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Effects of Various Inhibiting Conditions on the Early Embryos of Amphibia

(I) Effects of KCN and Dinitrophenol[1)]

By

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In the early embryos of amphibia it was pointed out that the region in which the oxidation of reduced dyes proceeded most rapidly corresponded to the region in which the conspicuous formative movements of gastrulation or neurulation took place intensively. And the existence of a close relation between the activity of dye-oxidation and the activity of formative movements of embryonic tissues was anticipated. However, before approaching this relation further, it seems necessary to find how the processes of formative movements are restrained in various noxious conditions.

For this purpose, the present experiments were performed: the early embryos of amphibia were treated with KCN which is known as the inhibitor of the respiration. On the other hand, they also treated with dinitrophenol and the hypertonic solutions of neutral salts. Then, the inhibition on the process of development, especially on the formative movements were observed.

In this paper the observations on the embryos treated with KCN and dinitrophenol are reported.

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Material and Method

KCN and dinitrophenol were used as the inhibitors of the development. KCN was diluted with distilled water in the molar concentrations of 1/500, 1/1,000, 1/5,000 and 1/10,000.

Dinitrophenol-solutions were prepared as follows: 1g. of dinitrophenol and 0.5g. of sodium bicarbonate were dissolved in 100c.c. of distilled water and this

1) This work was aided by the Scientific Research Expenditure of the Department of Education.

solution served as the stock solution. Then the stock solution was diluted with distilled water to become $1:5\times10^4$, $1:1\times10^5$, $1:2\times10^5$, $1:5\times10^5$ and $1:1\times10^6$.

Embryos of *Rana nigromaculata* were collected in the suburbs of Kyoto and were subjected to the experiments, when they became the blastulae and the gastrulae.

5~10 embryos with their vitelline membrane were immersed in the solutions of KCN and dinitrophenol contained in the glass dishes, 10 c. m. in diameter and 1 c. m. in depth for 24 hours. After the treatments the embryos were removed into the filtered pond-water and reared for about a week.

The experiments were carried out in May 1952 and in May 1953. The temperature at noon during the experiments stood between 20°C. and 22.8°C. Within the limits of these temperatures, the blastulae developed to the early gastrulae in four or six hours, and the early gastrulae developed to the early neurulae in about 16~18 hours.

Observations

The observations on the embryos 18 hours or 22 hours after the beginning of the treatments are arranged in the following 4 tables.

Table 1. Blastulae treated with KCN in various concentrations.

KCN conc.	treated individuals	22 hours after the beginning of the treatment		remarks
		available case	appearance of embryos	
1/500M	38	38	The lip of the blastopore appeared in the dorsal side. (early gastrula) Cytoplasm bursted forth in various regions of embryos.	All of them cytolysed in three days after the end of the treatment.
1/1,000M	26	12 (46%)	The lip of the blastopore appeared in the dorsal side. (early gastrula) Cytoplasm bursted forth in various regions of embryos.	All of them cytolysed in 5 days after the end of the treatment.
		14 (54%)	The lip of the blastopore appeard in the dorsal side. (early gastrula) Embryos appeared to be intact.	
1/5,000M	28	14 (56%)	The blastopore was not closed. The neural folds differentiated distinctly. (Fig. 2)	24 embryos survived more than 5 days.
		11 (44%)	The blastopore was closed. The neural folds differentiated distinctly. (early neurula)	
1/10,000M	29	28	They developed to early neurulae. In 5 embryos the neural folds contacted each other in the posterior region. External anomalies were not found.	28 embryos survived more than 5 days.
control	16	14	They developed to early neurulae. In 6 embryos the neural folds contacted each other in the posterior region. External anomalies were not found.	13 embryos survived more than 5 days.

Table 2. Early gastrulae treated with KCN in various concentrations.

KCN conc.	treated individuals	18 hours after the beginning of the treatment		remarks
		available case	appearance of embryos.	
1/500M	28	11 (41%)	The lip of the blastopore appeared in the dorsal side. (early gastrula)	All of them appeared intact through the treatment of 24 hours. 3 embryos cytolysed in 5 days after the end of the treatment.
		13 (48%)	The lip of the blastopore appeared circular. (middle gastrula) The yolk-plug was extraordinarily large and protruded.	
		3 (11%)	The lip of the blastopore appeared circular. (middle gastrula) The yolk-plug was normally formed.	
1/1,000M	26	20 (87%)	The lip of the blastopore appeared circular. (middle or late gastrula) The yolk-plug protruded. (Fig. 1)	23 embryos survived more than 5 days.
		3 (13%)	The lip of the blastopore appeared circular. (middle or late gastrula) The yolk-plug was formed normally.	
1/5,000M	22	21	They developed to early neurulae. External anomalies were not found.	18 embryos survived more than 5 days.
1/10,000M	28	25	They developed to early neurulae. In 8 embryos the neural folds contacted each other in the posterior region. External anomalies were not found.	25 embryos survived more than 5 days.
control	11	11	They developed to early neurulae. In 5 embryos the neural folds contacted each other in the posterior region. External anomalies were not found.	11 embryos survived more than 5 days.

Table 3. Blastulae treated with dinitrophenol in various concentrations.

DNP conc.	treated individuals	22 hours after the beginning of the treatment		remarks
		available case	appearance of embryos.	
$1:5\times10^{4}$	40	38	Gastrulation did not begin. Cytoplasm bursted forth in various regions of embryos.	All of them cytolysed in 3 days.
$1:1\times10^{5}$	36	21 (58%)	Gastrulation did not begin. Cytoplasm bursted forth in various regions of embryos.	All of them cytolysed in 3 days.
		15 (42%)	The lip of the blastopore appeared in the dorsal side. (early gastrula) Cytoplasm bursted forth in various regions of embryos.	
$1:2\times10^{5}$	40	35 (88%)	Gastrulation did not begin. Embryos appeared to be intact.	11 embryos survived more than 5 days.
		5 (12%)	The lip of the blastopore appeared in the dorsal side. (early gastrula) Embryos appeared to be intact.	
$1:5\times10^{5}$	27	23 (83%)	The lip of the blastopore appeared in the dorsal side. (early gastrula)	26 embryos survived more than 5 days.
		3 (12%)	The lip of the blastopore appeared circular. (middle gastrula) The yolk-plug protruded.	

$1:1\times10^{6}$	20	20	They developed to early neurulae. External anomalies were not found.	20 embryos survived more than 5 days.
control	18	17	They developed to early neurulae. In 6 embryos the neural folds contacted each other in the posterior region. External anomalies were not found.	17 embryos survived more than 5 days.

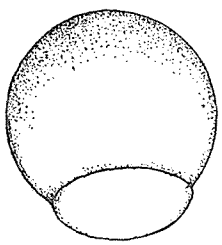

Fig. 1

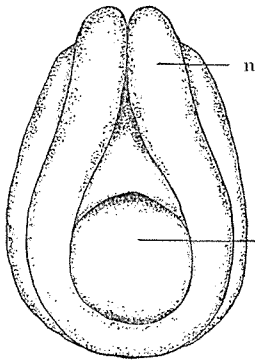

Fig. 2

Table 4. Early gastrulae treated with dinitrophenol in various concentrations.

DNP conc.	treated individuals	18 hours after the beginning of the treatments: available case.	18 hours after the beginning of the treatments: appearance of embryos	remarks
$1:5\times10^{4}$	36	23 (66%)	The lip of the blastopore appeared in the dorsal side. (early gastrula) Embryos appeared to be intact.	All of them cytolysed in 5 days.
		12 (34%)	The lip of the blastopore appeared in the dorsal and lateral sides. Embryos appeared to be intact.	
$1:1\times10^{5}$	31	12 (39%)	The lip of the blastopore appeared in the dorsal and lateral sides.	30 embryos survived more than 5 days.
		19 (61%)	The lip of the blastopore appeared circular. (middle gastrula) The yolk-plug was extraordinarily large and protruded.	
$1:2\times10^{5}$	28	22 (79%)	The lip of the blastopore appeared circular. (middle or late gastrula) The yolk-plug protruded.	26 embryos survived more than 5 days.
		6 (21%)	The lip of the blastopore appeared circular. (middle or late gastrula) The yolk-plug was formed normally.	
$1:5\times10^{5}$	17	12 (71%)	Embryos developed to early neurulae. The blastopore was not perfectly closed. (Fig. 2)	11 embryos survived more than 5 days.
		5 (29%)	Embryos developed to early neurulae. The blastopore was perfectly closed.	
$1:1\times10^{6}$	22	20	Embryos developed to early neurulae. In 11 embryos the neural folds contacted each other in the posterior region. External anomalies were not found.	20 embryos survived more than 5 days.

control	12	12	2 embryos developed to early neurulae. 10 embryos developed to middle neurulae. External anomalies were not found.	12 embryos survived more than 5 days.

Discussion and Conclusion

From the tables above enumerated it is clear that KCN and dinitrophenol inhibit the process of early embryonic development. These reagents, when they are used in higher concentrations, perfectly arrest the process of development, and at the same time they inflict the fatal injuries on the embryos, and cause the cytolysis of embryonic tissues. When employed in lower concentrations, they do not entail the cytolysis upon the embryos, although they can inhibit the processes of development.

In the abnormal processes of development caused by these reagents, two different patterns can be pointed out, one being the retardation of the developmental progress and another, the appearance of anomalies.

As shown in Table 3, the blastulae develop to the early or middle neurulae in 22 hours under the normal conditions, while when they are immersed in the solution of dinitrophenol of the concentration of $1:5\times10^5$, they develop only to the early or middle gastrulae in the same course of time. When immersed in the solution of $1:2\times10^5$, they hardly develop to early gastrulae, in 88 % of them the development is perfectly arrested. As shown in Table 4, the early gastrulae develop to the middle neurulae in 18 hours under normal conditions, while when they are immersed in the solution of dinitrophenol of the concentration of $1:5\times10^5$ they develop only to the early neurulae in the same course of time. When immersed in the solution of $1:2\times10^5$, the differentiation of the neural folds is never found within 18 hours. In Tables 1 and 2, the developmental progress of treated embryos are also retarded by KCN, and the retardation become severer as the concentration of KCN grow higher. From these facts it may be concluded that KCN and dinitrophenol similarly retard the developmental progress of blastulae and gastrulae, and that the higher the concentrations are, the severer the retardation is.

The following fact can be pointed out in Tables 3 and 4: when the blastulae are treated with dinitrophenol of the concentration of $1:1\times10^5$, 58 % of the treated embryos do not develop at all. Their development is perfectly arrested immediately after the beginning of the treatment. On the other hand, when the gastrulae are treated with the same reagent of the same concentration, the perfect blocking of the development do not occur at all in any embryos. In the concentration of $1:5\times10^5$, all of the treated gastrulae develop to the early neurulae in 18 hours, while 88 % of treated blastulae advance to the early gastrulae in 22 hours. This advancement is very slow, as compared with that

of the non-treated embryos, in which it takes 4 or 6 hours. From these facts the following conclusion can be drawn: blastulae are more susceptible than gastrulae to the effect of dinitrophenol in the point of retarding the progress of the development.

When the blastulae are treated with KCN of the concentration of 1/500M, the cytoplasm bursts forth as small droplets from the various regions of the embryos. On the other hand, when the gastrulae are treated with KCN in the same concentration, they stand intact throughout 24 hours of the treatment. In the concentration of 1/1,000M, the treated gastrulae stand intact throughout 24 hours of the treatment, while in the treated blastulae the cytolysing embryos are found in 22 hours. From these facts it can be concluded that blastulae are more susceptible than gastrulae to the cytolysing effect of KCN. The same conclusion can be drawn also from the experimental results with dinitrophenol, as is indicated in Tables 3 and 4.

LATINIK ('28) treated the embryos of *Rana fusca* in various developmental stages with the temperature of 32.5°C. At this temperature the abnormal embryos were yielded. When the treatment was performed on the blastulae for 3 hours, many abnormal embryos appeared, while, when the treatment was performed on the gastrulae, the treatment must be durated for 8 hours in order to obtain as many abnormal embryos as appeared with blastulae. Thus, he concluded that blastulae were more susceptible than gastrulae to the injurious effect of the high temperature.

His conclusion is in good agreement with mine. However, in my present experiments, the observations are performed concerning the susceptibility, not to the effects of yielding the abnormal embryos, but to the effects of retarding the developmental progress and of cytolysing the embryonic tissues. Therefore, it must be stated that susceptibility which LATINIK observed is not the same with my cases.

Besides the effect which causes the mere retardation of the embryonic development, these reagents possess another effect upon the process of the development, too. Looking through the four tables, three types of anomalies can be found in the embryos. The first is the extraordinarily large yolk-plug-formation in the late gastrulae. The second is the protrusion of the yolk-plug in the late gastrulae. The third is the incomplete closure of the blastopore in the embryos in which the neural folds are distinctly formed.

These three types of anomalies are considered to be resulted from the disharmonic performance of the development, because, when the various factors of the development are inhibited harmoniously, the anomalies would not occur, but only the retardation of the development would take place. Thus, the first two types are considered to be produced by the disharmonic performance of the various movements involved in the gastrulation, especially in the epiboly and the invagination. And the third type seems to be produced in embryos in which

the gastrulation is inhibited much more than the neurulation.

From these considerations it can be concluded that KCN and dinitrophenol inflict the similar disharmonic inhibition on the early embryonic development of amphibia.

In my another experiment in which the embryos are immersed in hypertonic solutions of neutral salts, similar types of anomalies are also found, i. e., neurulation also occurs when the epiboly and the invagination are hardly performed. Therefore, it seems probable that the inhibition of the movements of gastrulation is a common occurrence under the various noxious conditions. On this point, however, further discussion will be done in the following paper.

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