertilization and Activation of Fish Eggs

IV. Fertilization and Activation in Narcotized Eggs¹

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Previous studies on fertilization and activation of the fish (*Oryzias*) and the lamprey (*Lampetra*) indicate that the ripe unfertilized egg is of an irritable system with a gradient of excitation-conduction which is the highest at the animal pole and the lowest at the vegetal pole (YAMAMOTO 1939, 1944). It has been postulated that some sort of invisible change or an "impulse" may be provoked by the spermatozoon or activating agents at the cortex of the egg. This "impulse" propagates in wave-like manner as the cause of the visible breakdown of the cortical alveoli. The separation of the chorion is the consequence of the breakdown of the cortical alveoli. If this view is correct the cortical response may greatly be influenced by the change of irritability in the unfertilized egg. The present study has been undertaken in order to see the effect of narcosis on fertilization and activation of the unfertilized egg of *Oryzias latipes*.

Material and Method

Materials used were the orange-red type of the medaka (*Oryzias latipes*). Method for obtaining ripe eggs was the same as described in the previous papers. The ripe unfertilized eggs were kept in the isotonic (M/7.5) Ringer's solution (pH 7.3). Narcotics were dissolved in the isotonic Ringer's solution.

Fertilization in eggs narcotized with chloretone

It has been made shown that when the unfertilized eggs are completely narcotized with M/200 phenyl urethane no cortical change is provoked on insemination. When the narcosis is incomplete, insemination brings about the partial response. The cortical alveoli at the animal hemisphere are broken down while those at the vegetal hemisphere remained unchanged. (YAMAMOTO 1944c). The following experiment has been undertaken whether or not the same be valid with chloretone.

The ripe unfertilized eggs were treated with M/200 chloretone dissolved in M/7.5 Ringer's solution for various periods of time. They were

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inseminated immediately on return to Ringer's solution. The results are shown in Table 1. When the eggs are exposed to the solution of chloretone for short periods, the cortical response to insemination is nearly the same as in control eggs. Long exposure leads to complete inhibition of the cortical response. When the time of exposure is intermediate many eggs show partial breakdown of the cortical alveoli. The breakdown of the cortical alveoli begins at the animal pole and it propagates towards the vegetal pole. In many eggs, the cortical response ceases to propagate on

Table 1. Fertilization of the eggs narcotized with chloretone. The ripe unfertilized eggs of *Oryzias* were treated with M/200 chloretone-M/7.5 Ringer's solution and were subsequently inseminated on return to Ringer's solution. Experiments were performed at 28-28.5 C.

Time of exposure to chloretone (Min.)	Number of observations	Cortical response after insemination
0 (control)	3	20 out of 20 eggs : complete
5	3	20 " " 20 " : complete
15	3	{ 17 " " 20 " : complete 1 " " 20 " : partial 2 " " 20 " : no
25	3	{ 11 " " 21 " : complete 6 " " 21 " : partial 4 " " 21 " : no
35	3	{ 0 " " 21 " : complete 11 " " 21 " : partial 10 " " 21 " : no
45	3	{ 0 " " 20 " : complete 8 " " 20 " : partial 12 " " 20 " : no
60	3	{ 0 " " 20 " : complete 3 " " 20 " : partial 17 " " 20 " : no

Table 2. Recovery from narcosis. Ripe unfertilized eggs of *Oryzias* were narcotized with M/200 chloretone in M/7.5 Ringer's solution for an hour. They were inseminated after the return to Ringer's solution. Temperature 28-31°C.

Time after the return to Ringer's sol. (Min.)	Number of observation	Cortical response after insemination
0	3	18 out of 18 eggs : no
15	3	{ 13 " " 18 " : complete 5 " " 18 " : partial 0 " " 18 " : no
30	3	{ 19 " " 19 " : complete 0 " " 19 " : partial 0 " " 19 " : no

the way between the animal and the vegetal pole (Fig. 1). The partial breakdown of the cortical alveoli may be due the decrease of excitability by narcosis and the existence of the excitation-conduction gradient which is the highest at the animal pole and the lowest at the vegetal pole.

Fig. 1. 1, Unfertilized egg; 2, Partial breakdown of the cortical alveoli in fertilized egg which had been incompletely narcotized with chloretone in the unfertilized stage; 3, Complete breakdown of the cortical alveoli. The cortical alveoli are represented as dots and the oil droplets as small circles. A, Animal pole, V, Vegetal pole.

Inhibition of the response by chloretone is ready reversible. The unfertilized eggs were immersed in M/200 chloretone-M/7.5 Ringer's solution for an hour. They were inseminated at various time intervals after the return to Ringer's solution. As is seen in Table 2, most eggs showed the cortical response after 15 minutes. All eggs recovered the response after 30 minutes.

Stimulation with pricking in narcotized eggs

It has been shown in previous papers that the typical cortical response is provoked by the pricking of the unfertilized eggs with a glass needle. The following experiments were performed in order to see the effect of narcosis on the stimulation with pricking. The ripe unfertilized eggs were treated with M/200 phenyl urethane or M/200 chloretone in the isotonic Ringer's solution. They were pricked at the animal pole with a glass needle of 15μ in diameter. The results are shown in Tables 3 and 4. It will be seen that no egg can be activated after the exposure to the narcotics for 30 minutes. Eggs treated with narcotics for 15 minutes showed partial activation, namely, the partial breakdown of the cortical alveoli. In order to see the recovery from narcosis, egg narcotized with these agents for 30 minutes were returned to Ringer's solution and were pricked with a needle at various time intervals. It will be seen in Tables 3 and 4 that the eggs narcotized with phenyl urethane and chloretone recovered the typical cortical response after the recovery from narcosis.

Table 3. Pricking on unfertilized eggs narcotized with M/200 phenyl urethane-M/7.5 Ringer's solution. Eggs were pricked at the animal pole with a glass needle of $15\ \mu$ in diameter. Temperature 29-33°C.

Time of exposure to narcotic sol. (Min.)	Number of observations	Total number of eggs	Number of activated eggs	% of activation	Remarks
0 (control)	4	24	24	100	complete
5	3	26	26	100	„
15	3	26	15	58	partial
30	4	29	0	0	
Time after the return to Ringer's sol.					
5	3	21	0	0	
15	4	23	9	39	partial
30	4	30	24	80	complete
45	3	26	25	96	„

Table 4. Pricking on unfertilized eggs narcotized with M/200 chloretone-M/7.5 Ringer's solution. Temperature 28-32°C.

Time of exposure to narcotic sol. (Min.)	Number of observations	Total number of eggs	Number of activated eggs	% of activation	Remarks
0 (control)	4	20	20	100	complete
5	2	24	21	88	„
15	3	23	3	13	partial
30	5	25	0	0	
Time after the return to Ringer's sol.					
5	2	19	6	32	partial
15	3	19	19	100	complete
30	5	23	23	100	„

These experiments indicate that no response is provoked by pricking in completely narcotized eggs. In partially narcotized eggs the response is partial that is the breakdown of the cortical alveoli occur mainly in the animal hemisphere.

Chemical stimulation in narcotized eggs

It has been shown in previous papers that a number of surface active substance such as sodium-taurocholate, sodium glycocholate, sodium oleate, digitalin and saponin induce the activation of the unfertilized eggs. Amongst them saponin has been proved to be a very powerful stimulat-

ing agent. The following experiments have been undertaken in order to see the effect of narcosis on chemical activation.

The ripe unfertilized eggs were immersed in the solution of M/200 phenyl urethane or M/200 chloretone dissolved in the isotonic Ringer's solution for various periods of time. They were subsequently treated with 0.1 per cent solution of saponin (German) in the isotonic Ringer's solution for 4 minutes. Results are shown in Tables 5 and 6. The percentage of activated eggs decreases as the time of narcosis increases. It will be seen that relatively long exposure is necessary before the complete inhibition of activation. This is due to the strong stimulating action of saponin.

Table 5. Chemical (0.1 % saponin, 4') of unfertilized *Oryzias* eggs narcotized with M/200 phenyl urethane-M/7.5 Ringer's solution. Temperature 29-32°C.

Time of exposure to narcotic sol. (Min.)	Number of observation	Total number of eggs	Number of activated eggs	% of activation
0 (control)	3	40	40	100
30	3	40	20	50
60	3	40	6	15
90	3	37	3	8

Table 6. Chemical activation (0.1 % saponin, 4') of unfertilized *Oryzias* eggs narcotized with M/200 chloretone-M/7.5 Ringer's solution. Temperature 25-32°C.

Time of exposure to narcotic sol. (Min.)	Number of observation	Total number of eggs	Number of activated eggs	% of activation
0 (control)	3	28	28	100
30	3	28	6	21
60	3	28	6	21
90	3	28	2	7
120	3	30	0	0

To sum up, saponin fails to exert its stimulating effect on completely narcotized egg. Longer exposure of narcotized egg to saponin gives rise to the cytolysis without the breakdown of the cortical alveoli.

Thermal stimulation in narcotized eggs

It has been shown in previous papers that temporary rise in temperature leads to the typical activation in unfertilized *Oryzias* egg. When, however, narcotized eggs are exposed to the high temperature, no cortical change ensues. Tables 7 and 8 show the results of the experiments. The ripe unfertilized eggs have been treated with M/200 phenyl urethane or M/200 chloretone for various periods of time. They were subsequently treated with the high temperature after the return to Ringer's solution.

Table 7. Thermal activation (45°C, 2') of unfertilized *Oryzias* eggs narcotized with M/200 phenyl urethane-M/7.5 Ringer's solution. Temperature 28.5–32°C.

Time of exposure to narcotic sol. (Min.)	Number of observation	Total number of eggs	Number of activated eggs	% of activation
0 (control)	3	31	31	100
5	3	31	17	55
15	3	33	7	21
30	3	29	0	0

Table 8. Thermal activation (45°C, 2') of unfertilized *Oryzias* eggs narcotized with M/200 chloretone-M/7.5 Ringer's solution. Temperature 31°C.

Time of exposure to narcotic sol. (Min.)	Number of observation	Total number of eggs	Number of activated eggs	% of activation
0 (control)	3	45	45	100
5	3	45	14	31
15	3	45	0	0
30	3	43	0	0

It will be seen that the exposure to the narcotics for 30 minutes abolishes the cortical response to thermal stimulation. Treatment for 5–15 minutes induces the partial breakdown of the cortical alveoli.

Discussion

It has been shown that the unfertilized eggs of the medaka (*Oryzias*) and the lamprey (*Lampetra*) are of irritable system with the excitation-conduction gradient which is the highest at the animal pole and the lowest at the vegetal pole. It has also been postulated that the spermatozoon or activating agents stimulate the cortical protoplasm and some change or an "impulse" is provoked. This impulse propagates as the "wave of fertilization" which causes the breakdown of the cortical alveoli. The wave-like breakdown of the cortical alveoli is followed by the separation of the chorion (YAMAMOTO 1944a, b, c).

Our previous and the present experiments indicate that when the unfertilized eggs are completely narcotized with phenyl urethane or chloretone, no cortical response is provoked by spermatozoon or by activating agents such as electric, chemical, mechanical and thermal stimuli. In incompletely narcotized eggs such agents induce partial breakdown of the cortical alveoli. The effect of the narcotics is completely reversible. When the eggs recovered from narcosis, they show typical cortical response to spermatozoon or to artificial stimuli.

These facts strongly favour the view that the unfertilized egg is of an irritable system and the activation involves a process of excitation of

the egg cortex. When the unfertilized eggs are narcotized with phenyl urethane or chloretone the irritability of the egg decreases so that they fail to respond to the spermatozoon or to other stimulating agents.

Summary

1. Narcosis of the unfertilized *Oryzias* eggs with chloretone brings about reversible inhibition to fertilization reaction.

2. Narcosis of the unfertilized eggs with phenyl urethane or chloretone gives rise to the inhibition of activation on mechanical (pricking), chemical and thermal stimulation.

3. When the narcosis is not complete, partial cortical response occurs which begins near the animal pole and the vegetal pole.

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