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Effects of ionizing radiation on meiotic maturation of frog oocytes

I. *In vivo* studies

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SUMMARY

In an attempt to examine genomic function of the oocyte nucleus during meiotic maturation, the effects of X-irradiation on the oocyte of the frog (*Rana pipiens*) were studied. When oocytes were irradiated with 6000–36000 R before initiation of maturation, the ovulated eggs frequently failed to be fertilized and the jelly surrounding the unfertilizable eggs was always damaged. However, the egg itself proved to retain the capability of cleaving when a nucleus was transplanted from a blastula. Also, X-irradiated eggs when invested with the jelly from unirradiated frogs recovered their capacity for fertilization. The eggs thus fertilized developed and exhibited abnormalities characteristic of X-irradiation such as arrest of gastrulation and neurulation as well as production of haploidy. Irradiation of oocytes after completion of maturation brought about developmental abnormalities more frequently than irradiation before initiation of maturation. The Hertwig effect was found only when oocytes were irradiated after completion of maturation. However, no qualitative differences were found in the developmental abnormalities produced by irradiation before maturation and those produced by irradiation after maturation.

INTRODUCTION

Meiotic maturation of the oocyte is the terminal process in oogenesis during which the nucleus, the so-called germinal vesicle (GV), completes meiosis. In vertebrates, maturation starts concomitantly with ovulation under the influence of pituitary gonadotropin. However, in amphibians (Schuetz, 1967; Masui, 1967; Smith, Ecker & Subtelny, 1968; Thornton & Evennet, 1969) and fish (Dettlaff & Skoblina, 1969) it was demonstrated that the presence of follicle cells is a prerequisite for the action of gonadotropin to induce maturation, but some steroid hormones such as progesterone can induce maturation without the follicle cells. Consequently, it was proposed that the action of gonadotropin is mediated by release of a progesterone-like hormone from the follicle cells which in turn stimulates the oocyte to initiate maturation (Masui, 1967).

The first visible sign of maturation is germinal vesicle breakdown (GVBD). This is followed by condensation and segregation of the chromosomes and polar

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body formation. Along with these changes of the nucleus, a series of events occurs in the cytoplasm. In amphibian oocytes it has been shown that some cytoplasmic events during maturation take place independently of the nucleus. For example, protein synthesis occurs at the same magnitude in both enucleated and nucleated oocytes (Smith & Ecker, 1969). Also, both with and without the GV, the cytoplasm of maturing oocytes becomes capable of inducing maturation in untreated immature oocytes into which it is injected (Masui & Markert, 1971).

However, the oocytes deprived of a GV before its breakdown never develop even if a nucleus is later supplied by nuclear transplantation (Dettlaf, Nikitina & Stroeva, 1964; Smith & Ecker, 1969). In addition, synthesis of a significant amount of RNA was found to take place in the oocyte during maturation (Brown & Littna, 1964), suggesting that genomic activity of the nucleus takes place during this period. Furthermore, it has been conjectured that this RNA synthesis may play an important role in organization of the oocyte cytoplasm, as for instance in the formation of the germ plasm (Brachet & Malpoix, 1971).

Examination of genomic activity during maturation may be possible by comparing the development of two types of oocytes, one having the nucleus inactivated before initiation of maturation and the other having the nucleus inactivated after completion of maturation. Use of actinomycin D to suppress nuclear activity is not appropriate in this case, because it is difficult to distinguish developmental abnormalities caused by inhibition of genomic activity during maturation from those caused by the action of the chemical, which may still persist in the oocytes after completion of maturation. Inactivation of the genomic function of the GV by X-ray seems to be more adequate. It has been shown that X-ray at appropriate doses, 10000–40000 R (1000 R = 0·258C/1 kg air), preferentially inactivates the nucleus in loach eggs so that eggs irradiated give birth to androgenetic haploids in which the developmental pattern is very much the same as that of gynogenetic haploids derived from unirradiated eggs (Neyfakh, 1956, 1964). Possibly a similar dosage of X-ray could inactivate chromosomes in the frog oocyte while at the same time having a negligible effect on the other structures of the oocyte. If suppression of genomic function of the GV during maturation has a significant effect on the development of embryos, it would be expected that developmental patterns of androgenetic haploids produced by X-irradiation of oocytes prior to maturation would be different from that of the haploids produced by irradiation after completion of maturation. Thus, the role of genomic activity during maturation may be assessed.

Although a number of studies have investigated the effects of ionizing radiation on oocytes and early embryos, very few reports have dealt with meiotic maturation of amphibian oocytes (Rollason, 1949; Dettlaff, 1967). The present study re-examines the effects of X-ray on maturation of frog oocytes with the special aim of obtaining a clue to understanding the role of genomic activity during this period.

MATERIALS AND METHODS

Mature male and female *Rana pipiens* were purchased from dealers in Minnesota and Wisconsin, U.S.A. They were kept in containers with tap water at 4–5 °C until use. X-Irradiation was carried out with a Maxtron 250-X-ray (General Electric) at a dose rate of 2000 R/min (250 kV, 30 mA, HVL 2·25 mm Al, filter 0·25 mm Al). Doses delivered were varied, ranging from 6000 to 36000 R. After anaesthetization the frogs were placed in a rotating plastic bath containing ice and the belly was exposed to X-ray while the rest of the body was covered with Pb plates 5 mm thick. Anaesthetization was accomplished by immersing frogs in 0·03% MS 222 for 20 min. Maturation of oocytes and ovulation were induced by injecting a pituitary together with progesterone into a dorsal lymph sac (Wright & Flathers, 1961).

Transfer of body cavity eggs was carried out at varying times ranging from 18 to 30 h after hormone administration to the frogs. The following procedures were employed. In both donor and recipient frogs after anaesthetization a small incision was made in a dorsolateral area of the body immediately behind the forearm. Eggs were taken out from the donor with a pipette inserted into the body cavity through the incision. They were stained in a Ringer's solution containing 0·02% Nile blue sulfate for 10 min. After washing in Ringer's solution the eggs were introduced into the body cavity of the recipient through the incision with a pipette. The incision was sutured in both donor and recipient frogs before they recovered from anaesthesia.

Frogs were always kept at 18 °C for 72 h after hormone administration and then in the cold (4–5 °C) to prevent over-ripening of the oocytes. Artificial fertilization of uterine eggs was accomplished in a Petri dish containing a suspension of macerated testis in 10% Ringer's solution (Rugh, 1948). Embryos were reared at 18 °C in 10% Ringer's solution. Nuclear transplantation was carried out according to the method of Briggs & King (1953) except that enucleation of the recipient eggs was not performed. Androgenetic haploids were produced according to Porter's method (Porter, 1939) to compare development with those produced by X-irradiation. For chromosome examination a piece of tail tip cut out of an embryo was squashed under a coverslip after fixation with an aceto-orecin solution (DiBeraldino, 1962).

RESULTS

1. *Fertilization of oocytes X-irradiated before initiation of maturation.* Frogs were irradiated 1 or 2 h before hormone administration. Irradiated frogs often showed a skin color change on their backs from dark to light green so that the area exposed to X-ray is easily recognized. The color returns to normal within a few hours after irradiation. Hormones were administered after the irradiated frogs recovered from anaesthesia. At 18 °C all the frogs survived the

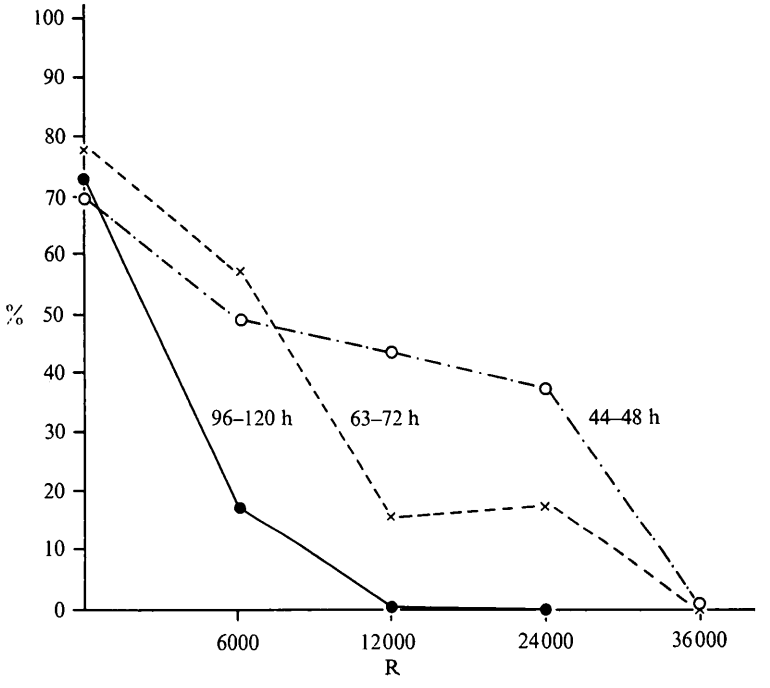

Fig. 1. Fertilizability of the oocytes X-irradiated before initiation of maturation (ovarian oocyte). Ordinate: Overall average of fertilizability. Each point represents more than one female. Fertilizability is then expressed as the average percentage of the oocytes which undergo cleavage after insemination, and for each female this was calculated from six different egg batches, each containing 100 eggs inseminated separately with sperm from different males. Only females which survived irradiation were used as donors. Abscissa: dose of X-ray (R). ○, ×, ●, Oocytes inseminated 48, 72 and 96 h after X-irradiation respectively. X-Irradiation was carried out immediately before hormone administration into mother frogs.

first 48 h, but within the next 24 h they started to die. Mortality was increased with increasing dose of irradiation as well as with increasing post-irradiation time. Between 48 and 72 h after irradiation, 2 out of 7 animals which received 24000 R and 5 out of 7 which received 36000 R died. After transferring to the cold (4–5 °C), still more frogs died. Before the end of the experiment, 120 h after X-irradiation, 2 out of 6 frogs given 6000 R, 2 out of 6 given 12000R, 5 out of 7 given 24000 R and all 7 given 36000 R died. None of the unirradiated control frogs died within this period.

Ovulation took place in all the frogs within 48 h after hormone administration except those irradiated with 36000 R. Of 7 animals given 36000 R, 4 failed to ovulate. Ovulation induced in the frogs which received 24000 R or 36000 R was not complete. Autopsy revealed that a number of oocytes had remained in the ovary. In some cases the oviducts swelled or haemorrhaged, and oocytes were obstructed in the middle of the oviduct.

The eggs from frogs that survived irradiation were tested for fertilizability.

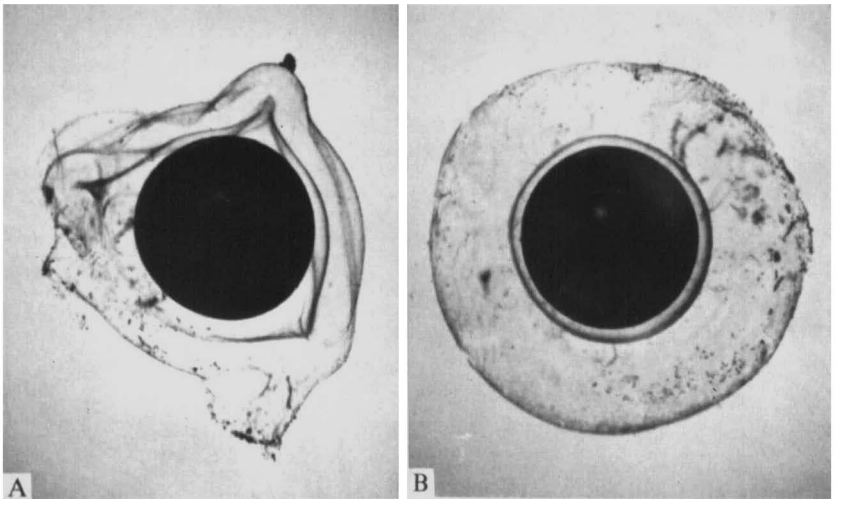

Fig. 2. (A) Unfertilized egg spawned from an X-irradiated frog showing an irregular jelly coat. Dose of X-ray: 24000 R. (B) Unfertilized egg spawned from an unirradiated frog showing a spherical jelly coat ×15.

Six different batches of eggs from the same female were inseminated separately with sperm from different males. The average percentage of the eggs which subsequently underwent cleavage was adopted as an indication of fertilizability. The percentage of fertilizable eggs was then compared among females receiving different radiation doses. Although a considerable variation in the fertilizability of oocytes was found among different individual females receiving the same dosage of irradiation, the overall fertilizability showed a tendency to decrease with increasing dosage of X-ray. This tendency became more acute as fertilization was delayed (Fig. 1). There was no appreciable decrease in the fertilizability of the eggs ovulated from unirradiated frogs.

A remarkable change was found in the jelly structure surrounding the eggs ovulated from irradiated frogs. The jelly of these eggs was usually stickier and swelled more extensively in 10% Ringer's solution than that of unirradiated eggs, and the shape was also different. The former becomes irregular in form after swelling while the latter becomes spherical (Fig. 2).

2. *Fertilization of oocytes X-irradiated after completion of maturation.* Since ovulation was always complete and uterine oocytes were found to be highly fertilizable by 48 h after the hormone had been administered into unirradiated frogs, X-irradiation of mature oocytes was carried out by exposing the frogs to X-ray 48 h after hormone administration. All the frogs were transferred to the cold 72 h after hormone administration and they survived for more than a week. Fertilizability of the irradiated eggs did not decrease within a few hours after X-irradiation as compared to those unirradiated except in those animals irradiated with 36000 R in which a significant immediate decrease was observed (Fig. 3). However, the fertilizability of all the X-irradiated eggs decreased

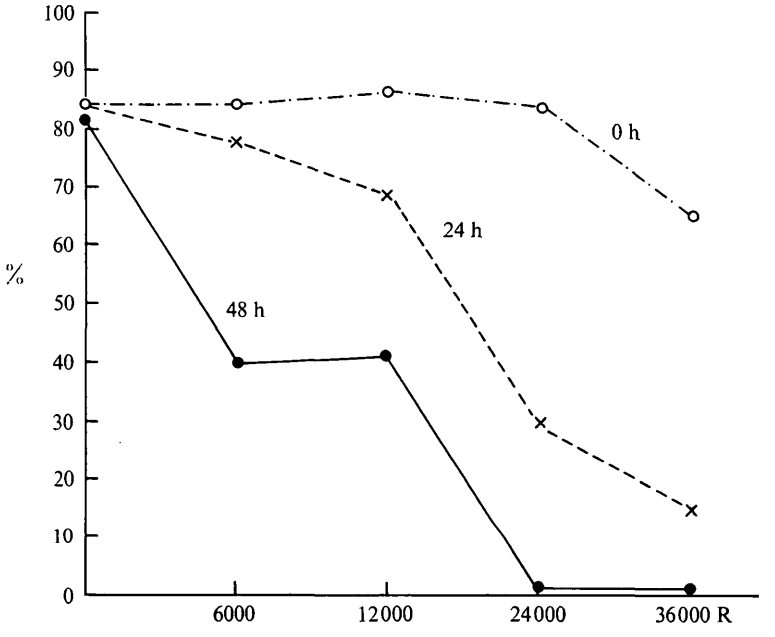

Fig. 3. Fertilizability of the oocytes X-irradiated after complete maturation (uterine oocyte). Ordinate: overall average percentage of the oocytes which undergo cleavage, which was calculated in the same way as in Fig. 1. Abscissa: dose of X-ray. ○, ×, ●, Oocytes inseminated 0, 24, and 48 h after X-irradiation respectively. X-Irradiation was carried out 48 h after hormone administration into mother frogs.

progressively within the next few days. Eggs irradiated with 24000 or 36000 R were no longer fertilizable 96 h after irradiation. The eggs deprived of fertilizability showed the same abnormalities of the jelly coat as described above.

3. *Transplantation of blastula nuclei into X-irradiated eggs.* To test whether the damaged jelly coat is responsible for the failure of fertilization of irradiated eggs, nuclei were transplanted into irradiated eggs which had proved no longer fertilizable by insemination. Recipient eggs were taken from the frogs which had been irradiated with 24000 or 36000 R 48 h after hormone administration and nuclei of normal blastulae were transplanted 96 h post-irradiation.

As indicated in Table 1, both irradiated and unirradiated recipient eggs which received the nucleus underwent cleavage to reach the blastula stage with the same frequency. In both cases, however, successful cleavage was less frequent than that reported by previous workers (Briggs & King, 1952). Probably this is due to the age of the eggs and the procedure adopted in the present experiment which avoided enucleation of the eggs. The result indicated that the irradiated eggs still retained the capability of initiating cleavage if provided with nuclei, while they were unfertilizable by insemination. Therefore it seems likely that inhibition of fertilization of the eggs by X-irradiation is caused by radiation-induced damage to the jelly coat.

Table 1. *Nuclear transplantation into uterine eggs irradiated with X-ray (96 h post-irradiation)*

X-ray dose (R)	Frogs examined	Oocytes examined	Unactivated (%)	Activated (%)	Cleaved (%)	Blastula (%)
			Nuclear transplantation			
0	2	40	0	100	43	35
24000	1	36	17	83	42	33
36000	2	40	5	95	45	35
			Insemination			
0	6	601	10	90	75	72
24000	1	261	100	0	0	0
36000	2	250	100	0	0	0

4. *Fertilization of X-irradiated oocytes after transfer into unirradiated frogs.* The possibility that the infertility of X-irradiated eggs can be alleviated by investing the eggs with normal jelly was tested by transferring body-cavity eggs from X-irradiated frogs into unirradiated frogs. In each transfer 100 eggs stained with Nile blue and from a donor were introduced into a recipient. When eggs were squeezed out from the recipient uterus, approximately 60–80 stained donor eggs were recovered, being found among unstained recipient eggs. They were all stained equally and easily distinguished from unstained eggs. There were no eggs stained at intermediate strength, suggesting that there is no transfer of the dye from the donor eggs to the recipient eggs. Moreover, it was observed that when donor eggs were of a different size than host eggs, the identification of the donor eggs based on the difference in the size exactly coincided with that based on the staining. These facts strongly indicate that the identification of the donor eggs based on their staining was reliable.

The eggs were tested for their fertilizability when the donor and recipient frogs both survived the operation. Some of the donor eggs recovered had been mechanically damaged so that no fertilization was expected and these were discarded from the estimation of fertilizability. The donor eggs recovered from the recipient mother frogs, eggs of the recipient frogs, and eggs squeezed out from the X-irradiated donor were inseminated at the same time with the same sperm suspension.

The oocytes transferred from unirradiated frogs showed no significant change in fertilizability in 3 out of 5 cases (A_1, A_2 and C_1), but a decrease in 2 cases (B_1 and B_2) in which the host's eggs showed lower fertilizability than the donor eggs (Table 2). From these control experiments it is obvious that the transfer procedure itself is rather disadvantageous to the fertilization of the donor eggs if fertilizability of the recipient's eggs is low. Nevertheless, fertilizability of the X-irradiated oocytes was significantly increased after transfer into unirradiated frogs in 5 out of 8 cases. In 2 cases (A_3 and A_4) the host's eggs

Table 2. *Fertilizability of X-irradiated oocytes which had been transferred into unirradiated foster-mother frogs*

Asterisks indicate cases in which increase in fertilizability of X-irradiated ooctyes is significant (statistical significance based on χ^2 test)

Time of insemination after hormone administration	Dose of X-ray (R) delivered to donors	Recipient frog	(*a*) Oocytes of X-rayed donors			(*b*) Oocytes of X-rayed donors transferred into unirradiated recipients			(*c*) Oocytes of unirradiated recipients		
			Oocytes examined	Activation (%)	Cleavage (%)	Oocytes examined	Activation (%)	Cleavage (%)	Oocytes examined	Activation (%)	Cleavage (%)
48 h (18 °C)	0	A_1	206	80	65	57	53	53	56	73	65
		A_2	189	90	73	40	73	73	97	97	95
	12000	A_3	179	72	54	112	78	62	176	60	51
		A_4	156	28	16	78	36	12	114	38	17
	24000	A_5*	172	38	19	73	93	74	206	99	79
72 h (18 °C)	0	B_1	156	100	94	31	90	65	61	82	79
		B_2	201	94	85	25	68	68	60	83	57
	12000	B_3*	32	34	25	35	80	49	109	75	54
	24000	B_4	174	72	38	57	75	37	68	96	84
		B_5*	105	32	10	73	92	81	199	100	89
	36000	B_6*	83	0	0	38	42	34	137	96	80
72 h (18 °C) plus 24 h (5 °C)	0	C_1	183	90	80	105	90	81	156	96	85
	12000	C_2*	126	70	32	63	86	71	60	83	73

Table 3. *Development of X-irradiated oocytes*

(Percentages of embryos were calculated against the total number of eggs which successfully cleaved after insemination.)

X-ray dose (R)	No. of frogs	No. of cleaved eggs	Failure in gastrulation (%)	Arrest at neurula stages (%)	Arrest at tail-bud stages (%)	Abnormal tadpole (%)	Normal haploid (%)	Normal diploid (%)
				X-irradiation before maturation				
6000	3	322	3	5	28	10	21	33
12000	3	292	17	21	16	18	21	7
24000	3	264	13	4	41	23	19	0
36000	3	17	65	35	0	0	0	0
				X-irradiation after maturation				
6000	3	350	67	16	11	0	5	1?
12000	3	466	39	19	34	4	6	0
24000	3	257	19	35	14	4	28	0
36000	2	124	56	9	24	2	9	9
				Unirradiated				
0	4	590	1	0	0	2	0	98

showed poor fertilizability so that no increase in the fertilizability of the donor eggs was expected. Only in one case (B_4) did fertilizability of the X-irradiated eggs remain unchanged in spite of high fertilizability of the host's eggs. In summary, fertilizability of X-irradiated eggs was significantly increased by transferring them into unirradiated hosts in 5 out of 6 experiments in which the fertilizability of the unirradiated host's eggs was comparatively high.

5. *Development of X-irradiated oocytes.* Fertilized eggs obtained from the frogs which had been X-irradiated before or 48 h after hormone administration were raised for 2 weeks. In all cases insemination was carried out 48 h after hormone administration. Almost all of the unirradiated oocytes passed gastrulation without difficulty and reached swimming tadpole stages (Table 3). All the tadpoles displayed diploid characteristics and 58 randomly chosen from 576 animals all had the diploid karyotype.

Most blastulae developed from X-irradiated eggs were arrested before reaching swimming tadpole stages. Blastulae which failed in gastrulation usually formed an amorphous structure consisting of a wrinkled ectodermal cap and a totally exposed yolk-laden mass of endoderm. Embryos arrested at neurula stages showed varying degrees of incomplete gastrulation and neurulation, and no sign of body elongation. In many cases the yolk-plug did not close, and a mass of yolk-laden tissue of various sizes was protruding. Some of them did not form a neural plate, but some formed incompletely or completely closed neural tubes. No strict correlation was found between incompleteness of gastrulation and that of neurulation. Complete gastrulation often occurred without resulting in neural plate formation.

Embryos arrested at tail-bud stages were often found to have suffered from disturbances of gastrulation. The body was usually short and crooked. A part of the endoderm was exposed and the neural tube was bifurcated near the caudal end. Many tadpoles also showed similar abnormalities to those encountered in the embryos arrested at tail-bud stages. Tadpoles without these abnormalities usually started swimming. Most of them exhibited the 'haploid syndrome' described by Porter (1939) and Briggs (1949), having a short trunk bent dorsally and a round belly, and showing a tendency towards edema (Fig. 4), but many tadpoles developed from X-irradiated oocytes were normal diploids.

Chromosomal examination carried out on 9-day-old tadpoles developed from X-irradiated oocytes revealed that 62 randomly chosen from 307 tadpoles with haploid syndrome were all found to have a haploid set of chromosomes (Fig. 5). On the other hand, all 25 chosen from 122 tadpoles which showed diploid external characteristics had a diploid set of chromosomes. Chromosome examination was also carried out with the embryos which were arrested at younger stages of development. However, no mitotic figure was found from any specimen, perhaps because mitotic activity of the embryos had ceased before their development was arrested. Therefore, no information was obtained of the ploidy of the embryos arrested at earlier stages of development.

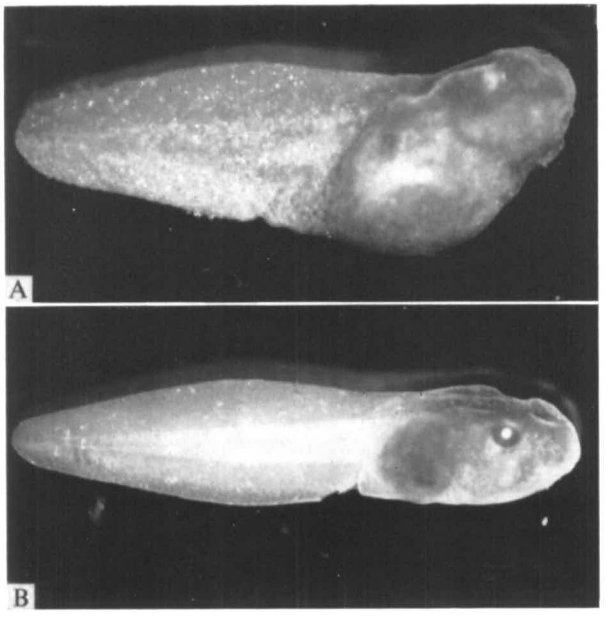

Fig. 4. (A) A haploid tadpole (9 days old) developed from an X-irradiated ovarian oocyte. X-Ray dose is 24000 R (× 12). (B) control diploid (× 10).

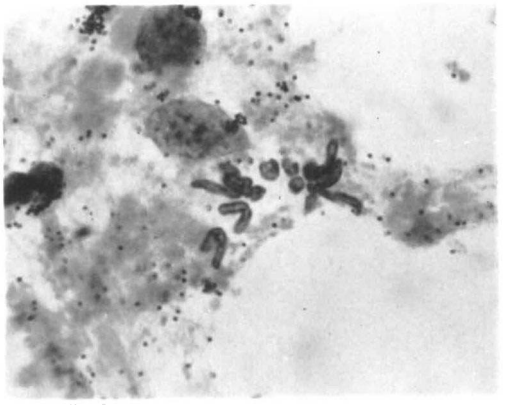

Fig. 5. Haploid set of chromosomes (n = 13) in a specimen of tailtip squash from a tadpole showing 'haploid syndrome' which developed from an X-irradiated ovarian oocyte. X-Ray dose: 24000 R (× 600).

Internal structures of 2-week-old tadpoles were examined by dissection after fixation with formalin. In haploid tadpoles the gut was still heavily laden with yolky cells and coiling had not started but the liver diverticulum had begun to form (Fig. 6). Primordial germ cells were found in all the haploid and diploid tadpoles examined, these being attached to the dorsal wall of the posterior part of the body cavity (Fig. 7).

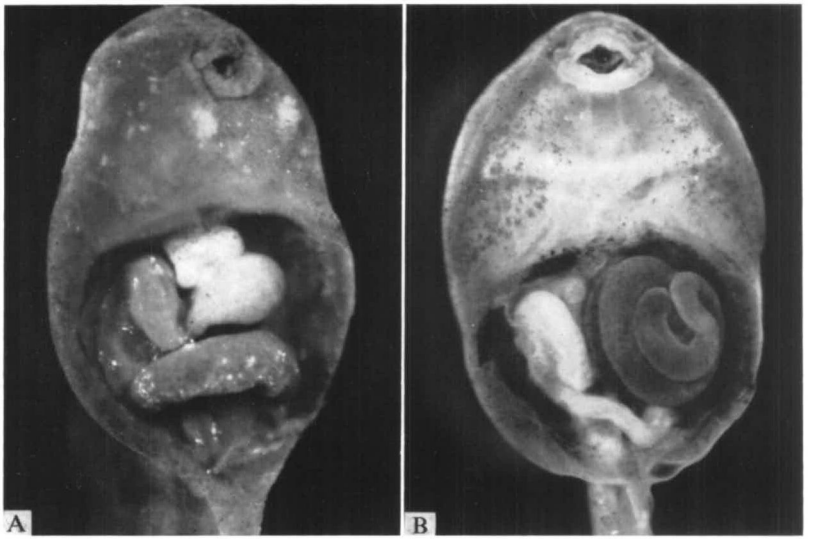

Fig. 6. (A) Internal organs of a haploid tadpole (12 days old) developed from an X-irradiated ovarian oocyte. X-ray dose: 24000 R. (B) Internal organs of a diploid control. ×15.

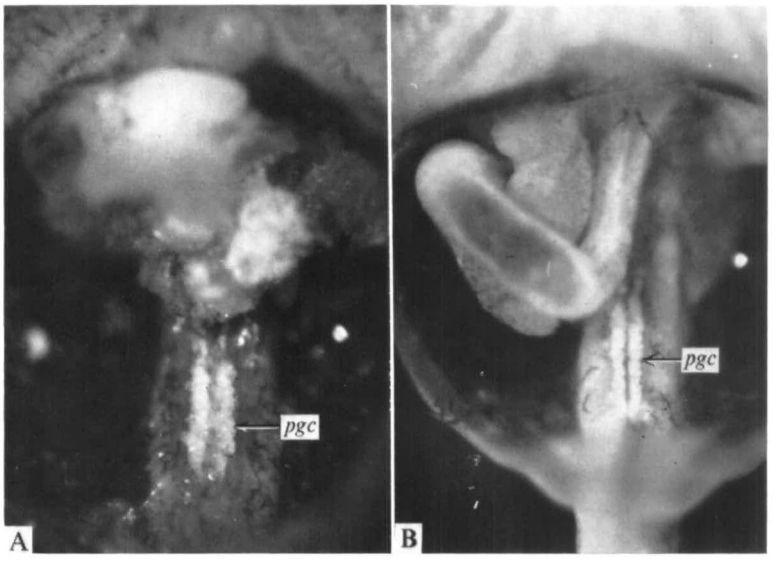

Fig. 7. Primordial germ cells (*pgc*). (A) Haploid tadpole (12 days old) developed from an X-irradiated ovarian oocyte. X-Ray dose: 24000 R. ×25. (B) Diploid tadpole developed from an unirradiated oocyte.

6. *Comparison of development of the oocytes X-irradiated before and after maturation.* As seen in Table 3, when ovarian oocytes were irradiated, about 80 to 90% of the cleaved eggs developed beyond the gastrula stage regardless of the dosage of X-ray delivered so long as the dose was below 24000 R, but

the embryos failed to gastrulate much more frequently at 36000 R. The percentage of the embryos which successfully developed to swimming tadpoles was progressively decreased with increasing dosage of X-ray. On the other hand, when uterine eggs were irradiated at low dosages, most of the eggs failed to gastrulate and a small proportion of the embryos developed into swimming tadpoles. However, with increasing dosage of X-ray, up to 24000 R, the percentage of embryos which failed to gastrulate progressively decreased and the percentage of those developing into tadpoles increased. At 36000 R gastrulation failure occurred very frequently.

Embryos which passed the gastrula stage often ceased their development between the neurula and tail-bud stages. No marked difference was found in the frequency of arrested development between the cases in which immature ovarian oocytes and mature uterine oocytes were irradiated. Many embryos hatched successfully. It was noted that all the tadpoles hatched from the oocytes irradiated after maturation were haploid, whereas those from the oocytes irradiated before maturation were often diploid. The frequency with which haploid tadpoles developed varied with the dose of X-ray. When mature oocytes were irradiated, the highest frequency was obtained at 24000 R, the same dose which brought about the lowest frequency of gastrulation failure. On the other hand, no remarkable variation in the frequency of haploid production was observed when immature oocytes were irradiated although development of diploid tadpoles was progressively decreased with increasing dosage of X-ray.

It was noted that no marked difference in the anatomical characteristics of haploids was found between the cases irradiated before maturation and those irradiated after maturation. Both haploids showed the same degree of gut abnormalities and they suffered from the characteristic edematous condition with similar frequency. All the haploids were found to form primordial germ cells. In addition, as far as the gross anatomical observations are concerned, no difference was found between radiation-induced androgenetic haploids and those produced by manual enucleation.

DISCUSSION

Bardeen (1909, 1911) first described effects of X-ray on ovarian, uterine and fertilized oocytes of *Rana pipiens*. He found that X-irradiation of ovarian and uterine oocytes brought about inhibition of ovulation as well as inhibition of fertilization. Rollason (1949) pointed out that radiation damage to the jelly surrounding eggs was responsible for inhibition of fertilization and it was brought about by X-ray only when irradiation was carried out before ovulation. She conjectured that radiation-induced dysfunction of the oviduct is responsible for the damage to the jelly. In her experiment the observation was made 48 h post-irradiation if irradiation took place before ovulation, but immediately if irradiation was accomplished after ovulation. In the present study the damage

to the jelly was found, whether irradiation was carried out before or after ovulation. However, the damage of the jelly caused by X-irradiation of uterine oocytes became apparent only 48 or 72 h after irradiation. Therefore, the discrepancy found between these results seems to be settled if the difference in the time after X-irradiation at which the observations were made is taken into consideration. This implies that the manifestation of the effect of X-ray on the jelly is delayed for some time after X-irradiation. From the present study it seems likely that radiation damage to the oviduct is not necessarily involved in the damage to the jelly, but rather it may be caused by the direct action of X-ray on the jelly substance itself.

Fertilization failure of X-irradiated oocytes is due mainly to the radiation-induced damage to the jelly. Cleavage can be induced by nuclear transplantation into eggs which were not fertilizable by insemination. This is evidence that the egg itself still retains the capability of initiating development. Furthermore, it was revealed that the fertilizability of X-irradiated eggs was increased to the same level as that of unirradiated eggs if transferred into the body cavity of unirradiated frogs. In this experiment, as pointed out before, the identification of the X-irradiated eggs based on their staining with Nile blue was reliable. Thus, it can be safely stated that the fertilizability of X-irradiated oocytes was recovered after passing them through the body of the foster mother. Thus, it is demonstrated that the loss of fertilizability of oocytes after X-irradiation is not caused by direct action of X-ray on the oocyte itself.

Although this experiment cannot single out a specific cause of the loss of fertilizability, the results described above altogether strongly suggest that it is radiation damage to the jelly which is responsible for the reduction in fertilizability of the X-irradiated oocytes, at least as far as the range of dosages employed in this experiment is concerned. The important role of the jelly coat in fertilization of amphibian eggs is well documented. In *Rana pipiens*, diffusible substances from the jelly are the factors which make the sperm potent while the bulk of structural elements of the jelly plays only a subsidiary role, possibly keeping the diffusible, active factors around the egg (Elinson, 1971). From this aspect, the loss of fertilizability caused by X-irradiation may be due to the loss of the diffusible, active factors through disorganization of the structural elements or through inactivation of the factors themselves.

As to the development of X-irradiated oocytes, a remarkable difference was found between the effect of irradiation on immature ovarian oocytes and that on mature uterine oocytes. It appears that developmental inhibition caused by irradiation of immature oocytes is progressively increased with increasing dosage. On the other hand, X-irradiation of mature oocytes gave a different result – that is, the inhibition of development of irradiated oocytes is decreased with increasing dose of X-ray until a certain dosage is reached. This characteristic relation between radiation dose and effect, called Hertwig effect (Hertwig, 1911), has been found when unirradiated oocytes are fertilized with irradiated sperm. The

Hertwig effect has been considered to be caused by the progressive inactivation of the sperm chromosomes (Rugh, 1939; Pogamy, 1971). It is conjectured that the presence of a disorganized haploid genome has a rather harmful, so-called dominant lethal effect, on the development of the embryo even if another intact haploid genome is present. Thus, if oocytes are subjected to the removal of developmental inhibitions by increasing the dosage of X-ray, it probably depends on both the intactness of the cytoplasm as well as the complete elimination of the disorganized chromosomes. The more intact the cytoplasm and the more the disorganized chromosomes are eliminated, the more frequently the development of viable haploids takes place.

Thus, the Hertwig effect observed when mature oocytes are irradiated may be due to the high radiation sensitivity of the chromosomes in relation to that of the cytoplasm. On the other hand, the absence of the Hertwig effect in the case of irradiation of immature oocytes may be due to either low sensitivity of chromosomes or high sensitivity of the cytoplasm or both. These speculations appear to be substantiated by the following observations. When mature oocytes were irradiated, the tadpoles were always haploids, while many of the tadpoles which developed from the oocytes irradiated before maturation were diploids, indicating that the chromosomes in immature oocytes are better protected against radiation than those in mature oocytes.

The differential sensitivity of chromosomes to radiation during meiotic as well as mitotic cell cycles has been well documented in different groups of animals. Low radiation-sensitivity of GV chromosomes and their increased sensitivity with the progress of meiotic maturation was reported in mice (Spalding, Wellnitz & Schweitzer, 1957) and in the insect (Bozeman & Metz, 1949; Whiting, 1955; Astaurov & Ostriakova-Varshaver, 1957; Koch, Smith & King, 1970; Murakami, 1971). In amphibians, high radiation sensitivity of mature oocytes in the uterus was reported as compared with immature oocytes in the ovary (Rollason, 1949). It was also found that radiation sensitivity of the fertilized eggs depends on the ploidy (Hamilton, 1967) as well as the phase of the cell cycle (Hamilton, 1969). According to this author, diploid nuclei are more resistant than haploid, and nuclei in late interphase and prophase are more resistant than those in metaphase. If this is a rule, then it is easy to understand why oocyte nuclei before maturation are more resistant than after maturation. The oocyte chromosomes are in meiotic prophase before maturation, but after maturation the haploid complement of chromosomes is arrested at meitoic metaphase II. Furthermore, it has been noted that the GV contain a high concentration of SH groups (Brachet, 1939), and while this may protect the immature oocyte chromosomes, mature oocyte chromosomes would lack this protection since GVBD probably results in a dilution of these SH-containing compounds.

On the other hand, it was pointed out that in amphibian oocytes cytoplasmic injury due to radiation was not always negligible (Duryee, 1949; Sambuichi, 1964). A high radiation sensitivity of the cytoplasm of the immature ovarian

oocyte is suggested by the following observation. When mature uterine oocytes were irradiated with 36000 R some viable haploid tadpoles developed, whereas no embryos developed beyond the neurula stage when immature ovarian oocytes were given the same dose. Since no diploid tadpoles developed in either case when the oocytes were irradiated with 24000 R, complete elimination of the maternal chromosomes must have occurred at 36000 R. Therefore, developmental inhibition caused by X-irradiation at this high dosage may be entirely referable to cytoplasmic damage of the oocyte. If so, radiation sensitivity of the cytoplasm of immature ovarian oocytes would be higher than that of mature uterine oocytes. This high sensitivity of the cytoplasm of immature oocytes may contribute to the absence of Hertwig effect in X-irradiation of immature ovarian oocytes.

As to development of haploid tadpoles from X-irradiated oocytes, no marked difference was found between the effects of the irradiation before and after maturation at least as far as their gross anatomical structures are concerned. Also, the haploids which developed from X-irradiated oocytes showed a striking similarity to the haploids which developed from manually enucleated eggs. It seems likely that destruction of oocyte chromosomes before maturation by X-ray does not have a significant influence on cytoplasmic maturation of the oocyte. High resistance of the process of oocyte maturation against ionizing radiation was also reported by Russian workers (Neyfakh & Shapiro, 1962; Dettlaff, 1967). It is known that while actinomycin D inhibits the response of oocytes to gonadotropin (Dettlaff, 1966; Brachet, 1967) it does not inhibit its response to progesterone (Schuetz, 1967), and oocytes injected with actinomycin D can complete meiosis and proceed to cleavage (Smith & Ecker, 1970). Moreover, the results of the present experiment showed that destruction of the oocyte chromosomes by X-irradiation before initiation of maturation does not disturb the development of the embryos at least up to the swimming tadpole stage. From these facts it seems very likely that genomic activity during meiotic maturation of the frog oocyte, if it does take place, is not obligatory either for the maturation process itself or for the further development of the embryos.

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