EFFECTS OF RADIATION ON FROG EGGS

I. THE DEVELOPMENT OF EGGS TRANSPLANTED WITH IRRADIATED NUCLEI OR CYTOPLASM

HAJIME SAMBUICHI

Zoological Laboratory, Faculty of Science, Hiroshima University, Hiroshima

Received July 25, 1964.

Concerning the biological damages evoked by ionizing radiation, an interesting problem is to elucidate the relative roles of irradiated nuclei and cytoplasm. It has been reported that in many cases the radiation damages are initially attributable to the irradiated cytoplasm (Miwa and others 1939; Whiting 1949; Duryee 1949; Nakao 1953), while in other cases they are principally due to the irradiated nuclei (Henshaw 1938, 1940; Ord and Danielli 1956 a, b; Errera and others 1958). Meanwhile, it is generally recognized by many investigators that the sensitivity of nuclei for radiation is very much higher than that of cytoplasm (Duryee 1949; Ord and Danielli 1956 a, b; Lasher and Rugh 1962).

The purpose of the present investigation is to elucidate which of the nucleus and the cytoplasm of a frog egg is the place where the main cause for destruction of development is turned out after irradiation. For the sake of analysis, comparisons were made in developmental capacities among three kinds of nuclear transfers which were different from one another in the irradiated part.

Before going further the author wishes to express his deep gratitude to Prof. T. Kawamura for his valuable advice and critical reading of the manuscript. And he also acknowledges the kindness of Prof. S. Tsuda, Faculty of Engineering, for permitting him to the facilities of γ -irradiation apparatus.

MATERIAL AND METHODS

Experiments were carried out in the breeding season of 1963. Eggs of the pond frog, *Rana nigromaculata* Hallowell, were mostly obtained after artificial ovulation induced by hypodermic implantation of frog pituitaries and artificially inseminated. The embryos of the experiment and control series were raised at $21 \pm 0.5^\circ\text{C}$.

Irradiation was made with γ -rays of ^{60}Co : a dose rate was 78.3 r/min at a distance of 18 cm from the source and total 783 r doses were given each egg or blastula. The procedure of nuclear transplantation was similar to that devised by Briggs and King (1952, 1953).

The irradiation of eggs was performed as follows: uterine eggs were laid on

a narrow glass plate, which was inserted into an exposure tube. The latter was packed with cotton in order to protect the eggs from drying during the irradiation. Immediately after the treatment, a half of these eggs were put to use for nuclear transplantation and the other half were made as the control.

The irradiation of blastulae was performed as follows: after their outer gelatinous membranes were removed they were inserted into an exposure tube with a small quantity of water. After the treatment a part of them were made use of a donor for nuclear transplantation; the remainders were allowed to develop as the control.

As shown in Figure 1, the following three kinds of nuclear transplantation were

Fig. 1. Diagram illustrating nuclear transplantation procedure for recombination between normal cytoplasm and an irradiated nucleus or irradiated cytoplasm and a normal nucleus.

made in combination between an egg cytoplasm and a blastula nucleus. 1) The cytoplasm of a normal unfertilized egg was combined with a normal blastula nucleus. 2) The cytoplasm of a normal unfertilized egg was combined with a nucleus of an irradiated blastula. 3) The cytoplasm of an irradiated unfertilized egg was combined with a normal blastula nucleus.

The chromosomes of embryos were examined by making use of the squash method. The developmental stages given by Shumway (1940) in *Rana pipiens* were applied to the present species.

For indicating the three kinds of nuclear transfers, following abbreviations are used in this paper.

Normal cytoplasm and a normal blastula cell nucleus.....NN

Normal cytoplasm and an irradiated blastula cell nucleus.....Nr
 Irradiated cytoplasm and a normal blastula cell nucleus.....rN

RESULTS

1. Control Series

1) Irradiated eggs: Most of the eggs which were irradiated before insemination normally cleaved and developed to the neural stage, although they were slightly inferior in fertilization ratio to the non-irradiated eggs. They were also quite the same with the latter in cleavage rate. Accordingly, any effect of irradiation was scarcely found in these eggs from the cleavage stage to the beginning of neurulation. However, some abnormalities began to appear when they reached the early neural stage. These abnormalities became severer as the development progressed and the developmental velocity of these embryos was extremely suppressed. A remarkable effect of irradiation was firstly found in the stage of neural folds as shown in Figure 2.

Fig. 2. Abnormalities as the neural folds stage of embryos developed from γ -irradiated eggs and blastulae. $\times 9$.

- A. Control embryos.
- B. Embryos from γ -irradiated blastulae.
- C. Embryos from γ -irradiated eggs.

Several percent of these embryos eventually became feeding tadpoles; a small number of them reached the metamorphosing stage about three months after insemination. The developmental rate of these embryos was presented in Table 1, together with that of the irradiated blastulae. Characteristic abnormalities found in them are shown in Figure 3. In the preparations of squashed abnormal embryos, the haploid number (13) of chromosomes was counted (Figure 4).

2) Irradiated blastulae: At the stage 9 about half of blastulae were exposed to γ -rays after their gelatinous membranes were removed. As presented in Table 1, the

Fig. 3. Abnormalities at 4 stages of embryos developed from γ -irradiated eggs and blastulae. $\times 5$.

- A. Normal embryos in the control series.
- B. Abnormal embryos developed from γ -irradiated eggs.
- C. Abnormal embryos developed from γ -irradiated blastulae.

Table 1. Development of γ -irradiated eggs and blastulae

Kind \ Stage	Total no. of eggs	No. of normally cleft eggs	No. of late blastulae	No. of late gastrulae	No. of neurulae		No. of tail-bud embryos	No. of muscular response embryos	No. of heart-beat embryos	No. of hatching embryos	No. of tadpoles
					early	late					
Normal eggs	398	384 (96)	384 (96)	380 (95)	376 (94)	376 (94)	367 (92)	359 (90)	353 (89)	348 (87)	345 (86)
Irradiated eggs	817	776 (92)	775 (92)	737 (90)	713 (87)	570 (70)	290 (35)	155 (19)	80 (10)	53 (6)	46 (5)
Normal blastulae	—	—	368	362 (98)	359 (97)	359 (97)	354 (96)	353 (96)	352 (95)	339 (92)	339 (92)
Irradiated blastulae	—	—	714	696 (97)	629 (88)	462 (64)	406 (56)	261 (30)	14 (2)	0	0

* Number in parentheses is percentage.

removal of the membranes did not seem to affect the developmental capacity of such blastulae. Accordingly, the decline of irradiated blastulae in the rate of development may be entirely attributable to the effects of irradiation. The irradiated blastulae gradually died at the same stages as the irradiated eggs did. There were no embryos developing beyond the heart-beat stage (St. 19). The characteristic abnormalities found in the embryos of this series are shown in Figure 3. In the preparations of squashed embryos, various abnormalities of chromosomes were observed, together with the diploid number of normally shaped chromosomes (Figure 5).

Fig. 4. Photographs of diploid (26) and haploid (13) chromosomes in mitotic figures of embryos. By the squash method. $\times 1500$

A. Diploid control embryos.

B and C. Abnormal embryos developed from eggs γ -irradiated before insemination.

2. Nuclear transplantation series

As described above, three kinds of nuclear transfers, NN, Nr and rN, were obtained. About one half of 120 transfers in the NN series cleaved normally and two-thirds of these cleft eggs became late gastrulae, as presented in Table 2. Some of the latter became abnormal with the progress of development, while less than 20 percent of the transfers of this series eventually grew into feeding tadpoles, most of which completed their metamorphosis.

The Nr transfers were the lowest in the ratio of cleft eggs among three kinds of transfers: they were at the level of about one half, as compared with NN ones. Thirty-five of 134 transfers cleaved normally and less than a half of the cleft eggs became late gastrulae. Eight of the latter completed neurulation and only four reached the muscular response stage (St. 18). No embryos could eventually develop beyond this stage. Abnormal embryos in this series are shown in Figure 6, together with those in the NN and rN series. There were various types of abnormalities including the haploid one.

The ratio of normally cleft eggs in the rN series was higher than that in the Nr: 39 of 118 eggs cleaved normally. However, only one-third of them could reach the late gastrula stage. Five of 14 gastrulae reached the muscular response stage (St. 18); three developed further, differing from the Nr transfers and became normally feeding tadpoles. Two of the latter grew into metamorphosing frogs. As shown

Fig. 5. Normal and abnormal chromosomes in mitotic figures of abnormal embryos developed from γ -irradiated blastulae. By the squash method. $\times 1500$

- A, B, C. Diploid chromosomes which appear normal in shape.
- D. Abnormal chromosomes with many constrictions.
- E. Haploid chromosomes which are abnormally shortened.
- F. Very numerous chromosomes found in one mitotic figure.

Table 2. Development of nuclear transplanted eggs

Kind	Stage	Total no. of transfers	No. of normally clef eggs	No. of late blastulae	No. of late gastrulae	No. of neurulae		No. of tail-bud embryos	No. of muscular response embryos	No. of heart-beat embryos	No. of hatching embryos	No. of tadpoles
						early	late					
Normal cytoplasm and normal nucleus		120	64 (53)	57 (49)	44 (37)	38 (31)	36 (30)	32 (27)	30 (25)	25 (21)	25 (21)	23 (19)
Normal cytoplasm and γ -irradiated nucleus		134	35 (26)	21 (15)	13 (9)	8 (6)	8 (6)	4 (3)	4 (3)	0	0	0
γ -irradiated cytoplasm and normal nucleus		118	39 (33)	26 (21)	14 (12)	9 (7)	7 (6)	6 (5)	5 (4)	3 (3)	3 (3)	3 (3)

* Number in parentheses is percentage.

Fig. 6. Abnormalities at 4 stages of embryos developed by the nuclear transplantation method. $\times 5$

Schematic figures showing the relationship between the nucleus and the cytoplasm in each transfer.

in Figure 6, there was no remarkable difference between the Nr and rN transfers in the kinds of abnormality found in embryos.

DISCUSSION

It has been already known that the biological damage produced by radiations is induced by irradiated nucleus in some cases, while in many other cases it is done by irradiated cytoplasm. Meanwhile, it has been generally recognized in several organisms that the sensitivity of a nucleus to radiation is much higher than that of cytoplasm.

The evidence that the effect of X-irradiation on the mitosis is limited to the nucleus has already been presented in marine invertebrate eggs. In the sea urchin (*Arbacia punctulata*), Henshaw (1938) found that there was no delay in the cleavage of enucleated egg fragments which were irradiated and afterwards fertilized with non-treated sperm. According to his later study (1940) on the cleavage in the same species, the delay of cleavage was brought about by the damage of the nucleus, not by that of cytoplasm, after X-irradiation. On the other hand, Miwa and others (1939) reported in the sea urchin, *Pseudocentrotus*, that delay of mitosis might be induced by the damage of the egg cytoplasm, when unfertilized eggs were irradiated with α -rays, as some of them showed marked delay after fertilization with non-treated sperm.

In *Habrobracon*, Whiting (1949) obtained androgenetic embryos derived from

irradiated cytoplasm and a non-treated sperm nucleus and found that the heavily irradiated cytoplasm killed such embryos. This author (1955) could not obtain the evidence that genetic changes in the non-treated male nucleus might be induced by the irradiated cytoplasm. On the other hand, Nakao (1953) showed that irradiated cytoplasm might bring radiation damages into non-treated male nuclear material in the silkworm eggs irradiated heavily before insemination.

Duryee (1949) examined, by microdissective procedure, the damage which was induced in the nuclei of salamander oocytes after exposure to a high dosage of X-rays. According to him, isolated nuclei immersed in irradiated cytoplasmic brei were damaged as the nuclei of irradiated oocytes were, while they resisted to radiation when they were alone.

Ord and Danielli (1952, 1956 a) searched for an action site of nitrogen mustard in amoebae by using the nuclear transplantation technique. They found that nuclei were much more readily damaged by injured cytoplasm than cytoplasm was by an injured nucleus, that amoebae with damaged cytoplasm usually did not divide, while those with a damaged nucleus could divide several times before death, if they had intact cytoplasm.

It has been known that, when unfertilized eggs are irradiated with X-rays before insemination, haploid embryos are obtained androgenetically (Rollason 1949). Similar haploid embryos can be produced gynogenetically from eggs fertilized with irradiated sperm (Rugh 1939). In the present experiment, androgenetically developed embryos were produced from γ -irradiated eggs by insemination with normal sperm. Although hatched haploid larvae were only six percent of fertilized eggs, most of them became feeding tadpoles. A small number of the latter reached their metamorphosing stage. Accordingly, it is assumed that the egg cytoplasm of such haploid was not damaged by the γ -irradiation, differing from the nucleus. This assumption is also supported by the results of the nuclear transplantation experiments. Some of the eggs in which the cytoplasm had been irradiated and the nucleus was introduced from a non-treated blastula cell developed normally; a few grew into metamorphosing frogs. On the other hand, all the eggs in which the nucleus was derived from an irradiated blastula cell and the cytoplasm was normal were remarkably reduced in developmental capacity; they did not develop beyond the heart-beat stage, just as irradiated blastulae did not. On the basis of these results, damage of the nucleus seems to be the main factor for the hindrance of development in an irradiated frog egg. Similar nucleo-cytoplasmic relationship was found in *Acetaburaria* (Errera and others 1958). The existence of a normal nucleus induced recovery of cytoplasmic damage while a damaged nucleus was not repaired even in normal cytoplasm.

SUMMARY

Frog eggs γ -irradiated before insemination developed normally during the early

stages, and then most of them became abnormal embryos. From the remainings a few metamorphosed haploid frogs and a few non-viable haploid tadpoles were obtained.

The transfers in which the cytoplasm had been irradiated and the nucleus was introduced from a normal blastula cell were very low in developmental capacity, as compared with the control transfers whose cytoplasm and nucleus had not been irradiated. However, two metamorphosed frogs and a tadpole were obtained from among rN transfers.

All the transfers in which the cytoplasm was normal and the nucleus was introduced from an irradiated blastula cell did not develop beyond the heart-beat stage, as irradiated blastulae did not.

LITERATURE CITED

- Briggs, R., and T. J. King, 1952 Transplantation of living nuclei from blastula cells into enucleated frog eggs. *Proc. Nat. Acad. Sci.* **38**: 455-463.
- Briggs, R., and T. J. King, 1953 Factors affecting the transplantability of nuclei of frog embryonic cells. *J. Exp. Zool.* **122**: 485-506.
- Duryee, R. W., 1949 The nature of radiation injury to amphibian cell nuclei. *J. Nat. Cancer Inst.* **10**: 735-795.
- Errera, M., A. Ficq, R. Logan, and Y. Skrebs, 1958 Nucleo-cytoplasmic relationships in irradiated cells. *Exp. Cell Res. (Suppl.)* **6**: 268-726.
- Henshaw, P. S., 1938 The action of X-rays on enucleated and non-nucleated egg fragments. *Am. J. Cancer* **33**: 258-264.
- Henshaw, P. S., 1940 Further studies on the action of roentogen rays on the gametes of *Arbacia punctulata*. I. Delay in cell division caused by exposure of sperm to roentogen rays. *Am. J. Roentogenol. Radium Therapy* **43**: 899-906.
- Lasher, R., and R. Rugh, 1962 The "Hertwig effect" in Teleost development. *Biol. Bull.* **123**: 582-588.
- Nakao, Y., 1953 Action of irradiated cytoplasm on untreated chromosomes of the silkworm. *Nature* **172**: 625-626.
- Miwa, M., H. Yamashita, and K. Mori, 1939 The action of ionizing rays on sea urchin. IV. The effects of alpha rays upon fertilized eggs. *Gann* **33**: 323-330.
- Ord, M. G., and J. F. Danielli, 1952 Site of action of mutagenic reagents, Nitrogen Mustard. *Nature* **170**: 921-922.
- Ord, M. G., and J. F. Danielli, 1956 a The site of damage in amoebae exposed to lethal concentration of methyl-di (β -chloroethyl)-amine (a "nitrogen mustard"). *Quart. J. Microscop. Sci.* **97**: 17-28.
- Ord, M. G., and J. F. Danielli, 1956 b The site of damage in amoebae exposed to X-rays. *Quart. J. Microscop. Sci.* **97**: 29-37.
- Rollason, G. S., 1949 X-irradiation of eggs of *Rana pipiens* of various maturation stages. *Biol. Bull.* **97**: 169-186.
- Rugh, R., 1939 Developmental effects resulting from exposure to X-rays. I. Effect on the embryo of irradiation of frog sperm. *Am. Philos. Soc.* **81**: 447-472.
- Shumway, W., 1940 Stages in normal development of *Rana pipiens*. *Anat. Record* **78**: 139-147.
- Whiting, A. R., 1949 Androgenesis, a differentiator of cytoplasmic injury induced by X-rays in *Habrobracon* eggs. *Biol. Bull.* **97**: 210.
- Whiting, A. R., 1955 Androgenesis as evidence for the nature of X-rays-irradiated injury. *Radiation Res.* **2**: 71-78.