The Effects of Ultraviolet Irradiation on Uncleaved Eggs of *Xenopus laevis*

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With three plates (figs. 1, 2, and 4)

Summary

The effects are described of ultraviolet (u.v.) irradiation upon the eggs of *Xenopus laevis*. The results obtained apply to fertilized eggs and also to unfertilized eggs into which blastula nuclei have been transplanted. Eggs were irradiated up to 20 min after laying, for periods varying from 15 to 50 sec.

The egg nucleus is completely inactivated by small doses of u.v. If fertilized eggs are used this gives rise to haploids, which the use of a nuclear marker has shown to be androgenetic. After irradiation the egg nucleus descends towards the centre of the egg, and comes to lie adjacent to the transplanted or sperm nucleus. At the first mitosis, however, it does not fuse but remains as a pycnotic clump in the centre of the spindle. Soon after this it disappears, without disintegrating into visible fragments. The transplanted or sperm nucleus appears to be unaffected by the irradiation and death of the egg nucleus.

The egg cytoplasm does not appear to be damaged, even after doses of u.v. which are considerably more than sufficient to kill the egg nucleus. The main reasons for this belief are that haploids obtained by other means develop no better than those obtained by u.v. An increase of u.v. treatment from 30 to 80 sec results in no increase in abnormalities sustained.

The jelly is broken down and the vitelline membrane weakened. This enables the egg to be penetrated by a micropipette without causing damage or preventing healing.

This investigation was undertaken to facilitate the analysis of nuclear transplantation experiments in *Xenopus*. The increased penetrability of eggs is of technical value for this purpose. The interpretation of these experiments is greatly facilitated by the knowledge that the egg cytoplasm need not be damaged by the u.v. and that the egg nucleus is completely inactivated so as not to interfere with the development of the egg.

Introduction

ULTRAVIOLET (u.v.) irradiation of newly laid *Xenopus* eggs has been found to kill the egg pronucleus without damaging the egg cytoplasm. This appears to be the most reliable and least damaging way of 'enucleating' the eggs of this species. The enucleation of eggs is a technique required for many different kinds of experiment; these include the enucleation of fertilized eggs for the production of androgenetic haploids, hybrid merogons, &c., and the enucleation of unfertilized eggs as in nuclear transplantation.

This paper describes the fate of the dying egg pronucleus after the u.v. treatment; this is of considerable importance in the interpretation of nuclear transplantation experiments which have been carried out with *Xenopus*

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(Fischberg, Gurdon, and Elsdale, 1958). The question whether u.v. damage has been sustained by the egg cytoplasm has been investigated.

Methods

U.V. irradiation

The u.v. treatment of unfertilized *Xenopus* eggs has been worked out for the purposes of nuclear transplantation (Elsdale, Gurdon, and Fischberg, 1961). After the jelly has swollen by uptake of water, eggs are placed on a dry glass slide, their animal pole facing upwards; it is important to remove free water from around them since their orientation may otherwise change. The eggs are now exposed to the u.v. beam for the appropriate time (see p. 302).

The u.v. source which has been used is a Hanovia 100-W, medium pressure, mercury arc lamp; it was used without a filter but with a quartz condenser. The eggs are placed about 13 cm from the source which is mounted so as to give a downwardly directed beam.

Enucleation of the egg by microdissection

Porter's (1939) method of enucleating eggs by lifting up the egg-spot with a needle cannot be reliably applied to the eggs of *Xenopus*, since the very elastic vitelline membrane impedes the formation of an exovate. The following method has therefore been developed. It enables eggs to be enucleated with considerable accuracy (97%), but may disturb the organization of the cytoplasm. A tungsten needle is pushed through the cortex of the egg so that its shaft lies just underneath the egg-spot. The needle is then lifted tangentially away from the egg, thus stretching the vitelline membrane, which carries the egg-spot and associated cytoplasm with it. Forceps are used to pinch off an exovate from the egg, and the needle disperses the exovate into the jelly, allowing the vitelline membrane to return to the surface of the egg. The use of forceps enables the size of the exovate carrying the egg nucleus to be easily controlled. Hamilton (1957) has also obtained androgenetic haploids in *Xenopus* by a modification of Porter's technique.

Criteria for determining the success of enucleation or of pronucleus inactivation

In order to determine the efficiency of these methods, fertilized eggs were used, and a resulting haploid individual was assumed to indicate a successful enucleation or irradiation. Though a haploid nucleus is known to occasionally become diploid, this occurs very seldom and the presence of a diploid was taken to indicate a failure of the operation. Chromosome numbers were estimated in different ways. The number of nucleoli per nucleus can be counted with the phase-contrast microscope from squash preparations of embryos. Nucleoli and chromosomes can also be counted after aceto-orcein or haemalum staining of embryos or tail-tips. Because the size of nuclei varies according to developmental stages as well as ploidy, and because the haploid syndrome of abnormalities is sometimes shown by diploids, these

criteria were used as an indication rather than proof of haploidy. Only those embryos which passed the blastula stage are included in the results given below.

In the case of transplant-embryos (i.e. embryos resulting from an irradiated unfertilized egg and an injected nucleus) the successful inactivation of the egg nucleus was confirmed by the use of a nuclear marker (Elsdale, Fischberg, and Smith, 1958; Fischberg and Wallace, in press). In transplantation experiments the donor nucleus possessed the nuclear marker, while the recipient egg did not. Analysis of the ploidy *and* nucleolar number of transplant-embryos shows clearly whether or not the egg pronucleus has participated in development. This only happens rarely, and when it does the transplant-embryos concerned can be excluded from the results.

Experiments and Conclusions

Evidence that u.v. irradiation kills the egg-nucleus and not the sperm nucleus

In order to investigate which pronucleus is inactivated by the u.v., experiments have been made with the *Xenopus* strain possessing a nuclear marker (Elsdale, Fischberg, and Smith, 1958; Fischberg and Wallace, in press). The principle of the experiments has been to mate a normal frog, all of whose (haploid) gametes are uni-nucleolate, to a heterozygous mutant frog of which half the gametes possess no nucleolus at all (o-*n*), and half are uni-nucleolate (1-*n*). The eggs were irradiated soon after fertilization, and all developed as haploids. One of two results is expected from such a cross, according to the way in which the cross is made. The haploid progeny may consist of both o-*n* *and* 1-*n* individuals; the o-*n* embryos could only have been derived from the mutant parent, while the 1-*n* embryos could have come from either parent. The gametes of a mutant parent are present in equal proportions of o-*n* and 1-*n* (Elsdale, Fischberg, and Smith, 1958). Thus if both kinds of embryos are present in about equal numbers in the progeny of this cross, it is reasonable to assume that all the 1-*n* individuals have also come from the gametes of the mutant parent. It is only if the o-*n*s are significantly fewer than the 1-*n*s that one must suspect that some of the 1-*n*s were derived from the non-mutant parent. The other expected result is for all the progeny of the cross to be 1-*n*s, with no o-*n*s present at all. The absence of o-*n*s means that none of the o-*n* gametes from the mutant parent have escaped irradiation, but it is nevertheless possible that some 1-*n* gametes from the mutant parent have contributed to the progeny of the cross. The extent to which this may have occurred becomes smaller, the greater are the number of embryos in the progeny, among which no o-*n*s were found.

In the first experiment to be carried out, a normal female was mated to a mutant male. The progeny of this cross contained 61 1-*n* haploids and 56 o-*n* haploids. These figures do not differ significantly from equality. In this experiment the o-*n* embryos were derived from the male gametes, and there is no reason to believe that any of the 1-*n* embryos were derived from the

egg pronucleus. This experiment was then repeated the other way round, by using a mutant female and normal male; the result was 117 1-*n* embryos and no 0-*n*s. This confirms the result of the previous experiment, since there is again no reason for believing that the pronucleus of any egg escaped u.v. inactivation.

Further confirmation of these results comes from nuclear transplantation experiments in which marked donor nuclei have been injected into unmarked recipient eggs. The nuclear marker shows that the great majority of the resulting embryos were derived from the injected nucleus alone. In less than 1% of cases does the egg nucleus participate in development; the reason for the failure of inactivation on these occasions is not clear, but may be due to rotation of the egg during irradiation, or to the presence of foreign matter overlying the egg nucleus.

The results of these experiments show that u.v. irradiation of the animal hemisphere of the egg selectively kills the egg pronucleus, while this is situated near the surface of the egg. Haploids which develop after such irradiation of fertilized eggs are apparently always androgenetic. The u.v. treatment in these experiments does not seem to affect either the sperm nucleus or the injected nucleus.

The effect of u.v. on the jelly and vitelline membrane of the egg

One effect of u.v. irradiation on eggs is to 'dissolve' the jelly and weaken the vitelline membrane. The latter effect of u.v. is very valuable for nuclear transplantation experiments, since the vitelline membrane cannot otherwise be penetrated by a micropipette without causing considerable damage to the egg. I wish to emphasize that this effect and its value for nuclear transplantation experiments were appreciated in collaboration with Dr. T. R. Elsdale. If too great a dose is given, the vitelline membrane fails to heal after penetration, and a large exovate is formed; too large a dose may also lead to the vitelline membrane drying on to the cortex of the egg, so causing abnormal development. With a moderate dose of u.v. the vitelline membrane heals well, but later breaks open, liberating the embryo—unharmed—a few hours before the usual hatching time. Thus it is necessary to choose an optimum u.v. dose which is sufficient to enable the egg to be penetrated without greatly distorting it, but which is not so great as to prevent healing of the injection wound. The optimum dose largely depends upon the rate at which the jelly 'dissolves' and that at which the vitelline membrane becomes penetrable. The duration of the dose, which must be determined experimentally, varies from 15 to 50 sec for the eggs of different frogs, though it is fairly constant for all eggs of any one ovulation. The reason for this variation is that eggs differ according to the ovulation in the amount of jelly they have, and in the extraneous matter attached to them. It seems to be necessary for the u.v. rays to break down the jelly before they can reach the egg nucleus, and it is probable that even in eggs which require very different optimum doses of u.v., the egg nucleus nevertheless receives the same dose.

The effect of u.v. upon the egg nucleus

Variation of the u.v. dose. The effect of u.v. upon the egg nucleus varies considerably according to the duration of the treatment (table 2), and also according to the length of time between fertilization and irradiation (table 1).

TABLE 1

The effect of u.v. irradiation on eggs at different intervals after fertilization

Time between fertilization and irradiation	*Total embryos analysed*	*Haploid*	*Diploid*	*Mosaics*
(min)				
5–11	31	29	2	0
12–20	53	53	0	0
21–29	23	11	11	1
30–39	23	0	23	0
40–50	18	0	18	0
Controls (no. u.v.)	187	0	187	0

TABLE 2

The effect of different doses of u.v. on the ploidy and survival of embryos derived from irradiated fertilized eggs

	Dose of U.V.				*Controls*	
	20–25 sec	*30–35 sec*	*40–50 sec*	*60–80 sec*	*Microdissection haploids*	*No treatment*
Ploidy of embryos:						
haploid	9	17	10	10	169 (97%)	0
diploid	0	0	0	0	6	34
Survival of embryos:						
total numbers	8	20	15	12	169	127
arrested late blastulae	12½%	10%	26½%	24½%	15%	0·75%
abnormal gastrulae	37½%	10%	..	..	20%	..
arrested neural folds	..	5%	6½%	..	10%	0·75%
slightly stunted post-neurulae	12½%	5%	..	8½%	20%	..
microcephalic or oedematous, (haploid syndrome)	37½%	70%	67%	67%	35%	..
normal tadpoles	..	..	..	..	..	98½%

Table 1, which includes experiments done on the eggs of 3 different frogs, shows that the nucleus of eggs irradiated within 20 min (at 20° C) of fertilization is almost always inactivated. Eggs irradiated more than 20 min after fertilization are often diploid, since the egg pronucleus has sunk far enough below the egg surface to be protected from the u.v. If the egg is not fertilized, the egg nucleus does not sink below the surface at once. Thus there is no need to irradiate unfertilized eggs for subsequent transplantation immediately after laying.

Another important respect in which the u.v. dose can be varied concerns its duration. It was mentioned above that an optimum u.v. dose is selected for each set of eggs according to their penetrability by a micropipette. It is fortunate that this same optimum dose is sufficient in nearly all cases to kill the egg nucleus, thus producing androgenetic haploids from fertilized eggs. It is of interest to find out whether this dose is the minimum capable of killing the egg nucleus or whether a reduction in it would still provide a high proportion of haploids. Table 2 shows the effect of varying the dose above and below the optimum, which in this case was 50 sec.

Table 2 and fig. 5 show that the survival of haploids from eggs irradiated for 30 to 80 sec is very similar, but that many more abnormalities are found among eggs irradiated for 20 to 25 sec. This almost certainly means that 20 to 25 sec of u.v. only partially inactivates the egg nucleus, which can then interfere with development. Embryos in the 20 to 25 sec group, though having only one nucleolus per nucleus, may well have been hyperhaploid, possessing extra chromosomes or chromosome fragments. The fact that the proportion of abnormalities found among the 3 groups of eggs irradiated for between 30 and 80 sec is the same, indicates that 30 sec or more of u.v. completely inactivates the egg nucleus, which does not then affect development. A similar effect was observed by Hertwig (1911) after X-ray treatment of amphibian eggs. Duryee (1949) has X-irradiated amphibian oocytes.

The observation that true haploids are obtained after little more than half the optimum u.v. dose, shows that this dose is more than sufficient for inactivation of the egg nucleus. It is, therefore, very unlikely that any chromosome fragments of the egg nucleus will escape complete inactivation, and then participate in development with the injected or sperm nucleus.

The fate of the irradiated egg nucleus. This investigation is based upon the analysis of 30 irradiated unfertilized eggs into which blastula nuclei were transplanted, and in which the transplanted nucleus appeared cytologically to be developing normally. The majority of these eggs were fixed during the first mitosis of the transplanted nucleus (50 to 80 min old), and the rest were

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FIG. 1 (plate). An arrow (→) indicates the egg pronucleus.

A, egg fixed 12 min after transplantation. The egg pronucleus has completed the second meiotic division.

B, egg fixed 23 min after transplantation, showing the pronucleus moving towards the centre of the egg.

C, egg fixed 25 min after transplantation, showing the egg pronucleus.

D, egg fixed 30 min after transplantation. The pronucleus is still morphologically normal.

E, egg fixed 40 min after transplantation. The dying pronucleus has a crinkled nuclear membrane and granular contents.

F, the next section of the same egg as in E, showing the transplanted nucleus (*n*) and donor cell yolk platelets (*dyp*). Part of the dying pronucleus (shown in E above) can be seen adjacent to the transplanted nucleus.

G, another egg fixed 40 min after transplantation, showing dying pronucleus. Part of the transplanted nucleus can just be seen in this section below the pronucleus.

H, the next section of the same egg as in G, showing the transplanted nucleus (*n*) and the dying pronucleus. The transplanted nucleus has a smooth nuclear membrane and a more homogeneous nucleoplasm.

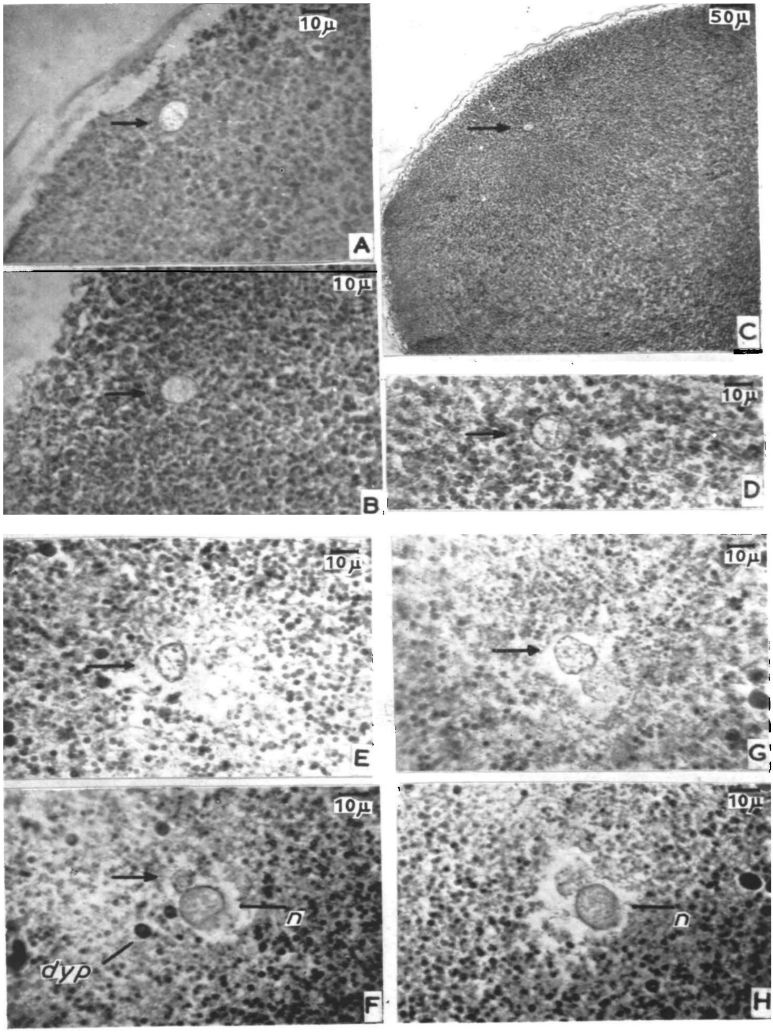

FIG. 1

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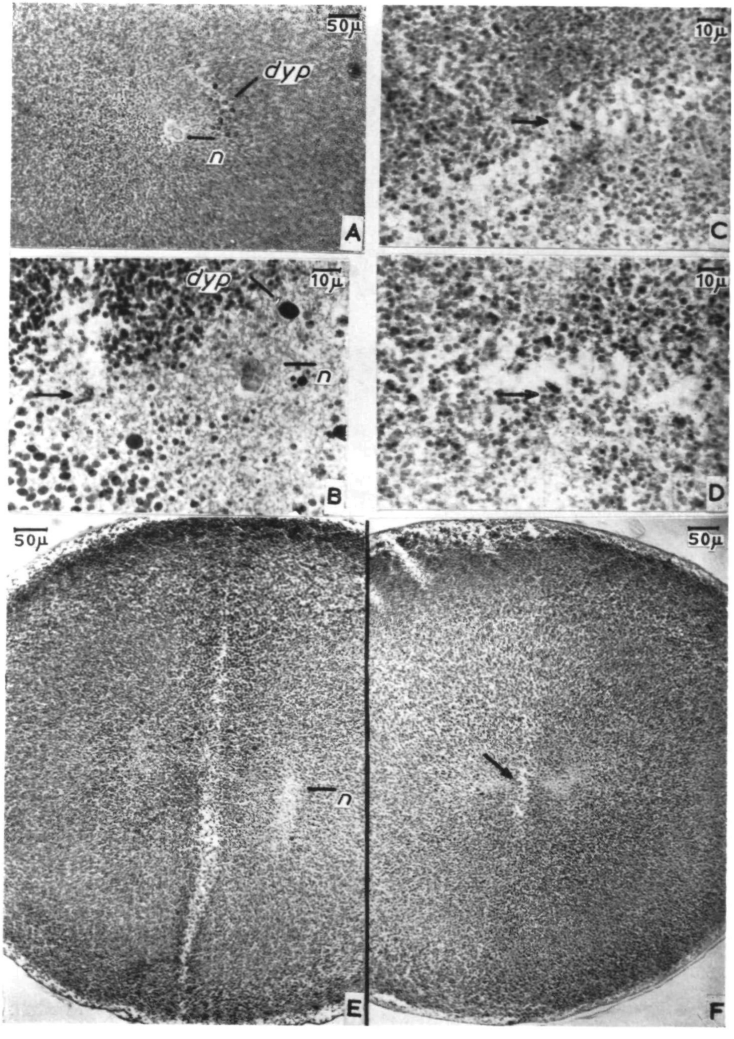

FIG. 2

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fixed at various times between 5 and 50 min after transplantation. As eggs were fixed at intervals between transplantation and the two-cell stage, it is in most cases not possible to tell whether any one egg would have developed at all, if it had not been fixed. In the main series of eggs with transplanted nuclei used for this analysis, 16 of the 20 eggs fixed appeared to have normally developing transplanted nuclei. The remaining 4 eggs had abnormal transplanted nuclei and would probably have cleaved abnormally. Of 28 unfixed control transplantations done at the same time, 6 did not cleave, 4 cleaved abnormally and the remaining 18 cleaved normally and became normal tadpoles. This means that the conclusions reached should be typical for eggs with a normally developing transplanted nucleus and irradiated egg nucleus.

Eggs were fixed in Perenyi's fluid for about 6 h, and washed out overnight in 70% alcohol. Before fixation most of the jelly was removed, and after fixation the vitelline membrane was removed from a small part of the vegetal pole, to allow free passage of fluids into the egg. As the egg is very fragile when fixed, it is desirable to leave the vitelline membrane to protect the rest of the egg. Serial sections at 10μ were cut parallel to the equator; they were then stained with Mayer's haemalum, and sometimes restained with Feulgen.

When analysing these sections the position of the nuclei distinguishes the developing nucleus from the egg pronucleus. However, the following criteria have been used to confirm their identification:

(1) The egg pronucleus is never surrounded by a clear yolk-free area of cytoplasm, which is always associated with the transplanted nucleus (compare fig. 1, A–D with fig. 1, F, H; in fig. 1, E, G the egg nucleus is inside the clear area associated with the developing nucleus).

(2) Nuclei of cells from the vegetal pole of the donor embryo have been used for transplantation; these nuclei have very large (vegetal) yolk platelets associated with them until their first mitosis after transplantation. These large yolk platelets are very conspicuous among the much smaller platelets of that part of the egg in which the transplanted nucleus lies (*dyp* in figs. 1, F and 2, A, B).

The events which take place after the successful irradiation of the egg

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FIG. 2 (plate). An arrow (→) indicates the egg pronucleus.

A, section showing donor cell yolk platelets (*dyp*) and yolk free region of cytoplasm around the developing transplanted nucleus. This is the same section as is shown in fig. 1, H.

B, egg fixed 59 min after transplantation, showing the pycnotic pronucleus and a daughter transplant nucleus (*n*); the latter, which is reforming after the first mitotic division, consists of small spheres at this stage.

C, egg fixed 61 min after transplantation. The pycnotic pronucleus is seen in the centre of the future cleavage furrow.

D, another egg, fixed 58 min after transplantation, again showing the pycnotic pronucleus left between the two daughter transplant nuclei (not visible in this section).

E, egg fixed 76 min after transplantation. One of the daughter transplant nuclei can be seen (*n*), having completed the first mitosis. The egg pronucleus has disappeared by this stage.

F, egg 58 min old; this is the same section as is shown in D, containing the degenerated pronucleus. The transplanted nucleus is at anaphase of its first mitotic division (not visible in this section).

nucleus are shown in figs, 1 and 2. At the time of irradiation the egg nucleus is at metaphase of the second maturation division; in spite of irradiation it completes that division, extruding the second polar body and forming an apparently normal pronucleus. The extrusion of the second polar body has only been observed in a single egg, and it is probable that in some irradiated eggs, the whole metaphase plate sinks into the egg as a diploid egg nucleus which then degenerates. The pronucleus now moves down towards the centre of the egg (fig. 1, A–D), just as it would if it had not been irradiated. 30 min after transplantation the irradiated pronucleus is still cytologically normal. After 40 min the transplanted nucleus has reached the position where pronuclei normally fuse—about one-third of the way from the animal pole to the vegetal pole. The pronucleus joins the transplanted nucleus; by this time it has begun to degenerate, as is apparent from its crinkling nuclear membrane and granular nucleoplasm as opposed to the smooth membrane and more homogeneous nucleoplasm of the transplanted nucleus. (In fig. 1, compare E with F, and G with H. E and F, and G and H show an almost identical situation in two different transplant-embryos.) The egg pronucleus, which remains adjacent to the transplanted nucleus but does not fuse with it, now becomes rapidly smaller and more condensed. About 60 min after injection the transplanted nucleus is at anaphase of its first mitosis. The pycnotic egg nucleus is now left between the two newly formed daughter nuclei in the equatorial plane of the spindle, which is also the plane of the future cleavage furrow (fig. 2, B, C, D, F). In fig. 2, B both the degenerating pronucleus and one of the daughter nuclei of the transplanted nucleus can be seen in the same section. When these sections were restained with Feulgen, no Feulgen-positive material could be seen in the cytoplasm, even in the precincts of the strongly staining pycnotic egg nucleus. This indicates that the egg nucleus does not give rise to chromosome fragments which then become incorporated into the transplanted nucleus. 70 to 80 min after transplantation, the daughter nuclei have reached the interphase stage (fig. 2, E); in 7 embryos of this stage, no trace could be seen of the egg nucleus, which must by now have completely disappeared.

A few fertilized eggs were irradiated and fixed at various times between fertilization and the 2-cell stage. Sections of these eggs showed that the irradiated egg nucleus behaves in the same way as in eggs with transplanted nuclei. The sequence of events taking place in non-irradiated fertilized eggs and in transplant-eggs with irradiated nuclei is shown diagrammatically in fig. 3.

The behaviour of the irradiated egg nucleus described above is very similar to what is already known to apply to the nuclei of tissues that have been heavily irradiated with X-rays or u.v. (Giese, 1950; Hollaender, 1955). The susceptibility of purines and pyrimidines to destruction by u.v. can account for the finding that the egg nucleus is killed long before the egg cytoplasm is damaged. Selman (1958) has described the effect of u.v. on fertilized newts' eggs (*Triturus palmatus*). He found that the same dose of u.v. could cause

incomplete inactivation of the egg nucleus as well as damaging the cytoplasm. The reason for this rather marked difference in the results with *Xenopus* and *Triturus* is not clear, but might be connected with polyspermy.

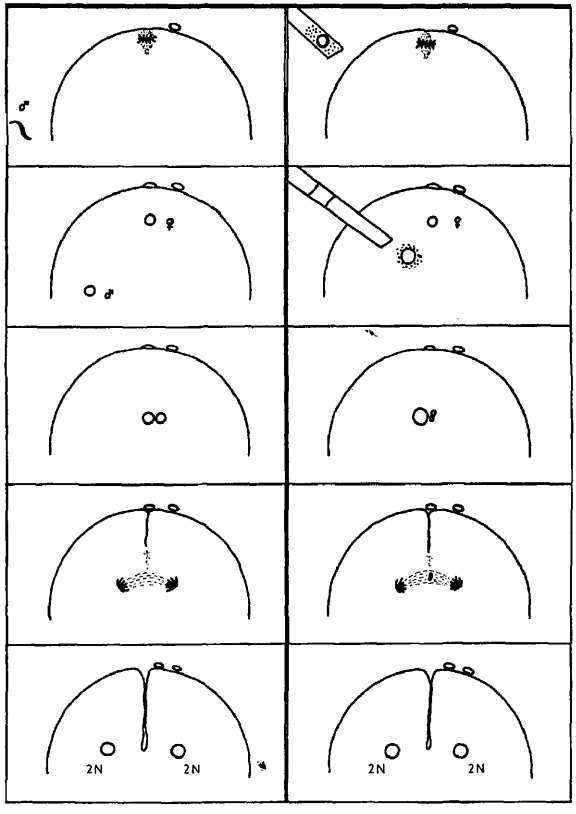

FIG. 3. Diagram comparing the development of normal fertilized eggs with irradiated eggs after nuclear transplantation. Left, normal fertilized egg. Right, u.v. irradiated egg with transplanted nucleus.

The effects of u.v. irradiation on the egg cytoplasm

The optimum dose of u.v. irradiation does not seem to cause any damage to the egg cytoplasm. This cannot, however, be demonstrated clearly by any single experiment, so several different ones are described which all support the same conclusion.

The survival of androgenetic haploids. The extent of u.v. damage can be estimated from the number of abnormalities among fertilized eggs after irradiation. However, eggs irradiated soon after fertilization develop as haploids, and it is difficult to be certain whether the irradiation has caused any abnormalities, since haploids are inherently abnormal owing to their reduced chromosome number. Table 2 and figs 5 and 6 show the frequency of abnormalities which have been found among embryos derived from u.v.-treated eggs, and also among haploids obtained by the microdissection method described on p. 300. In no case did an embryo differentiate further than the microcephalic and oedematous condition referred to as the haploid syndrome (fig. 4). This is generally true of *Xenopus* haploids obtained by other means (Rostand, 1951). Table 2 and Fig. 6 show that 70% of all u.v. haploids irradiated for between 30 and 80 sec reached the typical haploid syndrome. Since these embryos differentiated as far as is usually possible for haploids, this shows that 70% of eggs irradiated with the optimum u.v. dose do not suffer any cytoplasmic damage.

Figs. 5 and 6 show that the remaining 30% of u.v. haploids developed abnormally. These abnormalities may be due to cytoplasmic u.v. damage, or to considerable variation in the way in which haploids differentiate. Variation in the severity of haploid abnormalities would be expected in view of the fact that the haploid syndrome may be affected by exposed lethal genes (Subtelny, 1958). Owing to genetic variation these would be present in more lethal combinations in one spermatozoon than in another. Variation in the differentiation shown by haploids might also be expected to arise from poor quality eggs. Transplantation experiments (Gurdon, 1960) have shown that the quality of eggs varies considerably and poor eggs may result in abnormalities of development.

These considerations do not prove that u.v. has no harmful effect on the egg cytoplasm, but they constitute reasons for attributing abnormalities of development in fertilized irradiated eggs to other causes.

There is a tendency for eggs irradiated within 5 min of fertilization to suffer from more abnormalities of development than those irradiated after 5 min from fertilization. These abnormalities may be due to the chance irradiation of sperm nuclei or to a greater susceptibility of eggs to u.v. at this time. However, this source of abnormality is not thought to affect unfertilized eggs used for transplantation, since these are usually irradiated at about 20 min after laying. By this time they have undergone a kind of 'activation', which is also observed in fertilized eggs. This is recognized by rotation of the egg, and by a contraction of animal pole pigment; the egg cytoplasm also appears to become more solid in consistency.

The effect of increasing the u.v. dose. Table 2 shows that there is no increase

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FIG. 4 (plate). Photograph of androgenetic u.v. haploids (left) and diploid controls of same age (right). The haploids were irradiated for 40 to 50 sec. A similar amount of variation is found among haploid *Xenopus* obtained by other means.

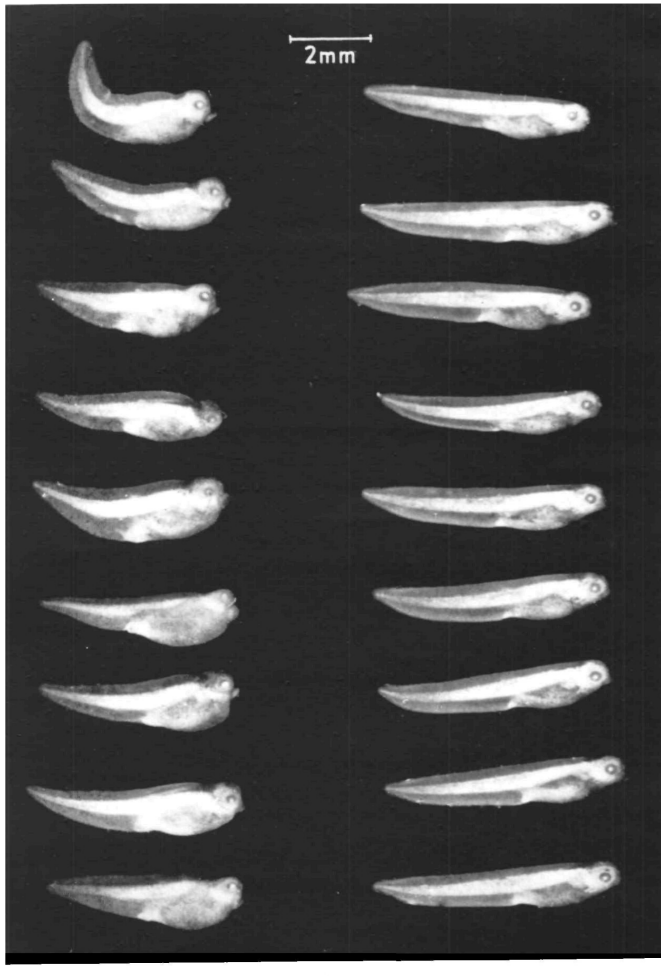

FIG. 4

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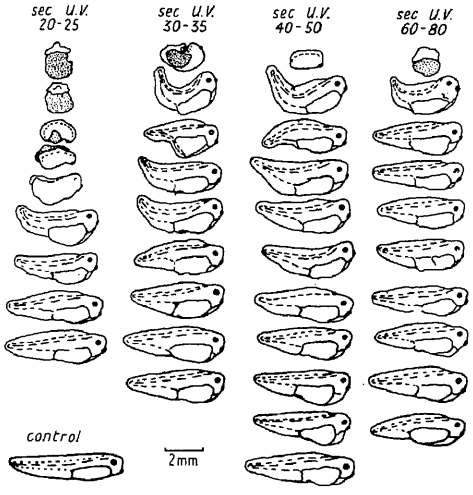

FIG. 5. Camera lucida drawings of haploids resulting from different amounts of u.v. irradiation. Many abnormalities of early development can be seen in the 20 to 25 sec group, while there is a much smaller proportion of early abnormalities in the next three groups.

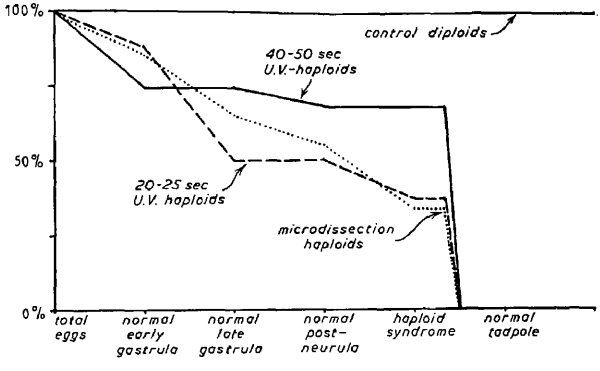

FIG. 6. Survival curves for haploids and controls, based on figures given in table 2. U.v. haploids of the 40 to 50 sec group have a very similar survival curve to those of the 30 to 35 and 60 to 80 sec groups, which are not shown. The method by which haploids are obtained causes different proportions of abnormalities in early development, but the furthest stage of differentiation reached by haploids is always the same.

in the abnormalities shown by u.v. haploids when the u.v. dose is increased from 30 to 80 sec. The egg nucleus appears to have been completely inactivated by 30 sec. The fact that this two to threefold increase in u.v. dose causes no increase in abnormalities is a very strong reason for believing that no cytoplasmic damage was incurred.

The survival of transplant-embryos. The survival of transplant-embryos varies considerably according to the condition of recipient eggs. If an early donor stage with undifferentiated nuclei is used and the recipient eggs are of good quality, it may happen that all transplant-embryos which pass the late blastula stage become normal tadpoles. This shows that at least in these cases the u.v. treatment of the recipient eggs could not have caused any cytoplasmic damage which has any effect after the late blastula stage.

Irradiated fertilized eggs which become diploids. If fertilized eggs are irradiated with u.v. more than 20 min after fertilization, the pronucleus of most eggs has sunk below the surface and is not affected by the u.v. The resulting embryos are therefore diploid and these develop into normal tadpoles. This shows that in these embryos the u.v. causes no cytoplasmic damage.

Eggs which are irradiated for 10 to 20 min after fertilization on the vegetal pole as opposed to animal pole, are diploid and suffer no externally visible abnormalities.

Conclusions of importance for the interpretation of nuclear transplantation experiments

The main purpose of this investigation has been to analyse the effect of u.v. irradiation on unfertilized eggs which were subsequently used for nuclear transplantation. The following comments therefore draw attention to the conclusions from this work which are particularly relevant to the interpretation of nuclear transplantation experiments. An optimum u.v. dose is chosen for the eggs of each female frog, such that a pipette can be pushed into the egg without causing damage or preventing healing. This dose has been found to be nearly double the minimum required to inactivate the egg nucleus completely. This shows that the egg nucleus is very thoroughly inactivated before the egg is provided with a transplanted nucleus. In the case of unfertilized eggs there is no need to irradiate them immediately after laying, since in contrast to fertilized eggs the egg nucleus remains on the surface. When the pronucleus degenerates it appears not to disintegrate into visible fragments, but to disappear gradually as a unit; this makes it unlikely that fragments from the pronucleus become attached to the developing transplanted nucleus and affect the future development of the egg.

Concerning u.v. damage to the egg cytoplasm, it is worth pointing out that in fertilized eggs irradiated for between 40 and 80 sec, only about 8% of eggs showed abnormalities after the late blastula stage (table 2). This means that with the u.v. doses used for nuclear transplantation experiments, most of the damage which might be attributed to u.v., results in arrested late blastulae. For most of our transplantation experiments it is desirable to consider only

those transplant-embryos which develop beyond late blastulae. Reasons have already been given (pp. 307–10) for believing that the egg cytoplasm suffers very little u.v. damage with the doses used; but in any case most of the developmental abnormalities which might be attributed to u.v. do not interfere with the interpretation of nuclear transplantation experiments.

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REFERENCES

DURYEE, W. R., 1949. 'Nature of radiation injury to amphibian cell nuclei'. J. nat. Cancer Inst., **10**, 735.

ELSDALE, T. R., GURDON, J. B., and FISCHBERG, M., 1961. 'A technique for nuclear transplantation in *Xenopus laevis*.' In press.

—— FISCHBERG, M., and SMITH, S., 1958. 'A mutation that reduces nucleolar number in *Xenopus laevis*.' Exp. cell Res., **14**, 642.

FISCHBERG, M., GURDON, J. B., and ELSDALE, T. R., 1958. 'Nuclear transfer in Amphibia and the problem of the potentialities of the nuclei of differentiating tissues.' Ibid., Suppl., **6**, 161.

—— and WALLACE, H., 1960. In press.

GIESE, A. C., 1950. 'Action of ultraviolet radiation on protoplasm.' Physiol. Rev., **30**, 431.

GURDON, J. B., 1960. 'Factors responsible for the abnormal development of embryos obtained by nuclear transplantation in *Xenopus laevis*.' J. Embryol. exp. Morph. In press.

HAMILTON, L., 1957. 'Androgenetic haploids of a toad, *Xenopus laevis*.' Nature, **179**, 159.

HERTWIG, G., 1911. 'Radiumbestrahlung unbefruchteter Froscheier und ihre Entwicklung nach Befruchtung mit normalem Samen.' Arch. mikr. Anat., **77**, 165.

HOLLAENDER, A. (editor), 1955. *Radiation biology*. Vol. 2, *Ultraviolet and related radiations*. New York (McGraw Hill).

PORTER, K. R., 1939. 'Androgenetic development of the egg of *Rana pipiens*.' Biol. Bull., **77**, 233.

ROSTAND, J., 1951. 'Parthénogénèse experimentale chez le xenope (*Xenopus laevis*).' C.R. Soc. biol. Paris, **145**, 1453.

SELMAN, G. G., 1958. 'An ultra-violet light method for producing haploid Amphibian embryos.' J. Embryol. exp. Morph., **6**, 634.

SUBTELNY, S., 1958. 'The development of haploid and homozygous diploid frog embryos obtained from transplantations of haploid nuclei.' J. exp. Zool., **139**, 263.