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SHORT COMMUNICATION

"Indent" Formation and Mitotic Delay of Sea Urchin Eggs Fertilized with X-irradiated Sperms

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When eggs of the sea urchin, *Clypeaster japonicus*, were fertilized with sperm irradiated with 4 kR X-rays, the first cleavage was delayed about 30 min. In addition, the disappearance of the indent and the end of the streak stage (marked by the disappearance of the nuclear membrane) were delayed by the same length as the first cleavage. From these observations, it is apparent that division delay induced by the irradiation is due to the prolongation of the streak stage.

In fertilized sea urchin eggs, the prophase stage of the first cleavage is prolonged by X-irradiation and this is known as radiation-induced mitotic delay[1]. Henshow[2] and Yamashita *et al.*[3] reported through examinations of fixed materials that both in the irradiated eggs fertilized with normal sperm and in the normal eggs fertilized with irradiated sperm, the early prophase is remarkably prolonged, but the late prophase is prolonged a little[3] or considerably.[2] Henshow[2] observed that mitotic delay in the living eggs is also due to prolongation of the streak stage.

In the present observation, it was examined under the light microscope how the progression of the eggs fertilized with the irradiated sperm differs from that of the eggs fertilized with non-irradiated during the first cleavage in *Clypeaster* eggs.

The present observations were performed during summer months of 1972 and 1973. Eggs and sperm of the heart sea urchin, *Clypeaster japonicus* were used. Cytoplasm of the egg is very transparent and available to observe the process of the first division in the living material. Dry sperm was irradiated with a X-ray machine operated at 170 kV and 25 mA with 2 mm aluminium filter. The dose rate was 400 R/min. and the total dose was 4 kR. Non-irradiated sperm served as control. Eggs were inseminated in a culture dish with a dilute sperm suspension within about 30 minutes after the irradiation. The culture dish was set under an inverted microscope and stages of the early development were observed at 20°C. The time when 50 per cent of the fertilized eggs had begun to divide was taken as the "cleavage time."

From the observation of the present study, whole period from the fertilization to the end of the first cleavage was divided into the following eight stages: Stage 1. The sperm head enters into the egg cytoplasm. Stage 2. The sperm aster becomes visible clearly and the sperm pronucleus moves toward the egg pronucleus. Stage 3. Fusion of pronuclei occurs. The sperm pronucleus is lost and the sperm aster becomes prominent. Stage 4. The streak from the sperm aster is formed in the center of egg and

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later lost. Stage 5. Prophase of nucleic division. Two new asters appear on the opposite sides of the nucleus and these asters also look like a streak when the egg is observed from the equator. This streak is observable till the end of the so-called early prophase which indicates the period from the beginning of Stage 5 till the disappearance of nuclear membrane. Stage 6. Metaphase. The chromosomes are alined in the equatorial plate. Stage 7. Anaphase. This stage begins with the separation of chromosomes and ends when they begin to dissolve. Stage 8. Telophase. The chromosomes dissolve and cell divides.

Stage 4 and the early prophase are generally called "streak stage" since a streak which in fact come from the different asters is observed in the center of the egg. It was difficult to distinguish these two kinds of streak in living egg under the light microscope. Consequently, in this observation the beginning of the streak stage was marked. As mentioned above, the streak stage begins a little before the beginning of the early prophase.

When the cell surface was observed, some dents began to appear randomly on whole egg surface just before Stage 3. In this paper, the dent was defined as the "indent." The indents enlarged and became clearly visible at Stage 4 (Fig. 1) and disappeared concurrently with the end of the streak stage. Then the egg surface became smooth at the end of Stage 5. Correlation between surface change and appearance of the streak was very close. It was difficult to determine strictly the beginning of appearance of the indents because this change becomes to be visible very slowly. In contrast, the indents disappeared within a very short time and corresponds to the end of the streak stage (Fig. 2). In degree of the development of "indent" there were some differences among the eggs from the different batches, but no clear differences were found among the eggs from the same batch. The eggs normaly developed to pluteus in spite of the grade of the indents.

Fig. 3 shows the typical results obtained from non-irradiated eggs fertilized with irradiated sperm. In regard to the egg fertilized with the irradiated sperm, radiation-induced delay of the first cleavage was measured to be approx. 30 minutes in comparison with

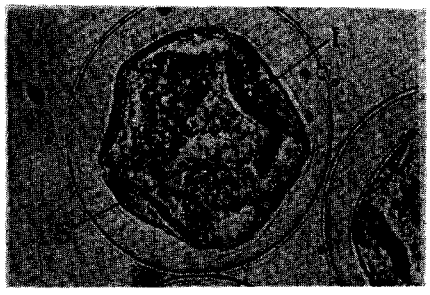

Fig. 1. Microphotograph showing the streak (S.) and indents (I.) of the egg (40 minutes after insemination).

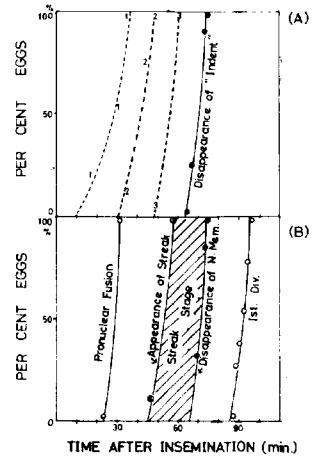

Fig. 2. Per cent eggs showing the indents during the first cleavage (A). Three broken curves indicate the degree of the indents: The beginning of appearance of the indents (—1—). The indents become clearly observable (—2—). The degree of the indents becomes extreme (—3—). Solid curve indicates disappearance of the indents.

The time course of the first cleavage of the eggs fertilized with non-irradiated sperm at 20°C (B).

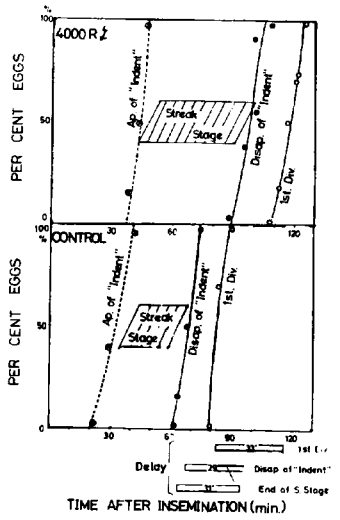

Fig. 3. Diagram showing effects of X-irradiation on the duration of the streak stage, and appearance and disappearance of the indents during the first cleavage.

the control eggs. In addition, the end of the streak stage (marked by the disappearance of the nuclear membrane) and the disappearance of the indent were delayed by the same length as the first cleavage. On the other hand, the beginning of the streak stage and the appearance of the indent were not delayed. Duration from the end of the streak stage or the disappearance of the indent till the first cleavage was not affected by radiation.

In short, it was confirmed by the present observation of the living cells under the light microscope that the X-ray-induced mitotic delay of *Clypeaster* egg results from the blockage of the cleavage in the streak stage, which especially becomes clearer when the indent formation is followed.

The duration of the appearance of the indents was prolonged in the egg fertilized with the irradiated sperm. This exactly corresponds to the prolongation of the streak stage. Therefore, the disappearance of the indent might be an usefull indicator to measure mitotic delay caused by X-ray induced prolongation of the streak stage in the living sea urchin egg. The formation of the "indent" may not be caused by the changes in the conformation of the membrane but resulted from various physical properties during one-cell stage as described by Hiramoto[4].

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