On the Insemination with Modified Spermatozoa in Urechis unicinctus

By Akira KOENUMA

Department of Biology, Faculty of Liberal Arts and Science, Shinshu Univ.
(received Dec. 16, 1959)

There are many experiments on the activation of the eggs of the gephyrean worm, *Urechis* (Tyler, '31a, '31b, '32a, '32b, Tyler and Schultz '32, Tyler and Bauer '37, Hiraiwa and Kawamura '34, '35, '36, Ohkawa, '52, '55, '58). In a previous paper (Koenuma, '56), it was shown that when normal eggs of *Urechis unicinctus* were inseminated with spermatozoa treated with uranyl nitrate solution, there occurred an appreciable abnormal cleavage resembled a polyspermic cleavage followed by the elevation of firtilization membrane taking place normally ('56). If polyspermy occurred in insemination with modified spermatozoa, it is unlikely to be explained by an assumption that the mechanism excluding supernumerary spermatozoa is involved only in the cortical reactions of the eggs upon fertilization without any interaction with spermatozoa concerned.

The present paper deals with confirmation of the fact that spermatozoa treated with uranyl nitrate participate in a polyspermic aspect of cleavage and further with the observation of abnormal cleavage induced by spermatozoa treated with acidified sea water.

I wish to express my thanks to Dr. Juro Ishida for his constant guidance and encouragement, and also thanks to Dr. Ichiro Tomiyama, director of the Institute of Misaki Marine Biological Station at which the work was carried out. Thanks are also due to Dr. Masumi Ohkawa, professor of Yokohama Municipal University, for supplying material used in the present work.

Material and Methods

The eggs used in the experiment were those of the gephyrean worm, *Urechis unicinctus*, which were obtained from Kanazawa-Hakkei in Tokyo Bay. In each experiment, unfertilized eggs were suspended and inseminated in normal sea water (pH 8.2) in a small glass dish. The glass dish was settled

under the microscope just after the insemination, observation being made at the definite interval in the same microscopic field and continued until 4-cell stage in most cases.

Experiments were carried out at room temperature, varying from 8.5°C to 16°C, but the change of temperature in each experiment was not found over 2°C.

Treatment of spermatozoa was carried out by means of dilution of sperm with uranyl nitrate or acid sea water. The effect of the reagents on eggs was enough to be neglected, because the final concentration of the reagents in the egg suspending medium at the time of insemination was too low to affect the fertilization of eggs as described in a previous paper (Koenuma '56).

The worm eggs were inseminated as follows:

(1) with spermatozoa treated with 5×10^{-4} M uranyl nitrate solution for 5 minutes at pH's 6.9, 7.2, 7.8, and 9.6.

(2) with spermatozoa treated with acid sea water for 5 minutes at pH 6.9, adjusted by the addition of diluted hydrochloric acid to sea water.

(3) once again with spermatozoa treated with acid sea water, 1 minute after the first insemination with normal spermatozoa.

(4) once again with spermatozoa treated with acid sea water, 1 minute after the first insemination with acid sea water treated spermatozoa.

(5) once again with spermatozoa treated with 5×10^{-4}M uranyl nitrate solution, 1 minute after the first insemination with normal spermatozoa.

(6) once again with normal spermatozoa, 1 minute after the first insemination with 5×10^{-4}M uranyl nitrate treated spermatozoa.

(7) with normal spermatozoa as the control experiment.

Results

The results of the experiment of *Urechis* are given in Table 1. Insemination was carried out in normal sea water in each experiment, cleavages of activated eggs being observed at the first and second cleavage stage. Abnormal activation refers to the total number of eggs cleaved abnormally at the first or second cleavage and not cleaveed with clearly polynuclear appearance.

As is shown in Table 1, abnormality of cleavage increased in the cases of insemination with spermatozoa treated with uranyl nitrate or acid sea water. When eggs were inseminated with treated spermatozoa earlier, the percentage of abnormality of cleavage reached high level regardless of the

pretreatment of spermatozoa with which eggs were inseminated later. When eggs were inseminated with normal spermatozoa earlier, however, little abnormality of cleavage occurred, regardless of the pretreatment of the spermartozoa with which the eggs were inseminated later. In alkaline solution, no effect of uranyl nitrate was found.

Table 1. Abnormality caused by the insemination with spermatozoa treated with uranyl nitrate or acid sea water (*Urechis unicinctus*)

Treatment of Spermatozoa	Eggs Observed	Membrane Formation		Cleaving Eggs Normal		Cleaving Eggs Abnormal		Uncleaving Eggs Normal		Uncleaving Eggs Abnormal		Abnormal Activation	
			%		%		%		%		%		%
N. S. W. 5 min. pH 8.2	43	43	100	41	95.3	0	—	2	4.7	0	—	0	—
5×10^{-4}M U. N. 5 min. pH 6.5	46	45	97.8	35	78	4	9	1	2	5	11	9	20
A. S. W. 5 min. pH 6.9	48	46	96	26	54	14	29	2	4	6	13	20	42
5×10^{-4}M U. N. 5 min. pH 7.8	47	47	100	38	81	9	19	0	—	0	—	9	19
A. S. W. pH 6.9 A. S. W. pH 6.9	62	62	100	30	48.5	32	51.5	0	—	0	—	32	51.5
5×10^{-4}M U. N. pH 7.8 N. S. W. pH 8.2	57	57	100	33	58	16	28	0	—	8	14	24	42
A. S. W. pH 6.9 N. S. W. pH 7.8	61	59	97	32	52	3	4	2	3	24	40	27	44
N. S. W. pH 8.2 5×10^{-4}M U. N. pH 7.8	37	37	100	34	94.5	0	—	2	5.5	0	—	0	—
5×10^{-4}M U. N. 5 min. pH 9.6	61	61	100	60	98.3	1	1.7	0	—	0	—	1	1.7

A. S. W. : acid sea water, $\frac{\text{A. S. W.}}{\text{A. S. W.}}$: the first insemination with spermatozoa treated with acid sea water before the second insemination with those treated with acid sea water, $\frac{\text{A. S. W.}}{\text{N. S. W.}}$: the first insemination with spermatozoa treated with acid sea water, before the second insemination with spermatozoa treated with normal sea water, N. S. W. : normal sea water, 5×10^{-4}M U. N. : 5×10^{-4}M uranyl nitrate, $\frac{\text{N. S. W.}}{5\times10^{-4}\text{M U. N.}}$: the first insemination with spermatozoa treated with normal sea water before the second insemination with spermatozoa treated with 5×10^{-4}M uranyl nitrate, $\frac{5\times10^{-4}\text{M U. N.}}{\text{N. S. W.}}$: the first insemination with spermatozoa treated with 5×10^{-4}M U. N. before the second insemination with spermatozoa treated with normal sea water.

Discussion

In present method used for the experiments, the small amount of solution with which spermatozoa were treated was poured into the medium where the eggs were inseminated, but its effect on activation was enough to neglect, because the final concentration of the medium was too low to affect the fertilization process of the eggs. Eggs of *Urechis unicinctus* did not given any abnormality upon fertilization in the medium which contained less than 10^{-5}M uranyl nitrate (Koenuma, '56).

It was reported that when eggs of *Urechis* were activated by various kinds of stimuli, polyspermic aspects of cleavage in which three-cell or four-cell occurred at the time of first cleavage took place in some cases (Tyler '31a, '31b, '32b, Hiraiwa and Kawamura '35, '36, Tylar and Bauer '37). On the one hand those phenomena were interpreted as the participation of the polar body nuclei which remained within the egg cytoplasm for the failure of polar body extrusion in cleavage (Tyler '31b, Tyler and Bauer '37). On the other hand, Hiraiwa and Kawamura described that there occurred the abnormal cleavage in which three or four-cell took place at first cleavage without the failure of the polar body extrusion ('35, '36).

In present results eggs of *Urechis unicinctus* inseminated with modified spermatozoa showed an abnormality resembled a polyspermic cleavage. The increase of abnormality induced by double insemination in which the eggs were inseminated with the modified spermatozoa earlier, then once again inseminated with spermatozoa later was clearly shown. Whether the abnormality of cleavage of *Urecis'* eggs inseminated with modified spermatozoa was caused from the parthenogenetic activation by modified spermatozoa or the abnormality was caused from real polyspermy given from the inhibition of polyspermy blocking mechanism, can not decide from present results. To clarify the cause of the abnormal cleavage induced by insemination of modified spermatozoa, it seems to be necessary to show the histological evidences.

Tyler and Schultz showed that when eggs of *Urechis caupo* were treated with acid sea water within three minutes after insemination, the fertilization process could be reversed, and such eggs could re-inseminated, and showed polyspermic cleavage ('32). Similar phenomenon is also known in the case of *Urechis unicinctus* (Ohkawa '58). Occurrance of abnormal cleavage in the case of *Urechis Unicinctus*, in which the eggs were inseminated with spermatozoa treated with acid sea water, is quite interesting in relation to the experiment of Tyler and Schultz ('32).

There is a paper on polyspermy in rabbit eggs, in which polyspermy increased when the eggs were fertilized by spermatozoa treated with X-ray irradiation (Amoroso and Parkes '47).

Summary

(1) An abnormal cleavage which resembled a polyspermic cleavage was induced by spermatozoa treated with 5×10^{-4}M uranyl nitrate or acid sea water (pH 6.9) at the time of fertilization in *Urechis unicinctus*.

(2) In the cases of the double insemination experiments, it was found that when the first insemination was carried out with spermatozoa treated with 5×10^{-4}M uranyl nitrate or acid sea water (pH 6.9), followed by appreciable number of abnormal cleavage, the second insemination caused a marked increase in abnormal cleavage, whether the spermatozoa were modified or not. When the first insemination was carried out with normal spermatozoa, no increase of abnormal division occurred, regardless of the treatment of spermatozoa at the second insemination.

(3) Whether the abnormal cleavage in the eggs inseminated with modified spermatozoa causes polyspermy or parthenogenetic activation of modified spermatozoa is not clear in present experiments. Because it is known that the abnormal cleavage resembled the polyspermic cleavage occurs in the case of artificial parthenogenetic activation of the eggs of *Urechis*.

References

(1) AMOROSO, E. C. and PARKES, F. R. S. (1947) *Proc. Roy. Soc.* L. B. 134, 57.
(2) HIRAIWA, Y. K. and KAWAMURA, T. (1934) *Kagaku* 4, 245.
(3) —— (1935) *J. Sci. Hiroshima Univ.* B. Div. 1. 4, 35.
(4) —— (1936) *Biol. Bull.* 70, 344.
(5) KOENUMA, A. (1956) *Zool. Mag.*, 65, 281.
(6) OHKAWA, M. (1952) *J. Yokohama Mun. Univ.*, Ser., C-3, No. 4, 1.
(7) —— (1955) *Zool. Mag.* 65, 210.
(8) —— (1958) *Bul. the Yokohama Mun. Univ. Soc.* 10 N. S. 1, 105.
(9) TYLER, A. (1931 a) *Biol. Bull.*, 60, 187.
(10) —— (1931 b) *Biol. Bull.*, 61, 45.
(11) —— (1932 a) *Biol. Bull.*, 63, 218.
(12) —— (1932 b) *J. Exp. Zool.*, 63, 155.
(13) TYLER, A. and BAUER, H. (1937) *Biol. Bull.*, 73, 161.
(14) TYLER, A. and SCHULTZ, T. (1932) *J. Exp. Zool.*, 63, 509.